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Andrés Díaz Lantada *Editor*

Microsystems for Enhanced Control of Cell Behavior

Fundamentals, Design and Manufacturing Strategies, Applications and Challenges

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Editor
Andrés Díaz Lantada
Mechanical Engineering
Universidad Politecnica de Madrid
Madrid
Spain

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To my beloved Melike,
How can my muse want subject to invent,
While thou dost breathe, that pour'st into my verse
Thine own sweet argument, too excellent
For every vulgar paper to rehearse?
O! give thy self the thanks, if aught in me
Worthy perusal stand against thy sight;
For who's so dumb that cannot write to thee,
When thou thy self dost give invention light?
Be thou the tenth Muse, ten times more in worth
Than those old nine which rhymers invocate;
And he that calls on thee, let him bring forth
Eternal numbers to outlive long date.
If my slight muse do please these curious days,
The pain be mine, but thine shall be the praise.

William Shakespeare

To our daughter Seda,
If you can keep your head when all about you
Are losing theirs and blaming it on you;
If you can trust yourself when all men doubt you,
But make allowance for their doubting too:
If you can wait and not be tired by waiting,
Or, being lied about, don't deal in lies,
Or being hated don't give way to hating,
And yet don't look too good, nor talk too wise;

If you can dream, and not make dreams your master;
If you can think, and not make thoughts your aim,
If you can meet with Triumph and Disaster
And treat those two impostors just the same…

…If you can fill the unforgiving minute
With sixty seconds worth of distance run,
Yours is the Earth and everything that's in it,
And—which is more—you'll be a Woman, my Daughter!

Rudyard Kipling

Preface

Last decades have seen remarkable advances in computer-aided design, engineering and manufacturing technologies, multi-variable simulation tools, medical imaging, biomimetic design, rapid prototyping, micro- and nano-manufacturing methods, and information management resources, all of which are expanding the horizons of most biomedical engineering fields and the related medical device industry. Emerging areas such as tissue engineering and biofabrication, which depend on biomedical microsystems or microdevices capable of interacting with cells and tissues, such as cell culture systems, scaffolds, and advanced implants, are directly bound to these technological advances.

The present book covers such topics in depth, with an applied perspective and providing several case studies that help to analyze and understand the key factors of the different stages linked to the development of biomedical microsystems aimed at interacting at a cellular level, from the conceptual and design steps, to the (rapid) prototyping, validation, and industrialization phases. Main current research challenges and future potential are also discussed, taking into account relevant social demands and a growing market already exceeding billions of dollars. In time, advanced biomedical microdevices will decisively change procedures and result in the medical world, dramatically improving diagnoses and therapies for several types of pathologies. But if these biodevices are to fulfill present expectations, today's engineers need a thorough grounding in related design, simulation, testing and manufacturing technologies, and intimate cooperation between experts of different areas has to be promoted, as is also analyzed within this handbook.

The text is aimed at anyone working or simply interested in biomedical engineering, in the tissue engineering and biofabrication areas and in the medical devices industry, including physicians, scientists, and industrial, biomedical, chemical, electrical, and materials engineers. It is also a comprehensive introduction for students studying biomedical engineering at masters level, as well as for researchers planning to carry out a Ph.D., linked to the development of biomedical microdevices for interacting with cells and tissues, and pursuing improvements in tissue engineering materials, methods and (bio)devices, towards

effective biofabrication strategies. Designed for maximum readability, without compromising scientific rigor, this handbook provides a broad overview of these rapidly evolving disciplines, also discussing main breakthroughs and expectations.

I truly hope it might be of help for students and researchers and even motivate them to follow some of the research directions outlined.

Madrid
December 2015

Andrés Díaz Lantada

Acknowledgements

No book is ever the product of one person's efforts, and certainly this one was no different. It would never have become truth without the help and suggestions of many supportive relatives, friends, and colleagues, only a proportion of which I have space to acknowledge here.

I owe a great deal to my colleagues and students at Universidad Politécnica de Madrid who through their own research, comments, and questions have encouraged, supported, and enlightened me. It has been a pleasure to work together with them in research tasks, which have led to interesting solutions detailed in several chapters of the handbook.

Professor Dr. Pilar Lafont Morgado has enlightened my career with her particular conception of university, in its broadest sense, and of the connections between teaching, learning, and research. Professor Dr. Josefa Predestinación García-Ruíz has been a cheerful co-worker and I have learned from her experiences and wisdom, not only about cells, but very especially about life. Professors Alisa Morss Clyne and Jürgen Stampfl gave me the opportunity of living extraordinary experiences in their research labs, at Drexel University and at the Technical University of Vienna respectively, and made me feel at home, while helping me to expand my horizons. Professor Jose Luis Endrino has been an admirable research colleague and is now a good friend, always ready for interesting discussions and hands-on tasks.

I am very grateful to Mr. Pedro Ortego García, whose expertise has been of great help for the manufacture and trials of many of the prototypes included, as case studies, in the different chapters of the handbook. Researchers including Hernán Alarcón, Miguel Ángel de Alba, Adrián de Blas, Gillian Begasse, Alberto Bustamante, Enrique Colomer, Diego Curras, Sébastien Deschapms, Graciela Fernández, Axel Michel, Javier Mousa, Beatriz Pareja, Miguel de la Peña, Jeff Resnick, Miguel Téllez, Santiago Valido, and Beatriz del Valle have supported me with engineering tasks.

Of course my parents, Andrés and Piedad, with a whole life of dedication and love, have been watching me in every breath I took and looking after me in every step I took. I hope they are proud of their son.

Above all my wife Melike Erol (the essence of joy made person) and my daughter Seda (whose laughter is continuous source of inspiration) are my life.

Madrid Andrés Díaz Lantada
December 2015

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About the Editor

Andrés Díaz Lantada studied Industrial Engineering and specialized in Mechanical Engineering at Universidad Politécnica de Madrid (UPM), Spain (www.upm.es). He obtained his Ph.D. in Mechanical Engineering in 2009 with a thesis, directed by Chair Prof. Dr. Pilar Lafont, on "Methodology for the structured development of medical devices based on active polymers," which received UPM Extraordinary Prize and Second Prize from The Official Association of Industrial Engineers of Madrid. He has worked for 10 years as researcher at the Mechanical Engineering Department of this university and collaborated actively with its Product Development Laboratoty, both in research and teaching tasks. During 2011 spring–summer, he worked at the Institute of Materials Science and Technology at the Technische Universität Wien, as a postdoctoral researcher in the Additive Manufacturing Laboratory under the guidance from Prof. Dr. Jürgen Stampfl, for the development of biomimetic microsystems for tissue engineering. In 2013 he carried out his postdoctoral stay at the Vascular Kinetics Lab at Drexel University, under the guidance of Prof. Dr. Alisa Morss Clyne, where he collaborated as visiting research professor and took part in biomedical microsystem projects linked to modeling the blood–brain barrier.

Now he works as Associate Professor Doctor at UPM and teaches subjects, both at graduate, postgraduate (Industrial Engineering Degrees) and doctoral levels (Masters and Ph.D. programs on mechanical engineering and Masters and Ph.D. programs on biomedical engineering), as well as specialization courses. His main teaching activities are related with the subjects "Computer-aided Mechanical Engineering," "Design and Manufacturing with Polymers," "Development of Medical Devices," and "Biomechanics & Bioengineering." At the same time he is actively researching in different areas related to product development, specially focused on medical devices, including rapid prototyping technologies, CAD–CAE–CAM tools and active materials for improving diagnostic and therapeutic applications of biodevices. Recently he is aiming at exploring novel ways of interacting at a cellular level with the help of advanced design and manufacturing tools.

He has been a researcher in six public national research projects funded by the Spanish Ministry or Science and Education and by Madrid's Regional Government, including the Spanish Singular Strategic Project "IBE-RM" on rapid manufacturing, in two international projects for the development of medical devices funded by Chile and Peru Research & Innovation Ministries, in six private-funded research projects, and in 16 teaching innovation projects, mainly linked to the implantation process of the "European Area of Higher Education" and to project-based learning (PBL) activities. Currently, he participates as UPM Coordinator in the European Project "ToMax" (Factories of the Future), led by Prof. Dr. Jürgen Stampfl.

He is co-founder of the UPM—Research Group on "Machines Engineering" (since 2007) and of the UPM—Innovative Teaching Group for "Integrated Mechanical Engineering Teaching" (since 2006), both groups under the leadership of Chair Prof. Dr. Pilar Lafont. Among some teaching proposals he has edited a special number on "Learning through Play in Engineering Education," a special number on "Impact of collaboration between Academia and Industry on Engineering Education" and a special number on "Engineering Education: Beyond Technical Skills," the three of them for the International Journal of Engineering Education. He is currently focused on promoting collaborative research in the field of tissue engineering and biofabrication and acts as UPM contact researcher in the European Virtual Institute of Knowledge-Based Multifunctional Materials and in the COST Action NEWGEN: New Generation Biomimetic and Customized Implants for Bone Engineering.

As a result of his research activities, Andrés Díaz Lantada has been awarded the following prizes: Medal of the Spanish Royal Academy of Engineering for young researchers under 40 years, 2015; UPM Young Researcher Award, 2014; UPM Educational Innovation Award, 2014; Prize to the Best business ideas from the Actua-UPM Spin-off Creation Programme, UPM (among 250 ideas); Second Prize to the Best business plans from the Actua-UPM Spin-off Creation Programme, UPM (among 80 business plans); Ph.D. Extraordinary Prize from the ETS Industrial Engineering at Universidad Politécnica de Madrid, 2010; Second Ph.D. Prize from the Association of Industrial Engineers of Madrid, 2011.

Dr. Andrés Díaz Lantada has published more than 45 papers indexed in journals from the JCR and more than 125 papers in conference proceedings, with more than 450 citations in the last 5 years (h-index of 11, i10-index of 12), according to Google scholar. He has also published several books (4) and book chapters (42) including, as main author, the "Handbook on Active Materials for Medical Devices: Advances and Applications" (2012, Book Citation Index, 1st edition 25,000 copies), under appointment by PAN Stanford Publishing, and the "Handbook on Advanced Design and Manufacturing Technologies for Biomedical Devices" (2014), under appointment by Springer, (with 1,000 copies recently translated into arabic). He is co-inventor of 10 patents related to the use of active materials for improving sensing/actuating capabilities of medical products and with improved solutions for tissue engineering. He has organized and chaired the Special Session on "Active Materials for Medical Devices" and the Special Session on "Rapid Prototyping for Improving the Development of Biodevices" at the

Biostec—Biodevices 2009 and 2010 “International Conferences on Biomedical Electronics and Devices,” promoted by the IEEE-EMBS. He has been co-chair of the 5th Industrial Workshop of the European Virtual Institute on Knowledge-based Multifunctional Materials.

Part I
Fundamentals

Chapter 1
Some Introductory Notes to Cell Behavior

Andrés Díaz Lantada

Abstract The cell is the most basic functional, structural and biological unit of life as we understand it. Cells are able to perform coherent functions and make up all tissues and organs of all living multicellular organisms; in consequence, there are usually referred to as life's building blocks. They can replicate independently and contain the hereditary information necessary for regulating cell functions, including growth, metabolism, apoptosis, protein synthesis, movement and replication, for transmitting information to the next generation of cells. As present handbook will focus on the development strategies for biomedical microsystems aimed at interacting with human cells, which constitute our tissues and organs and play a fundamental role in health and disease, it is important to provide a brief introduction to the cell as complex multi-scale and multi-physical/chemical living organism, to its structure and subunits and to its main functions, before focusing on the field of biomedical microsystems. In addition, as tissue repair and tissue engineering are directly connected to stem cells and several chapters of present Handbook will cover development strategies for biodevices linked to tissue engineering, which constitute a very relevant part of the biomedical microsystems designed for interacting at a cellular level, it is very important to provide an introductory note to cell types and to cell differentiation processes. This chapter pretends to serve as introduction to the handbook, as regards the nature, structure and main types and functions of cells.

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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Personal note

My first approach to The Cell, as the fundamental unit for life, took place in our living room at home, when I was a 12-year old student facing the challenging 6th school year, in which for the first time we had different teachers for each of our subjects.

The Natural Sciences teacher, Prof. José María Piñero (who was also in charge of Sports Education and somehow managed discipline among us), was fond of project-based learning methods and of students' personal implication in their own learning process. One day he told us that we should prepare a 20-page written report with hand-made illustrations on a very special topic: the eukaryotic cell.

We had only 2 weeks for handling the report and the task seemed overwhelming; it was our first serious project and, at that time, we did not count with the internet as a valuable resource for the rapid, easy and cheap access to almost universal knowledge.

However I found the help of a sorcerer: my father Andres Diaz Fernandez, Doctor in Medicine, Professor of Internal Medicine at the Complutense University of Madrid, and passionate for medical research and practice. He helped me to structure and to write that first project (and many subsequent ones), he introduced me to the differences between prokaryotic and eukaryotic cells, he highlighted the marvels of the cellular microcosmos, with a basic but rigorous approach, including the search and reference of relevant information sources.

The final result was nice, but the best part of the project was its implementation, between several books of Biology and Medicine and with the support of a 20-year collection of the Lancet and the New England Journal of Medicine, working at home, but conscious of starting something special, which would turn out to be a vocation for science and discoveries.

Now, almost three times older, I face a similar challenge: trying to introduce some relevant aspects about the cells and their behavior. My background is in the field of Industrial and Mechanical Engineering and my limited understanding about cells and their phenomenology has been recently acquired, as a consequence of my interest for Biomedical Engineering, through the development of biodevices for studying cells and thanks to stimulating conversations with colleagues.

The task is in any case complex, but I will follow the same approach I learned with my father and proceed step by step, as in the poem from Machado: "... caminante no hay camino, se hace camino al andar."

1.1 The Cell: A Complex Multi-scale and Multi-physical/Biochemical Living System

The cell is the most basic functional, structural and biological unit of life as we understand it. Cells are able to perform coherent functions and make up all tissues and organs of all living multicellular organisms; in consequence, there are usually referred to as life's building blocks. They can replicate independently and contain the hereditary information necessary for regulating cell functions, including growth, metabolism, apoptosis, protein synthesis, movement and replication, for transmitting information to the next generation of cells (Maton 1997).

In short, living organisms can be divided into unicellular, made of just one cell such as bacteria, archaea and most protists, and multicellular, made of multiple cells, including some protists, fungi, plants and animals.

Cell structure is made of cytoplasm, which contains many biomolecules such as proteins and nucleic acids, enclosed within a membrane typically surrounded by flagella, pili or cilia, which protect the cell and facilitate movement and communication with neighbor cells. Cells with inner compartimented membrane—bound organelles, in which more specific functions and metabolic processes take place and including a cell nucleus, a special organelle where the DNA is stored, are called eukaryotic cells and constitute the basis of the more complex organisms such as fungi, plants and animals. Cells without a nucleus and with the DNA in direct contact with the cytoplasm are called prokaryotic cells and were the first form of life in our planet. Prokaryotic cells have typical sizes of 1–5 μm, while eukaryotic cells are typically 10–100 μm. In consequence, interacting with single cells requires working in the micro-scale, as discussed in several chapters and cases of study along the Handbook.

The typical cell structure is detailed under these lines, explaining about the membrane, cytoplasm, cytoskeleton and genetic material, which are common to all cell types (except for red blood cells), and detailing the more typical organelles from eukaryotic cells. It is important to note that, in spite of being life's basic unit, cells are complex multi-scale and multi-physical/biochemical living systems made of a micro-cosmos of components.

Cell membrane. The cell membrane surrounds the cytoplasm of living cells and separates the intracellular cosmos from the extracellular environment. The membrane is also necessary for anchoring the cytoskeleton, which provides shape to the cell, and for attaching to neighbor cells and to the extracellular matrix, in order to form complex tissues and organs. The membrane, being selectively permeable to ions and organic molecules, controls the traffic of substances in and out of the cell. It is made of a phospholipid bilayer with embedded proteins that behave as channels and pumps to move the molecules in and out of the cell. The membrane participates also in processes such as adhesion, ion conductivity, signaling and motility. It also maintains the cell potential and mainly protects the cell.

Cytoplasm. The cytoplasm is made of cytosol, a gel-like or sol/gel-like substance encapsulated within the membrane, and organelles. All contents of

prokaryotic cells are contained within the cytoplasm; while, in the eukaryotic cells, the genetic content of the nucleus is separated from the cytoplasm by the nuclear membrane, which surrounds the nucleoplasm. The cytoplasm is made of water in around an 80 % and is the region where most cellular processes occur, including metabolic processes and reproduction.

Cytoskeleton. The cytoskeleton provides structural integrity to the cell and maintains its shape, anchors organelles in place, helps during the absorption of external materials by a cell, supports during the separation of daughter cells after cell division and moves parts and components of the cell along its growth and during its motility. In eukaryotic cells, the cytoskeleton is made of microfilaments, intermediate filaments and microtubules, mainly formed by proteins (with the monomeric subunit called actin making up the microfilaments, with the dimeric subunit called tubulin making up the microtubules and with varied subunits for the intermediate filaments, such as vimentin, lamin and keratin, among others).

Genetic material. The two types of genetic material are: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), which help cells to perform different fundamental functions. DNA is used for long-term information storage, as the DNA sequence encloses the biological information contained in an organism. RNA is used for enzymatic functions and for information transport. In prokaryotic cells, the genetic material is made of a simple circular DNA molecule located in the nucleoid region of the cytoplasm. Eukaryotic cells, on the contrary, structure their genetic material into different linear molecules called chromosomes inside the nucleus and include some additional genetic material within some organelles, such as the mitochondria in human eukaryotic cells for energy production.

Organelles. The organelles are specialized sub-units with the cells, which are specialized for carrying out specific vital functions. Eukaryotic and prokaryotic cells have several organelles, but those from the prokaryotics are simpler and not membrane-bound.

In eukaryotic cells we find organelles including: the cell nucleus, acting as main information centre and storing the genetic material; mitochondria and chloroplasts, which generate cell's energy respectively by oxidative phosphorylation and photosynthesis; the endoplasmatic reticule, a transport network for molecules targeted at specific destinations; the Golgi apparatus, for processing and packaging macromolecules; the lysosomes and peroxisomes, with enzymes for digestion and for getting rid of toxic components, respectively; the centrosome, for producing the microtubules, for organizing the cytoskeleton and for directing the transport through the endoplasmatic reticule and the Golgi apparatus; and the vacuoles that sequester waste products and store water in plants.

In eukaryotic and prokaryotic cells, the ribosomes are a large complex of RNA and proteins, in which the RNA from the nucleus is employed to generate proteins from amino acids.

Figure 1.1 provides an image of the anatomy of an animal eukaryotic cell to highlight the level of complexity of this unit block of life.

The mentioned structures and subunits detailed help cells to perform their vital functions, including: metabolism, the process by which cells process nutrients and

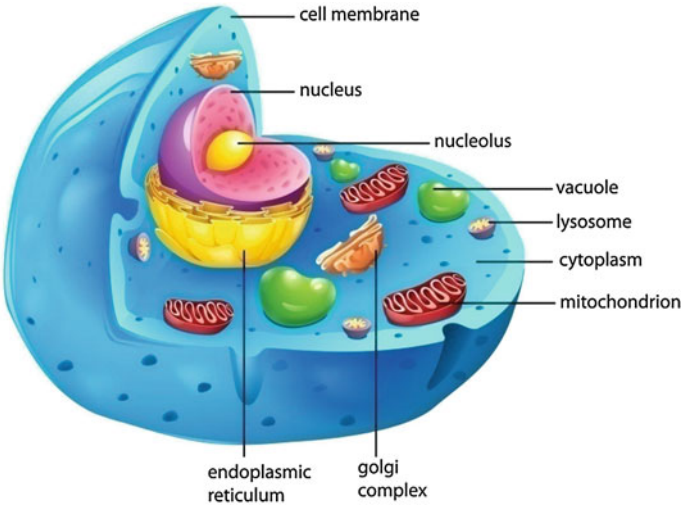

Fig. 1.1 Anatomy of an animal eukaryotic cell. Purchased under standard license agreement: [blueringmedia]© www.123RF.com

grow between successive divisions; replication or division, the process by which a single cell divides into two daughter cells, eventually leading to complex multi-cellular organisms; protein synthesis, the process, involving transcription and translation, by which cells create new proteins from amino acid building blocks to modulate and maintain cellular functions; and movement.

Present Handbook will focus on the development of biomedical microsystems for interacting with eukaryotic cells, which constitute our tissues and organs and play a fundamental role in health and disease. In consequence, when talking about "cells" we will normally refer to human eukaryotic cells. Next section provides a more detailed explanation of eukaryotic cell biological functions and of eukaryotic cell types within our organism.

1.2 Cell Differentiation and Cell Types

A human being comes into life by the formation of the zygote, the eukaryotic cell formed by fertilization of two gametes, which gives rise to the more than 200 types of all human stem and non-stem cells, which conform our different tissues and organs. Stem cells, capable of transforming into different tissues and typical from the developing embryo and fetus, can be also found in post-natal tissues and play a fundamental role for our lifetime maintenance, integrity and homeostasis (Shamadikuchaksaraei et al. 2014; Lanza et al. 2014).

As tissue repair and tissue engineering are directly connected to stem cells and several chapters of present Handbook will cover relevant development strategies for biodevices linked to tissue engineering, which constitute a very relevant part of the biomedical microsystems designed for interacting at a cellular level, it is very important to provide an introductory note to cell types and to cell differentiation processes.

In general, stem cells are defined as cells with self-renewal abilities and with the capability of differentiation into specialized cell types. Self-renewal is linked to proliferation and to the generation of stem cells with the same features as the original parent cell, while maintaining an undifferentiated state. Potency is the ability to differentiate into specialized cell types. The differentiation potential of stem cells helps to divide them into different categories, including: totipotent stem cells, such as the zygote and its descendants up to the eighth-cell stage in mammals, which can produce the embryo and the placenta; pluripotent stem cells, including cells from the inner cell mass of the blastocyst, embryonic stem cells and induced pluripotent stem cells (iPS), which can turn out into all the cells of the three embryonic layers; multipotent stem cells, such as mesenchymal stem cells (MSCs), which can generate bones, cartilage and adipose tissue, but not all tissue types; bipotent, which can produce two cell types; and unipotent, which only differentiate into one mature cell lineage.

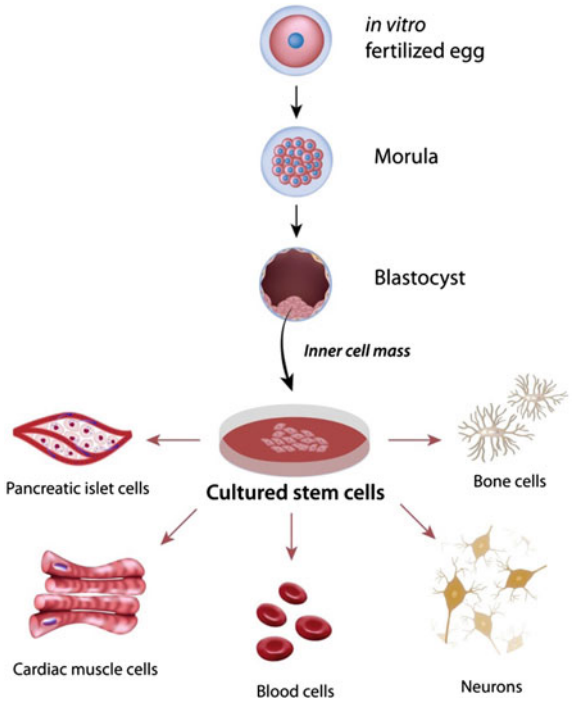

Fig. 1.2 Stem cells cultivation and differentiation. Purchased under standard license agreement: [alila]© www.123RF.com

Figure 1.2 schematically presents a process of pluripotent stem cell culture and differentiation, by which differentiated cells from the three embryonic layers and derived tissues can be artificially obtained. These layers include: the mesoderm, which forms muscles, bone, cartilage, connective tissue, adipose tissue, lymphatic system, cardiovascular system, blood cells, genitourinary system, dermis, serous membranes and notochord; the endoderm, which forms the stomach, the colon, the liver, the pancreas, the urinary bladder, the epithelial parts of trachea, the lungs, the pharynx, the thyroid, the parathyroid, and the intestines; and the ectoderm, which forms the epidermis, hair, nails, cornea, retina, enamel, and all cells from the central and peripheral nervous systems, among other cell types.

It is important to put forward that the behavior and fate of stem cells is not just dependent on genetic information, but is also regulated by other biochemical and mechanical cues and signals (epigenetic cues), which come from their micro-environment.

Such local micro-environment that provides physical and chemical support and signals for survival and regulation is commonly referred to as stem-cell niche, and will be mentioned along the Handbook in all chapters and cases of study linked to the development of biomimetic tissue engineering constructs and microsystems, mainly for bone, cartilage and muscle repair using mesenchymal stem cells (h-MSCs) (Caplan 1991, 2008). In consequence, for adequately replicating the 3D stem cell niche, chemical components including cytokines, growth and trophic factors, generated by cells themselves or by their surrounding companions, and other chemical factors have to be taken into account. In addition, several physical forces from the surrounding environment, such as tensile and compressive stresses, vibratory excitations, fluid shear stresses and the presence of electro-magnetic fields and gravitational forces have to be considered. Other elements, such as the presence of companion stem cells and the differentiated cell types from the adjacent tissue, clearly affect stem cell dynamics, overall behavior and fate. Finally, the physical and chemical properties of the extracellular matrix, such as stiffness, porosity, viscoelasticity, roughness or surface topography and composition, regulate stem cell function (Shamadikuchaksaraei et al. 2014; Lanza et al. 2014).

1.3 Cell Structure and Mechanics

Once differentiated, cell structure and function are intimately linked and affect their mechanics, responses and abilities to interact with the surrounding micro-environment. As previously mentioned, an adult human being is formed by more than 200 types of cells with different structures and functions, although all of them being eukaryotic cells with similar basic components. For instance, Fig. 1.3 provides an illustration of 8 different cell types whose structure is critical for their adequate aggregation into relevant tissues and for their efficient and collaborative performance.

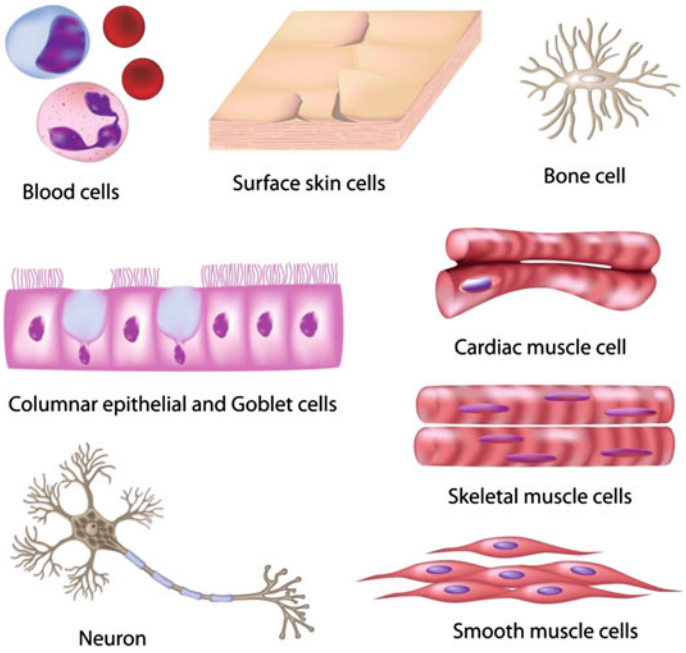

Fig. 1.3 Human cell collection and their typical structures. Purchased under standard license agreement: [alila]© www.123RF.com

As reviewed by Fletcher and Mullins (2010), the ability of an eukaryotic cell to resist deformation, to transport intracellular cargo and to change shape during movement depends on the cytoskeleton and both internal and external physical forces can act through it to affect local mechanical properties and overall cellular behaviour and adopted geometry and structure. Apparently, the cytoskeleton structures and the structures from surrounding cells, together with the physical properties of the extracellular matrix, act as epigenetic determinant cues to regulate cell shape, function and fate.

Mechanical cause-effect relationships leading to special aggregations of cells and cellular geometries can be easily found in post-natal tissues. For instance, it is very interesting to note that long bones, typically subject to bending stresses, have developed an outer compact geometry and an inner porous topology, which is clearly in accordance with the best possible distribution of material for the existing stresses. Cell geometries and aggregations in different muscles seem also to be the consequence of external mechanical factors. For example, skeletal muscle cells, aimed at performing and withstanding axial stresses, develop a linear geometry and aggregate in aligned bundles, while cardiac muscle cells, with multi-axial stimuli, form interwoven structures. Skin cells, aimed at providing protection against impact and penetration from external menaces, adopt a more planar tile-like and multi-layered structure, as modern knowledge-based engineered materials aimed at

providing excellent wear resistance. Of course, these geometries and structures are not just promoted by mechanical cues, but also affected by biochemical signals from the niche (i.e. the presence of oxygen gradients inside bone structure during growth and differentiation), as a complement to genetic issues.

In any case, the fact that cellular development and fate is very dependent, not just of the biochemical signals of the environment and of their genetic history, but also of the mechanical properties and mechanical stimuli acting within the extra cellular matrix, has promoted the birth of a new field of science and technology at the interface of biology and engineering, that of mechanobiology.

Mechanobiology focuses on the way that physical forces, stresses and strains, and changes in cell or tissue mechanics contribute to cell and tissue development, to the success of physiological interactions and even to the appearance of disease. A major challenge in the field is linked to understanding the complex mechanisms by which cells sense and respond to mechanical signals: the mechanotransduction properties of cells (Jacobs et al. 2012).

If these response mechanisms are clearly understood, the future development of cell-based sensors and actuators, capable of performing highly selective tasks with nanometric precision, will be a much more straightforward procedure and reach clinical translation in a more direct way, than using state-of-the-art trial and error approaches based on systematic variations involving several potential factors of influence.

The use of biomedical microsystems, particularly developed to obtain valuable information about the impact of all possible external mechanical stimuli on (stem) cell behavior, gene expression, differentiation and fate, constitutes a promising approach to that end, as will be detailed in forthcoming chapters. However, the development of such microsystems faces challenging design, manufacturing and testing issues, which must be necessarily taken into account from the product planning and conceptual design stages.

1.4 Cell Adhesion, Migration and Contraction

As previously mentioned, the cytoskeleton provides structural integrity to the cell and maintains its shape, anchors organelles in place, helps during the absorption of external materials by a cell, supports during the separation of daughter cells after cell division and moves parts and components of the cell along its growth and during its motility. The cytoskeleton is made of microfilaments, intermediate filaments and microtubules, mainly formed by proteins (with the monomeric subunit called actin making up the microfilaments, with the dimeric subunit called tubulin making up the microtubules and with varied subunits for the intermediate filaments, such as vimentin, lamin and keratin, among others).

This cytoskeleton, hidden within the cell's surface membrane, not only helps to stabilize the cell, but also actively generates contractile forces through an acto-myosin filament-shortening mechanism similar to that responsible of muscular movements. Cells apply these forces to their adhesions to other cells, as well as to

extracellular matrices or scaffolds that hold the cells together within the living tissues of different organs. These tensional forces also promote structural rearrangements within the cytoskeleton that govern multiple cellular activities, including movement, migration, contraction, among other process taking place at the molecular level. In fact, cells can be modeled by using simple tensegrity structures, in which elastic strings and sticks interact by means of tensile connections. Because they are prestressed, when these tensegrity models are not anchored, they take on a more rounded shape. However, both the cell and nucleus flatten out and spread in a coordinated way when they are attached to a rigid substrate. In addition, when their anchors are clipped, both the cell and the nucleus spontaneously retract again into a round shape. This is exactly what is observed when cells adhere to and detach from a culture substrate. Analysis of these structural models also reveals that applying stress locally on the surface of a hierarchical tensegrity results in global structural rearrangements in various locations and on several levels. Even movement can be explained in terms of slight changes of tensions within the filaments and fibres of the cytoskeleton (Ingber 2004).

These active fibres also play a relevant role in the development of filopodia or actin-rich plasma-membrane protrusions that function as antennae or sensors for cells to probe their environment, detect potential dangers and promote movement by attaching to microtextures. In consequence, these filopodia have an important role in cell migration, neurite outgrowth and wound healing and serve as precursors for dendritic spines in neurons (Mattila and Lappalainen 2008).

However, the mentioned actuation forces are not only mechanically, but also chemically driven. In fact the cytoskeleton acts as a mechanochemical scaffold that is both structure and catalysts. Cells adhere, move, migrate and contract by means of changing their level of internal prestress, shifting forces back and forth and also by using these localized forces to drive biochemical remodeling events (Ingber 2004), which constitute the basis of cell mechanochemical transduction as explained in the following section.

1.5 Cellular Mechanochemical Transduction

According to the previously mentioned features, cells and tissues can be seen, from the perspective of Materials Science and Engineering, as “smart materials and structures”. In fact, cells and tissues are able to perceive and respond to a wide set of environmental stimuli and gradients of them, including the presence of biochemical cues and microorganisms, the mechanical and topographical properties of the extra cellular matrix and surfaces upon which they lie, the application of vibrations and the surrounding electromagnetic fields, to cite just a few cells, as well as other microorganisms, by integrating structural networks with biochemical assemblies and information processing networks, can function both as sensors, processors and actuators, while at the same time moving, growing, and producing the energy required for these processes (Ingber 2004).

It is relevant to understand that the mechanical information is transduced into genetic and biochemical changes at the cellular and tissue levels and that these mechanochemical transduction phenomena drive the basic principles of life, as they are responsible of: cellular geometrical changes, adhesion and detachment to and from surfaces, generation of filopodia, motility, migration and interaction with potential dangers and companion cells, gene expression, reproduction, differentiation into relevant tissues, apoptosis (programmed cell death), anoikis (form of programmed cell death that is induced by anchorage-dependent cells detaching from the surrounding extracellular matrix), tumoral processes, metastasis and also repair (Ellis et al. 1991; Indran et al. 2011; Frisch and Screaton 2001).

Although all the involved phenomena are not yet understood, not only due to their intrinsic complexity, but also due to their mutual interactions, there is clear evidence that mechanical forces alter cell membrane ion permeability and leads to the activation of growth factors and hormone receptors. In addition, stress-induced conformational changes in the extracellular matrix can modify integrin structure and promote the activation of several secondary messenger pathways within the cell. In short, changes in the equilibrium between internal and external forces acting on extracellular matrices and changes in mechanochemical transduction processes at the cellular level appear to be important mechanisms by which our bodies adjust their needs to store, transmit and dissipate the energy that is required during development and movement (Silver and Siperko 2003).

Several of the microsystems presented as cases of study along this Handbook are designed for studying the influence of mechanical, biochemical and combined mechano-chemical inputs on cell behavior. Biodevices with microchannels for studying cell motility under gradients of chemicals, microtextured biodevices for assessing the impact of surface topography on cell adhesion and movement, biodevices with interconnected pools for studying interactions between different cell types, tissue engineering scaffolds with gradients of mechanical properties and loaded with extracellular matrix components, growth factors and cell-conditioned media, among other biomedical microdevices, will be covered in detail.

1.6 Main Conclusions and Future Research

The cell is the most basic functional, structural and biological unit of life as we understand it, but constitutes also a micro-cosmos of complex interactions and components. Cells are able to perform coherent functions and make up all tissues and organs of all living multicellular organisms; in consequence, there are usually referred to as life's building blocks. They can replicate independently and contain the hereditary information necessary for regulating cell functions, including growth, metabolism, apoptosis, protein synthesis, movement and replication, for transmitting information to the next generation of cells. They are key in all disease procedures and fundamental for any approach to therapy.

As present Handbook focuses on the more relevant development strategies for biomedical microsystems aimed at interacting with human cells, which constitute our tissues and organs and play a fundamental role in health and disease, it has been important to provide a brief introduction to the cell as complex multi-scale and multi-physical/chemical living organism, to its structure and subunits and to its main functions, before focusing on the vast field of biomedical microsystems.

Finally, as tissue repair and tissue engineering are directly connected to stem cells and several chapters of present Handbook will cover development strategies for biodevices linked to tissue engineering, which constitute a very relevant part of the biomedical microsystems designed for interacting at a cellular level, present introductory chapter has aimed to provide an introductory note to cell types and to cell differentiation processes, as well as to the more relevant cues for driving such processes, which can be exploited by several microdevices.

References

Caplan AI (1991) Mesenchymal stem cells. J Orthop Res 9(5):641–650

Caplan AI, Mullins RD (2008) All MSCs are pericytes? Cell Stem Cell 3(3):229–230

Ellis RE, Yuan JY, Horvitz HR (1991) Mechanisms and functions of cell death. Annu Rev Cell Biol 7:663–698

Fletcher DA (2010) Review article: cell mechanics and the cytoskeleton. Nature 463:485–492

Frisch SM, Screaton RA (2001) Anoikis mechanisms. Curr Opin Cell Biol 13(5):555–562

Indran IR, Tufo G, Pervaiz S, Brenner C (2011) Recent advances in apoptosis, mitochondria and drug resistance in cancer cells. Biochim Biophys Acta 1807(6):735–745

Ingber DE (2004) Mechanochemical basis of cell regulation. Biotechnol Revolution 34(3)

Jacobs CR, Huang H Kwon RY (2012) Introduction to cell mechanics and mechanobiology. Garland Sci

Lanza R, Langer R, Vacanti J (2014) Principles of tissue engineering. 4th edn. Elsevier

Maton A (1997) Cells building blocks of life. Prentice Hall, New Yersey

Mattila PK, Lappalainen P (2008) Filopodia: molecular architecture and cellular functions. Nat Rev Mol Cell Biol 9:446–454

Samadikuchaksaraei A, Lecht S, Lelkes PI, Mantalaris A, Polak JM (2014) Stem cells as building blocks. In: Lanza R, Langer R, Vacanti, J (eds) Principles of tissue engineering, 4th edn. Elsevier

Silver FH, Siperko LM (2003) Mechanosensing and mechanochemical transduction: how is mechanical energy sensed and converted into chemical energy in an extracellular matrix? Crit Rev Biomed Eng 31(4):255–331

Chapter 2
Brief Introduction to the Field of Biomedical Microsystems

Andrés Díaz Lantada

Abstract Biomedical microsystems are reshaping the way researchers and physicians carry out their diagnostic, preventive, therapeutic and surgical tasks and, therefore, impacting Healthcare in a very relevant way. In general, the miniaturization of biomedical devices promotes the speed, sustainability and fiability of diagnostic tasks; prevents damages to surrounding tissues in surgical procedures, hence shortening recovery time; allows for the interaction at a cellular level for a better understanding of biological phenomena and supports advanced research, among other positive aspects. Although the handbook's core topic is the more specific field of biomedical microsystems for interacting with cells, together with the use of advanced design and manufacturing strategies for their efficient development, it is important to provide a context for such microsystems within the whole ground of biomedical microdevices. This chapter provides a brief introduction to the more relevant types of biomedical microdevices, including microsystems for efficient diagnostic purposes, microsystems for personalized therapeutic purposes, microsystems for minimally invasive surgical procedures and the emerging microsystems for interacting with (and even controlling) cells. Main current research trends are also outlined.

2.1 A Historical Perspective of Microsystem Technologies

Micro-manufacturing technologies, capable of manufacturing devices with details in the typical range of 1–500 μm, started in the late 1950s mainly linked to the electronic industry, for producing circuits with improved performance, without a dramatic increase of final device size. Such beginning was very connected to the properties of silicon, which can be easily micro-machined using chemical attacks through specially designed patterns or masks.

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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The lecture "There's plenty of room at the bottom", given at Caltech in 1959 by Richard P. Feynman (reprinted 1992, revised 1993), focused on the possibility of improving and expanding the use of micro- and even nano-manufacturing technologies for obtaining more efficient, multifunctional and scalable systems, and made researchers aware of the related socio-economical importance.

The progressive adaptation of micro-techniques for micro-machining alternative materials, including metals, ceramics and polymers, and the introduction of novel manufacturing technologies, including laser micro-manufacturing, electron/ion beam milling, micro-replication tools and high-precision additive manufacturing, have since the 1960s greatly promoted final quality of the obtained microsystems, as well as the incorporation of additional features and the combination of materials, in many cases using thin-film technologies for special contact phenomena.

The applications of microsystems in the biomedical field are indeed remarkable and continuously evolving thanks to progresses in the aforementioned micro-technologies, as explained in detail in this chapter. As living organisms are made up with cells, whose dimensions typically range from 10 to 100 μm, micro-manufactured devices (with details precisely in that range) are very well-suited to interacting at a cellular level for promoting innovative diagnostic and therapeutic approaches.

More recently, nanotechnology, with the possibility of controlling the structure of materials at even smaller scales, is also benefiting microsystems and biomedical microdevices. For instance, the capability of structuring materials at the nano-scale helps to improve the mechanical performance, corrosion resistance, long-term biocompatibility, and other relevant properties, of many biodevices and biomedical microsystems, which are already commercially available.

This chapter provides a brief introduction to the more relevant types of biomedical microdevices, including microsystems for efficient diagnostic purposes, microsystems for personalized therapeutic purposes, microsystems for minimally invasive surgical procedures and the emerging microsystems for interacting with (and even controlling) cells. Additional details linked to micromanufacturing technologies for biodevices will be provided in Chap. 8, while Chap. 9 will focus on nanomanufacturing resources and biofunctionalization techniques.

2.2 Main Applications for Biomedical Microsystems

According to the United States Food and Drug Administration FDA, a medical device can be defined as: Medical machine, contrivance, implant, in vitro reagent, or other similar or related article, including a component part, or accessory that is:

- Recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them;
- intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals;

- intended to affect the structure or any function of the body of man or other animals, and does not achieve any of its primary purpose through chemical action within or on the body of man or other animals and does not depend on metabolic action to achieve its primary purpose.

According to European Union legal framework, a medical device is: Any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, together with any accessories, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes and necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of:

- Diagnosis, prevention, monitoring, treatment, or alleviation of disease;
- diagnosis, monitoring, treatment, alleviation of, or compensation for an injury or handicap;
- investigation, replacement, or modification of the anatomy or of a physiological process;
- and control of conception.

Biomedical microsystems, being medical devices, can therefore be designed and produced for different applications, according to previous definitions, such as: diagnostic purposes, surgical purposes and therapeutical purposes. In recent years, the development of novel strategies and microsystems to interact at cellular level and the advances linked to using cells, incorporated to biomedical microdevices, for enhancing the performance of traditional devices or even studying and solving health issues in radical new ways, is reshaping the medical device industry. Such microsystems capable of interacting at a cellular level constitute the core topic of present Handbook. In consequence, we will also consider them, as a different class of biomedical microdevices and provide a brief introduction to them in the last section of this chapter, after detailing some basic issues about the more classic biomedical microsystems aimed at diagnostic, surgical and therapeutical purposes in the following sections. Clearly, biomedical microsystems capable of interacting at a cellular level will also pursue diagnostic, surgical and therapeutical objectives, but achieve them in novel manners, as the cases of study included along the whole Handbook will try to clarify.

2.3 Biomedical Microsystems for Diagnostic Purposes

Adequate disease management requires the support of effective, robust and rapid diagnostic systems, for providing patients and physicians with the relevant information for selecting the adequate therapy, either if resorting to medicines or if requiring surgical procedures. Preventive strategies also require from systems capable of performing rapid diagnoses and monitoring tasks. If these evaluations

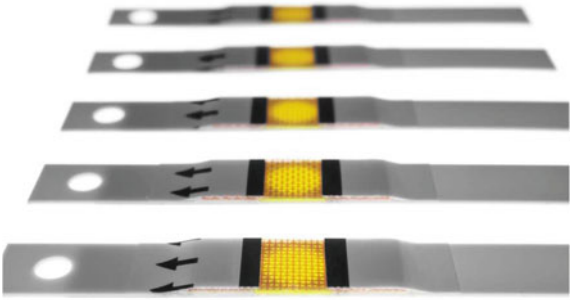

Fig. 2.1 Unused test strips with micrometric pattern for the analysis of blood glucose. Purchased under standard license agreement: [excitator]© www.123RF.com

can be carried out in non-invasive ways, patients' life quality can be importantly promoted, especially for chronic health issues. Performing them by means of low-cost systems and at the patient' point-of-care, without requiring expensive and highly specialized equipments and personnel, is fundamental for working towards the democratization of Healthcare.

Biomedical microsystems for diagnostic purposes, in many cases based on the use of microfluidic-based principles, promote multi-plexation, speed of response, sustainable mass-production and low-cost solutions, hence constituting interesting alternatives to existing diagnostic procedures for fulfilling the previously detailed specifications. Among the different microsystems with diagnostic capabilities, which are reshaping Healthcare, it is important to mention examples including: low-cost HIV testing strips, test strips for the analysis of blood glucose and for the consequent management of diabetes, pregnancy strips, microfluidic chips for urine infection testing and rapid antibiogram kits, to name just a few. Figure 2.1 shows some unused test strips with micrometric patterns for the rapid analysis of blood glucose. Some chapters of the handbook will be devoted to disease management by means of microfluidic devices, "labs-on-chips" and "organs-on-chips", all of which may benefit from the incorporation of patients' cells for personalized and biomimetic approaches.

2.4 Biomedical Microsystems for Surgical Purposes

The application of miniaturization technologies to the surgical field and the combination of the resulting microsystems for surgical purposes with all sorts of medical imaging resources, has promoted the avent of minimally invasive surgical procedures. Minimally invasive surgery is a revolution for Healthcare, which turns out to be more effective, efficient and sustainable.

In minimally invasive surgery, surgeons use a wide variety of technology-based procedures to operate with less injury to the body than with more traditional open surgery approaches. In almost all cases, it is much safer than open surgery and it allows the patients to recover faster and to heal with less pain. After recovery, the sequelae are also of less relevance. In addition, minimally invasive surgery is usually done on an outpatient basis or requires only a short stay in the hospital, which promotes the sustainability (both social and economical) of Healthcare systems worldwide.

Endoscopy, arthroscopy, laparoscopy, thoracoscopy, urethroscopy and bronchoscopy, among other minimally invasive surgeries, are performed through one or more small incisions, using small tubes and tiny video cameras and with the support of micro-surgical instruments. In some cases, ad hoc developed robots, with special micro-actuators, are used to support or even substitute the surgeon for high-precision tasks. These surgeries provide magnified and even 3D views of the surgical environment and all this gives the surgeon enhanced precision, versatility and control of the whole procedure.

Among the wide set of biomedical microdevices for surgical purposes, we can cite some relevant examples including: ballon expandable stents and self-expandable stents for different vascular issues, micro-pincers or micro-grippers for enhanced biopsy, micro-anastomosis devices for rapid and non-invasive suture, remotely controlled micro-surgical actuators with tactile feedback for the accurate targeting of cancerous tissues, wire-less controlled microrobots for hyperthermia therapy, among others. By means of example, Fig. 2.2 provides an illustration of a minimally invasive surgical procedure called angioplasty, in which a balloon and a stent, guided and controlled by an active catheter, are used to create a bigger opening in the vessel to increase blood flow. This procedure is common-practice nowadays and saves between 500,000 and 1,000,000 patients a year, only in the United States.

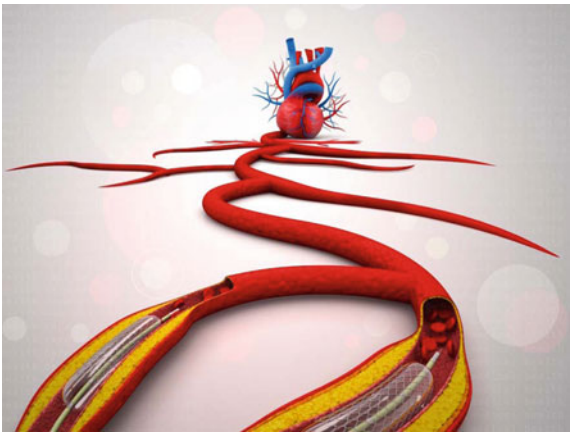

Fig. 2.2 Illustration of stent placement with the help of an active catheter. Purchased under standard license agreement: [R. Rajendran]© www.123RF.com

The shift from traditional surgery to minimally invasive approaches, based on the use of advanced surgical microsystems and related infrastructures, requires of course different abilities, not just linked to surgeons' hands-on expertise, but also regarding the use of complex supporting equipments and software resources, which may constitute a challenge for experienced professionals. Again life-long learning proves to be necessary in most professions, especially those continuously evolving linked to the biomedical and engineering fields.

For learning purposes, it is very important to remark the possibility of using anatomical models, which in many cases provide adequate biomimetic geometries and mechanical features, even better than those attainable by using dead tissues and organs, typically acquired at the butcher shop. The use of anatomical models is also much more sustainable than resorting to animal models for surgical training purposes. We would like to remark some very interesting models of organs and tissues commercialized by Simulab Corporation (www.simulab.com).

In some cases more complex biostructures and systems can be built, normally for anatomical studies, surgical training and planning, which can also be used for in vitro evaluating biomedical microdevices before resorting to trials with animal models. Surgical implantation can be mimicked normally aimed at studying if a particular geometry is implantable, if a minimally invasive approach is possible, if different designs of a biodevice behave in a similar way or not, or even if active implantable devices based on intelligent materials work as predicted in the computer-aided design and engineering stages (Díaz Lantada 2013).

Virtual reality and second life are also being explored as a way of promoting training in novel surgical procedures enabled by surgical microdevices (Wiecha et al. 2010). These combinations of technological resources enable the rapid creation of computer generated worlds and immersive environments, which are then used to develop new models, training methods for the biomedical (surgical) sector, among other industries.

2.5 Biomedical Microsystems for Therapeutic Purposes

Animal tissues and organs perform their complex functions in many cases by means of microstructures, capable of unconscious or conscious movements, and in many occasions involving combinations of static and dynamic responses, which benefit from special multi-scale geometries and features, continuously reshaped through evolution and adaptation to the environment. In consequence, when trying to develop biomimetic systems for replacing biological structures and functions, it is important to take into account multi-scale and multi-physical/chemical aspects for finding adequate solutions.

Medical devices for therapeutic purposes, such as passive and active implants, clearly benefit from the incorporation of micro-components capable improving their interaction with surrounding tissues or the performance of dynamic actions, as

Fig. 2.3 Model of artificial human heart with micro-actuated valves. Purchased under standard license agreement: [satori]© www.123RF.com

support to the structural ones. Having micro-components, these biodevices can be considered microsystems and their development processes involve the use of advanced design, manufacturing, biofunctionalization and testing resources. The use of micro-actuators and micro-sensors promotes the precision and efficacy of their responses, as well as energetically efficient and long lasting performances.

Among the wide set of biomedical microdevices for therapeutic purposes, we can cite some relevant examples including: artificial heart valves, implantable drug-delivery systems, glaucoma valves or cerebrospinal fluid shunts, to name just a few. As example, Fig. 2.3 provides an illustration of a model of artificial human heart with micro-actuated valves. Some of these biomedical microsystems may benefit from the incorporation of biomimetic microtextures for enhanced interaction with surrounding tissues and even from the integration of patients' cells for personalized responses, as will be discussed along the Handbook.

2.6 Biomedical Microsystems for Interacting with Cells

As previously introduced, the development of new strategies and microsystems to interact at cellular level and the advances linked to using cells, incorporated to biomedical microdevices, for enhancing the performance of traditional biodevices or even for studying and solving health issues in radical new ways, is reshaping the whole medical device industry. Such microsystems capable of interacting at a cellular level constitute the central issue of this Handbook for the following key aspects.

Understanding how cells behave and interact with surrounding cells, tissues, microorganisms and all types of biological, biochemical and biomechanical cues from their environment, constitutes a relevant research challenge and requires the support, not only of advanced manipulation and imaging technologies, but also of specifically designed biomedical microsystems with micrometric and even

nanometric details for enabling interactions at a cellular and molecular level. The clear comprehension of how the basic life unit behaves and interacts with other cells and surrounding tissues is fundamental for the future of Medicine and is starting to impact the development of new drugs, the approach to disease management and the treatment of tisular damage due to aging and accidents.

These types of microsystems, together with the use of advanced design and manufacturing strategies for their efficient development, constitute the core topic of present Handbook. Among the biomedical microsystems aimed at interacting with and studying the behavior of cells, it is important to mention the following areas of research and application: microsystems for disease management, microsystems for understanding cell activities, scaffolds for tissue engineering, cell-based sensors & actuators and microsystems for modeling life by controlling cells using microfluidic environments.

In order to develop microsystems capable of interacting at a cellular level, a wide set of technologies is being combined to support the design, modeling and manufacturing processes involved in modern engineering design approaches. The use of computer-aided design and engineering resources is relevant for the design of biomimetic features and for in silico assessing the potential performance of novel biomedical microsystems. The employment of advanced micro- and nano-manufacturing tools allows the incorporation of tiny features for affecting cell adhesion, motility and fate, towards effective, efficient and sustainable biomedical microsystems. The use of biofunctionalization procedures and post-processes is also becoming common-practice for an enhanced performance, which must be clearly assessed by systematic testing procedures before deciding to tackle mass-production and commercialization.

Most chapters of present Handbook will be devoted to detailing these design, modeling, manufacturing, biofunctionalization, testing and final mass-production resources and to providing cases of study linked to the complete development of biomedical microdevices capable of interacting with cells (and tissues) and of covering the research and application areas mentioned in the previous paragraph.

A key aspect for the successful development of these advanced devices is the need of tight collaboration between experts in biomedical and technological sciences along the whole process, as well as the use of a common language for avoiding mismatches between the initial specifications and the final performance, as further discusses in Chap. 5 when dealing with the systematic methodologies for the straight-forward development of biomedical microdevices.

By means of examples, Fig. 2.4 shows the computer-aided design and rapid prototype of a microsystem for cell co-culture with an integrated cantilever micro-porous membrane for separating the different cell-types and for the promotion of a biomimetic response. The physical model is obtained in epoxy resin by laser stereolithography, a technology used along the Handbook for the development of several cases of study. Additional introductory examples are provided in Fig. 2.5, which presents a couple of physical models of tissue engineering scaffolds for sphincter and tendon repair, again obtained for using laser stereolithography.

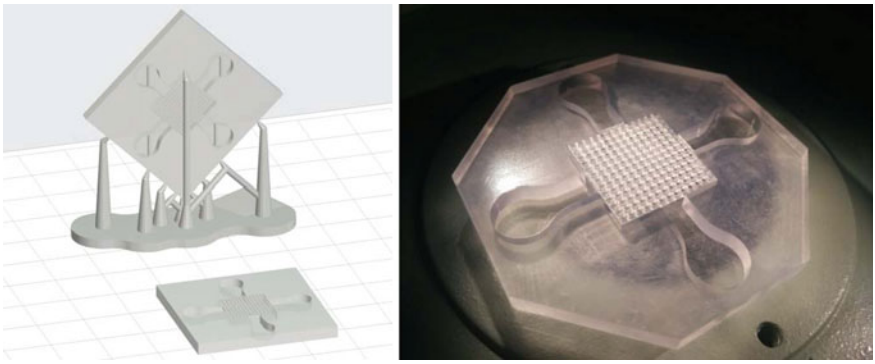

Fig. 2.4 Computer-aided design and rapid prototype of a microsystem for cell co-culture with integrated cantilever micro-porous membrane. Physical model obtained in epoxy resin by laser stereolithography

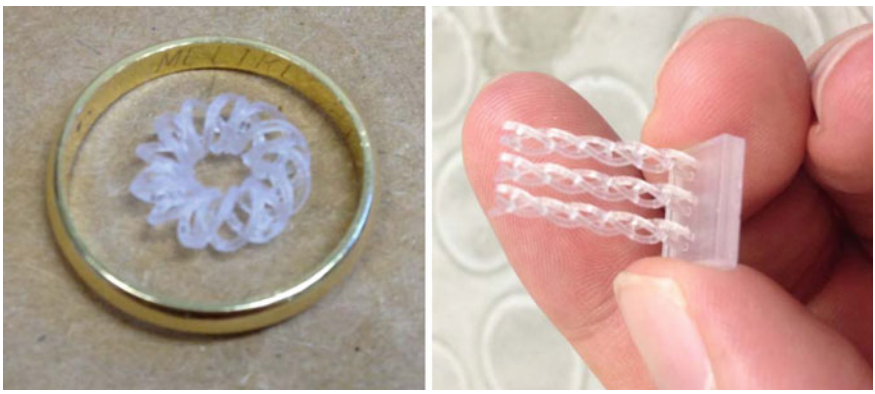

Fig. 2.5 Tissue engineering scaffolds for sphincter and tendon repair. Physical models obtained in epoxy resin by laser stereolithography

2.7 Main Conclusions and Future Research

Biomedical microsystems are reshaping the way researchers and physicians carry out their diagnostic, preventive, therapeutic and surgical tasks and, therefore, impacting Healthcare in a very relevant way. In general, the miniaturization of biomedical devices promotes the speed, sustainability and fiability of diagnostic tasks; prevents damages to surrounding tissues in surgical procedures, hence shortening recovery time; allows for the interaction at a cellular level for a better understanding of biological phenomena and supports advanced research, among other positive aspects.

Although the Handbook's core topic is the more specific field of biomedical microsystems for interacting with cells, together with the use of advanced design and manufacturing strategies for their efficient development, it is important to have provided here a context for such microsystems within the whole ground of biomedical microdevices.

This chapter has provided an introduction to the more relevant types of biomedical microdevices, including microsystems for efficient diagnostic purposes, microsystems for personalized therapeutic purposes, microsystems for minimally invasive surgical procedures and the emerging microsystems for interacting with (and even controlling) cells. Main related current research trends have been also outlined.

References

Díaz Lantada A (2013) Handbook of advanced design and manufacturing technologies for biodevices. Springer

Wiecha J, Heyden R, Sterntal E, Merialdi M (2010) Learning in a virtual world: experience with using second life for medical education. J Med Internet Res 12(1)

Chapter 3
Brief Introduction to Biomedical Microsystems for Interacting with Cells

Andrés Díaz Lantada

Abstract Understanding how cells behave and interact with surrounding cells, tissues, microorganisms and all types of biological, biochemical and biomechanical cues from their environment, constitutes a relevant research challenge and requires the support, not only of advanced manipulation and imaging technologies, but also of specifically designed biomedical microsystems with micrometric and even nanometric details for enabling interactions at a cellular and molecular level. These types of microsystems, together with the use of advanced design and manufacturing strategies for their efficient development, constitute the core topic of present Handbook. Among the biomedical microsystems aimed at interacting with and studying the behavior of cells, it is important to mention the following areas of research and application: microsystems for disease management, microsystems for understanding cell activities, scaffolds for tissue engineering, cell-based sensors and actuators and microsystems for modeling life by controlling cells using microfluidic environments. This chapter provides an introduction to these different types of biomedical microdevices and to the related basic concepts, to which we will get back in subsequent chapters linked to design, manufacturing, biofunctionalization and testing strategies and to the complete development of different cases of studies linked to the aforementioned families of biomedical microdevices. Main current research trends are also outlined.

3.1 The Challenge of Interacting at a Cellular Level

Natural materials, tissues and organs are consequence of an evolutionary process, amidst a changing environment, in which resources are limited, aimed at the fulfilment of several complex functions, including survival, structural stability, access to and processing of nutrients, elimination of debris and waste, protection against

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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external dangers, communication, overall energetic efficiency and even self-regeneration. In fact, the whole process can be seen as a multi-objective dynamic optimization for enhanced performance facing several, mainly energetic, constraints and contradictions (i.e. improved mechanical strength may require more nutrients, organism speed can be opposed to mechanical endurance, and superior adaptability to environmental changes may need lighter materials), hence bearing some resemblance with most engineering problems and related design solutions. Therefore, resulting geometries of living organisms are highly complex (Place et al. 2009), as a consequence of the several functions and constraints to be accomplished; non-intuitive, as solutions provided by Nature have not followed human hypotheses and simplifications, used for "methodical" development processes, neither our mistakes, consequence of limited knowledge of natural phenomena; and extremely varied, accounting for the wide set of macro and microenvironments that surround their materials, tissues and organs.

Tissue engineering and regeneration faces the ambitious challenge of recreating in laboratory the natural processes of cell expansion, differentiation and tissue formation, for improved diagnostic approaches and personalized therapeutic solutions, benefiting from the replacement of damaged or lost tissues, and pursues the overwhelming task of reconstructing entire organs. Pioneer developments in the field of tissue engineering have been based on very simple devices and geometries, aimed at solving very specific problems, such as supplying a drug in an enhanced way, regenerating small portions of damaged tissues or in vitro addressing cell response against toxic agents, pathogens or medicines. Biodegradable polymeric discs or planar substrates, microspheres, and woodpile structures marked the dawn and first years of tissue engineering, although more complex porous foam-like geometries have also been used since the beginnings of such recent research area.

In many cases, biodevices with quite simple geometries work properly for their desired purposes, such as studying cell behavior and fate for a deep understanding of life, and are more likely to reach market than other much more over-engineered products. However, to promote further developments in tissue engineering and cell biology, the complexity of biomaterials has to be adequately addressed. Aspects such as mechanical properties, surface topography, porosity and pore distribution are difficult to control in synthetic biomaterials but essential for interacting at a cellular level and for the success of extracellular matrices for cell-culture, as they are key issues linked to cell dynamics and evolution and also necessary for the proper access to nutrients and elimination of waste.

Therefore, related geometries are especially complex to design and manufacture, and there are still several interconnected parameters of influence needing adequate assessment. In addition, even if cell growth is obtained in 3D scaffolds, final tissue viability requires adequate vascularization, as diffusion can only provide transport of nutrients within a few hundred microns. However, vascularization induction within artificial tissues is an unresolved challenge, laying at the centre of bone regeneration research strategies, demanding even more complexity to the geometries of scaffolds and implants linked to tissue regeneration, so as to promote biomimetic responses.

Clearly, it is interesting to note that biomimetic approaches are marking trends and opening new horizons in fields such as energy, transport and information and communication technologies, but are not still impacting health-related sectors as would be expected, possibly due to the aforementioned intrinsic complexity of biological materials. Fortunately, recent advances in computer-aided engineering, materials science and technology, micro and nanomanufacturing resources, and surface functionalization approaches (Yao et al. 2013), are helping to control the three-dimensional geometries and the surface properties of multiple materials and biodevices, with a remarkable degree of precision and with the possibility of defining desired properties from design stage for interacting at a cellular and sometimes even molecular level.

Nevertheless, such advances have not yet been applied cooperatively and in a systematic way for the resolution of current challenges in tissue engineering and cell biology, hence being their potential beneficial synergies unexploited. Among main current tissue regeneration challenges, it is important to cite the urgent need for synthetic extracellular niches (or advanced scaffolds) capable of adequately mimicking the complex geometries and mechanical performances of the different tissues and organs of interest. The incorporation of defined surface topographies and chemistries to such extracellular matrices for improved biochemical response and the generation of different types of tissues upon a single tissue regeneration biodevices, by correctly combining cells, growth factors, drugs and extracellular matrices (Yao et al. 2013), are also important requirements. Such a cooperative and methodic application of technological breakthroughs will promote our capability of interacting with cells and understanding their behavior, which is indeed relevant for the success of several novel diagnostic and therapeutic strategies.

Researchers in the field should confront the relevant and yet unsolved challenges mentioned in the previous paragraph and manage them by methodically combining complex geometries, design processes, manufacturing technologies, materials, growth factors and drugs, on the basis of biomimetic approaches, whose application in the biomedical field will be reinvented. The shift from more traditional trial and error combinations, between materials and technologies to provide very specific solutions, to a more systematic integral and novel combination of advanced materials, structures, design, manufacturing, biofunctionalization and assessment technologies, will be a key aspect to support a knowledge-based future of tissue regeneration, cell biology and medicine in general.

To systematically develop and assess novel biomimetic strategies in tissue regeneration based on the advancement and integration of advanced bioinspired geometries, materials, technologies and processes for multi-scale, multi-material, multi-phase, time-responsive cell culture platforms and scaffolds (in fact advanced biofunctional niches), will allow us to improve the knowledge about cells and about tissues. To employ the knowledge generated with the help of biomedical microsystems and to apply the integral tissue regeneration strategies to the development of biomimetic diagnostic and therapeutic devices in the fields of tissue

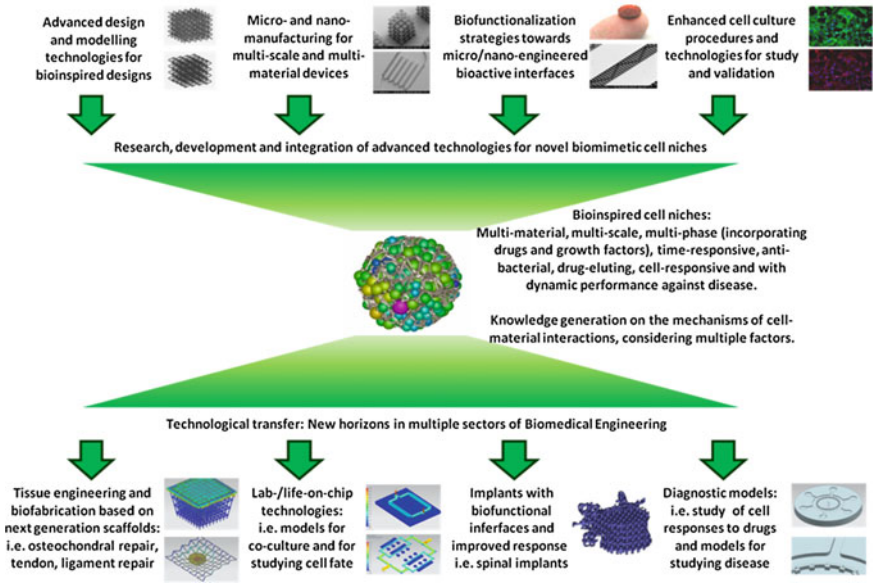

Fig. 3.1 Schematic representation of the potential of systematically applying recent technological advances to the development of biomedical micro-devices for the promotion of interactions at a cellular level

engineering, biofabrication, biomedical microsystems, implantable devices and in vitro models for drug screening, will allow us to go beyond tissue regeneration and reach the most general concept of integral disease management and personalized knowledge-based medicine. Figure 3.1 schematically represents the potential of systematically and cooperatively applying recent technological advances to the development of biomedical micro-devices for interacting at a cellular level.

Next sections introduce the different types of biomedical microsystems, aimed at interacting at a cellular level, which will benefit from these approaches. The whole Handbook is devoted to explaining and providing cases of study linked to the development of such microsystems. The following research trends are also present along the whole Handbook and drive our efforts:

1. The generation of resources (libraries of designs and properties) for an adequate biomimetic multi-scale modeling of the 3D structures and the surface topographies of biomaterials and structures.
2. The incorporation of advanced lattice structures based on the use of mechanical metamaterials and non-Euclidean (fractal) geometries for promoting novel ways of controlling the properties and performance of synthetic biomaterials for the biomedical field.
3. The systematic combination of several advanced micro and nanomanufacturing technologies with surface modification resources towards multi-scale, multi-

material, biomechanically and biochemically improved cell culture platforms and scaffolds.
4. The generation of new procedures for the suitable incorporation of growth factors and drugs upon the surfaces and into the structures of multi-phase cell culture platforms and scaffolds.
5. The assessment of the combined impact of materials, structures, surfaces, growth factors, microorganisms and supporting drugs on cell behaviour and fate and selection of the most-adequate combinations for the promotion of different tissue formation.
6. The objective and systematic comparative study of cell culture platforms and tissue engineering scaffolds (advanced biofunctional niches) by means of cell culture experiments.
7. The comparative evaluation of microsystem and implant surface structures with data base entries on existing successes and failures.
8. The development of the tissue engineering scaffold of the future, which will be based on functional gradients of properties, on the use of composite materials, on the incorporation of adequately distributed growth factors and drugs for delivery strategies and on special anchorages for promoting cell motility, proliferation and differentiation. Such next generation 4D scaffolds will not only promote adequate three-dimensional cell adhesion and proliferation, but will take into account that the process of musculoskeletal tissue regeneration needs the expression of different phenotypes in a dynamic environment, which must adapt to the requirements of the cells along the regeneration process and include dynamic response to disease.
9. The search for novel applications of the generated knowledge in bioengineering areas including biofabrication (beyond tissue regeneration), biomedical microfluidics "lab-on-chips", "organs-on-chips" and "life-on-chips", other biomimetic implantable devices and diagnostic models for drug screening and disease modeling.
10. The performance assessment, by means of cell culture experiments, of the different applications proposed and developed.

But first it is necessary to introduce the different areas of application and the types of biomedical microsystems aimed at interacting with cells for improving our understanding about the basic units of life.

3.2 Microsystems for Disease Management

The integrated study of biomechanical and biochemical issues in disease is usually carried out with the essential support of fluidic microdevices and microfluidic diagnostic platforms, as fluids enable the transport of nutrients, debris, gases,

pathogens and drugs to and from cells, help to control the movement of microorganisms in vitro and make the application of controlled stresses in culture systems possible. In fact the field of microfluidic systems for diagnosis has experienced an explosive growth in the last two decades, promoted by the convergence of clinical diagnostic techniques and mature microfabrication technologies capable of producing submillimeter-size fluidic channels and reservoirs in several materials (Jenkins and Mansfield 2013).

These advances have led to the development of versatile and self-sufficient lab-on-chip microfluidic devices or "labs-on-chips", aimed at integrating the complex operations and procedures typical from biochemical and biological laboratories in just a few cm^2, by taking advantage of microfluidic operation, which promotes reaction speed, sustainability due to the use of low fluid and sample volumes, and repeatability, thanks to multiplexing and automation. At present, most lab-on-chip devices are in fact "chip-on-lab" systems, as these complex microfluidic platforms still require from several support actuation and characterization technologies for a correct operation (Jenkins and Mansfield 2013). Even if further research in the field will promote additional miniaturization and integration of capabilities, lab-on-chip devices incorporating cells or tissue samples are already very interesting for studying and modeling disease.

Chapter 11 covers the applications for disease management based on the more conventional microfluidic devices and "labs-on-chips", while the development of biomedical microdevices for studying cell behavior and cell-to-cell interactions is detailed in Chaps. 12–14 and 21. Further on, the development of biomimetic cell culture scaffolds (see also Sect. 3.3), which can be also applied to the study of diseases and the testing of new drugs, is explained in Chaps. 15–20. Finally the more recent (and complex) organ-on-chip and life-on-chip approaches are discussed in Chaps. 20 and 22 and the potential of biofabrication is introduced in Chap. 23.

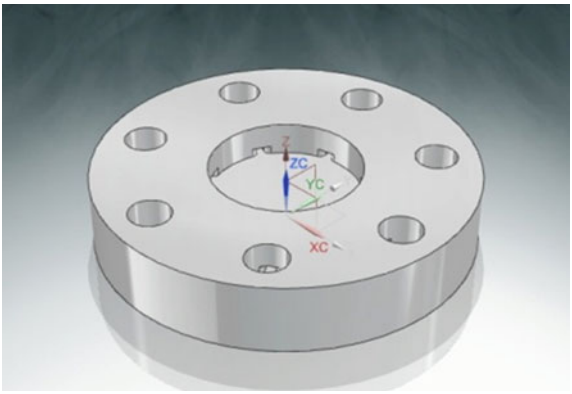

Fig. 3.2 Example of a diagnostic lab-on-chip actuated by capillary action

Figure 3.2 shows an example of a lab-on-a-chip for illustrating the concept and providing an idea of the typical geometries and components involved in these systems.

3.3 Microsystems for Understanding Cell Behavior

The use of conventional cell culture upon Petri dishes is not able to emulate the complex biochemical and biomechanical interactions present in living organisms that drive cell dynamics, differentiation and eventual tissue formation and are, therefore, inadequate for precisely studying and modeling disease, as well as for evaluating the actual potential of novel drugs and therapies. Cell performance is linked to their microenvironment, including the surrounding extra cellular matrix, other cells and soluble factors, such as growth factors and cytokines. Cells respond to biomechanical stimuli and parameters, such as topography of the surrounding material, stiffness of the substrate or environment, effects of vibrations, among others; but also interact biochemically according to the surrounding materials' compositions, to the presence of other cell signals and to gradients of nutrients, drugs and pathogens (Yao et al. 2013). In consequence, more complex devices are needed to assess, model and understand cell behavior.

Microfluidic systems combined with or integrating advanced scaffolds and platforms for cell culture, are ideally suited for an optimized control of cell growth, interactions and motion, by means of adequately producing biomechanical and biochemical phenomena. Cell movement can be mechanically oriented, using channels, walls, holes, bridges, textures and patterns. The fluids in motion can be used to apply the desired shear stresses needed to promote certain cellular differentiations into relevant tissues. Including additional inlets and outlets to a microfluidic disease model can help to introduce nutrients, disease initiators and drugs for therapy. The use of support chambers can facilitate the establishment of chemical gradients to induce cell motility. Producing mechanical and chemical modifications of certain zones of a biodevice, by changing surface topography or stiffness, by patterning surfaces with ligands…, can affect and help to control cell shape, cell size, cell-material adhesion, differentiation and other events necessary for a more complete understanding of cells and their behavior.

Details regarding design, manufacturing and testing strategies for the straightforward development of biomedical microdevices for the study and assessment of cell behavior and of cell-to-cell interactions is detailed in Chaps. 12–14.

Figure 3.3 provides a couple of examples, linked to the design of a micro-textured microsystem for studying cell adhesion and motility, for illustrating the typical geometries used in these kind of devices for analyzing cell behavior.

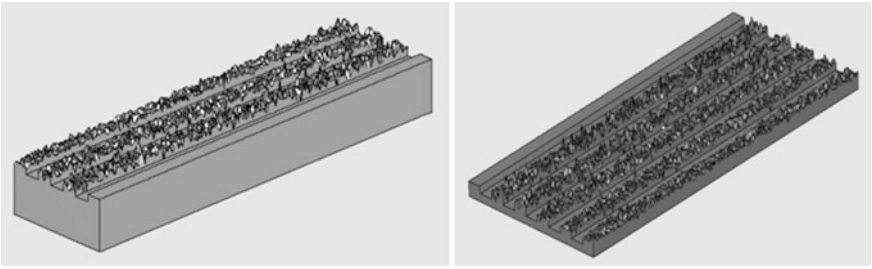

Fig. 3.3 Microtextured microsystems for studying cell adhesion and motility

3.4 Scaffolds and Microsystems for Tissue Engineering

An essential element involved in tissue engineering processes is the extra cellular matrix or scaffold which serves as substrate or framework for cell growth, aggregation and tissue development (Langer and Vacanti 1993). These scaffolds must be porous so as to allow cell migration during the colonization process as well as the transport of nutrients and waste to and from cells, but they have to be also resistant enough to withstand possible mechanical demands, especially if final scaffold (or device) implantation is desired. In many cases biodegradability of the scaffold may be a relevant and desired property, although in many repair strategies the scaffold may act as an optimized, active or "intelligent" implant, seeded with cells from the patient for an improved integration, but remaining within the body of the patient as a bioinert or bioactive element.

Additionally, as cells are able to feel their microenvironment and substrate elasticity and texture upon which they lie by modifying their morphology, their cytoskeleton configuration, and by intra- and extracellular signaling, increasing efforts are continuously being focused on the application of advanced biomimetic design and manufacturing technologies, so as to generate and modify the structures and surfaces of biomaterials. Aspects such as scaffolds' elasticity, porosity, pore size, and surface microtexture promote cell adherence, migration and proliferation within the scaffold, for subsequent differentiation into relevant cell types. Thus, tissue progenitor cells and the scaffold plays a fundamental role in most tissue engineering strategies as its properties can deeply influence the global success of new tissue formation and the controlled fabrication of the scaffold structures is becoming increasingly important for novel approaches within regenerative medicine (Thomas et al. 2010; Chen et al. 2010; Buxboim and Discher 2010).

Several strategies for the design, modeling and manufacture of biomimetic "knowledge-based" tissue engineering scaffolds, together with cases of study linked to the repair and regeneration of hard and soft tissues, will be detailed in Chaps. 15–19. Here we have just provided a brief definition and Fig. 3.4 helps to illustrate the typical morphologies of tissue engineering scaffolds. The use of multi-scale design

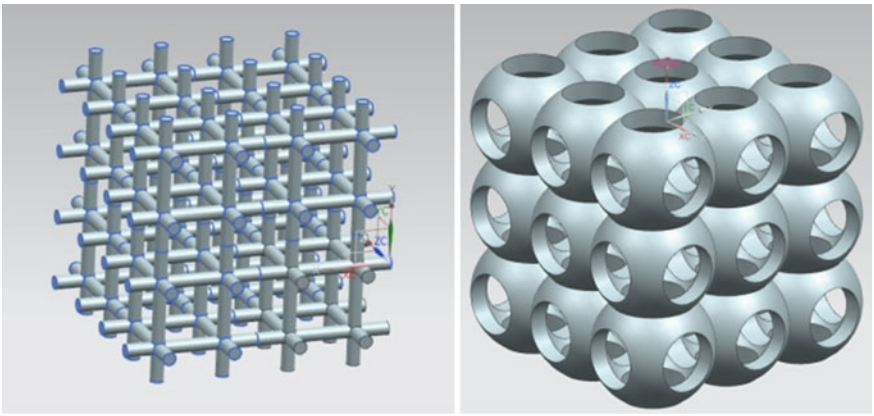

Fig. 3.4 Examples of typical geometries used as tissue engineering scaffolds

approaches or the employment of functional gradients of porosity and mechanical properties is also common and helps to promote biomimetic approaches, as the biological materials and structures are normally anisotropic.

3.5 Cell-Based Sensors and Cell-Based Actuators

Cells and tissues can be seen, from the perspective of Materials Science and Engineering, as "smart materials and structures". In fact, cells and tissues are able to perceive and respond to several environmental stimuli and gradients of them, including the presence of biochemical cues and microorganisms, the mechanical and topographical properties of the extra cellular matrix and surfaces upon which they lie, the application of vibrations and the surrounding electromagnetic fields, to cite just a few, as already detailed in several chapters of the Handbook.

Advances in technologies for manipulating, culturing and monitoring single cells, together with progress in the fields of modeling, simulation, prototyping and testing, have led to a better understanding of how cells respond to several types of stimuli and accurate predictions about the behavior of cells and tissues are already possible. In consequence, cells and tissues can be employed as living transducers for the development of (micro-)sensors and (micro-)actuators, as it is possible to predict and control their responses.

Due to the micro- and nano-geometries of cells and tissues and to the highly specificity and speed of several of their biochemical and biological responses, the sensors and actuators based on them are extremely precise and can provide high throughput, thanks to the possibilities of multi-plexing. Such biohybrid cell-based devices have the potential to outperform most types of already existing sensors and

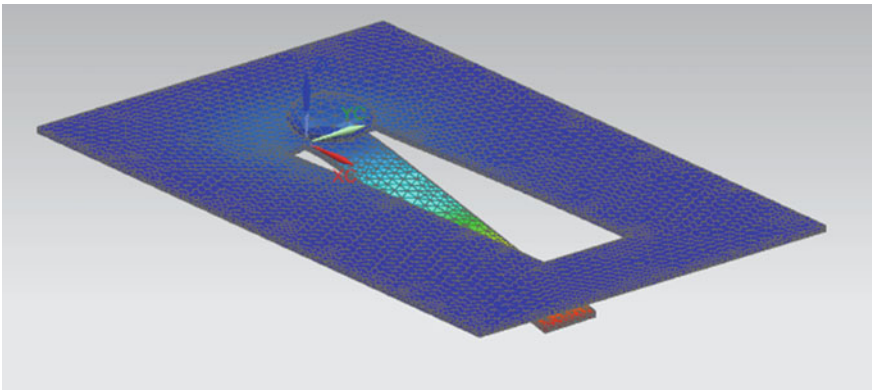

Fig. 3.5 Schematic simulation of micro-valve actuated by cell expansion

actuators based on inorganic components, although important research in the field is yet necessary.

Chapter 21 provides and introduction to the development of cell-based sensors and actuators and to current main challenges in this novel area. Once such challenges are solved, the frontiers between biological systems, machines and synthetic engineering systems in general will start to fade away. Figure 3.5 provides, as example of these types of solutions, a conceptual design of a micro-valve actuated by means of cell expansion simulated with the help of finite-element modeling.

3.6 Microsystems for Modeling Life by Controlling Cells

Counting with simple biomimetic microsystems capable of mimicking the behaviour of complex organs, or at least of some of their significant functionalities, constitutes a realistic and very adequate alternative for disease modeling and management, capable of providing even better results than more conventional animal models. These simplified replicas of human organ functionalities are being developed in the form of advanced lab-on-chip devices generically called "organs-on-chips", and are already providing interesting results (Huh et al. 2011, 2013).

Most of the already developed organs-on-chips in fact focus on specific interactions among a couple of cell types cultured together, help to assess the effect of chemicals and drugs on cells cultured upon channel networks resembling the organization of more complex organs, or mimic concrete fluid-cell interfaces.

Among the most remarkable experiences published so far, we would like to highlight studies linked to replicating, to some extent, the behaviour of several human organs and physiological structures including: liver (Ho et al. 2006), heart (Domian et al. 2009), lung (Huh et al. 2011) and blood-brain barrier (Wilhelm et al. 2011), among other interesting proposals. Disease development has been also

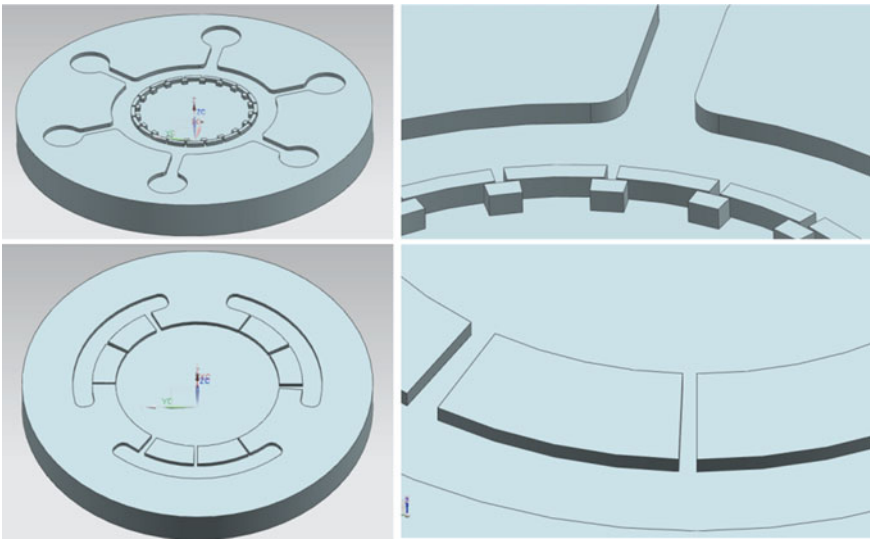

Fig. 3.6 Examples of versatile platforms for biomimetic cell co-culture

studied and predicted by means of organs-on-chips, as some experiences linked to real-time monitoring of kidney stone formation show (Wei et al. 2012). Main strategies for the design, manufacturing and testing of these biodevices will be provided in Chap. 22; here we have just introduced the concept. Figure 3.6 helps to illustrate it by showing a couple of versatile cell culture platforms with multiple chambers, separated by micropillars or connected by microchannels, for the co-culture of different cell types and the potential modeling of physiological interactions.

3.7 Main Conclusions and Future Research

Understanding how cells behave and interact with surrounding cells, tissues, microorganisms and all types of biological, biochemical and biomechanical cues from their environment, constitutes a relevant research challenge and requires the support, not only of advanced manipulation and imaging technologies, but also of specifically designed biomedical microsystems with micrometric and even nanometric details for enabling interactions at a cellular and molecular level.

These types of microsystems, together with the use of advanced design and manufacturing strategies for their efficient development, constitute the core topic of present Handbook. Among the biomedical microsystems, aimed at interacting with and studying the behavior of cells, it is important to consider the following types of devices, according to their area of application and research: microsystems for

disease management, microsystems for understanding cell activities, scaffolds for tissue engineering, cell-based sensors and actuators and microfluidic systems for modeling life by controlling cells.

This chapter has provided an introduction to the different types of biomedical microdevices and to the related basic concepts, to which we will get back in subsequent chapters linked to design, manufacturing, biofunctionalization and testing strategies and to the complete development of different cases of studies linked to the aforementioned families of biomedical microdevices. Main current research trends have been also outlined.

References

Buxboim A, Discher DE (2010) Stem cells feel the difference. Nat Methods 7(9):695

Chen WL, Likhitpanichkul M, Ho A, Simmons CA (2010) Integration of statistical modeling and high-content microscopy to systematically investigate cell-substrate interactions. Biomaterials 31:2489

Domian IJ, Chiravuri M, Van der Meer P, Feinberg AW, Shi X, Shao Y, Wu SM, Parker KK (2009) Generation of functional ventricular heart muscle from mouse ventricular progenitor cells. Science 326(5951):426–429

Ho CT, Lin RZ, Chang WY, Chang HY, Liu CH (2006) Rapid heterogeneous liver-cell on-chip patterning via the enhanced field-induced dielectrophoresis trap. Lab Chip 6:724–734

Huh D, Hamilton GA, Ingber DE (2011) From 3D cell culture to organs on chips. Trends Cell Biol 21(12):745–754

Huh D, Kim HJ, Fraser JP, Shea DE, Khan M, Bahinski A, Hamilton GA, Ingber DE (2013) Microfabrication of human organs-on-chips. Nat Protoc 8:2135–2157

Jenkins G, Mansfield CD (2013) Microfluidic diagnostics: methods and protocols. Springer, New York, Heidelberg, Dordrecht, London

Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920

Place ES Evans ND, Stevens MM (2009) Complexity in biomaterials for tissue engineering. Nature

Thomas WE, Discher DE, Shastri VP (2010) Mechanical regulation of cells by materials and tissues. MRS Bull 35:578

Wei ZJ, Amponsah PK, Al-Shattic M, Nie ZH, Bandyopadhyay BC (2012) Engineering of polarized tubular structures in a microfluidic device to study calcium phosphate stone formation. Lab Chip. doi:10.1039/C2LC40801E

Wilhelm I, Fazakas C, Krizbai IA (2011) In vitro models of the blood-brain barrier. Acta Neurobiol Exp 71:113–128

Yao X, Peng R, Ding J (2013) Cell-material interactions revealed via material techniques of surface patterning. Mater Views 25:5257–5286

Chapter 4
State-of-the-Art Bioengineering Resources for Interacting with Cells

Andrés Díaz Lantada

Abstract The detailed study of cell behavior and of the interactions between the cells and with their surrounding environment can be achieved by biomedical microdevices specifically designed to assess and even to control cellular responses, as explained in several chapters of present Handbook. In fact, the development and use of biomedical microsystems capable of interacting with cells and of helping researchers to obtain relevant information from cell behavior and interactions constitutes the central topic of the Handbook. However, for enabling such studies, a set of already common technologies for the micro-manipulation, culture, labelling, monitoring and visualization of cells and their (mutual) interactions are needed. This chapter provides a brief approximation to such technologies, as they are support resources for the developments detailed in forthcoming chapters. The use of supporting software and ad hoc developed programs for the real-time control and for the automated assessment of cell behaviors, on the basis of the images obtained by adequate labelling and visualization, also help to promote the development of more systematic studies for understanding cells and ultimately life.

4.1 Interacting at the Micro- and Nano-scale

Basic disease mechanisms rely on complex interactions at the micro- and nano-scale, which can only be globally understood by using resources and technologies capable of imaging, sensing, monitoring and acting at such scale levels. Healthy tissues and organs also depend on adequate interactions among different cell types and microorganisms, whose behavior and mutual relations can only be effectively assessed by employing ad hoc developed systems again capable of reaching the micro- and even nano-scale, while providing also information about the behavior of larger portions of tissues and organs. Multi-scale approaches and

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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technologies based on different functional principles are typically used, as happens with the combined use of micro-computed tomography and positron emission tomography for improved cancer diagnosis (Rodt et al. 2012).

Apart from using the medical technologies typically available at state-of-the-art hospitals and medical centres, such as ultra-sound imaging, (micro-)computed axial or helical tomography, positron emission tomography, more conventional radio-imaging resources and all sorts of surgical equipments and instruments, for the overall diagnosis and treatment of patients, the future of Medicine and Healthcare depends on further research linked to novel approaches. These approaches will help with solving current challenges and with providing answers to emerging health threats. In many cases, the development of novel strategies for addressing health problems will be linked to better understanding how cells behave and how they interact with their environment. Much research is needed and requires the help of low-cost and sustainable devices for the development of very systematic studies, typically in controlled laboratory environments, before safely applying the generated knowledge to solving real patients' issues.

The detailed study of cell behavior and of the interactions between the cells and with their surrounding environment can be achieved by biomedical microdevices specifically designed to assess and even to control cellular responses, as explained in several chapters of present Handbook. In fact, the development and use of biomedical microsystems capable of interacting with cells and of helping researchers to obtain relevant information from cell behavior and interactions constitutes the central topic of the Handbook.

However, for enabling such studies, a set of already common technologies for the micro-manipulation, culture, labelling, monitoring and visualization of cells and their (mutual) interactions are needed. This chapter provides a brief approximation to such technologies, as they are support resources for the developments detailed in forthcoming chapters.

The use of supporting software and ad hoc developed programs for the real-time control and for the automated assessment of cell behaviors, on the basis of the images obtained by adequate labelling and visualization, also help to promote the development of more systematic studies for understanding cells and ultimately life.

4.2 Technologies for Mechanical Interaction with Cells

Studying cell behaviour typically requires from technologies capable of picking cells from living tissues or from bio-banks and further transferring them to cell culture systems. Syringes, scalpels, microtomes and minimally invasive surgical resources are typically employed for such purposes, either at the bedside or in lab environments. Normally, with these tools, small portions of tissues are selected and manipulated and an additional degree of precision is required for interacting at single cellular level.

Mechanically interacting with single cells is interesting indeed, as provides very relevant information regarding cell behaviour and responses to the nearby environment and can be also used to assess healthy tissue states and disease.

In addition, the fact that cellular development and fate is very dependent, not just of the biochemical signals of the environment and of their genetic history, but also of the mechanical properties and mechanical stimuli acting within the extra cellular matrix, has promoted the birth of a new field of science and technology at the interface of biology and engineering, that of mechanobiology.

Mechanobiology focuses on the way that physical forces, stresses and strains, and changes in cell or tissue mechanics contribute to cell and tissue development, to the success of physiological interactions and even to the appearance of disease. A major challenge in the field is linked to understanding the complex mechanisms by which cells sense and respond to mechanical signals: the mechanotransduction properties of cells (Jacobs et al. 2012).

In order to perform adequate studies in the field of mechanobiology, the use of ad hoc designed biomedical microsystems is a very appealing approach, as will be further discussed in Chap. 13. However, several state-of-the-art technologies also allow for the acquisition of relevant information regarding cell mechanics and mechanical properties and complement the aforementioned tools for manipulating small portions of tissues.

For instance, micropipette aspiration of living cells can be used for transferring them from an expansion platform to a Petri dish for further studies, but also as a very direct way of studying their mechanical properties. With such tool, soft cells, including neutrophils and red cells, and more rigid cells, such as chondrocytes and endothelial cells, can be studied. The interpretation of the measurements obtained with basic continuum models leads to relevant information, regarding the values of cells' elastic and viscous properties, as previously detailed (Hochmuth 2000).

An interesting, although much more complex and expensive alternative, is based on using the points of atomic force microscopes (AFM) for pinching cells and assessing their elastic and viscous properties by monitoring their deformation and vibrations (Weisenhorn et al. 2000). The use of optical traps (laser tweezers) has also provided interesting results (Dai and Sheetz 1995). All these resources can be effectively complemented by microsystems aimed at addressing cell behavior and dynamics, as will be detailed in several of the cases of study presented along the Handbook.

4.3 Technologies for Electromagnetical Interaction with Cells

Naturally occurring electromagnetic (EM) fields are not just important for cell-surface and cell-material interactions, but also for the normal development of the organism and its physiological functions (Funk and Monsees 2006).

Consequently, artificially produced electromagnetic fields can be used for tuning the response of cells under culture and its development towards relevant tissues in vitro. In fact, living cells are electromagnetic units and electromagnetism is present in the origin of many tumoral processes and biomimetic cell culture may be supported by controlled electromagnetic fields.

Among state-of-the-art devices for the application of controlled EM fields, it is important to mention electrophoretic systems. Electrophoresis is the motion of dispersed particles within a fluid due to the influence of a typically uniform electric field and can be used to separate particles by size or by charge, hence being the basis of several analytical chemistries. Electrophoretic procedures can be also performed using non-uniform fields and upon gels, which results interesting for interacting with cells embedded within gels, for the promotion of dynamic cell cultures and for studying the secreted proteins by cells (Wimmer et al. 1994). These electrophoretic systems can be also used for cell micro-manipulation to create microsystems that separate cell mixtures into its component cell types or act as electrical grippers to transport cells and to place or fix them in very specific locations using devices previously reviewed (Rosenthal and Voldman 2005; Voldman 2006).

Another technique for electromagnetically interacting with cells is linked to carrying out cell culture processes supported by electromagnetic levitation (Souza et al. 2010). Although conventional 2D cell culture, typically performed upon Petri dishes (see Sect. 4.4), is an essential tool in drug discovery and evaluation, in tissue engineering and repair and in all areas of stem cell research, it is also true that conventional tissue culture produces two-dimensional cell growth with gene expressions, inter- and intra-cellular signals and overall morphology that differ from those found in vivo. In consequence, the fiability of the information obtained by means of 2D cell culture is not always as useful as desired. Cell culture by electromagnetic levitation is one of the alternative approaches to perform more three-dimensional cell culture environments and consequent more biomimetic cell cultures and studies. In these systems, cells are typically encapsulated within and hydrogel with nanoparticles. By spatially controlling the applied magnetic field, the geometry of the cell mass can be manipulated, and multicellular clustering of different cell types in co-culture can be achieved, which proves adequate for the development of fiable cancer growth models (Souza et al. 2010; Haisler et al. 2013). The great potential of these systems has even led to commercial products, such as the Bio-Assembler™ by n3DBioSciences (www.n3dbio.com).

4.4 Technologies for Culturing Cells and Obtaining Tissues

The use of adequate environments for culturing, expanding and studying cells and microorganisms is necessary for effective diagnostic tasks and for studying the basic mechanisms of disease. Biomimetic and even personalized approaches should

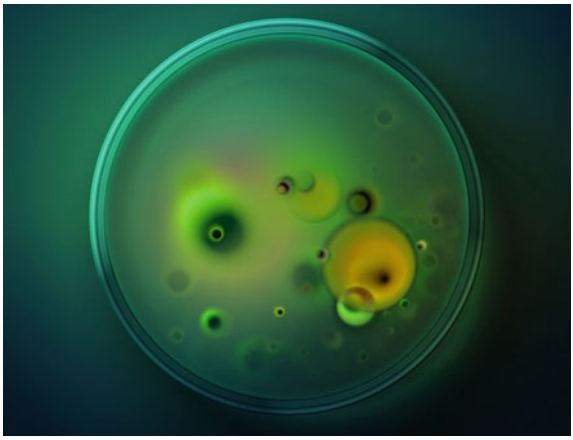

Fig. 4.1 Petri dish with bacteria. Purchased under standard license agreement: [Andrey Alyukhin]© www.123RF.com

be promoted, while considering also aspects linked to mass-production and sustainability for the development of systematic studies.

Among the most used environments for studying cells, tissues and pathogens and their mutual interactions, it is important to highlight cell-culture dishes or plates typically referred to as Petri dishes, which are named after the German scientist Julius Richard Petri and constitute one of the more common technologies present in any molecular biology and micro-biology laboratory.

In short, Petri dishes are shallow cylindrical glass or plastic lidded dishes that biomedical professionals use to culture cells and pathogens, as schematically shown in Fig. 4.1. Modern Petri dishes usually incorporate rings or slots on their lids and bases so that they can be more easily stacked.

Petri dishes are in many cases filled with liquid containing agar and a mixture of nutrients, blood, salts, carbohydrates, dyes, indicators, antibiotics and other bio-fluids to carry out microbiology studies. Once the agar cools and gelates, the dish can be inoculated with pathogens, such as bacteria and viruses, although studies with viruses are typically performed with bacteria used as hosts for the viral inoculum. Eukaryotic cells can be also studied upon Petri dishes, either expanding them in a liquid medium or in agar.

Many advanced Petri dish designs are based on incorporating or integrating several plates or wells into a single plastic part to create a "multi-well" plate, as happens with ELISA-devices (enzyme-linked immunosorbent assays) for carrying out detailed antibiograms. ELISA is also a popular format of micro-fluidic device that uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample (urine, blood, sweat, mucus, among other biofluids). The diagnostic process using ELISA devices includes the following steps: Antigens from the sample are attached to a surface (in form of solutions added to the micro-wells). Then, a further specific antibody is applied over the surface so it can bind to the antigen covering the well. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's

substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate for visual purposes. Typical ELISA multi-well plates include 96 wells for carrying out very systematic studies, although these must usually be performed by very well trained professionals. Recent point-of-care testing approaches, as discussed in Chap. 11, are focusing on the development of rapid and easy diagnostic microsystems, as alternative to more complex diagnostic resources requiring relevant infrastructures and experienced professionals.

In spite of the relevance of Petri dishes, ELISA devices and similar micro-plate related devices for studying pathogens, cells and tissues, there are some relevant controversial issues regarding their effectivity and potential for the future. Being completely planar surfaces, it is clear that the cell-culture environment achieved by using a Petri dish is not a real 3D cell-culture system. In consequence, cells do not interact with their environment (pathogens, other cells, extra cellular matrix components…) as within the body and the quality of the information obtained is not as perfect as desired.

As an alternative, much attention is being paid and great research efforts are being applied to the development of biomimetic platforms for human cell research (Vunjak-Novakovic and Scadden 2011), in many cases resorting to the concept of tissue engineering scaffolds and 3D cell-culture materials and environments or 3D cell-culture niches. Indeed, the niche composition and 3-D structure play an important role in stem cells state and fate, as well as the incorporation of adequate growth factors and conditioned media (Chan et al. 2009).

These alternative scaffold-based solutions, especially focusing on the design and manufacturing strategies for the incorporation of the desired biomimetic and even personal properties for enhanced performance and response, will be further discussed in Chaps. 15–19.

4.5 Main Microscopy Resources and Cell-Imaging Processes

Apart from biomedical devices aimed at manipulating and culturing cells, their adequate study and assessment of interactions with their surrounding environment could not be achieved without adequate microscopy resources and cell-imaging processes. The origins of high-quality cell imaging and live cell imaging date back to the beginning of the XX Century, with the first films of living cells performed by Ries (1910) (Landecker 2006) and some advances linked to colour photography carried out by Santiago Ramón y Cajal for visualizing the neurons (Márquez 2004), among other pioneers.

Nowadays, state-of-the-art cell-imaging includes a wide set of microscopy technologies, supported by several cell-staining procedures. Among the most common ones, it is important to mention the spread use of: confocal laser scanning

microscopy, digital holographic microscopy, fluorescent microscopy, multi-photon fluorescent microscopy, two-photon excitation microscopy, scanning electronic microscopy and even atomic force microscopy, all of them with their own advantages and drawbacks, including remarkable quality/cost ratios.

With some of the more advanced, such as confocal microscopy, multi-photon fluorescent imaging and two-photon excitation microscopy, 3D imaging of tissues is possible, which constitutes an excellent support for the development and final validation of 3D cell culture niches and tissue engineering constructs. However, in many cases the use of more modest and straight-forward optical or fluorescent microscopes is enough for performing adequate analyses. An in-depth comparative study of these resources for cell imaging has been recently performed (Tayebi et al. 2012).

In any case, many of these resources are based on adequately applying some sorts of fluorescent dyes to highlight some of the structures of the cells under study, such as the cytoskeletons, the nuclei and the organelles. In fact, fluorescent products provide a unique toolbox for studies of cell proliferation, migration, chemotaxis, gene expression, invasion, apoptosis, mitosis and differentiation into relevant tissues. Labeling strategies range from expressing fluorescent proteins to loading long-lasting probes or cell-permeant cytoplasmic labels to some fixable membrane tracers. The energetic behavior of cells can be perceived and even the movements of single or multiple cells can be traced and studied.

The use of specialized supporting software, as companion of modern microscope working stations (see Fig. 4.2), is of special interest for dynamically tracking cell

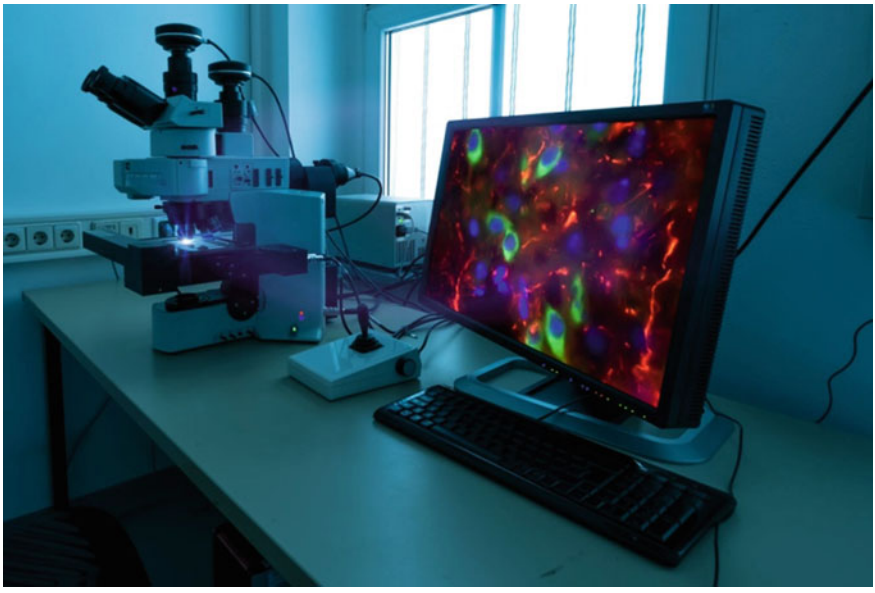

Fig. 4.2 Modern fluorescent microscope working station. Purchased under standard license agreement: [Ana Ivanova]© www.123RF.com

movements, for individualized monitoring of cells or for automated cell counting. There are even open-access easy to use options, such as CellProfiler from the Broad Institute, whose remarkable applications in indentifying and quantifying cell phenotypes has been put forward (www.cellprofiler.org; Carpenter et al. 2006). The vast community of Matlab users has also developed a wide set of codes for cell counting and cell segmentation (www.mathworks.com, The Mathworks Inc.).

4.6 Main Conclusions and Future Research

Basic disease mechanisms rely on complex interactions at the micro- and nano-scale, which can only be globally understood by using resources and technologies capable of imaging, sensing, monitoring and acting at such scale levels. Healthy tissues and organs also depend on adequate interactions among different cell types and microorganisms, whose behavior and mutual relations can only be effectively assessed by employing ad hoc developed systems again capable of reaching the micro- and even nano-scale, while providing vital information about the behavior of larger portions of tissues and organs.

This chapter has provided an overview of some of the most common tools used for the micro-manipulation, culture, labelling, monitoring and visualization of cells and their (mutual) interactions. These technologies support and complement the research presented along the Handbook. Their current limitations and present challenges inspire exploration tasks, aimed at the development of more efficient or biomimetic alternatives, as happens with the strategies towards 3D cell culture niches, as potential substitutes for Petri dishes, or with the design and manufacture of novel microsystems for interacting with single cells.

References

Carpenter A, Jones TR, Lamprecht MR, Clarke C, Kang IH, Friman O, Guertin DA, Chang JH, Lindquist RA, Moffat J, Golland P, Sabatini DM (2006) CellProfiler: image analysis software for identifying and quantifying cell phenotypes. Genome Biol 7:R100

Chan CKF, Chen CC, Luppen CA, Kraft DL, Kim JB, De Boer A, Wei K, Helms JA, Kuo CJ, Weissman IL (2009) Endochondral ossification is required for hematopoietic stem cell niche formation. Nature 457(7228):490

Dai J, Sheetz MP (1995) Mechanical properties of neuronal growth cone membranes studied by tether formation with laser optical tweezers. Biophys J 68:988–996

Funk RHW, Monsees TK (2006) Effects of electromagnetic fields on cells: physiological and therapeutical approaches and molecular mechanisms of interaction. Cells Tissues Organs 182:59–78

Haisler WL, Timm DM, Gage JA, Tseng H, Killian TC, Souza GR (2013) Three dimensional cell culture by magnetic levitation. Nat Protoc 8(10):1940–1949

Hochmuth RM (2000) Micropipette aspiration of living cells. J Biomech 33:15–22

Jacobs CR, Huang H, Kwon RY (2012) Introduction to cell mechanics and mechanobiology. Garland Science

Landecker H (2006) Seeing things: from microcinematography to live cell imaging. Nat Methods 6:707–709 Historical Comment

Márquez MB (2004) Santiago Ramón y Cajal: Algo más que un fotógrafo. Ámbitos 11–12:139–153

Ries J (1910) Chronophotographie de la fécondation et de la segmentation. Travaux de l'Association de l'Institute Marey 2:225–228

Rodt T, Luepke M, Boehm C, Hueper K, Halter R, Glage S, Hoy L, Wacker F, Borlak J, Von Falck C (2012) Combined micro-PET/ micro-CT imaging of lung tumours in SPC-raf and SPC-myc transgenic mice. PLoS ONE. doi:10.1371/journal.pone.0044427

Rosenthal A, Voldman J (2005) Dielectrophoretic traps for single-particle patterning. Biophys J 88:2193–2205

Souza GR, Molina JR, Raphael RM, Ozawa MG, Stark DJ, Levin CS, Bronk LF, Ananta JS, Mandelin J, Georgescu MM, Bankson JA, Gelovani JG, Killian TC, Arap W, Pasqualini R (2010) Three-dimensional tissue culture based on magnetic cell levitation. Nat Nanotechnol Lett

Tayebi L, Nozari A, Vashaee D, Mozafari M (2012) Current opinion in tissue engineering microscopy techniques. In: Méndez-Vilas A (ed) Current microscopy contributions to advances in science and technology. Formatex

Voldman J (2006) Electrical forces for micro-scale cell manipulation. Annu Rev Biomed Eng 8:425–454

Vunjak-Novakovic C, Scadden DT (2011) Biomimetic platforms for human stem cell research. Cell Stem Cell 8(3):252–261

Weisenhorn AL, Khorsandi M, Kasas S, Gotzos V, Butt HJ (2000) Deformation and height anomaly of soft surfaces studied with an AFM. Nanotechnology 4:106–113

Wimmer K, Harant H, Reiter M, Blüml Gaida T, Katinger H (1994) Two-dimensional gel electrophoresis for controlling and comparing culture supernatants of mammalian cell culture productions systems. Cyto-technology 16(3):137–146

Part II
Design and Manufacturing Technologies and Strategies

Chapter 5
Systematic Methodologies for the Development of Biomedical Microdevices

Andrés Díaz Lantada

Abstract The application of systematic engineering design methodologies, together with relevant advances in computer-aided design, engineering and manufacturing (CAD-CAE-CAM) technologies, novel materials and micro-/nano-manufacturing resources, have reshaped product development in the last three decades, greatly improving aspects such as time-to-market and overall project costs, as well as final product or device quality and overall performance. These methodological and technological improvements have also a remarkable impact in the development of novel medical devices and all kinds of products in the biomedical field, but very especially in the area of biomedical microsystems for interacting at a cellular and even a molecular level. This chapter is focused on providing a general description of the product development process, but taking into consideration specific aspects for the field of biomedical microsystems. The typical product development stages are covered: (detection of a relevant need, planning and specifications, conceptual design, basic engineering, detailed engineering, production and product market life) and the systematic methodologies commonly applied are also analyzed, providing a historical perspective, together with an overall view of additional methods for ensuring end-quality. The present introduction to modern product development is complemented by the several cases of study included in the Handbook, which have been developed following the proposed steps of systematic procedures. The overview of advanced design, modeling and manufacturing technologies provided in Chaps. 6–10 help to additionally support the methodological aspects of present chapter with very relevant resources used along the Handbook. This chapter constitutes and adapted and improved version of "Chap. 1: Introduction to modern product development", from Springer's "Handbook on Advanced Design and Manufacturing Technologies for Medical Devices" also by Andrés Díaz Lantada.

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2,
28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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5.1 The Relevance of Systematic Development Strategies

According to the main language academies a "product" (from the Latin *productus*), in its principal meaning, is "anything that has been produced", or an expanded definition might be, "anything useful manufactured or made that contributes economic value". If we look more deeply at the meaning of "Engineering", we can take the definition provided by "ABET—Accreditation Board for Engineering and Technology", according to which: "Engineering is the profession in which a knowledge of the mathematical and natural sciences gained by study, experience and practice is applied with judgement to develop ways to utilize, economically, the materials and forces of nature for the benefit of mankind". In line with this designation and bearing in mind the reality of industry, we can enumerate the main jobs done by engineers in the course of their work:

- Design and calculation of products, processes, facilities and plants in every area of industry.
- Research, development and innovation in products, processes and industrial methods.
- Preparing, leading and managing projects in every area of industry.
- Managing, planning and supervising multidisciplinary teams.
- Strategic planning of quality systems, production systems and environmental management.
- Financial management of projects and industrial concerns.
- General management or technical work or research and development projects in the plants and factories of industrial concerns.

According to the degree of dedication required by the work set out, three types of engineer can be defined for the present-day world:

- Production engineers—They produce goods and services. They keep the means of production in working order and manage them efficiently.
- Design engineers—They design new products and new processes. They take an active part in research, development and innovation.
- Management engineers—They decide and control the techno-economic and political processes on an entrepreneurial level in local, national and global contexts.

In whichever case, many of the problems facing engineers in their jobs are closely linked with the design and development work of new products, a large percentage of which are intended to help solve other more complex problems or evaluate the performance of certain functionalities of a system. In other cases, particularly in research centres and universities, the development process is more oriented towards showing the feasibility or usefulness of a new function, geometry, material, technology or process concerning a product. Bioengineers face with the special aspects devoted from developing product for interacting with tissues and organs of the human body, what constitutes an additional challenge.

In research-related situations, the process usually ends up proving such feasibility or usefulness as a result of a prototype being obtained that is not only capable of rousing the interest of the scientific community but also that of the main companies in the sector concerned that might be willing to start up production and market the concept. Thus, design and development engineers, often referred to as designers, contribute with their work to finding solutions and developing specific products. They also have important responsibilities as their ideas, knowledge and skills are decisive in deciding the technical, economic and safety aspects of a product. It is important to emphasize that due to the complexity of modern technology only on very rare occasions can an entire product development process be carried out by an individual organisation. This task usually requires considerable human and technical resources which involve difficulties of organisation and communication, very especially in the biomedical field.

To increase a new product's chances of success, the development process must be methodically and exhaustively planned and systematically executed. Not only must technical and financial feasibility be considered but also concepts like the end-safety associated with using the product, and the environmental impact that its use might have in addition to the human factors that can influence the different stages of the design process. Using wide-ranging information sources and following the recommendations laid down in regulations is also highly recommended for a successful outcome.

A development methodology should, therefore, integrate different issues so that the overall process is logical and comprehensible, as the following section will explain. To achieve this, it is essential for the process to be divided into stages and tasks, each with its own individual method and way of going about the job.

The following section sets out the main stages to be found in most general theories of design engineering and product development (Roozenburg and Eeckels 1995; Pahl and Beitz 1996; Muñoz-Guijosa et al. 2005), also applicable to more complex projects (De Cos castillo 1999a, 1999b).

Although this work is focused on the design of biomedical microdevices, it is important to point out that the main stages of development of these types of biomedical products basically coincide with the conventional stages proposed by the systematic methodology about to be explained.

Nevertheless, several additional considerations need to be borne in mind due to the specificity of the field, together with the considerable modifications derived from working with novel design and manufacturing technologies, as well as with new biomaterials, that may help optimize applying this general methodology to the specific case of biomedical microdevices for interacting at a cellular level.

In any case, depending on the variety of the problems and tasks involved in developing products, design actions have multiple facets and the need for systematic methodologies arises. Clearly, design tasks are dependent on basic scientific and technological know-how, but also on the individual experience of different design engineers and their specific knowledge in the area related to the product under development, from household devices and toys, to biomedical microsystems.

It should be remembered that designers have the prime responsibility for a product's technical and economic aspects and also for the efficient development process of its commercial aspects by adapting it to limited schedules and costs. It is therefore important to have designed a process or methodology that will lead to appropriate guaranteed solutions. This methodology must be flexible but at the same time capable of being planned, optimized and verified. However, this approach can only lead to success if all those taking part in the design have the necessary knowledge and work systematically. It is important to make a distinction between the science of design and design methodology. The science of design uses scientific method to analyze the structure of technical systems and how they relate to the environment, with the purpose of developing rules for these technical systems by taking the system components and examining how they are related.

However, design methodology is a specific way of acting to design technical systems that get their knowledge from the teachings of design science and cognitive psychology as well as from practical experience in different sectors. It includes: action plans for connecting the different work and design stages in accordance with content and the organisation envisaged; strategies, rules and principles for reaching general and specific goals; and methods for solving the problems of individual design or partial tasks. In line with this approach, a design methodology should:

- Encourage a direct approach to problems.
- Foster creativity and understanding.
- Facilitate the search for optimal solutions.
- Be based on the methodical application of knowledge.
- Be compatible with the methods and discoveries of other disciplines.
- Maintain the interest of the participants.
- Be easily learned and taught.
- Reduce times, costs and errors.

The approach set out should lead those involved in design to find possible solutions more quickly and directly than if they were working purely from intuition or experience. These two qualities are obviously important for any design process. In any case the use of a systematic methodology is not at loggerheads with intuition or experience, but simply attempts to expand and channel the potential of talented designers, while demonstrating that successful solutions do not only depend on creativity or intuition or experience but on a whole range of factors. If the problem and design-linked tasks are structured, we also manage to recognize that existing solutions can be applied to solve concrete problems and speed up the stages of the overall process. It also lets us use powerful computer-aided design tools suited to optimizing specific tasks. These tools will be discussed along the chapter. On the other hand, it is essential to use systematic procedures, whose typical stages are described in the following section, to adequately organize the information flows resulting from the design project and to prepare all the paperwork required to start up product production and any after-sales procedures.

5.2 Typical Stages Involved in Systematic Development Strategies

The outcomes of previous research, satisfactorily proven through numerous developed products, led to a slightly modified work structure (Roozenburg and Eeckels 1995; Pahl and Beitz 1996; Muñoz-Guijosa et al. 2005) which includes: planning, conceptual design, basic engineering and detailed engineering, although a clear dividing line cannot always be set between these stages.

Defining objectives and planning. This broadly consists of the strategic decision taken by a company, university or research centre, as to which products or ideas must be developed to satisfy the new social needs, taking account of the scientific-technological and socio-economic circumstances of the time. To set about a product idea that will be successful the state of the market has to be fully understood and especially customers and their needs. Thus, market and customer requirements become the major stimuli for developing new products.

However, these stimuli frequently have other origins, the most important of which are politics, the appearance of new technologies, processes, materials, discoveries or research results and environmental issues. Neither should the role played by internal stimuli be underestimated (arising in the company, university or technology centre itself) when it comes to making a decision about a new product. Among these internal stimuli are new ideas or outcomes related to research activity and the implementation of new means of production as well as production being made more rational and diversified. Depending on the stimuli mentioned, the main tasks to be included in the "defining objectives and planning" stage are:

- Situation analysis—by carrying out an in-depth study of the company and its products, together with market analysis and other possible information sources, a thorough analysis can be reached of the starting out point.
- Drawing up search strategies—by bearing in mind the companies' aims, strengths and weaknesses, as well as market gaps and needs, certain areas or promising fields can be discovered where ideas can be sought to be applied.
- Finding product ideas—from the search in the chosen field for new applications, functions, principles of functionality, geometries, materials, energy management methods and other alternatives, a set of product ideas can be found.
- Choosing product ideas—depending on the company's aims and market needs, the set of ideas found are evaluated in order to choose the most attractive product idea.
- Defining the product to be developed—by evaluating the different alternatives against a list of requirements a product proposal or definition is reached together with some initial objectives concerning costs, prices and schedules.

In the field of biomedical microdevices, it is necessary from the very beginning to take into consideration releval medical needs, either diagnostic or therapeutic, as

first source for defining the objectives and for finding the product (biomedical microsystem) to be developed.

Conceptual design. This is the stage where a decisive global principle is reached or a basis for reaching a satisfactory solution based on identifying crucial problems and choosing the right functional principles that in combination will attain the set objective. If this stage is to be properly tackled a series of prerequisites must be fulfilled linked to a correct conclusion of the previous stage. The objective must therefore be clearly stated and, in principle, be technically and financially viable. In addition, the designer must be informed of the needs of this conceptual design stage and the existence of possible solutions that allow proceeding directly to the design or basic engineering stage. The scope and depth required for the conceptual design stage must also be pre-established. Related to the above, the main tasks included in this stage are listed below:

- Abstraction for identifying basic problems. The decisive designs and principles based on traditional methods cease to provide optimum responses in the face of scientific-technological advances concerning technologies, materials or procedures, which adequately combined usually provide the key to more effective new solutions. On the other hand, every industry, company or research centre has countless experiences, which, although valuable, can lead to prejudice and hinder the creative process. For this reason, particularly at the outset of a new product design, designers must make an effort of abstraction and distance themselves from the influences of conventional ideas and focus on analysing the list of requirements and setting out the fundamental problem or problems in an objective manner.
- Setting functional frameworks. Having set out the basic problem to be solved, a global function must be obtained based on energy flows, mass and signals so that a relationship between the inputs to, and outputs from the plant, machine, part or object to be designed can be established. This global function can then be divided into less complex sub-functions and a lower level of abstraction, all of which can be individually dealt with to facilitate the search for solutions. Combining and relating these sub-functions leads to the so-called functional framework. It is advisable to draw up several functional frameworks depending on whether it is wished to optimize costs, functionalities, quality, development time or other factors.
- Designing functional frameworks. After establishing the different functional frameworks the principles of functionality for each of the sub-functions need to be sought. When they have been found, they should be properly interconnected to produce all the different possible functional frameworks that fulfil the global function. In line with the different preferences (cost, timeframe, quality and others) a table of choices can be made to choose the most suitable functional frameworks.
- Obtaining the decisive principle. By taking the functional frameworks the different decisive principles to be evaluated can be obtained based on the different techno-economic criteria and preliminary calculations that can lead to the choice

of the most adequate decisive principle (proposal for a preliminary solution or product concept) that can be worked on.

Basic engineering. When the decisive principle has been decided it is time to specify the underlying ideas behind this preliminary proposal for a solution or product concept. During the basic engineering stage (also often called basic design) the design engineers have the task of defining the basic shapes and geometries that characterize the product, and must also choose the preliminary materials and appropriate manufacturing processes. It is at this stage when technical, technological and economic considerations become of vital importance. In other words the mission of this stage is to provide a definitive general outline of the product to be developed, on which an effective analysis can be performed concerning: function, duration, manufacture, assembly, functionality, costs and safety.

Unlike the conceptual design stage, the basic engineering stage is subject to numerous checks, which means the work of analysis and synthesis constantly alternate and complement each other. An enormous effort also needs to be made regarding the compilation of information to make it easier to evaluate solutions, identify errors and continuously optimize.

The complexity of this stage is also greater because many actions have to be performed simultaneously. Sub-tasks need to be repeated when high levels of information are reached and because any change in an area or sub-area has repercussions on all the rest. For these reasons, it is impossible to set a series of steps to be strictly adhered to that will ensure the basic engineering will come to a successful conclusion. However, the following approach may be followed in general terms:

- Choose the relevant product requirements.
- Make scale drawings with the existing spatial constraints and evaluate the required free spaces.
- Draw up a basic outline to decide which components will be required to fulfil the main functions.
- A preliminary design of the parts and components that fulfil these main functions.
- Draw up a basic outline to decide which components will fulfil the remaining secondary functions.
- Draw up the preliminary designs of parts and components that fulfil these secondary functions.
- Evaluate the designs using both technical and economic criteria.
- Decide the overall preliminary design.
- Optimize the chosen design, eradicating any weak points that may have arisen during evaluation.
- Make proposals for improvement and checking if cost and quality objectives are met.
- Prepare a basic preliminary parts and documentation list for production and assembly. This documentation comprises the starting point for the detailed engineering stage.

During the basic engineering stage it is very useful to use check lists to ensure that when designing the different parts intended for the main product functions, all the various aspects have been taken into account. Of these aspects the most important are:

- Function.
- Principle of functionality.
- Design.
- Safety.
- Regulations.
- Ergonomics.
- Manufacturing.
- Quality control.
- Assembly.
- Transport.
- Operation.
- Fault detection.
- Recycling.
- Maintenance.
- Cost.
- Timescale.

Alongside this stage, as part of the work to compare designs and check geometries and functionalities, it is very useful to manufacture prototypes that will aid decision-making and help reduce the number of design iterations and minimize both the timescales and costs associated with product development. Currently a distinction is made between virtual prototypes, the result of computer aided design, simulation, calculation and manufacturing programs ("CAD-CAE-CAM" programs) and physical prototypes that coincide with the traditional concept of "original product sample for testing and checking". Supporting design, simulation and manufacturing technologies are described in the following sections.

However, the end of the basic engineering stage and the beginning of the detailed engineering stage cannot be precisely delimited as there is always some overlap that is to the benefit of the overall process.

Detailed engineering. Once the final basic design has been obtained, work must be begun on the requirements of the shape, properties, size and tolerances of the different parts. The final choice of manufacturing and assembly must also be done as well as final cost evaluation. The outcome of this stage is the definitive technical specifications of the product: a list of functionalities, production plans and the specifications including the instructions for assembly, disassembly and operation. Based on this information or technical documentation, production start-up can be undertaken as well as the placing of the product on the market. According to the above, detailed engineering work can be divided into the following:

- Finalising the end design. The different parts are fully defined by means of plans or 3D geometry CAD files, and materials, tolerances, adjustments and other details are specified.
- Parts integration. By means of full comprehensive plans or CAD assembly files which define the product as a whole.
- Finalising paperwork. For an unambiguous definition of the product and be able to launch production.
- Final checks. As to compliance with general regulations and company standards. Precision of size and tolerances, the availability of standard or catalogue parts and other checks.

The basic and detailed engineering stages can often be brought together in one single design stage with a global focus where the level of detail is gradually added. The ever more generalized use of CAD-CAE-CAM technologies and the already mentioned PLM tools has promoted this gradual fusion between stages, which also simplifies any information exchange between the agents involved in product design. Other authors with a similar outlook to that set out (Roozenburg and Eeckels 1995) also include production and marketing planning actions in the methodology they put forward, since the overall design of a product requires considerable human resources and materials to be assigned as well as production, distribution and sales strategies and other after-sales services. Figure 5.1 shows a full design process diagram with explanations (objectives and planning, conceptual design, and basic and detailed engineering).

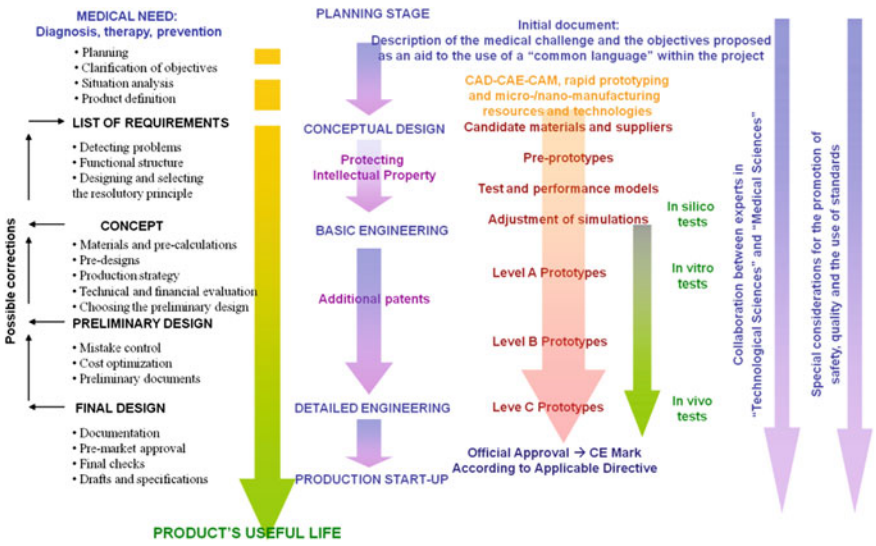

Fig. 5.1 Overview of the typical stages involved in the systematic development process of novel biomedical microdevices

5.3 State-of-the-Art Design Technologies for Microsystems

The appearance of support "software" for engineering design work and its gradual incorporation into industry since the end of the 80s, together with growing operational and calculating capacity, have caused major changes to the way design processes are carried out. Information exchange has become easier enabling countless effects in combination to be taken into account using multivariable simulations and enabling forecasts to be made concerning the influence of parameters such as the material or the manufacturing process on the end quality.

All these "software" tools can be included in a set of computer tools for managing the life-cycle of a product or "PLM programs—Product Lifecycle Management" (Stark 2004; Saaksvuori and Immonen 2008). These capabilities enable a company to effectively manage and develop their products and related services throughout their economic life. All companies also need to manage the communications and information with their customers ("CRM tools or programs—Customer Relationship Management"), with their suppliers (programs called "SCM—Supply Chain Management") and company resources (programs referred to as "ERP—Enterprise Resource Planning").

These three groups of software programs together with the PLM programs complete the four cornerstones of the information technology infrastructure that enable the main needs of a company to be addressed.

More directly linked to product development in line with the approach taken here, PLM tools that include the following types of software programs come to the fore for performing tasks like:

- PPM—Product and Portfolio Management. These are programs aimed at helping determine the optimal combination or sequence for the projects proposed for the company to successfully achieve it objectives in accordance with its economic and technological strategy and actual market requirements. These tools help analyse resources, costs, investment, production schedules and how one project affects another.
- CAD—Computer-Aided Design. These programs support design engineers, architects and other design professionals in their work, which is to make their designs a reality. They usually have 2D and 3D drawing systems for creating files or have all the information on a product's geometry and its different parts, as well as its plans. Changes can be made, symmetries are included, scale designs and numerous operations that can help make changes to the design.
- CAE—Computer-Aided Engineering. These computer programs allow simulating designs that have usually been made with CAD programs, and apply kinematic, dynamic, thermal or fluid mechanics considerations to the geometries designed and, above all, the chosen materials. They allow analysing how changes will affect the product or its parts and help optimize the number of prototypes or tests required.

- CAM—Computer-Aided Manufacturing. These programs lend support to prototype manufacturing work and end products by converting the information on part geometry from a CAD program into a code that can be understood by numerical control, manufacturing or rapid prototyping machines. On occasions it has a similar mission to CAE programs, letting part quality be simulated according to the manufacturing process used as well as allowing a study on geometries and materials.
- PDM—Product Data Management. These are programs focused on facilitating the records and paperwork of the processes to create modify and revise any of the parts of a product. The information stored ranges from specifications, CAD file diagrams, plans, manufacturing documents, assembly documents, tenders, test specifications and quality control, as well as financial reports.

In recent years the boundaries between these types of software are shrinking with the ever more frequent appearance of packs that combine different modules to provide a global response to all the aforementioned needs. As explained, these technologies can provide assistance at every product design stage as well as production start-up, market placement and after-sales services. The benefits of using them become obvious at the basic engineering stage where their use is even more justified in the detailed engineering stage where the amount of information handled increases rapidly, as will be explained further on and further understood when going through the cases of study included in the different chapters of the Handbook. Additional information and cases of study, regarding state-of-the-art and also advanced design technologies, with remarkable impact in the field of biomedical microsystems, can be found in Chap. 6, linked to biomimetic design resources, and in Chap. 7, linked to multi-scale and multi-physical/chemical modeling of biomedical devices.

5.4 State-of-the-Art Manufacturing Technologies for Biomedical Microsystems

Regarding prototypes, the industrial importance taken on over the last decade by the so-called “manufacturing and rapid prototyping technologies” should be emphasized. These technologies enable physical parts to be directly obtained in a short time (hours or a few days) from the designs made with the help of a computer using “CAD-CAE-CAM” programs. They are of great help in optimising design iterations, help the early detection of errors and speed up production start-up. They are usually either based on additive manufacturing approaches (like laser stereolithography or selective laser sintering) or on material elimination manufacturing processes (high speed numerical control machining). The different technologies available mean that prototypes can be obtained in a wide range of metal, ceramic and polymeric materials with remarkable precision (Freitag and Wohlers 2003;

Kucklick 2006; Lafont Morgado et al. 2000; Díaz Lantada et al. 2007; Díaz Lantada and Lafont Morgado 2009; Díaz Lantada 2012).

Depending on the objective and the similarity to the end product, the physical prototypes are usually divided into the three following levels:

- Level "A" prototypes (commonly called "A-samples"). These are demonstration prototypes for analysing shapes, geometries and other more subjective aspects (like aesthetics, visual impact or ergonomics) related to the product under development.
- Level "B" prototypes (commonly called "B-samples"). These are functional prototypes intended for checking the behaviour of different product parts and their functionalities. Although they are generally made of non-final materials, these tests are usually performed with limits on certain applications.
- Level "C" prototypes (commonly called "C-samples"). These are prototypes with similar materials and behaviour to the end product although the manufacturing methods used to obtain them do not coincide with the methods used in production. These level "C" prototypes are usually manufactured for final checks, to prepare production start-up and for obtaining official approval as part of the detailed engineering stage which will be dealt with further on.

Additional information and cases of study, regarding state-of-the-art and also advanced manufacturing technologies, with remarkable impact in the field of biomedical microsystems, both for the development of rapid prototypes and end parts, can be found in Chap. 8, linked to micro-manufacturing tools, and in Chap. 10, linked to the mass-production of biomedical devices.

5.5 State-of-the-Art Technologies for Functionalizing Biomedical Microsystems

Biointerfaces play a very relevant role in the success of implantable devices and in any kind of biomedical microdevice designed to interact with living tissues and cells. Biocompatiblity can be promoted by the incorporation of special surface coatings and aspects such as cell adhesion, motility and even fate can be to some extent controlled by means of inducing changes to the material and to the surface properties of biodevices.

In consequence, the employment of successful (bio-)functionalization tools and strategies may be a basic aspect for obtaining a biomedical device with improved performance during its life-cycle and for attaining competitive advantages also capable of converting a novel medical concept on a successful entrepreneurial adventure.

Among the most relevant technologies capable of modifying the surfaces of biomedical microdevices towards and enhanced performance, it is important to mention:

- Thin-film deposition technologies. Allow the deposition of micrometric polymeric, metallic or ceramic films by means of chemical-vapour deposition, physical vapour deposition, sol-gel processes, electrospinning, spin and dip coating and even self-assembly. Applications are normally aimed at providing special properties to a biodevice (biocidal properties, enhanced biocompatibility…) or as support for subsequent etching processes.
- Micromachining and chemical etching. Some of these technologies are based on processes similar to those used in conventional manufacturing (milling, drilling, lathing…) although with much more precise tools and capable of reaching detail levels in the range of a few microns. Other even more precise technologies also eliminate material from a substrate by using focused highly energetic beams from different sources, including lasers, electron-beams, ion-beams, X-rays or even water. In other cases, chemical micromachining (etching) is used to engrave substrates by chemical attacks (using acids or bases), after some parts have been protected by a mask.
- Direct structuring of surfaces with topographies controlled from desing. A recent option for controlling surface topography is the use of biomimetic design procedures (see some examples from Chaps. 6, 8 and 13) for obtaining a CAD model of the biodevice, which can be directly manufactured by means of high-precision additive manufacturing technologies, capable of obtaining very complex geometries in several materials. The designs can be even controlled using a multi-scale approach and different technologies can be combined for obtaining final features with different precisions, as also detailed in Chap. 8.

Additional information and cases of study, regarding state-of-the-art and also advanced technologies for (bio-)functionalization, with remarkable impact in the field of biomedical microsystems, can be found in Chap. 9, linked to nano-manufacturing tools and surface functionalization resources.

5.6 State-of-the-Art Resources for Operating Biomedical Microsystems

Apart from the design, manufacturing and functionalization resources detailed in previous sections, the technologies for interacting at a cellular level, including microscopy and micro-manipulation resources, presented in Chap. 4 are key tools for the adequate development and operation of biomedical microsystems aimed at interacting with cells. However, for a successful life-cycle of any device, the use of methods for the systematic promotion of quality and safety are of great value, as described in the following paragraphs. The history of industrial design usually recognizes three quality leaps regarding design approach and the fundamental reasons behind that design; these changes of approach are directly related to the three following concepts (whose application also changes product development methodologies):

- Productivity. The main objective at the start of the Industrial Revolution since the very existence and survival of emerging industry depended on this concept.
- Safety. A concept that has gradually taken on importance throughout the 20th century as society became more aware, with increasing economic growth together with the technological progress that enabled safer systems to be introduced. At first, it was considered to be a factor that hampered production, but later it was shown that productivity and safety contributed synergy, and so, manufacturing safe products safely became paramount.
- Quality. A notion that especially over the last three decades has become a basic goal of production processes and developed products. Referring to product design it can be understood to mean "the set of properties of the design process that enables products to be set in production that fulfil the needs envisaged at the outset".

Whatever the circumstances, reaching acceptable levels of quality involves more and more the company as a whole and product design means taking account of quality issues throughout the entire design process already explained. A good starting point is the ISO 9000 Series set of standards which set out the basis for applying quality procedures in different organisations and the associated tasks, such as product design, production and manufacturing processes and commercial activities. The standards advocate that the best way to attain top quality is to take measures that will avoid failure by systemising processes and quantifying the parameters that have most bearing on quality.

The implications involved in this set of standards and how they relate to the European Union's so-called "New Approach Directives" will be dealt with in the discussion of standards included towards the end of the following chapter, with a special emphasis on the design of medical devices. Another key factor for attaining high levels of quality in product design tasks is to correctly interpret the customers' requirements and be exact in defining the initial specifications. In principle, the key factors are:

- Systemizing the design process by using structured methodologies.
- Identifying potential faults and taking counter-measures.
- Identifying any potential disturbances to the input parameters that might affect the output parameters and take counter-measures.
- The participation of all departments (design, production, testing, quality, sales, purchases, commercial and any others) in the aforementioned tasks.
- Learning based on the defects of previous products.

Tools to ensure design quality. To ensure quality throughout the design stage and methodically take account of the key factors, the use of various tools is becoming widespread, of which the most important are:

- "QFD—Quality Function Deployment" and related methodologies. Such tools help to take into account market and user demands when tackling the development of a new product. By using several matrixes for quantifying the need of relevant changes linked to the enterprise services and production system,

materials and processed used, global quality optimization is promoted and final results improved. "QFD" is designed to help planners focus on characteristics of a new or existing product or service from the viewpoints of market segments, company strategies, or new technology-development needs. It is applied in a wide variety of services, consumer products and emerging technology products.

- The use of "Failure Trees". This is a tool for systemising and enhancing the process and for detecting potential faults and disturbance factors. It is incorporated at the conceptual design stage once the product's functional framework is available with the general function and all the sub-functions involved in the product's proper working, all of which must be checked. The different functions and sub-functions are checked one by one, thinking of how they could fail and searching out any possible faults and then looking for the possible causes and disturbances that could lead to those faults. When any possible causes of faults have been assessed, counter-measures are designed for each one. If necessary, the concept is redesigned, the design is improved or the procedures for manufacture, assembly, logistics, quality, maintenance and others are modified. As the work required to complete a fault tree for an entire product is considerable, this method is usually limited to decisive issues and critical processes. It is advisable for designers to make this way of working part of their everyday activities and so apply these concepts almost by intuition.
- "FMEA Method—Failure Mode and Effects Analysis". This method, originally designed for the "Apollo" program is more powerful than the fault tree since it quantifies the absolute importance of every fault mode by using the so-called "RN"—risk number, which is quantified according to the probability of fault occurrence and the probability of its being detected. Therefore, risks can be classified in order of importance and priorities set for searching for and executing counter-measures. It is widely used nowadays in all industrial sectors. However, the use of this method requires expert staff in all departments. The "FMEA" is usually reviewed several times during product design and possible counter-measures, responsible persons and control dates are set. This method helps ensure the quality but above all the safety of the product right from the design stage, which has previously proven to be of great help in fields such as machine design (Muñoz Sanz et al. 2007).
- Quality meetings. Specially designed to avoid difficult-to-solve faults in the advanced stages of development. The starting point is usually a check-list based on questions concerning the experience of previous designs. Members of all departments usually take part in these meetings where counter-measures are suggested and persons are proposed for being responsible for applying the measures in the set timeframe. Once again, it is essential to emphasize the importance of fluid communication between all those involved in the product design process if a successful outcome is to be reached.

Additional tools for ensuring quality include several standards, which provide systematic descriptions of methodologies for direct application. Among such standards it is important to cite the ISO 9000 family for quality management, the

ISO 14000 and 19000 families for quality audits and environment, the OHSAS 18000 standards on security and health and the ISO 28000 family on supply chain quality.

In the Biomedical field, quality and security esurance are intimately linked to assessing the effectiveness and risks related to a novel device, including aspects such as biocompatibility testing and incorporated to the global biodevice development systematic methodology.

Additional information and cases of study, regarding the complete development process of biomedical microsystems, including testing and operation, can be found in Chaps. 11–23, which cover several types of biomedical microdevices capable of interacting at a cellular level, from tissue engineering scaffolds and cell-culture platforms, to microfluidic devices and organs-on-chips.

5.7 Main Conclusions and Future Research

This chapter has focused on providing a general description of the modern product development process, covering its typical stages (detection of a relevant need, planning and specifications, conceptual design, basic engineering, detailed engineering, production and product market life) and the systematic methodologies commonly applied, together with an overall view of additional methods for ensuring end-quality.

Such systematization, together with relevant advances in design and manufacturing technologies, as well as in materials science and engineering, which are central part of the present Handbook, have reshaped product development and deeply influenced the medical device sector, improving the quality, performance and capabilities of all types of biomedical microdevices, together with a more adequate control or aspects linked to the viability of every project, including time-to-market and overall development costs.

The present introduction to modern product development is complemented by the additional information provided in the following chapter, regarding specific relevant aspects to be taken into account when the development process is linked to a medical device and, more specifically, to biomedical microdevices.

The overview of advanced design, modeling and manufacturing technologies provided in Chaps. 6–10 help to additionally support the methodological aspects of present chapter with very relevant resources used along the Handbook.

The cases of study detailed in Chaps. 11–23 and linked to a wide set of final applications of biomedical microdevices aimed at interacting at a cellular and even molecular level, covering from tissue engineering scaffolds and cell culture platforms, to microfluidic devices and organs-on-chips, are developed on the basis of the presented methodology.

References

De Cos Castillo M (1999a) Teoría general del proyecto. Volumen I: Dirección de proyectos. Editorial Síntesis, Madrid

De Cos Castillo M (1999b) Teoría General del Proyecto. Volumen II: Ingeniería de proyectos. Editorial Síntesis, Madrid

Díaz Lantada A (2012) Handbook of active materials for medical devices. PAN Stanford Publishing

Díaz Lantada A, Lafont Morgado P (Advisor) (2009) Metodología para el desarrollo de dispositivos medicos basados en el empleo de polímeros activos como sensores y actuadores. PhD Thesis, Universidad Politécnica de Madrid

Díaz Lantada A, Lorenzo Yustos H, Lafont P, Munoz-Guijosa JM, Echavarri Otero J, Muñoz Sanz JL (2007) Teaching applications for rapid prototyping technologies. Int. J. Eng. Educ 23 (2):411–418

Freitag D, Wohlers T (2003) Rapid prototyping: state of the art. Manufacturing technology information analysis centre, Chicago

Kucklick TR (2006) The Medical Device R and D Handbook. CRC Press

Lafont Morgado P, Lorenzo Yustos H et al (2000) Rapid tooling: moldes rápidos a partir de estereolitografía. Revista de plásticos modernos 524:150–157

Muñoz Sanz JL, Leal Wiña P, Erades García-Toledano G, Echavarri Otero J, Díaz Lantada A (2007) "Metodo cuantitativo para la evaluación de la seguridad en máquinas en la fase de diseño". Actas del 8º Congreso Iberoamericano de Ingeniería Mecánica. Pontificia Universidad Católica del Perú. Cuzco

Muñoz-Guijosa JM, Bautista Paz E, Lafont Morgado P, Muñoz Sanz JL, Muñoz García J, Echavarri Otero J, Lorenzo Yustos H, Díaz Lantada A (2005) Proyecto de Máquinas: Apuntes de Clase. Sección de Publicaciones de la E.T.S.I. Industriales – Universidad Politécnica de Madrid. Madrid

Pahl G, Beitz W (1996) Engineering design: a systematic approach, 2nd edn. Springer, London

Roozenburg N, Eeckels J (1995) Product design: fundamentals and methods. John Wiley and Sons, New York

Saaksvuori A, Immonen A (2008) Product lifecycle management, 3rd edn. Springer, Heidelberg

Stark J (2004) Product lifecycle management: 21st Century paradigm for product realisation. Springer, Heidelberg

Chapter 6
Addressing the Complexity of Biomaterials by Means of Biomimetic Computer Aided Design

Andrés Díaz Lantada

Abstract The degree of optimization achieved by biological materials and their very special properties, their hierarchical designs and their multi-scale structures, continue to be great sources of inspiration for engineers and materials scientists world-wide. Fortunately, the development, in the last decades, of advanced computer-aided design, engineering and manufacturing technologies and the groundbreaking manufacturing paradigm consequence of the advent of additive manufacturing technologies, which enable solid free-form fabrication, have provided extremely relevant resources for the development of new knowledge-based multifunctional materials following biomimetic approaches for enhanced performance. This chapter covers some of the new design and manufacturing strategies that promote biomimicry and their advantages will be also put forward by means of several cases of study included in the following chapters, linked to the complete development process of tissue engineering scaffolds, organs-on-chips and other microfluidic biomedical devices benefiting from bioinspired designs. Section 6.1 introduces the term biomaterial and compares it to the concept of biological material, also detailing main differences between synthetic and biological materials, which constitute challenges as well as sources of inspiration for materials scientists and engineers. Sections 6.2 and 6.3 cover different strategies for obtaining biomimetic designs using the information from imaging techniques as principal input, while Sects. 6.4 and 6.5 detail procedures based on direct modeling by means of advanced computer-aided design, recursive and Boolean operations and models based on precise mathematical descriptions of living organisms, tissues and biological structures.

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2,
28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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6.1 Biomaterials and Conventional Man-made Materials

The term "biomaterial" generally designates materials used in the manufacture of devices that interact with biological systems and that are applied in the different branches of medicine (Wong and Bronzino 2007; Peterson and Bronzino 2008). This definition includes materials with very different properties and classifiable into different families, such as metals, ceramics, polymers and composite materials. According to their origin they can also be classified as natural or synthetic. Another possible classification is based on the influence the biomaterial has on the body or the extent of the reaction it produces on surrounding tissues, the following division being generally accepted:

- Bioinert materials. Characterized by their low reactivity in the body, which means they can co-exist with the surrounding tissue without any apparent change to the functions and properties of this tissue. Typical materials of this kind used in implantable devices are, tantalum, titanium, aluminium, magnesium and zirconium oxide.
- Biodegradable or bioabsorbable materials. They have the capability to be body-compatible and to degrade a certain time after implant, giving rise to non-toxic products that can be eliminated or metabolized by the body. Some materials of this family are porous hydroxyapatite, the salts of calcium phosphate and some polymers, such as poly-(lactic acid) and poly-(vinyl alcohol).
- Bioactive materials. They have the ability to form direct chemical ties with the surrounding tissue allowing this tissue to grow freely on their surface. Some examples of these materials are high density hydroxyapatite and tricalcium phosphate.

Some authors identify the term "biomaterial" with that of "biological material", although we will use the aforementioned definition of biomaterial, which is more versatile, as it includes all types of materials used for the successful development of biodevices, including those natural and synthetic, as well as those coming from living organisms (biological materials) and those obtained from inert sources.

In any case, for the progressive improvement of synthetic biomaterials, towards more effective performances in the field of biomedical microdevices, it is very important to take into account the main differences between biological materials and man-made materials.

Biological materials typically include special features, such as: (a) functional gradients of density and mechanical properties consequence of their anisotropic lattice and porous structures; (b) self-healing properties thanks to the presence of cells able to re-configure the extra cellular matrix and tl correct problems related to ageing or damages; (c) optimal performance considering the energy employed to develop the biomaterial, the energy consumed during its life-cycle and the strains and stresses required to adequately interact with the environment.

Many biological materials also incorporate self-sensing abilities and the biological materials and structures can be considered smart systems with sensing and actuating capabilities. Regarding even more specific features and properties of some biological materials, it is interesting to highlight aspects such as the control of contact phenomena achieved by means of incorporating multi-scale geometries and surfaces with micro-/nano-topographies, which has a very relevant impact in the development of advanced functionalities including self-cleaning properties (as happens with the lotus flower leaves to cite just an example), improved self-healing abilities and enhanced endurance to external dangers.

On the other hand, traditional man-made or synthetic materials have normally mainly focused on a progressive improvement of yield stresses, Young's moduli, hardness and toughness, in some cases taking also into account materials density and trying to optimize the mechanical endurance/density ratio. The development of composite materials and foams, the application of thermal processes such as tempering or the application of surface hardening coatings, among other already common materials and industrial processes, has enabled additional degrees of freedom for designers and led to the concept of "knowledge-based materials" and "knowledge-based multifunctional materials".

According to the European Virtual Institute of Knowledge Based Multifunctional Materials, KMM-VIN AISBL, these "knowledge-based (multifunctional) materials" are designed for enhanced performance in very demanding loading and environmental conditions like thermo-mechanical and impact loading, high strain rates and temperature regimes, aggressive chemical environment, and possible combinations thereof. Such regimes are typical of applications in health, aerospace and automotive transport, energy, turbo-machinery industry, tribology, chemical industry, electronic devices and microsensors. These materials (abbreviated as KMM) include inter alia advanced ceramics, metal-ceramic composites, other composites, functionally graded materials, intermetallics, shape memory alloys, coatings, high temperature steels, biomaterials and other types of smart materials, capable of responding in a desired way to external stimuli (http://kmmvin.eu/).

However, the degree of optimization achieved by biological materials and their very special properties, continue to be great sources of inspiration for engineers and materials scientists world-wide. Fortunately, the development, in the last three decades, of advanced computer-aided design, engineering and manufacturing technologies and the new manufacturing paradigm consequence of the advent of additive manufacturing technologies, which enable solid free-form fabrication, have provided extremely relevant resources for the development of new "KMM" materials following biomimetic approaches for enhanced performance.

This chapter covers some of the new design and manufacturing strategies that promote biomimicry and their advantages will be also put forward by means of several cases of study included in the following chapters, linked to the complete development process of tissue engineering scaffolds, organs-on-chips and other microfluidic biomedical devices benefiting from bioinspired designs.

But before entering into details, it is important to consider the main differences between of Euclidean geometry, used by most computer-aided design software for product development, and non-Euclidean geometry, which is better suited for describing natural objects. The fact is that Euclidean geometry is limited for describing and modeling the complexity of our Universe. Some of Euclid's axioms were in fact revised during XIX and XX centuries, by the introduction of alternative geometries, more adequate for describing complex objects and phenomena, such as elliptic and hyperbolic geometries for describing planets and perceptual distorsions (Bolyai and Lobachevsky) or even studying the nature of light (Einstein's Theory of General Relativity). More recently, the complexity and auto-similarity of several natural systems and natural occurring phenomena has given birth to the field of fractal geometry.

The use of fractal models for mimicking such natural surfaces can prove to be useful for design tasks. Fractals are rough or fragmented geometric shapes that can be split into parts, each of which is (at least approximately) a reduced-size copy of the whole. The term *fractal* was coined by Benoît Mandelbrot in 1975 and derives from the Latin *fractus* meaning "broken" or "fractured"; benchmark handbook on fractal geometry and nature explains the birth of this novel geometry in depth (Mandelbrot 1982). The term is used to describe complex geometries that are too intricate to be formulated in conventional Euclidean terms, with properties like self-similarity and defined usually with simple recursive procedures. The mathematical equations defining fractals are "nowhere differentiable" and cannot be measured in conventional terms. A fractal usually has a "fractal dimension" exceeding its topological dimension and that may fall between the integers. For instance fractal surfaces, due to their roughness and intricate appearance (when looked at close range) are more than bidimensional, even though their overall appearance (when looked from the distance) is planar. Additionally fractal and random paths, even though their unifilar appearance, can end up covering the whole reference plane, when the path length increases, thus being sometimes even bidimensional. Several definitions of fractal dimensions can be found in the references (Mandelbrot 1982; Falconer 2003) and the details are out of the scope of present Handbook although, in some models used latter on, we will refer to the fractal dimension of some surfaces (with fractal dimensions between 2 and 3), normally directly connected with a parameter of the defining equation.

Since the early works linked to fractal geometry, it became clear that they could be used for describing the geometries, patterns and roughness of natural objects. Although fractals are commonly considered to be infinitely complex (due to their usual recursive definitions) "approximate fractals" are easily found in nature, which usually display self-similar structure over an extended, but finite, scale. By limiting the steps applied in a recursive definition of a conventional fractal, approximate fractals can be obtained, which mimic very complex natural geometries. Natural objects that are approximated by fractals include clouds, mountains, lightning bolts, coastlines, snowflakes, various vegetables and several corporal and animal geometries (Mandelbrot 1982; Falconer 2003).

6.2 Medical Images as Source for Inspiration

The advances seen in recent decades in different medical image capture systems (mainly, computed tomography (CT), Doppler echo scans, nuclear magnetic resonance (NMR) or magnetic resonance imaging (MRI) and positron emission tomography (PET), as well as more novel combinations PET/CT) have led to a remarkable increase in the diagnostic capabilities of these machines as well in the reliability of the diagnoses made based on this information and the therapeutic decisions taken as a result. Main differences between the different medical imaging (MI) technologies can be explained by means of the type of radiation they use, of the final precision and of their several application fields.

For example nuclear magnetic resonance imaging uses non-ionizing radiation, while computed tomography or positron emission tomography use ionizing radiation. Normally NMR is more linked to obtaining images from soft-tissues, while CT usually focuses on hard-tissues, even though such conventional separation has blended in the last decade. PET is typically more used as a diagnosis support tool in oncology and neurology, usually together with an additional result from more precise anatomical imaging. In fact PET scans are increasingly read alongside CT or magnetic resonance imaging (MRI) scans, with the combination (called "co-registration") giving both anatomic and metabolic information (what the structure or organ is, and what it is doing biochemically). More modern PET scanners are now commercially available with integrated high-end multi-detector-row CT scanners (so-called "PET/CT"). CT and MRI scanners are able to generate multiple two-dimensional cross-sections (called tomographs, or "slices") of tissue and further three-dimensional reconstructions. Early PET scanners had only a single ring of detectors; hence the acquisition of data and subsequent reconstruction was restricted to a single transverse plane. More modern scanners include multiple rings, essentially forming a cylinder of detectors.

The medical community is also currently benefitting from the opportunity to exchange information from different medical image capture systems among centres and researchers. This is thanks to the "DICOM" (Digital Imaging and Communication in Medicine) standard and its generalised usage as a working format for different three-dimensional image reconstruction software, particularly with the introduction of version DICOM 3.0 in 1993.

Software resources like "MIMICS" (Materialise NV) have also appeared (see the list provided below), which not only enable three-dimensional reconstruction to be performed from medical images, but also basic operations on these images and their conversion to other more universal formats usable by "CAD-CAM" design, engineering and manufacturing programs. As already explained, these CAD-CAM programs (Solid Edge, Catia, NX-8.5, Autodesk-Inventor, I-DEAS, Rhino, Solid Works and others) comprise a wide range of computer tools that assist engineers,

architects and design professionals in their work. Simulations for in silico assessment of designs can also be performed with the help of CAE resources.

The power of these software packages quoted, and their being able to be used to handle information from medical imaging as a basis for the designs, means that currently the design of personalised prostheses and bimiemetic biomedical devices can be performed in a question of hours while also making easier comparisons between alternative designs (Hieu 2002; Harryson 2007). In addition, the considerable industrial expansion experienced in recent years by a range of technologies called "rapid prototyping (RP) technologies", normally based on high-speed computer numerical control machining or on additive manufacturing approaches, that enable schedules and costs to be reduced by manufacturing parts directly from geometric information stored in CAD-CAM program files, are presenting new opportunities for a personalised response to the development of implants, prostheses and biodevices in general, the social impact of which could turn out to be highly positive (Schwarz 2005; Kucklick 2006).

Progressive linkage between CAD tools, MIMICS-like software and CAM assisted manufacturing, is resulting benefitial for the promotion of bioinspired or biomimetic approaches in all kinds of products and industries, and very especially in the biomedical field. The more remarkable software resources, together with applications in the product development sector, have been previously reviewed (Díaz Lantada and Lafont Morgado 2011) and are actualized further on, providing some examples of how the use of CT-imaging is indeed versatile.

There are several software tools, for handling the information obtained from medical imaging technologies, and enabling computer-aided design, engineering and prototyping tasks. They are usually referred to as "MIMICS-like" programs (due to the relevance of MIMICS (Materialise NV). Among such programs, due to their industrial impact and quality of results, it is important to mention at least:

- MIMICS (Materialise NV), for general purpose applications.
- Simplant & Surgiguide (Materialise NV), oriented to Odontology.
- 3D Doctor, for bone modeling from CT scan and soft tissue from MRI.
- Analyze (Mayo Clinic), for handling images from MR, CT and PET.
- MRIcro Software, for converting medical images to Analyze format.
- Biobuild, for converting volumetric imaging data to RP file formats.
- Volume Graphics, for general purpose applications.

Listed below are the main applications of computerized tomography (as a representative technology within the medical imaging sector), together with software for processing medical images and "CAD-CAE-CAM" tools, for optimizing product design and development activities:

- Personalized designs (Bibb and Brown 2000; Chang et al. 2003; Díaz Lantada et al. 2010a, b, c, d).
- Reverse engineering (Flisch 1999; Vasilash 2009).

- Object reconstruction (Effenberger et al. 2008; Vasilash 2009).
- Prototyping and trials (Flisch 1999; Effenberger et al. 2008).
- Inspection of manufacturing defects (Losano et al. 1999; Effenberger et al. 2008).
- Inspection of crack propagation (Losano et al. 1999; Effenberger et al. 2008).
- Non-destructive evaluations (Losano et al. 1999; Effenberger et al. 2008).

These technological combinations provide novel ways of tackling more efficiently the design process, but also for validating manufacturing processes and verifying service life. It is very important to mention that the whole process is economical and non-destructive.

The case study detailed in this section as an example details the process for producing a customized hip prosthesis design from the helpful information of medical images. The aim was to produce a non-cemented prosthesis where the metal part is pressure-mounted inside the femur and must therefore be made to fit the available space. More detailed information may be found in the references (Osuna 2008; Ojeda et al. 2009). Just as a brief revision, hip replacement is a surgical procedure in which the hip of the patient is replaced by a prosthetic hip. Such joint replacement orthopaedic surgery is generally conducted to relieve arthritis and related pain or to fix severe physical joint damage as part of hip fracture treatment. A total hip replacement (total hip arthroplasty) consists of replacing both the acetabulum and the femoral head while hemi- (or half) arthroplasty generally only replaces the femoral head. The prosthesis used in hip replacement consists of different parts, the acetabular cup, the femoral component, in which this case study focuses and the articular interface. The femoral component is designed to fit in the femur, normally by removing a part of the bone and shaping the remaining part to accept the prosthetic component. There are two main types of femoral components, cemented, based on adhesive fixation between prosthesis and bone, and uncemented, based on friction for promoting stability. Final prosthesis type selection depends on several factors, including age of the patient, mechanical strength of the bone, as assessed with the help of medical imaging, life expectancy, among others.

Even though the potential benefits of personalized femoral components for hip replacement is still controversial, it is clear that, if taking the geometry of patient's femur as design input for the femoral component, required bone adaptation (through boring, milling and cutting) during surgical intervention should be lower and lighter. In addition, the final manufacture towards a final porous structure could lead to enhanced osseointegration, allow the incorporation of antibiotics for improved recovery and lead to mechanical properties mimicking those of bone, hence promoting a biomimetic and biomechanical performance.

Regarding the design and manufacturing strategy, the usual procedure to carry out a customized examination, with a view to using a biomimetic device, usually begins either by taking a computerized tomography—CT or a nuclear magnetic resonance—MRI /NMRI of the patient needing the prosthesis. Then, with the aid of

.dicom or .dcm (Digital Communications in Medicine) format, the information from the CT or MRI can be transferred to a program such as "Mimics", so that it can be displayed in 3D. These programs usually include modules for selecting parts of the patient's bone geometry and storing them in .stl or .igs formats that can be read by other CAD programs, for ad hoc design operations, after processing the images "slice by slice".

Figure 6.1 provides an example of the damaged geometry of a hip joint, as seen using nuclear magnetic resonance and as three-dimensionally reconstructed by means of CAD resources.

After having selected the relevant part of the biological component, in this case of study the internal cavity of the patient's femur, to which the metal part of a customized prosthesis must be adapted, this 3D geometry can be transferred to a valid format for a design program and this femoral zone can be used as the basis for a customized prosthesis design. Final Boolean operations with previously designed lattice or porous structures lead to a final porous biomimetic and biomechanical implant (Fig. 6.2).

A similar process is used in other cases of study along the Handbook (see Chaps. 16 and 19 for the design of tissue engineering scaffolds for tibial and vertebral repair respectively).

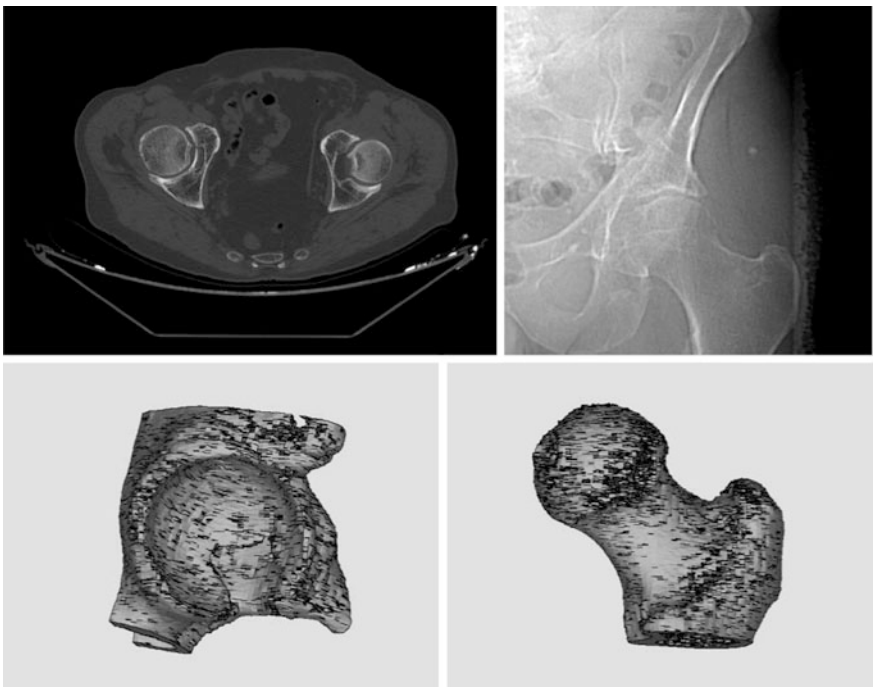

Fig. 6.1 The damaged geometry of a hip joint, as seen using nuclear magnetic resonance and as three-dimensionally reconstructed by means of CAD resources

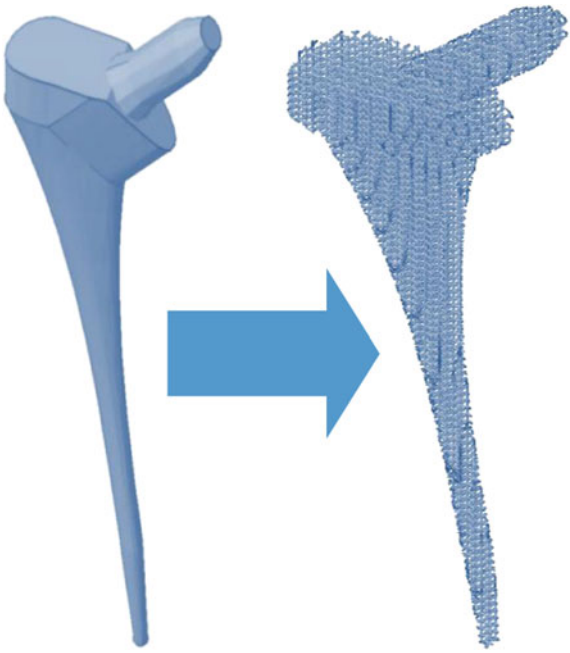

Fig. 6.2 Personalized design of the femoral component of a hip prosthesis and porous prosthesis obtained, by means of Boolean operations, for the promotion of osseo-integration and biomechanical performance, thanks to elasticity matching

6.3 Digitalizing Biological Materials for Design Purposes

Digitalizing biological materials, either resorting to imaging technologies based on optical scanners, laser systems or penetrating radiation, or by developing and employing mathematical models capable of describing the degree of complexity of biostructures and biosurfaces, has relevant implications in the final development of biomimetic biomedical microdevices.

The use of mathematical models to generate biomimetic surfaces (see Sect. 6.5), especially thanks to the gradual employment of recursive and fractal models, often yields good approximations to the microtopography of living organisms, though it poses certain limitations when generating 3D CAD files for subsequent use in conducting simulations and obtaining physical prototypes using computer-aided engineering and manufacturing (CAE-CAM) tools. However, said biological surfaces, generated from mathematical models, present sometimes excessive homogeneity or self-similarity. In consequence, the slight imprecisions of living organisms, which are responsible for certain interesting properties, cannot be adequately represented and their further connection with final applications in the field of biomedical devices is sometimes complex.

Therefore, processes using imaging techniques for the digitalization of biological materials may be preferred in many cases, although some technologies may be extremely expensive for small and medium design enterprises. Depending on the

desired level of precision, low-cost imaging resources (desktop scanners, photo-based reconstructions) may also provide rewarding results.

In this section we provide an example of a fast, low-cost and efficient method to yield biomimetic 3D CAD files of the microtopography of biological materials, files that can subsequently be used as an aid for simulating various interactions (mechanical, thermal, fluid, etc.) between the environment and the biomaterial, as well as for the micromanufacturing of small specimens, whose texture resembles that of the model. The process is applied to reconstructing a small skin patch and a superhydrophobic patch of cloth.

The process relies on a high-resolution photo-camera to obtain images of the area being analyzed and on converting the resulting images into height matrixes, which are then used to construct the CAD files that imitate the tiny details of the original three-dimensional geometries. Using a similar process several microtopographies and micro-textures of living organisms can be mimicked and further used for designing biomedical microdevices with improved features for interacting at a cellular level.

Just as a brief revision, the skin is the body's most extensive organ, forming the main barrier between internal organs and the external environment. It accounts for around 16 % of the body's weight. It has a surface area of some 2 m^2, and it varies in thickness between 0.5 mm at the eyelids and 4 mm at the heels. As the body's first line of defense, it is constantly exposed to potentially harmful environmental agents, including solid, liquid and gaseous materials, sunlight and microorganisms. Although the skin can be bruised, lacerated, burned or infected, its unique properties allow it to engage in a constant cycle of healing, exfoliation and cellular regeneration. To fulfill its protective role, the skin is home to a permanent flora of microorganisms. There are relatively innocuous strains that protect the skin's surface from other, more virulent microorganisms. A thin layer of lipids covers the skin and contains oily bactericidal acids that protect against penetration by harmful microorganisms. The skin, thus, also doubles as an immunological barrier. It also has other important functions such as temperature regulation, somatosensation and the synthesis of vitamin D.

There is a great amount of variation between the different body parts in terms of the skin's structure. This makes a description of the "normal skin" covering each body surface difficult. There are clear differences in the properties of skin; for example, the thickness of the layers, the distribution of sweat glands and the amount and size of hair follicles. Nevertheless, skin does have certain structural properties that are common to all parts of the body. It always consists of three layers: the epidermis (outer layer), the dermis (internal layer) and the subcutaneous adipose layer (hypodermis). The basement membrane separates the first two layers, while the subcutaneous tissue, a layer of loose connective tissue and adipose tissue, connects the dermis to the body's underlying tissues (Simandl 2009).

The skin's functions depend greatly on the properties of its outermost layer, the epidermis, meaning that properly simulating its surface microtopography is necessary in order to conduct studies on the interactions between the environment and the human body. However, most recent studies on the computer-aided graphical

generation of human skin have involved simulations of large areas of the human body, avoigint the incorporation micrometrical details in almost every case, as this would have entailed time- and computer-intensive calculations.

Some researchers have focused on modeling wrinkles and the effects of aging (Boissieux et al. 2000; Yang and Zhang 2005; Zhuo et al. 2006) in an effort to enhance the appearance of animated characters in entertainment programs and in the videogame industry, as well as to simulate the effects of various cosmetic products. Leading studies have resorted to generating wrinkles along vector fields so as to incorporate additional textures to surface meshes (Bando et al. 2002). In order to take into effect biomechanical aspects, recent research has resorted to using the boundary element method to simulate skin defects and to analyze their effect on other anatomical structures (Tang 2002), though detailed effects of the surface topography were omitted. On occasion, physical prototypes have also been constructed to simulate the mechanical features of the epidermis, the dermis and subcutaneous fat. These models used polymeric materials of different rigidity and hardness to complement surgical training simulators, especially as these relate to devices for minimally invasive laparoscopic surgery (Munro et al. 1994).

In terms of the biomimetic design of anatomical elements, numerous researchers have resorted to the use of medical imaging tools (mainly computerized tomography and nuclear magnetic resonance), in combination with software to process said images (such as MIMICS, Materialise NV) and CAD programs. The availability of CAD files with the geometry of body structures, both muscular and bone tissues, has thus served to aid in the development of personalized implants (Kucklick 2006; Díaz Lantada et al. 2010a, b, c, d), especially when combined with rapid prototyping techniques (Winder and Bibb 2005; Kim 2008). The accuracy of the aforementioned medical imaging systems, however, still does not allow for a faithful reproduction of the details associated with the surface microtopography of tissues, though new advances in micro-CT technology are constantly yielding significant improvements (Shi et al. 2008; Guo et al. 2010). The use of CAD-CAE-CAM (computer-aided design/engineering/manufacturing) tools is also applicable to tissue engineering, having given rise to a new field of study called computer-aided tissue engineering, a field that was initially associated with anatomical imaging, modeling and simulation and with surgery planning (Sun and Lal 2002). This, in conjunction with new advances in biomanufacturing (see also Chap. 23) and associated biomaterials-based additive manufacturing tools (bioplotters), points to the manufacture of small body structures in the not-to-distant future. In any case, in order to benefit from the advantages of high-precision aided manufacturing systems aimed at producing artificial biostructures, we have to take into account all aspects related to the generation of surface microtopographies, the effects of which are crucial to the proper operation of the tissues that we wish to mimic.

The biomimetic processes typically employed involve the use of mathematical models, such as fractals (Mandelbrot 1982), and can output surface textures to CAD files, which can be converted to formats that can be exported to CAE-CAM software. These files in adequate formats can then be used, in conjunction with finite

element analysis techniques, to conduct simulations as a prelude to the manufacture of physical prototypes (Díaz Lantada et al. 2010a, b, c, d, Biocoat), though imitating the desired topography is not always simple (Sect. 6.5).

In this section, as already metioned, we resort to a low-cost process based on high-resolution photography. The skin surface photographed for present case study measures 9 mm × 6 mm, yielding 640 × 480 pixel images, meaning that the size of the details captured is on the order of 20 μm, which is sufficiently precise for the majority of micromanufacturing techniques currently available, (see Chap. 8), as well as for analyzing any kind of cutaneous pathology. The area photographed corresponds to a section of the fingerprint of the author. The images are processed using Adobe Photoshop to convert them to gray scale, followed by filtering to soften highlights. The images, saved in the .raw format, are then input to a program that converts the gray scale to an altitude scale, similar to some open-access programs used for 3D printing of photographs. The program also converts the files into the .stl format for use in computer-aided design.

Different options are available for the CAD software used to convert from surface meshes, in .stl format, to conventional solid CAD pieces. Particularly important is the use of software specifically designed to handle .stl files (Materialise Magics, VisCAM, Solid View, MeshLab, among others) or the use of so-called mesh-to-solid programs, which transform .stl meshes into formats typically recognized by other CAD software. In our case, we used the CAD-CAE-CAM NX-8.5 software (Siemens PLM Solutions) to represent the .stl surfaces and the solid CAD pieces, with the final rendering. Subsequent conversions to .iges format allows for an additional exchange of information with more specific calculation programs, such as Ansys or Abaqus, as well as with programs specifically designed for additive rapid prototyping with 3D Lightyear by 3D Systems.

As mentioned earlier, the design process starts by converting the image of a photograph, expressed as a matrix with information on the colors (or the gray scale) for each coordinate pair (x,y) on the plane, into a matrix in which the colors or gray scale are replaced by data on the altitude of each point on the photo. To achieve this, the darkest pixels in the image are assigned a zero altitude, representing the bottom of the folds in the skin. The image's brightest pixels are assigned an altitude based on reference information and models (Boissieux et al. 2000; Jacobi et al. 2004; Yang and Zhang 2005) that provide different typical values for the height of wrinkles, depending on region of the body and age of the subject. In our case we use a maximum difference of 160 μm for the fingerprints. Values between these maximum and minimum values are linearly interpolated. An additional scaling along the x- and y-axes contained in the plane of the original image may also be necessary to adapt the size of the meshed surface to the actual dimensions of the area photographed, which in this case was 9×6 mm^2.

Once the height matrix is obtained, it is converted into a .stl format surface mesh, which allows for subsequent processing by specific CAD software. Since surfaces with negligible thickness, like the intermediate meshes in .stl format, cannot be manufactured with the aid of RP technologies, nor do they allow for simulations based on the application of finite element analysis, they must first be converted into

solid pieces with a non-zero thickness. The first step in this process can be achieved with the typical CAD tools used to automatically generate molds (core and cavity) from surfaces.

In the second step, the core can be cut at the desired distance to obtain the desired surface but with a certain thickness. Or a prismatic block can be used on which to imprint the wrinkled surface before finishing the process by combining the block and the cavity. Figure 6.3 shows the high-precision picture of the skin patch and CAD model showing the three-dimensional reconstruction. The model has been obtained by converting the colours of the two-dimensional image, into the values of

Fig. 6.3 High-precision picture of a skin patch and CAD model showing the three-dimensional reconstruction. The model has been obtained by converting the colours of the two-dimensional image, into the values of height over a grid planar

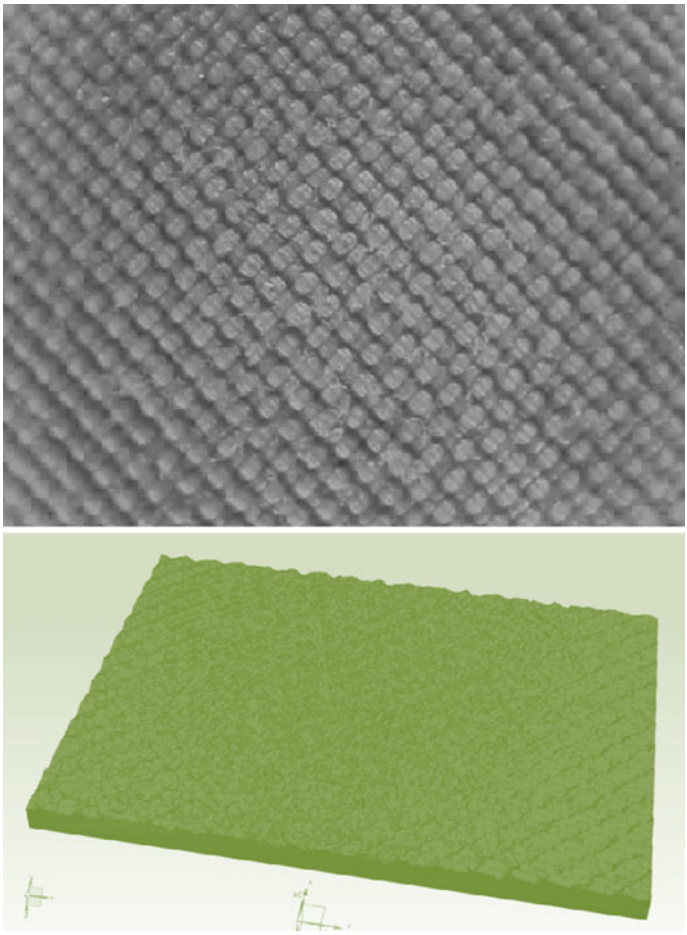

Fig. 6.4 High-precision picture of a super-hydrophobic cloth patch and CAD model showing the three-dimensional reconstruction

height over a grid planar. Figure 6.4 provides and additional example, obtained using a similar procedure, related to the three-dimensional reconstruction of a super-hydrophobic cloth patch.

The 3D images shown help to demonstrate the simplicity and effectiveness of the process described. They also help to validate its applicability for producing biomimetic designs of the surfaces of biological and biomimetic object for further bioinspired design tasks. This proposal also has applications in the field of tissue engineering, since it can aid in producing CAD files with geometries that imitate the surface characteristics of different fabrics for subsequent simulation of their behaviour with the aid of FEM-CFD software, as has already been done with certain biomimetic surfaces. It should prove interesting to employ this type of files to assess

the response of tissues with different designs in terms of their surface texture and the response of different fluids so as to analyze their hydrophobic and impermeability characteristics.

Regarding the manufacture of biological and bioinspired surfaces, the proposed design method, with the help of high-precision additive manufacturing, may well be an important complement to current biofabrication and bioreplication tools, such as biotemplating, sol-gel, atomic layer deposition, physical-/chemical-vapour deposition or imprint lithography and casting, for several industrial applications (Pulsifer et al. 2010; Lakhtakia et al. 2009). For large series of parts and devices soft-lithographic approaches and micro-replication techniques, such as micro hot-embossing and micro injection molding, may also be good choices, as detailed in Chap. 10.

After having detailed in Sects. 6.2 and 6.3 different strategies for obtaining biomimetic designs using the information from imaging techniques as principal input, Sects. 6.4 and 6.5 detail procedures based on direct modeling by means of advanced computer-aided design, recursive and Boolean operations and models based on mathematical descriptions of living organisms and tissues.

6.4 Computer-Aided Design for Controlling the Structure and Density Distribution of Materials

The structure and density distribution of biomedical devices for interacting with human tissues, from prostheses and tissue engineering scaffolds, to micromembranes for microfluidic devices and organs-on-chips, can be in fact easily controlled with the use of common computer-aided desing resources.

The process, for biomimetic lattice structures, normally includes combination of solid operations (cylinders, piles…) for obtaining a unit cell. Subsequently, a pattern operation or a periodic replication of such solids and unit cells leads to a 3D portion of the space being filled with the desired lattice structure. Intersecting the obtained lattice with a solid device leads to the final biodevice with controlled inner structure. In the case of porous structures, the process instead of additive is subtractive. It normally begins with a cube, sphere or cylinder, from which smaller spheres and cubes are usually subtracted. The porous structure (or metamaterial) obtained can additionally be intersected with the geometry of a solid prosthesis, for finally obtaining a porous implant.

Using different sizes of lattices and pores, just by employing 2D patterns in the XY plane, and by changing the dimensions of each 2D pattern along the z axis, leads to functional gradients of density and mechanical properties. With this approach, another typical property of biological materials, which usually include functional gradients of properties, can be obtained. Pore size and lattice thickness

can be also controlled from the inside to the outside, with applications in the development of artificial bone models.

Hierarchical and multi-scale approaches can be also used, working recursively for the development of fractal-like structures, with pores at different scale levels, as also happens in biological materials. Modeling human vasculature, which has a typical hierarchical fractal-like geometry and developing advanced types of tissue engineering scaffolds, in which the generation of vasculature is promoted, are just some examples of potential applications.

Even though all conventional CAD programs already commented (Solid Edge, NX-8.5, Catia v.5, Solid Works, Autodesk-Inventor…) include several operations for designing unit cells and replicating them, for applying pores to solid objects and Boolean operations for applying an outer geometry to a lattice structure, novel CAD resources are being specifically developed for promoting the application of meta-materials to product development.

Among ad hoc CAD software resources oriented to the design of lattice and porous structures, for improved control of aspects such as density, stiffness and resistance of final geometries, we would like to cite "Within" (www.within-lab.com), "Inspire" (www.solidthinking.com), and "Netfabb" (www.netfabb.com), as the most advance ones, and with direct application in Biomedical Engineering and in the development of biomedical microdevices for interacting at a cellular and even molecular level.

Recent advances in topological optimization, a mathematical approach that optimizes material layout within a given design space, for a given set of loads and boundary conditions, are also helpful for deriving into lattice and porous structures and progressively being incorporated to conventional CAD resources (Bendsoe and Sigmund 2003; Schramm and Zhou 2006).

Several cases of study linked to the use of porous geometries, lattice structures, multi-scale and hierarchical designs for the development of advanced biodevices, including: tissue engineering scaffolds for bone, muscle, cartilage and ligament repair; microfluidic devices with microporous membranes, for static and dynamic cell culture procedures; and organs-on-chips, for modeling relevant physiological interactions benefiting from the use of hierarchical and multi-scale microtextures, micromembranes and micropillars, are included in Chaps. 15–23.

Figure 6.5 introduces some three-dimensional CAD structures, with controlled distributions of density and mechanical properties, which can be physically obtained by means of additive manufacturing technologies in a wide set of materials, from (bio)polymers and (bio)ceramics, to metals, alloys and composites. Some of them will be further employed and detailed as complete development cases of study in the aforementioned chapters. In many cases these geometries are also referred to as cellular structures.

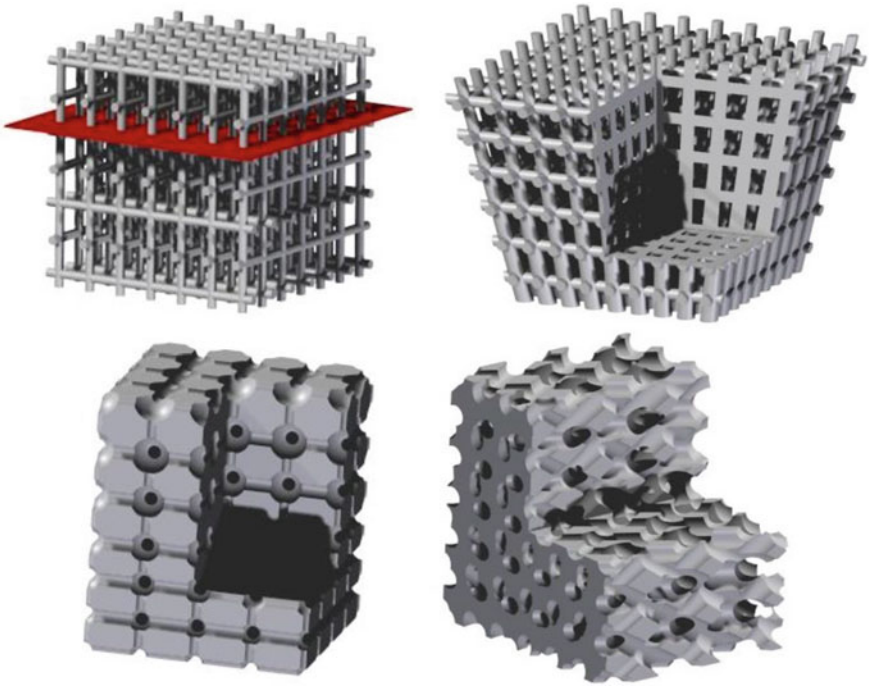

Fig. 6.5 Examples of three-dimensional structures, with controlled distributions of density and mechanical properties and with direct applications in the field of biomecial microdevices for interacting at a cellular level (Téllez, M., Díaz Lantada (advisor), 2015, see Chap. 15 for additional details)

6.5 Computer-Aided Design for Controlling the Textures and Topographies of Materials

Several studies have focused on the importance of surface topography and microtexture for promoting positive effects in all kinds of biomedical devices, from implantable prosthesis to extra cellular matrixes and scaffolds for cell growth and tissue engineering. These textures have a significant influence in osseointegration of prosthesis, cell proliferation and tissue growth given that those cells and tissues seem to be more "comfortable" and spread more quickly when faced with biodevices with similar surface properties.

In addition the use of biomimetic surfaces can help to introduce numerous desirable phenomena in machine, mechanical and structural elements, thus improving contact between parts, reducing wear or even obtaining self-cleaning objects (Barthlott and Neinhuis 1997; Groenendijk 2007). However, the process of introducing desired roughness on the surfaces of man-made objects is still mainly linked to carrying out machining operations, laser processing or chemical attacks.

In all these cases, post-processing operations can be difficult to control and it would be very positive to directly impose special topographies from the design stage.

During the last decade, increasing attention has been paid to using fractals for promoting modeling, design and simulation tasks in several areas of Biomedical Engineering, some of them also linked to the development of novel biomedical microdevices for interacting at a cellular and even molecular level. The most remarkable ones include:

- Modeling the behaviour of microorganisms. Several studies have been reported on the use of fractal models for describing the growth and expansion rate of bacteria and for evaluating the dynamics of coexisting species of microorganisms (Tsyganov et al. 2007).
- Modeling complex organisms and their systems. Regarding complex organisms (including human anatomy) fractals have been applied to modeling systems of pulmonary and blood vessels and vascular networks, as well as for carrying out subsequent fluid mechanics simulations (Lin et al. 2004).
- Modeling the surfaces of organs and tissues. Recent interest has appeared in the use of fractals for mimicking the surfaces of organs and tissues and thus improving the designs and in vivo performance of several prosthetic devices (Longoni and Sartori 2010).
- Designing biomimetic biodevices, such as scaffolds for tissue engineering or prostheses with improved tribological properties (Díaz Lantada et al. 2010a, b, c, d, 2012a, b).

In fact, very recent interest has appeared in the use of fractals for mimicking the surfaces of organs and tissues and thus improving the designs and in vivo performance of several prosthetic devices, although some limitations linked to the design procedure still have to be overcome (Díaz Lantada et al. 2013).

We explain in this section the use of mathematical fractal models for designing the complex and highly irregular surfaces of biomimetic objects. In this way, some parameters including roughness, waviness, skewness… can be controlled from the design stage and adapted in a more efficient way to the requirements of the final applications. The final bioinspired or biomimetic multi-scale surfaces z(x,y) can be considered as the sum of two different types of surfaces (z_m (x,y) for the micro-textures and z_n (x,y) for the nanotextures), each providing a relevant components at a different scale level. Fractal models can be applied to controlling both micro- and nano-textures or just for providing a micro or nano-texture upon already available geometries. Therefore the process offers the possibility of tailoring the surfaces micro-/nano-textures (in combination with high-precision manufacturing technologies) for inducing contact phenomena determinant for the success of biomedical microdevices, including superhydrophobicity, superhydrophilicity, self-cleaning properties, enhanced osseointegration, improved lubrication, among other features.

The surfaces and substrates, as a basis for the deisgn of prostheses, implants, tissue engineering scaffolds, cell culture platforms and biomedical microdevices can be based either on static or on dynamic fractal models. Among static models, it is

important to note the use of fractional Brownian fractal surface models and of Mandelbrot-Weierstrass equations, while the dynamic models are developed upon Kardar-Parisi-Zhang and Langevin equations. Information regarding the different terms of such equations and main design parameters can be found in the relevant references of the field of fractal geometry (Weierstrass 1886; Mandelbrot and Ness 1968; Mandelbrot 1982; Berry and Lewis 1980; Kardar et al. 1986; Falconer 2003; Coffey et al. 2004). The use of fractional Browninan fractal surfaces is also additionally detailed in Chaps. 8, 10, 12 and 13, when describing the complete development process of micro-textured microdevices for the assessment of cell adhesion, motility and overall behavior.

In short, a fractal model is evaluated above a grid according to the degree of precision of the final manufacturing process. The multi-scale surface is obtained as sum of the micro- and nano-textures defined accordingly and, in our case, stored in form of Matlab surface or surfaces. Once the Matlab surfaces have been obtained, using some of the fractal models previously detailed or alternative ones, the related geometrical information can be stored in the form of a [X, Y, Z] matrix and can be further converted into .stl or a similar universal format, so that the surface can be recognized and imported with a CAD program, for additional design operations (i.e. providing the surface with a thickness different to zero, copying the surface atop a previously designed geometry…).

Figure 6.6 shows the multi-scale design of a surface mimicking the micro- and nano-topography of self-cleaning biological surfaces similar to those of the lotus leaves. The Matlab code used can be found in the Annexes of the Handbook and is based on the incorporation of a fractal "noise" on top of trigonometric functions in the form:

$$Z(x, y) = A \cdot \sin(x) \cdot \sin(y) + B \cdot \sin(x/10) \cdot \sin(y/10) + \text{Brownian motion}.$$

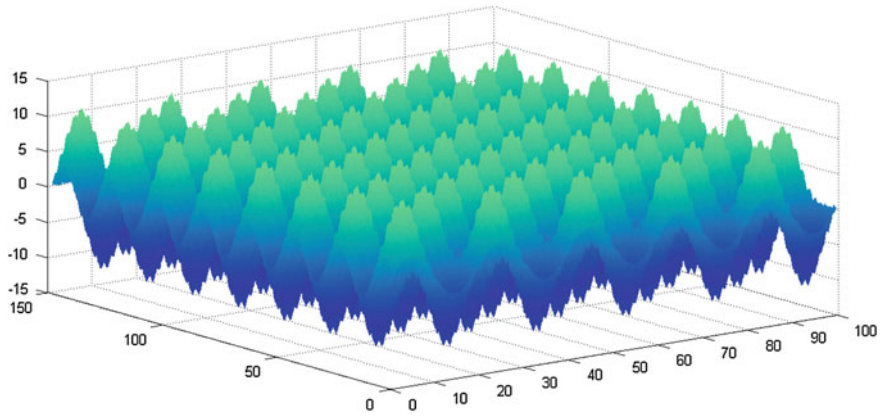

Fig. 6.6 Multi-scale design of a surface mimicking the micro- and nano-topography of self-cleaning biological surfaces similar to those of the lotus leaves

The three terms allow for the surface control at different scale levels, with a couple of "wavy" terms of different frequency and a final more random noise for the incorporation of irregularities typical from biological materials.

Figure 6.7 shows the scheme for the fractal-based computer-aided design of surfaces for biofabrication and biomimetic purposes, according to a patented process by our team (Spanish Patent and Trademark Office P201030956).

Some additional biodevices based on this process for fractal-based biomimetic design, including scaffolds for cell growth and Tissue Engineering related procedures, as well as microsystems for studying cell motility, can be found in Chaps. 10–13, when focusing on the manufacture of biodevices with micro and even nano features.

Previous paragraphs have focused on the design process of biodevices based on fractal surfaces, thus leading to textured devices but with an overall geometry clearly "planar", which may be somehow limited for obtaining three-dimensional implants and prostheses. Fractal models may also be of help for reproducing the spatial morphology of tissues and organs and for providing a way of controlling aspects such as porosity, surface/volume ratio, stiffness…, which are decisive for promoting some chemical reactions and biological processes.

We introduce here the use of "fractal spheres" or "fractal seeds", whose spatial distribution for filling the 3D space and subsequent Boolean combination with the solid objects of prostheses, organs or biostructures, can lead to three-dimensional porous structures for being used as support for 3D cell growth, both in tissue engineering and in the novel field of biofabrication. The process is schematically described in Fig. 6.8 and is based on combining "fractal spheres", which can be defined by the equations detailed below. A fractal sphere, by adapting the definition

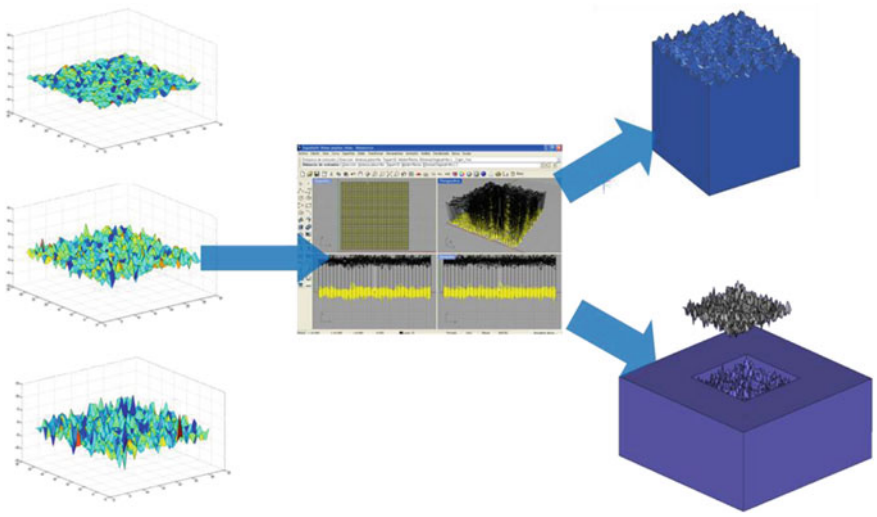

Fig. 6.7 Scheme for fractal-based design of surfaces for biofabrication. Patented process: Spanish Patent and Trademark Office P201030956

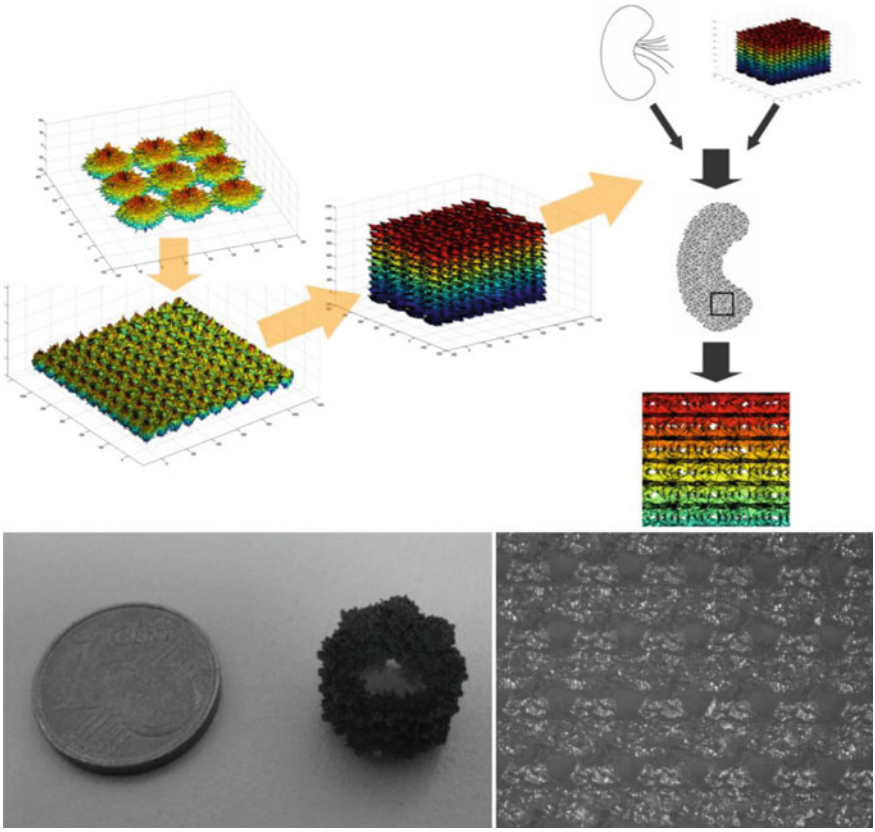

Fig. 6.8 Scheme for fractal-based design of structures for biofabrication. Patented process: Spanish Patent and Trademark Office P201030957. Rapid prototypes of three-dimenstional structures of microtextured scaffolds for tissue engineering and cell culture platforms based on fractal seeds

of fractional Brownian fractal surface, can be defined by an almost-randomly changing radius in the form:

$$r(\vec{x}) = r_0 + \sum_{k=1}^{\infty} C_k \cdot \lambda^{-\alpha k} \cdot \sin(\lambda^k \cdot |\vec{x}| + A_k)$$

Such expression describes the fractal sphere radius as a function of the position vector ($\vec{x}$) of each point of an initially regular spherical mesh (as can be obtained for instance with the "sphere" command of Matlab). Applying the expression to the initially regular sphere, the initial radius r_0 is forced to change by the summatory of terms including random functions (A_k, C_k) and control parameters (α, λ). The summatory must again be limited, so as to avoid an infinite loop, but the approximate fractal sphere obtained may well be of use for several applications.

Additional details on the computation of fractal spheres can be found by having a look at the Matlab (The Mathworks Inc.) code of the different programs included in the Annexes of the Handbook.

Similar and novel ways of extending the texturization process, based on fractal biomimetic models, to the external features of several prostheses and to the features of tissue engineering scaffolds, may promote very interesting biological and contact phenomena. The progressive incorporation as an additional command (i.e. "apply roughness" or "apply fractality") to conventional CAD programs is also matter of research and can be already achieved in modeling programs present in the cinematographic industry.

Of course these complex geometries can be even impossible to manufacture with conventional subtractive procedures, due to their inner porosity, irregular features and fractality. However, the use of additive manufacturing technologies, as thoroughly done along the Handbook, constitutes a right approach.

Similar procedures are applied along the Handbook for the design of several cases of study linked to biodevices aimed at interacting at a cellular level and at the assessment of the impacts of surface topographies on cell behavior and fate (see Chaps. 8, 10, 12 and 13).

Alternative uses of such fractal spheres and fractals applied to modifying the surfaces of three-dimensional objects are also linked to conventional prostheses, for instance for trying to provide additional roughness and increase friction coefficient, for the promotion of primary stability and subsequent osseointegration, also promoted by textures and edges to which osteoblasts typically attach properly.

We have focused here on the use of fractals for the design of irregular complex surfaces and of fractal three-dimensional objects such as spheres, perhaps not having adequately focused on linear fractal models, as they are easier to generate and widely covered in the literature and websites.

In fact fractal paths and related models (diffusion limited aggregation, lattice random walks, random branching processes, among others) can be also helpful for several tasks linked to biomedical engineering and have great potential for the development of microsystems, lab-on-a-chip devices and appliances linked to tissue engineering (prototypes for electrophoresis, prototypes with controllable capillarity, among others). They are also commonly applied to modeling cell motility and their usual random walks on top of planar surfaces. as further discussed in Chap. 12, when focusing on micro-manufacturing technologies and related photo-lithographic approaches.

In the near future biofabrication will also benefit from CAD designs based on fractal paths and similar models (i.e. branching processes for mimicking bronchia and blood capillaries…), as well as from advances on high-precision medical imaging technologies. Certainly biomimetic approaches will further benefit from the input of several disciplines, as mathematical modeling, computer-aided design process and reverse engineering technologies (including the combined utilization of medical imaging and CAD), for providing more versatile solutions.

6.6 Main Conclusions and Future Research

The degree of optimization achieved by biological materials and their very special properties, their hierarchical designs and their multi-scale structures, continue to be great sources of inspiration for engineers and materials scientists world-wide. Fortunately, the development, in the last decades, of advanced computer-aided design, engineering and manufacturing technologies and the groundbreaking manufacturing paradigm consequence of the advent of additive manufacturing technologies, which enable solid free-form fabrication, have provided extremely relevant resources for the development of new knowledge-based multifunctional materials following biomimetic approaches for enhanced performance.

This chapter has covered some of the new design and manufacturing strategies that promote biomimicry and their advantages will be also put forward by means of several cases of study included in the following chapters, linked to the complete development process of tissue engineering scaffolds, organs-on-chips and other microfluidic biomedical devices benefiting from bioinspired designs.

Main detailed strategies for the promotion of biomimicry and of biomimetic designs can be grouped in two main categories: those based on the use of imaging resources for the digitalization of biological structures and those based on the use of mathematical and software resources for the construction of geometries with properties similar to those found in biological materials. Both categories have been covered and different cases of study have been introduced and will be additionally detailed in following chapters of the Handbook.

References

Bando Y, Kuratate T, Nishita T (2002) A simple method for modeling wrinkles on human skin. In: Proceedings of the 10th pacific conference on computer graphics and applications (PG'02), pp 1–10

Barthlott W, Neinhuis C (1997) Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta 202:1–8

Bendsoe MP, Sigmund O (2003) Topology optimization: theory, methods and applications. Springer

Berry MV, Lewis ZV (1980) On the Mandelbrot-Weierstrass function. Proc R Soc London A, 370:459–484

Bibb R, Brown R (2000) The application of computer aided product development techniques in medical modeling topic: rehabilitation and prostheses. Biomed Sci Instrum 36:319–324

Boissieux L, Kiss G, Thalmann NM, Kalra P (2000) Simulation of skin aging and wrinkles with cosmetics insight. In: Proceedings of the eurographics workshop on computer animation and simulation, pp 15–27

Chang CC, Lee MY, Ku YC (2003) Digital custom dentare design with new abrasive computer tomography and rapid prototyping technologies. Biomed Eng Appl Basis Commun 15(3):115–119

Coffey WT, Kalmykov YP, Waldron JT (2004) The Langevin equation: with applications to stochastical problems in physics, chemistry and electrical engineering, 2nd edn. World Scientific Publishing Co

Díaz Lantada A, Lafont Morgado P et al (2010a) Substrato cuasibidimensional para crecimiento de células y tejidos y método de obtención del mismo. Spanish Patent and Trademark Office, Patent application number P201030957 (patent pending after positive state-of-the-art study)

Díaz Lantada A, Lafont Morgado P et al (2010b) Soporte tridimensional para producción artificial de órganos y otras estructuras del organismo humano y método de obtención del mismo. Spanish Patent and Trademark Office, Patent application number P201030956 (already granted)

Díaz Lantada A, Endrino JL, Mosquera AA, Lafont P (2010c) Design and rapid prototyping of DLC-coated fractal surfaces. J Appl Phys—Conference Series, 252(1):012003

Díaz Lantada A, Del Valle Fernández R, Lafont Morgado P, Muñoz García J, Muñoz Sanz JL, Munoz-Guijosa JM, Echávarri Otero J (2010d) Development of personalized annuloplasty rings: combination of CT images and CAD-CAM tools. Ann Biomed Eng 38(2):280–290

Díaz Lantada A, Lafont Morgado P (2011) Enhancing product development through CT images, computer-aided design and rapid manufacturing: present capabilities, main applications and challenges. In: Theory and applications of CT imaging. InTech, pp 269–290

Díaz Lantada A, Endrino JL, Sánchez Vaquero V, Mosquera AA, Lafont Morgado P, García-Ruíz JP (2012a) Tissue engineering using novel rapid prototyped diamond-like carbon coated scaffolds. Plasma Processes Polym (4 Oct. 2011) Online (DOI: 10.1002/ppap.201100094), 9(1):98–107

Díaz Lantada A, Lafont Morgado P, Echávarri Otero J, Chacón Tanarro E, De la Guerra Ochoa E, Munoz-Guijosa JM, Muñoz Sanz JL (2012b) Biomimetic computer-aided design and manufacturing of complex biological surfaces. In: Biodevices 2012—international conference on biomedical electronics and devices. IEEE-EMBS, Vilamoura, Algarve

Díaz Lantada A, Pareja Sánchez B, Gómez Murillo C, Urbieta Sotillo J (2013) Fractals in tissue engineering: toward biomimetic cell-culture matrices, microsystems and microstructured implants. Expert Rev Med Devices 10(5):629–648

Effenberger I, Kroll J, Verl A (2008) New solutions and applications of 3D computer tomography image processing. In: Proceedings of the SPIE, 6813, 68130A

Falconer K (2003) Fractal geometry: mathematical foundations and applications. Wiley

Flisch A (1999) Industrial computed tomography in reverse engieering applications. In: International symposium on computerized tomography for industrial applications and image processing in radiology. DGZfP Proceedings

Groenendijk M (2007) Self cleaning Lotus leaf imitated in plastic by using a femtosecond laser. University of Twente, source: www.physorg.com

Guo X, Liu X, Zhang B, Hu G, Bai J (2010) A combined fluorescence and microcomputer tomography system for small animal testing. IEEE Trans Biomed Eng 58:2876–2883

Harrysson O (2007) Custom-designed orthopaedic implants evaluated using FEM analysis of patient computed tomography data. BMC Musculoskelet Disord 8(91)

Hieu LC (2002) Design and manufacturing of personalized implants and standardized templates for cranioplasty applications. Ind Technol IEEE ICIT'02, 2(11–14):1025–1030

Jacobi U, Chen M, Frankowski G, Sinkgraven R, Hund M, Rzany B, Sterry W (2004) In vivo determination of skin surface topography using an optical 3D device. Skin Res Technol 10:207–214

Kardar M, Parisi G, Zhang YC (1986) Dynamic scaling of growing interfaces. Phys Rev Lett 56:889–892

Kim M (2008) Rapid prototyping: a new tool in understanding and treating structural heart disease. Circulation 117:2388–2394

Kucklick TR (2006) The medical device R&D handbook. CRC Press

Lakhtakia A, Martín-Palma RJ, Motyka MA, Pantano CG (2009) Fabrication of free-standing replicas of fragile, laminar, chitinous biotemplates. Bioinspir Biomim 4:034001

Lin DW, Johnson S, Hunt CA (2004) Modeling liver physiology: combining fractals, imaging and animation. In: Proceedings of the 26th Annual International Conference of the IEEE-EMBS, San Francisco CA USA, 3120–3123

Longoni S, Sartori M (2010) Fractal geometry of nature (bone) may inspire medical devices shape. Nat Proc (in press), received 02 June 2010, posted 03 June 2010
Losano F, Marinsek G, Merlo AM, Ricci M (1999) Computed tomography in the automotive field. Development of a new engine head case study. In: Computerized tomography for industrial applications and image processing in radiology. DGZfP Proceedings
Mandelbrot B (1982) The fractal geometry of nature. W. H. Freeman, San Francisco
Mandelbrot BB, Van Ness JW (1968) Fractional Brownian motions, fractional noises and applications. SIAM Rev 10(4):422–437
Munro A, Park KG, Atkinson D, Day RP, Capperauld I (1994) Skin simulation for minor surgical procedures. J R Coll Surg Edinb 39(3):174–176
Ojeda Díaz C, Osuna López J, Lafont Morgado P, Díaz Lantada A (2009) Estudio de la estabilidad primaria, influencia en el diseño de vástagos de prótesis femorales personalizadas: Aplicación a paciente específico. 3° Congresso Nacional de Biomecânica. Sociedade Portuguesa de Biomecânica. 11–12 February 2009 in Braganza
Osuna JE (2008) Combinación de tecnologías para la optimización del desarrollo de prótesis. Master Thesis. Universidad Politécnica de Madrid
Peterson D, Bronzino J (2008) Biomechanics. Principles and applications. CRC Press, Taylor and Francis Group. Florida
Pulsifer DP, Lakhtakia A, Martín-Palma RJ, Pantano CG (2010) Mass fabrication technique for polymeric replicas of arrays of insect corneas. Bioinspir Biomim 5(3):036001
Schramm U, Zhou M (2006) Recent developments in the commercial implementation of topology optimization. Solid Mech Appl 137(6):238–249
Schwarz M (2005) New materials processes, and methods technology, 1st edn. CRC Press, Taylor & Francis Group
Shi H, Farag AA, Fahmi R, Chen D (2008) Validation of finite element models of liver tissue using micro-CT. IEEE Trans Biomed Eng 55:978–985
Simandl G (2009) Disorders of skin integrity and function. In: Porth (ed) Pathophysiology: concepts of altered health states. Lippincott Williams & Wilkins
Sun W, Lal P (2002) Recent development on computer aided tissue engineering—a review. Comput Methods Prog. Biomed 67(2):85–103
Tang YM (2002) Modeling skin deformation using boundary element method. Comput Aided Des Appl 7(1):101–108
Téllez M (2015) Díaz Lantada (advisor) Diseño, simulación y caracterización de andamios para la reparación de tejido osteocondral. Final degree thesis, Universidad Politécnica de Madrid
Tsyganov MA, Kresteva IB, Aslanidi GV, Aslanidi KB, Deev AR, Ivanitsky GR (2007) The mechanism of fractal-like structure formation by bacterial populations. J Biol Phys 25:165–176
Vasilash G (2009) Ford accelerates product development with additive technology. In: Time Compression (www.timecompression.com)
Weierstrass K (1886) Abhandlungen aus der Functionenlehre. Springer
Winder J, Bibb R (2005) Medical rapid prototyping technologies: state of the art and current limitations for application. J Oral Maxillofac Surg 63:1006–1015
Wong J, Bronzino J (2007) Biomaterials. CRC Press, Taylor and Francis Group. Florida
Yang XS, Zhang JJ (2005) Modeling and animating hand wrinkles. In: Workshop on computer graphics and geometric modeling—CGGM 2005, pp 199–206
Zhuo S, Chen J, Luo T, Zou D, Zhao J (2006) Multimode nonlinear optical imaging of the dermis in ex vivo human skin based on the combination of multichannel mode and Lambda mode. Opt Express 14:7810–7820

Some Interesting Related Websites

On fractals with demonstrations:

http://mathworld.wolfram.com/Fractal.html
http://mathworld.wolfram.com/FractalDimension.html
http://mathworld.wolfram.com/HausdorffDimension.html
http://demonstrations.wolfram.com/fractals

On Euclidean and non-Euclidean geometries:

http://mathworld.wolfram.com/EuclideanGeometry.html
http://mathworld.wolfram.com/Non-EuclideanGeometry.html

Note: For using some Matlab (The Mathworks Inc.) programs for constructing fractal surfaces and fractal spheres, please have a look at the Annexes of the Handbook.

Chapter 7
Multi-scale and Multi-physical/Biochemical Modeling in Bio-MEMS

Andrés Díaz Lantada

Abstract Multidisciplinarity is intrinsic to Biomedical Engineering, as the products, processes and systems of the biomedical industry, aimed at continuously improving the diagnosis, treatment and prevention of pathologies, are normally developed by large teams of physicians, biologists, materials scientists and engineers. In the field of biomedical microsystems (bio-MEMS) for interacting at a cellular and even molecular level, several physical, chemical and biological phenomena are present and an adequate comprehension of the behaviour of such microdevices also requires studying interactions between the microdevices and the surrounding environments at different scale levels. In such complex systems, the use of modeling resources may be a key aspect towards a straightforward and successful development process. As modern (bio)engineering systems usually exploit phenomena at different scales for improving functionalities of traditional systems, linking the different scales and using multi-scale modeling approaches can increase the predictive capability and applicability of modeling to a wide range of applications. In addition, as modern (bio)engineering systems typically involve different areas of Physics and Chemistry, understanding and modeling their behavior requires the use of multi-physical/chemical modeling approaches. Only by being able to describe the behavior of such (bio)engineering systems at different scale levels and taking account of the physical and chemical phenomena involved in their operation, can we benefit from the advantages of (computer-aided) modeling regarding cost saving, reduction of time-to-market and overall understanding of the products, processes and systems under development. This chapter details methods and examples and provides some cases of study linked to the use of multi-scale and multi-physical/chemical modeling approaches in the field of biomedical microsystems for interacting at a cellular and even molecular level, as introduction to procedures used thoroughly along the Handbook.

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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7.1 The Relevance of Multi-scale and Multi-physical/Biochemical Modeling

Multidisciplinarity is intrinsic to Biomedical Engineering, as the products, processes and systems of the biomedical industry, aimed at continuously improving the diagnosis, treatment and prevention of pathologies, are normally developed by large teams of physicians, biologists, materials scientists and engineers. In the field of biomedical microsystems for interacting at a cellular and even molecular level, several physical, chemical and biological phenomena are present and an adequate comprehension of the behaviour of such microdevices also requires studying interactions between the microdevices and the surrounding environments at different scale levels. In some cases the microdevices may interact with single cells, in others they may be designed to promote special molecular interactions, but in most cases, some features of these microdevices may work at a cellular level and some areas may contact larger tissues and biological structures.

In such complex systems, the use of modeling resources may be a key aspect towards a straightforward and successful development process. The most crucial issue related to modeling in industrial and specifically in biomedical applications is in the formulation of models that produce realistic and robust results.

In general, modeling and simulations can serve as the eyes and hands of the experimentalists, researchers and developers, helping them to access information that would not be available otherwise and interpret the experimental results. Modeling provides also valuable predictions for the development of a system in a quicker or cheaper way than with trial and error methods. The adequate support by means of prototyping and testing facilities, so as to validate and adjust simulation results, for further use in subsequent developments, is also a common and advisable practice.

According to the report for the European Commission "What makes a material function? Let me compute the ways" (Rosso and De Baas 2012), a convenient way of approaching materials modeling consists in separating the models according to the entities whose behaviours are being studied and described (i.e. electrons, atoms, nanoparticles and grains and continuum unit cell). In consequence, models at different scales appear:

- Electronic models describe the behavior of electrons, focusing on a length scale from 0.1 to 10 nm and relying on basic rules of nature, mainly those derived from quantum mechanics.
- Atomistic models concentrate on the behavior of atoms and describe their interactions using quantum mechanics, interatomic potentials and molecular dynamics, working on a length scale from 0.1 to 100 nm.
- Mesoscopic models focus on the behavior of nanoparticles or grains, going down to 10^{-6} m. At this supraatomic scale, the details of the atomic motions are averaged out or replaced by stochastic terms and the use of soft potentials and thermodynamic models is adequate.

- Continuum models describe the behavior of a continuum unit cell. At this level material is assumed to be continuously distributed throughout its volume. Models at this scale disregard the discrete particle-based structures and smaller details. At this larger scale level, the models concentrate on how materials are seen and touched. Modeling at this scale can predict material failure, defect formation, crack propagation, solidification, plastification and other important variables for industrial manufacturing.

In any case, as modern (bio)engineering systems usually exploit phenomena at different scales for improving functionalities of traditional systems, linking the different scales and using multi-scale modeling approaches can increase the predictive capability and applicability of modeling to a wide range of applications. In addition, as modern (bio)engineering systems typically involve different areas of Physics and Chemistry, understanding and modeling their behavior requires the use of multi-physical/chemical modeling approaches.

Only by being able to describe the behavior of such (bio)engineering systems at different scale levels and taking account of the physical and chemical phenomena involved in their operation, can we benefit from the advantages of (computer-aided) modeling regarding cost saving, reduction of time-to-market and overall understanding of the products, processes and systems under development. The possibility of linking information between the different scale levels and between the different domains of Physics and Chemistry promotes enhanced results.

It is important to highlight that the different modeling approaches always provide an approximate description of the complex products, processes and systems being studied. Currently there are no completely accurate models or powerful computational resources capable of providing a perfect description of the multidisciplinarity, versatility and potential of a single eukaryotic cell. In consequence, when modeling more complex microdevices, in which millions of cells interact with micro- or nano-metric features or in which novel tissues are being formed, only the most relevant aspects will be considered for obtaining an overview of the devices' performance. Normally modeling will serve to optimize just a design or operation parameter, in order to enhance the final results obtained with the device.

The following sections detail methods and examples and provide some cases of study linked to the use of multi-scale and multi-physical/chemical modeling approaches in the field of biomedical microsystems for interacting at a cellular and even molecular level. Some of the principles and methods introduced here will be used thoroughly along the Handbook.

7.2 Multi-scale Modeling of Cell Behavior

The cell is the most basic functional, structural and biological unit of life as we understand it. Cells are able to perform coherent functions and make up all tissues and organs of all living multicellular organisms; in consequence, there are usually

referred to as life's building blocks. They can replicate independently and contain the hereditary information necessary for regulating cell functions, including growth, metabolism, apoptosis, protein synthesis, movement and replication, for transmitting information to the next generation of cells (Maton 1997).

In short, living organisms can be divided into unicellular, made of just one cell such as bacteria, archaea and most protists, and multicellular, made of multiple cells, including some protists, fungi, plants and animals. The complex tissues and organs of multicellular organisms, dependent on the collaborative performance of differentiated cell types, are intrinsically multi-scale systems. Cells themselves are also a multi-scale micro-cosmos of subsystems, mechanisms and components, with dimensions ranging from few nanometers to several microns.

Cell structure is made of cytoplasm, which contains many biomolecules such as proteins and nucleic acids, enclosed within a membrane typically surrounded by flagella, pili or cilia, which protect the cell and facilitate movement and communication with neighbor cells. Cells with inner compartmented membrane—bound organelles, in which more specific functions and metabolic processes take place and including a cell nucleus, a special organelle where the DNA is stored, are called eukaryotic cells and constitute the basis of the more complex organisms such as fungi, plants and animals. Cells without a nucleus and with the DNA in direct contact with the cytoplasm are called prokaryotic cells and were the first form of life in our planet. Prokaryotic cells have typical sizes of 1–5 μm, while eukaryotic cells are typically 10–100 μm. In consequence, interacting with single cells requires working, at different levels of detail among the micro- and nano-scale. Several orders of magnitude are involved when studying the cell, not only as regards its size and the size of its subsystems, but also concerning the elasticity, viscosity and mechanical properties in general of the materials (solids and fluids) making up a cell.

Therefore, in spite of being life's basic unit, cells are complex multi-scale and multi-physical/biochemical living systems, which require multi-scale and multi-physical/biochemical modeling strategies. It is also important to mention that with state-of-the-art computational resources and modeling methods, the complete behavior of a single cell, with all its subsystems and possible interactions with the surrounding environment, cannot yet be perfectly predicted. In consequence, much of the research involving cells and tissues requires experimental approaches using biomedical microdevices for the assessment of cell behavior and fate.

However, very adequate approximations, to some relevant behaviors of cells and tissues and to the performance of several cellular mechanisms, can be obtained using multi-scale modeling approaches and multi-physical/biochemical simulation strategies.

As regards multi-scale modeling of cells, different scales can be combined with the appropriate computational modeling techniques and available software permits building and simulating multi-scale models without having to become involved with the underlying technical details of computational modeling, as previously reviewed (Meier-Schellersheim et al. 2009). Top-down and bottom-up (from the system to the subsystems and viceversa) approaches co-exist, as well as deductive

and phenomenological terms in the typical hybrid models for the more comprehensive descriptions of cell behavior.

Ordinary and partial differential equation models can usually help researchers to model interactions from the macro-scale down to micrometric scale, helping to understand tissue mechanics, to model surgical procedures, to approximate the mechanical properties of cells, among other interesting issues. Cellular and tissular performances can be studied at the mesoscopic level by means of thermodynamic models, i.e. for modeling the sodium-potassium pump in muscles or the transmission of nerve impulses. Thermodynamic, soft potential and stochastic models help to address molecular interactions such as DNA transitions, cellular locomotion (see Sect. 7.5), the kinetics of enzymes and their behavior as molecular machines or gene expression processes and their random variations, among other phenomena (Nelson 2004; Meier-Schellersheim et al. 2009).

Open-access software resources, such as CompuCell3D, CellDesigner™ and SBW, allow researchers with basic programing experience to quickly understand how to construct and execute complex virtual cellular and tissue simulations for modeling development, homeostasis, toxicity and disease in tissues, organs and organisms, covering sub-cellular, multi-cell and even continuum tissue scales (www.compucell3d.org; www.systems-biology.org; www.celldesigner.org; Swat et al. 2012).

Several cellular subsystems can be perceived as molecular machines and using similar artificially built nanomachines, performing in an extremely precise way for transmitting forces, motion or energy, can open new horizons in the field of nanotechnology (Drexler 1986, 1990, 1991; Piccolino 2000; Mavroidis et al. 2004; Luskin 2010). Adequately modeling their performance and being able to fabricate them artificially are challenges progressively being solved (Leach 1996).

Main issues regarding the multi-scale modeling of cells and tissues lay, not just on the development of more efficient models and powerful computational tools, but also in the conception, design, implementation and final operation of adequate validation systems by means of in vitro and in vivo studies, capable of providing the correct inputs and adjustments to in silico strategies.

Even though multi-scale modeling of cells and tissues is beyond the scope of present Handbook, we expect that the references provided may be of help for researchers interested in the topic. As for the multi-physical modeling of cellular and tissular behavior and of biodevices for interacting with them, next section tries to provide an introduction and several examples are included along the Handbook, most of them linked to supporting the development of successful biodevices.

7.3 FEM-Based Simulations for Multi-physical Modeling of Cell Behavior and of Biomedical Microsystems (Bio-MEMS)

Computer-aided engineering (CAE) refers to the general use of software to aid in engineering tasks, in its broadest sense even including computer-aided design and computer-aided manufacturing; although in product design, CAD is perceived as the starting point for designing a part, CAE involves the simulations carried out upon a CAD part, in order to verify geometries and materials, and CAM is linked to the simulations realized upon a CAD part to prepare manufacturing processes and to the automated control of machine tools during production.

Although CAE can involve the use of all kinds of software and computer-aided calculations, in product development and linked to computer-aided design, such calculations are normally carried out by application of the finite element method (FEM), whose generalization in the final decades of the 20th Century has been essential for promoting the incorporation of CAE analysis tools together with CAD software packages.

Such method allows solving complex engineering problems by using a mesh discretization of a continuous domain into a set of discrete elements (connected by nodes) and by transforming initial partial differential equations, as well as integral equations, into an approximate system of ordinary differential equations (forced to be valid in the nodes) for final numerical integration. This method is specially well-suited for solving partial differential equations over complicated domains or geometries, when the domains change during the whole simulation, when the desired precision varies over the systems under study or when the solutions lack smoothness. Its applications in multi-physical modeling are outstanding.

The foundations of the FEM method are out of the scope of present Handbook and are well detailed in pioneer works elsewhere (Zienkiewicz et al. 2005). Being a matrix-based calculation method, it is very adequate for programming and most engineers using FEM simulations resort to commercial software, although for research tasks it is also common that researchers themselves develop ad hoc programs for their particular problems. In any case, through present Handbook we will be using NX-8.5 (Siemens PLM Solutions) for design and FEM-based simulation tasks.

When using software resources for FEM-based simulations, it is necessary to understand the working methodology, divided into pre-processing, analysis and post-processing, as detailed below:

Pre-processing. Is the stage focused on preparing the model, normally starting from a previous CAD geometry. During this stage the geometry is discretized into elements and nodes (meshing) and the material and physical properties, as well as the loads and boundary conditions are applied.

Analysis solver. Is the real computational stage, oriented to verifying the prepared model and to developing the calculations (frequently iterative) and finally storing

the results from stresses, strains, displacements, temperatures, fluid flows, velocities…

Post-processing. Allows users to represent and study the solutions obtained, which are normally represented upon the geometries of interest in form of colormaps, each color showing a different level of stress, strain, displacement, flow, temperature, velocity, among other results.

It is very relevant to note that "red" colors do not mean that a part is breaking and "green" colors do not mean that a part is properly prepared for service. The colormaps are just visual helps and the actual values for instance of stress, have to be adequately compared to the values the material is capable to resist, so as to verify is part is prone to failure in such simulated conditions, simulations which must be somehow validated or verified. Therefore it is always very advisable to have a preliminar estimation of final simulation results, possibly carried out analytically using simplified models, so as to help verification during the post-processing stage. Further detailed validation of simulation results, with the help of real trials carried out using rapid prototypes and different characterization resources, is an excellent way of increasing our confidence in simulation results and of helping with optimization tasks. It is also very necessary to note the importance of using a coherent units system during the pre-processing stage, so that calculation leads to adequate results. In many cases, when the post-processing stage represents strange or "impossible" results, the actual cause is linked to an incorrect use of units.

In the last decades, thanks to the progressive advances on CAD tools and to the continuous incorporation of novel simulation features for helping designers, many CAD-CAE software offer very comprehensive FEM-based resources for studying different physical fields and for multi-physical and even multi-chemical modeling (Lorenzo Yustos et al. 2010), as detailed in the following further on.

The different modules of conventional CAE software are oriented to solving the most typical problems of Engineering, normally linked to evaluating the mechanical performance of a system, thermal and fluidic phenomena that may occur during service life, failure prevention and overall optimization.

Stress calculation modules are the most used for solving static problems, as loads (forces, pressures, accelerations) and boundary conditions (clamped, fixed displacements, fixed rotations) can be defined and final results provide information on stress and strain fields, displacements and even security factors if materials yield strength is defined properly.

Dynamic response calculations are also possible, as most FEM-based simulation software include modules for studying natural vibration modes and related frequencies of parts and structures, so as to analyze which kinds of cyclic loads may promote resonance phenomena and improve service life of structures and mechanical systems.

Thermal phenomena can also be studied with the help of the finite element method, what is highly useful for analyzing the in service behaviour of mechanical systems and structures and predicting the steady-state temperature field they can develop, due to the heat coming from motors, installations, inner or outer

phenomena, fluids and other systems from the environment…, as well as the transitory evolutions of such heatings.

Heat transmission through conduction, convection and radiation can be analyzed and normal results are temperature fields and thermal fluxes. The temperature maps obtained can be also connected to mechanical simulation modules, so as to analyze the mechanical effects of temperature changes.

Fluid simulations generally allow obtaining velocity fields, pressure fields and convection coefficients along a system, from previously defined inlet and outlet fluids entering and exiting the control volume. Coupled thermal-fluid simulations can also be carried out. Normally these modules help also to optimize geometries by realizing aerodynamic/hydrodynamic related considerations.

Other more specific modules include impact and fatigue calculations, for assessing part life and unexpected phenomena; topological optimization tools, for optimizing geometries; electromagnetic simulations, more linked to telecommunications and electronics and some additional resources focused on micro- and nano-technologies.

In Biomedical Engineering, the use of FEM-based simulations helps greatly along the completed product development process, for analyzing different physical phenomena and interactions. Normally there are three main application fields of FEM in Biomedical Engineering, as detailed below.

First of all FEM-based simulations are used to assess in silico the performance of (bio)medical (micro)devices, from prostheses to microsystems, so as to validate or optimize the CAD design. Secondly, FEM-based simulations are used to study the behavior of human organs, tissues and biological structures, so as to increase our comprehension about how the different mechanical, thermal or fluidic phenomena affect them, aiming also at the discovery of novel diagnostic or therapeutic alternatives. Finally FEM-based simulations are very helpful to model and analyze the multi-physical interaction between biomedical devices and living tissues (even at a cellular level), so as to predict their effects on the patients. We aim to provide some representative examples of these applications.

Along the Handbook we employ FEM-based simulations to analyze the overall performance of several biomedical microdevices, including the mechanical properties of tissue engineering scaffolds, the dynamic response of active cell culture microsystems, the fluidic behavior of different labs-on-chips and organs-on-chips, among other applications detailed in Chaps. 12–23.

Here we focus on a couple of examples linked to multi-physical modeling in the field of tissue engineering. By means of FEM simulations, the next pages provide: (1) a preliminary approach to the study of cell growth within tissue engineering scaffolds; and (2) a procedure for the evaluation of velocity and temperature fields, for designing dynamic cell culture processes within scaffolds placed in bioreactors and selecting the adequate fluidic and thermal inputs. This procedure helps to select the optimal flow rates leading to a biomechanic shear rate upon the cells being cultured and to decide the power required for the heating elements within the bioreactor, in order to achieve a more homogeneous and physiological temperature during cell growth.

7.3.1 *Multi-physical Modeling of Tissue Engineering Scaffolds*

(a) *Preliminary approach to cell colonization*

During the last decades, in general with the intention of optimizing design, manufacturing and testing costs associated to the development of novel solutions for the tissue engineering field, several researchers are focusing research efforts to the development of cellular and tissular growth models. These cell colonization models help to predict the behavior of biomaterials and implants and, after the adequate adjustment of simulations, to optimize the design processes of more complex biodevices. These cell growth processes may be affected by the physical properties of the biomaterial, scaffold or implant being colonized, by the presence of fluids in motion surrounding and stimulating the cells, by other heat transfer processes and by the electromagnetic fields of the environment, among potential factors of influence. In consequence, the use of FEM-based modeling resources, which are specially well-suited to multi-physical phenomena, also provides very valuable results for modeling cell-material interactions. In some cases, as the basic equations governing cell behavior may have a similar structure to those linked to common physical phenomena, the employment of commercially available FEM-based modeling software may be almost direct, normally supposing just a change of scale. In other cases, when focusing on more specific non-linear behaviors and interactions, ad hoc developed codes may be required.

In this sub-section, by means of example, we simulate the evolution of cell concentration within a tissue engineering scaffold with a conventional wood-pile structure. We resort to a partial differential equation for modeling the evolution of cell concentration (which will be subsequently numerically solved using the finite element method) according to:

$$\frac{\partial C}{\partial t} = \left(\frac{\partial^2 C}{\partial^2 x} + \frac{\partial^2 C}{\partial^2 y} + \frac{\partial^2 C}{\partial^2 z}\right) \cdot \alpha + (C - C_\infty) \cdot \beta + C^* \cdot \gamma$$

Being:

C	The concentration of cells, i.e. number of cells per unit volume
C*	The cell generation rate
C_∞	The concentration of cells in the surrounding environment
α, β, γ	Model constants

The equation is a simplified version of previously published models (Ho et al. 2008) and bears important resemblance to the basic equations of heat transfer in three-dimensional spaces. In consequence, the thermal-FEM calculation module by NX-8.5 may be used, with an adequate change of scale, to model the processes of

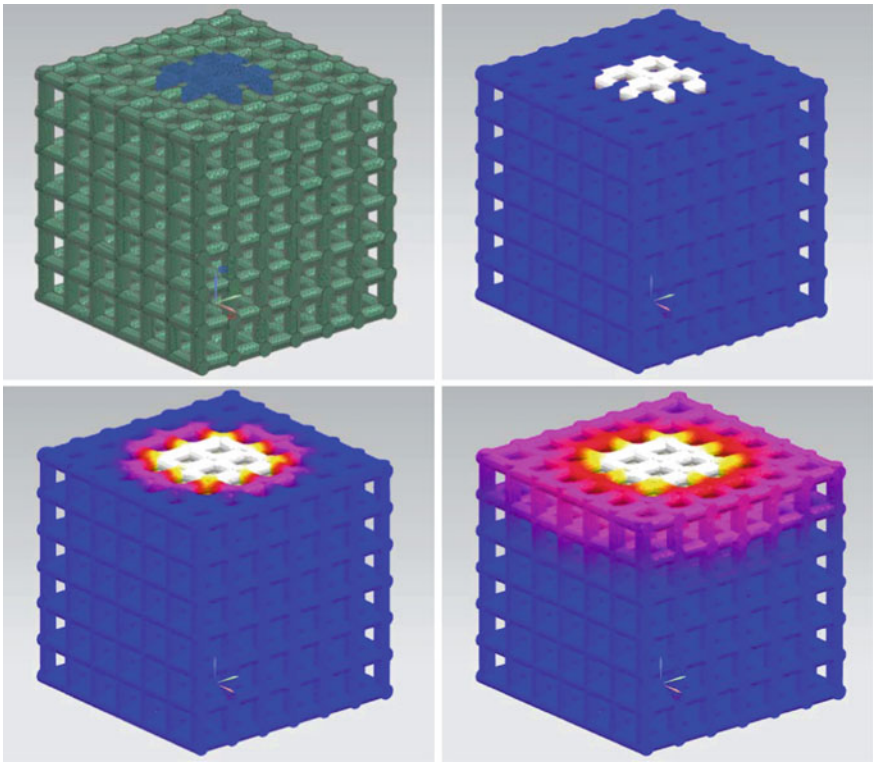


Fig. 7.1 FEM simulation of the colonization process of a tissue engineering scaffold by cultured cells. The evolution of cell concentration is appreciated. Simulation carried out with NX-8.5 (Siemens PLM Solutions)

cell growth and diffusion in tissue engineering scaffolds and related biomaterials, whose colonization rate is interesting regarding their use as therapeutic repair or regeneration constructs. Figure 7.1 shows the implemented FEM model upon the scaffold's geometry and main results from a transitory simulation, in which the evolution of cell concentration within the scaffold can be clearly appreciated.

These models are usually an overall approximation to the real complexity of cell behavior and to the performance of biomaterials. In consequence, the use of real prototypes submitted to real cell culture processes is still the most adequate procedure for obtaining information regarding cell behavior and about cell-material interactions. Such ex vivo or in vitro trials can also provide very valuable information for adjusting the parameters of colonization models, as the one used here, so as to progressively implement more realistic simulation tools. However, repeatability of the in vitro trials, which is also challenging, constitutes a key issue for empirically adjusting the simulations.

(b) *Dynamic cell culture process in bioreactor*

Cells and tissues respond, not just to their genetic information and surrounding biochemical cues, but also to several mechanical properties and to the presence of physical stimuli of their extra cellular matrix and surrounding environment, as well as to gradients of them, including Young's modulus, surface topography, hardness, presence of vibrations, fluids in motiong, electromagnetic fields, among other external influences studied in the evolving field of cell mechanobiology with the help of biomedical microdevices capable of interacting at a cellular (sometimes even subcellular) level.

Curiously clear differences, regarding gene expression, differentiation into final tissues and fate, are perceived between cell culture processes carried out in static and dynamic conditions. The use of fluid flows for the generation of adequate biomimetic dynamic culture conditions is common, typically resorting to the use of bioreactors with inserted tissue engineering scaffolds or constructs and to the employment of microfluidic systems, for controlling these processes.

In fluidic simulations, we typically operate with a control volume of fluid, which surrounds the physical object being studied. In our case, using the CAD-software with which the scaffold is designed, we carry out a Boolean operation subtracting the scaffold from a block of material. Subsequently the block is meshed and water is applied as "material".

Boundary conditions including an inlet flow of 5 g/s and an outlet flow of 5 g/s are applied (taking into account mass conservation) and the model is then solved for obtaining the desired pressure and velocity fields. Such velocities act upon the cells being cultured and promote the appearance of shear stresses, which have very positive effects, if adequately tuned. Simulation conditions can be easily changed in order to select the most adequate culture conditions, depending on the desired shear stresses upon the cells.

Figures 7.1 and 7.2 show the FEM model and simulation of the fluid flow along a bioreactor with a tissue engineering scaffold placed inside. The fields of velocities are appreciated. These can be tuned to promote desired shear stresses upon the cells being cultures, as will be further discussed in Chaps. 20 and 22, when dealing respectively with biomedical microfluidic devices and with microfluidic devices for organ-on-chip modeling approaches.

Another relevant issue, when culturing and monitoring cells during several hours or days, is the use of an adequate culture temperature within the bioreactor, for which purpose the implemented cell culture system may incorporate heating resistances or plates. The design of these systems is more complex, as coupled thermal-fluidic problems appear. Again the use of FEM-based simulations helps to obtain relevant information and to optimize the designs. The adequate selection of heating power and resistance distribution for a physiological and homogeneous temperature field can be carried out with the support of these software resources.

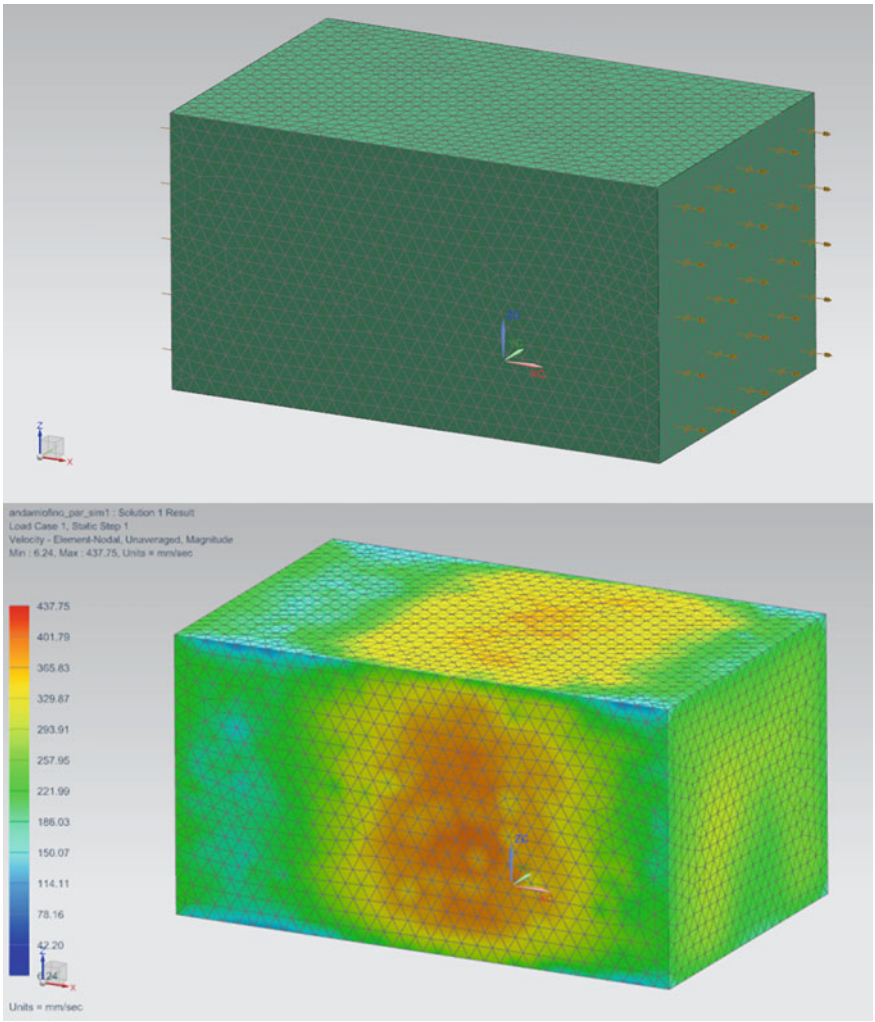


Fig. 7.2 FEM model and simulation of the fluid flow (5 g/s) along a bioreactor with a tissue engineering scaffold inside. The field of velocities is appreciated. Simulation carried out with NX-8.5 (Siemens PLM Solutions)

Figures 7.4 and 7.5 show the FEM model and simulation of the fluid flow along a heated bioreactor with a tissue engineering scaffold inside. The fields of velocities and temperatures are appreciated. In this case, the use of a thermal plate seems inadequate, as the temperature is not as homogeneous as expected, due to the low temperature of the fluid entering the bioreactor. It would be much more advisable to use a preheated fluid, so as to reach a more physiological temperature nearer to body conditions (Fig. 7.3).

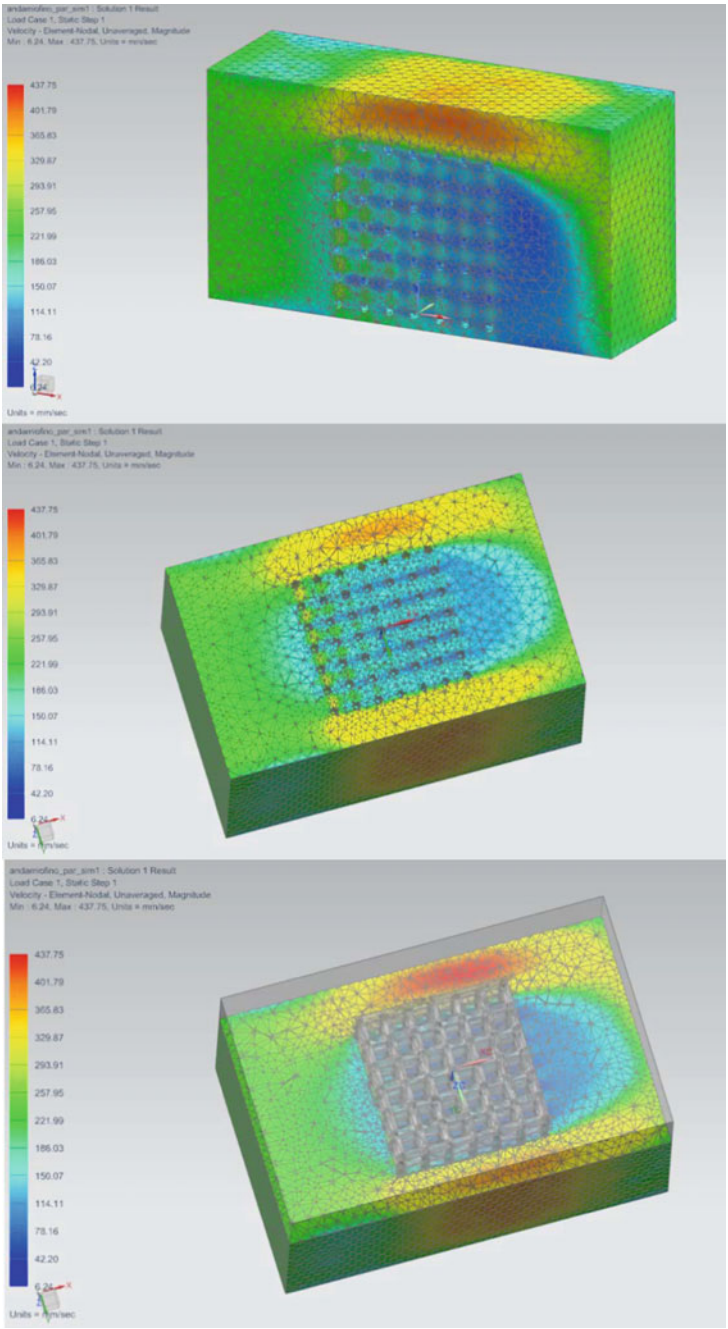
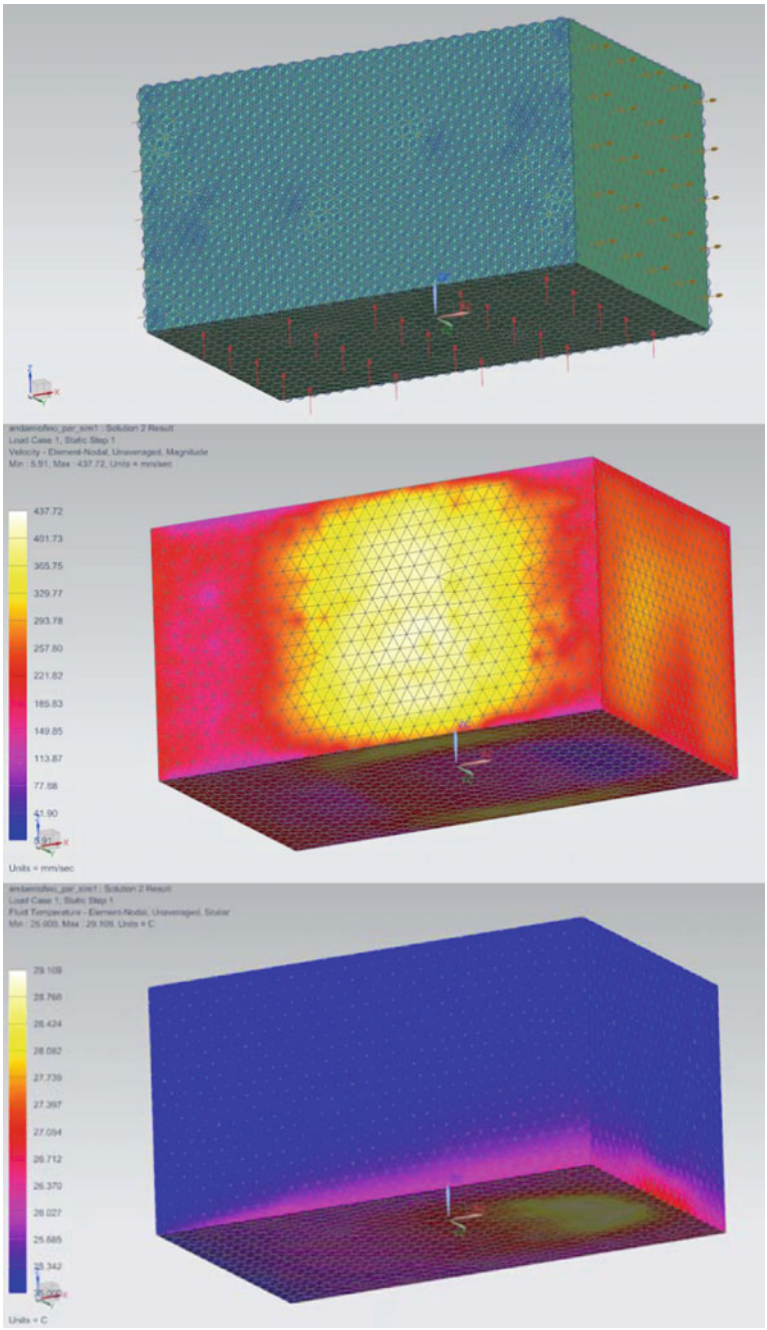


Fig. 7.3 FEM model and simulation of the fluid flow (5 g/s) along a bioreactor with a tissue engineering scaffold inside. The field of velocities is appreciated. Simulation carried out with NX-8.5 (Siemens PLM Solutions)


Load Case 1, Static Step 1
Velocity - Element-Nodal, Unaveraged, Magnitude
437.72
401.73
365.75
329.77
293.78
257.80
221.82
185.83
149.85
113.87
77.88
41.90
Units = mm/sec
Load Case 1, Static Step 1
Fluid Temperature - Element-Nodal, Unaveraged, Scalar
29.109
28.766
28.424
28.082
27.739
27.397
27.054
26.712
26.370
26.027
25.685
25.342
Units = C

◀ **Fig. 7.4** FEM model and simulation of the fluid flow along a bioreactor with a tissue engineering scaffold inside and placed upon a thermal conditioning plate. The fields of velocities and temperatures are appreciated. Simulation carried out with NX-8.5 (Siemens PLM Solutions). Main simulation parameters include: Flow rate = 5 g/s. Power = 5 W. Convection coefficient = 4 W/(m^2/°C). Ambient temperature = 25 °C

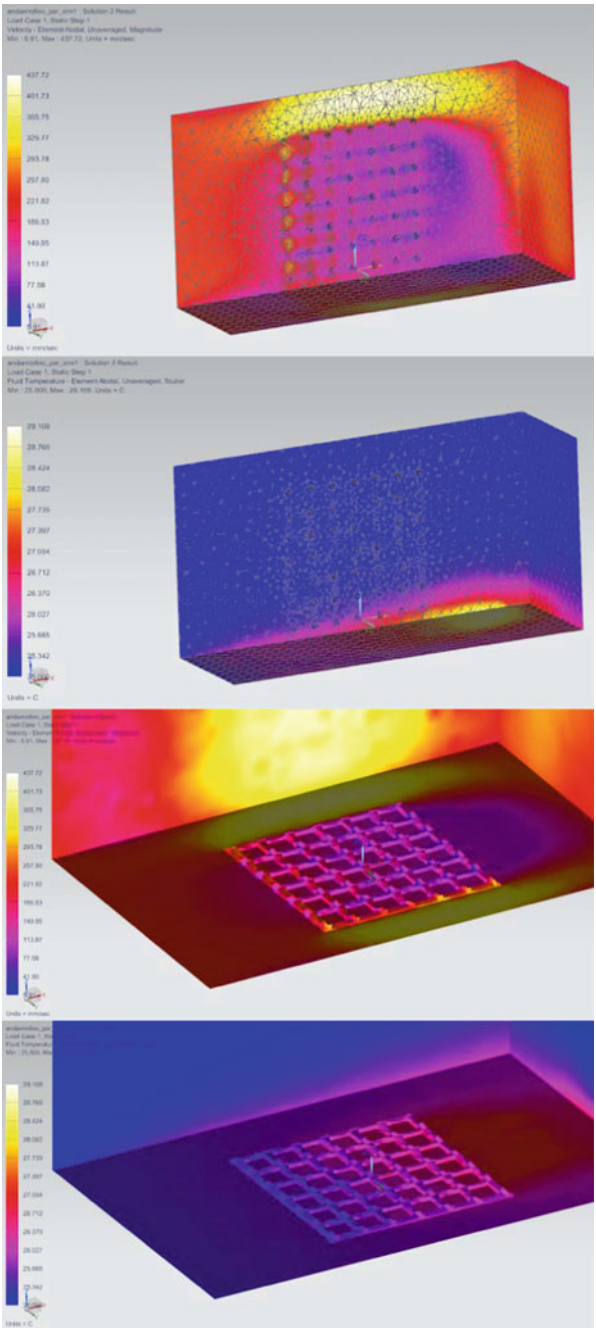
7.4 Resources for Multi-biochemical Modeling of Cells and Biomedical Microsystems (Bio-MEMS)

Apart from the impact of physical interactions, either mechanical, or fluidical or electromagnetical, between cells and their surrounding environment, it is clear that genetic and biochemical cues play also a relevant role in their behaviour. The great complexity of cells and their subsystems and all the possible inter- and intra-cellular communication pathways, based on biochemical reactions, have promoted the development of novel strategies and techniques for modeling subcellular processes and obtaining a better understanding about the basic mechanisms of life and its fundamental unit.

Not just multiple scale levels have to be taken into account, but also several and simultaneously occurring chemical processes, in many cases collaborative, which can be addressed with the help of resources from disciplines such as biochemical systems theory, systems biology and systems engineering. Usually, the complex networks and processes involved require the implementation of specific software for modeling the multiple biochemical issues present and solving the problem in terms of dynamic evolutionary networks. Such dynamic evolutionary networks are broadly recognized as a universal approach to modeling complex systems, from cells, to organisms, ecosystems, social groups and even market economy.

Among the most interesting software for modeling complex phenomena in cells, it is important to note Virtual Cell (www.nrcam.uchc.edu), developed by the University of Connecticut, which has been successfully applied to the study of several intracellular signalling processes even associated with the study of disease (Brown and Loew 2012). In some cases, physical and biochemical phenomena have been taken into account by merging different models (Brown et al. 2011).

Cellular automata also constitute a basic method for the dynamic modeling of complex networks and interactions, with applications in Biology and Medicine (Kier et al. 2005). A cellular automaton consists of a regular grid of cells, each in one of a finite number of states, typically “active” and “inactive”, which in biological systems may be related to the presence of an enzyme, a protein, a pathogen, among other possibilities. The information, mass or energy from a cell may move to an adjacent cell depending on probabilistic rules, defined in accordance with the system under study, as well as based on the available trajectories. The whole network is calculated iteratively. An initial state is selected by assigning a state for each cell. A new generation or iteration is created (advancing one unit of time), according to the fixed rules (generally, a mathematical function) that determine the new state of each cell in terms of the current state of the cell and the states of the



◀ **Fig. 7.5** EM model and simulation of the fluid flow along a bioreactor with a tissue engineering scaffold inside and placed upon a thermal conditioning plate. The fields of velocities and temperatures are appreciated. Simulation carried out with NX-8.5 (Siemens PLM Solutions). Main simulation parameters include: Flow rate = 5 g/s. Power = 5 W. Convection coefficient = 4 W/(m^2/°C). Ambient temperature = 25 °C

cells in its near environment. The cells of the automaton may correspond to real cells or to different parts of cellular subsystems.

They may also correspond to different locations of a biomedical microsystem, where living nutrients, chemicals, cells, tissues and even pathogens co-exist and mutually interact, hence providing interesting information for the design of bio-MEMS and bioengineering systems (Song and Kinney 2002; Lemon and King 2006).

7.5 Modeling Cell Dynamics and Tissue Formation

Adequate models of cell dynamics and tissue formation are powerful resources for the in silico assessment of the potential performance of biomedical systems and devices under development. These tools prove especially relevant for devices such as implants and microsystems aimed at interacting with cells and at affecting their dynamics and behaviour, as the ways cells move within them or colonize them may be a key for adequately modeling a disease or for the success of a novel therapeutic approach. Previous sections have highlighted the use of FEM based simulations and put forward the potential of cellular automata for modeling cell colonization processes. The detailed FEM-based colonization models are linked to deterministic partial differential equations solved numerically, while the cellular automata used for solving dynamic network problems include iteration rules and random terms. When focusing on cell dynamics and tissue formation other interesting options are possible for modeling cell behaviour in an even more straight-forward way.

Possibly the more direct way of modeling the movement of a cell, cultured upon a planar surface or within a Petri dish, is the use of random walk models, also related to Brownian and aleatory movements. In short, a random walk is a walk or series of steps where each of the steps taken by the object is independent of the previous steps. Typically all steps have the same length and are taken with a fixed frequency. In a two-dimensional space, each cell modeled using a random walk approach, can only move along the "X" and "Y" axes, hence along vectors: [1, 0], [0, 1], [−1, 0] and [0, −1]. In a three-dimensional space, at each iteration the cell can move along "X", "Y" and "Z" axes, thus along vectors: [1, 0, 0], [−1, 0, 0], [0, 1, 0], [0, −1, 0], [0, 0, 1] and [0, 0, −1]. These random movements can be programmed; selecting the movement vectors randomly every iteraction, in our case using Matlab (see Annex A.VII.7).

Additional terms or rules can be used to develop more realistic models or to take into account existing boundaries, if the cells interact with the surfaces of a

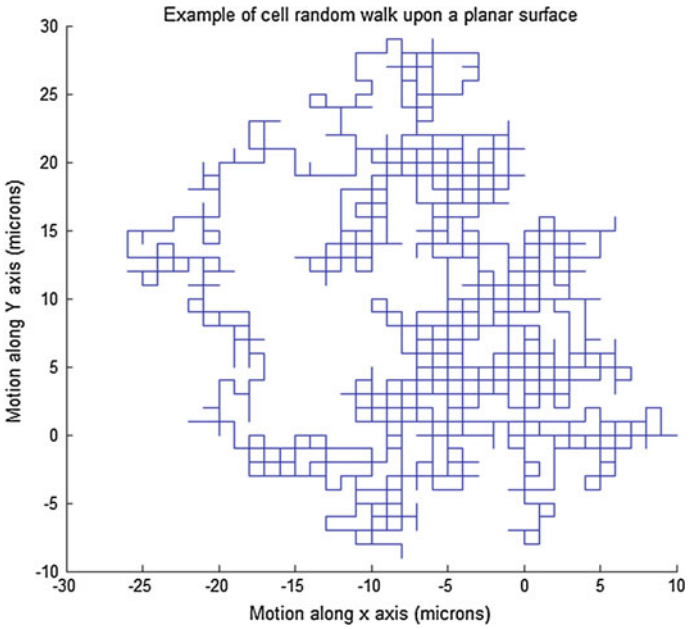


Fig. 7.6 Example of cell random walk upon a planar surface

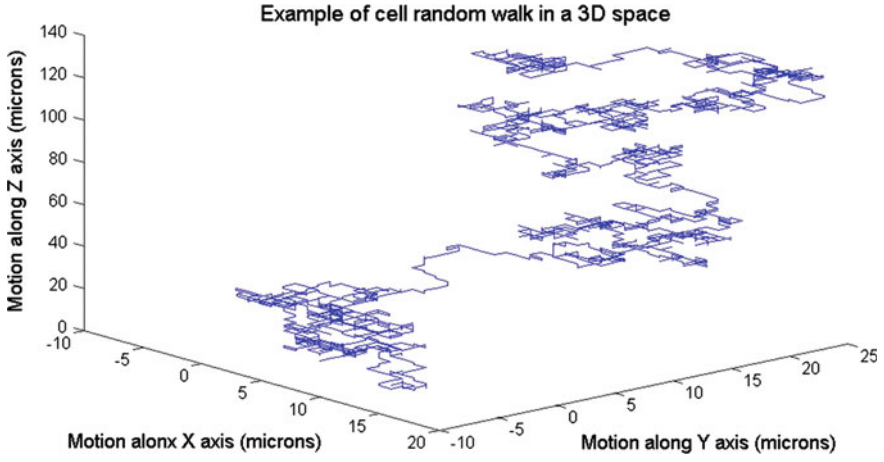


Fig. 7.7 Example of cell random walk in a 3D space

biomedical microsystem or implant, or the presence of nutrients, gradients of chemicals and even pathogens. Figures 7.6 and 7.7 provide basic examples of cell random walks upon a planar surface and within a three-dimensional environment respectively.

Apart from their application to modeling cell movement and propagation upon surfaces or within implants, scaffolds and extracellular matrices, these random walk models can be also applied to processes at a molecular level. For instance, random walk models with auto-avoidment have been used for modeling the self-diffusion and cross-linking of DNA strands as an example of biological diffusion and polymeric conformation processes (Smith et al. 1995; Maier and Radler 1999; Nelson 2004).

Other options for modeling cell movements and tissular growths involve fractal growth models and the use of cellular automata (Sengers et al. 2007). The use of the cellular automata has been already mentioned in the previous section, as example of the strategies linked to systems biology and to the use of dynamic evolutionary networks. Regarding the application of fractal models, it is important to recall the relevance of fractal geometry for modeling a wide set of natural phenomena. In fact, the use of fractal growth models for the simulation of cellular and tisular growth constitutes one of the more successful applications of fractal geometry to the field of Cell Biology and cancer research (D'Onofrio 2009). Other interesting studies have been reported on the use of fractal models for describing the growth and expansion rate of bacteria and for evaluating the dynamics of coexisting species of microorganisms (Tsyganov et al. 2007).

In any case, it is necessary to remark again that these models, simulations and all the information obtained using them are important indeed for a more efficient design and development process of biomedical microsystems aimed at interacting with cells. For instance, when developing a biodevices with cell culture chambers for studying cell dynamics, the use of random walk models as those from previous paragraphs may help to define the required chamber size, according to the desired duration of the cell culture experiment.

At the same time, medical microsystems, capable of interacting at a cellular level, can be very useful for carrying out cell culture and tissue growth studies, with which detailed information can be gathered and used to finally adjust several models and simulations and further use them for subsequent designs with a higher degree of confidence in the simulation results.

7.6 Main Conclusions and Future Research

Modern bioengineering systems usually exploit phenomena at different scales for improving functionalities of traditional systems. In consequence linking the different scales and using multi-scale modeling approaches can importantly increase the predictive capability and applicability of modeling to a wide range of applications.

In addition, as modern bioengineering systems typically involve different areas of Physics and Chemistry, understanding and modeling their behavior requires the use of multi-physical/chemical modeling approaches. Only by being able to describe the behavior of such bioengineering systems at different scale levels and

taking account of the physical and chemical phenomena involved in their operation, can we benefit from the advantages of (computer-aided) modeling regarding cost saving, reduction of time-to-market and overall understanding of the products, processes and systems under development.

This chapter has provided methods and detailed examples and some more complete cases of study, linked to the employment of multi-scale and multi-physical/chemical modeling approaches in the field of biomedical microsystems for interacting at a cellular and even molecular level. It provides an introduction to procedures and resources, which will be used thoroughly along the Handbook when detailing other complete development cases of study in the areas of tissue engineering scaffolds, implants, microfluidic devices, labs-on-chips and organs-on-chips, among others.

Future research will be focused on improving our capability of modeling the cell, as a multi-scale and multifunctional system, as well as its interactions with other cells, with surrounding tissues and with complex devices, which may benefit from cells as transducers towards improved cell-based sensors and actuators. A brief introduction to these research trends is provided in Chap. 21.

References

Brown SA, Loew LM (2012) Computational analysis of calcium signaling and membrane electrophysiology in cerebellar Purkinje neurons associated with ataxia. BMC Syst Biol 6–70

Brown SA, Moraru II, Schaff JC, Loew LM (2011) Virtual NEURON: a strategy for merged biochemical and electrophysiological modeling. J Comput Neurosci 31(2):385–400

D'Onofrio A (2009) Fractal growth of tumors and other cellular populations: linking the mechanistic to the phenomenological modeling and vice versa. Chaos Solitons Fractals 41(2):875–880

Drexler E (1990) Enginers of creation: the coming era of nanotechnology. Anchor Books, 1986 (Oxford University Press, 1990)

Drexler E (1991) Molecular machinery and manufacture with applications to computation. PhD Thesis, MIT

Ho SY, Yu MH, Chung CA (2008) Simulation of cell growth and diffusion in tissue engineering scaffolds. IFMBE Proc 23:1745–1748

Kier LB, Seybold PG, Cheng C-K (2005) Modeling chemical systems using cellular automata. Springer

Leach G (1996) Advances in molecular CAD. Nanotechnology 7(3)

Lemon G, King JR (2006) Multiphase modeling of cell behaviour on artificial scaffolds: effects of nutrient depletion and spatially nonuniform porosity. Math Med Biol 24(1):57–83

Lorenzo Yustos H, Lafont Morgado P, Díaz Lantada A, Fernández-Flórez Navidad A, Muñoz Sanz JL, Munoz-Guijosa JM, Muñoz García J, Echávarri Otero J (2010) Towards complete product development teaching employing combined CAD-CAE-CAM technologies. Comput Appl Eng Educ 18(4):661–668

Luskin C (2010) Molecular machines in the cell. Discovery Institute

Maier B, Radler JO (1999) Conformation and self-diffusion of single DNA molecules confined to two dimensions. Phys Rev Lett 82:1911–1914

Maton A (1997) Cells building blocks of life. Prentice Hall, New Yersey
Mavroidis C, Dubey A, Yarmush ML (2004) Molecular machines. Annu Rev Biomed Eng 6:363–395
Meier-Schellersheim M, Fraser IDC, Klauschen F (2009) Multi-scale modeling in cell biology. Wiley Interdisc Rev Syst Biol Med 1(1):4–14
Nelson P (2004) Biological physics. Energy, information, life. W.H. Freemand and Company, New York
Piccolino M (2000) Biological machines: from mills to molecules. Nat Rev Mol Cell Biol 1:149–153
Rosso L, De Baas A (2012) What makes a material function? Let me compute the ways. In: Modeling in FP7 NMP Programme, European Commission
Sengers BG, Please CP, Oreffo ROC (2007) Experimental characterization and computational modeling of two-dimensional cell spreading for skeletal regeneration. R Soc: Interface 4(17):1107–1117
Smith DE, Perkins TT, Chu S (1995) Self-diffusion of an entangled DNA molecule by reptation. Phys Rev Lett 75(22):4146–4149
Song J, Kinney KA (2002) A model to predict long-term performance of vapor-phase bioreactors: a cellular automaton approach. Environ Sci Technol 36(11):2498–2507
Swat M, Thomas GL, Belmonte JM, Shirinifard A, Hmeljak D, Glazier JA (2012) Computational methods in cell biology. Methods Cell Biol 110:325–366
Tsyganov MA, Kresteva IB, Aslanidi GV, Aslanidi KB, Deev AR, Ivanitsky GR (2007) The mechanism of fractal-like structure formation by bacterial populations. J Biol Phys 25:165–176
Zienkiewicz OC, Taylor RL, Zhu JZ (2005) The finite element method: its basis and fundamentals, 6th edn. Butterworth-Heinemann

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Chapter 8
Rapid Prototyping of Biomedical Microsystems for Interacting at a Cellular Level

Andrés Díaz Lantada, Jeffrey Resnick, Javier Mousa, Miguel Ángel de Alba, Stefan Hengsbach and Milagros Ramos Gómez

Abstract The applications of microsystems in the biomedical field are indeed remarkable and continuously evolving thanks to recent extraordinary progresses in the area of micromanufacturing technologies, capable of manufacturing devices with details in the typical range of 1–500 μm. As living organisms are made up with cells, whose overall dimensions typically range from 5 to 100 μm, micro-manufactured devices (with details precisely in that range) are very well-suited to interacting at a cellular level for promoting innovative diagnostic and therapeutic approaches. This chapter provides an overview of the more relevant micromanufacturing technologies with special application to the development of advanced micro-medical devices and to the manufacture of rapid prototypes, as several of these manufacturing technologies will be applied thoroughly along the Handbook for the development of different cases of study linked to microfluidic biodevices for disease modeling, to cell culture platforms for understanding cell behavior, to labs-on-chips and organs-on-chips and to tissue engineering scaffolds. The different technologies detailed in present chapter are also illustrated by means of application examples related to the aforementioned types of biomedical microdevices aimed at interacting at a cellular level. The possibility of combining technologies for the promotion of multi-scale and biomimetic approaches is also analyzed in detail and some current research challenges are also discussed.

A. Díaz Lantada (✉) · J. Resnick · J. Mousa · M.Á. de Alba
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

S. Hengsbach
Institute of Microstructure Technology, Karlsruhe Institute of Technology,
Hermann von Helmholtz Platz 1, Eggenstein-Leopoldshafen, Germany

M. Ramos Gómez
Centre for Biomedical Technology, Universidad Politécnica de Madrid UPM
Scientific and Technological Park, Montegancedo Campus, Crta. M40 - km 38,
28223 Pozuelo de Alarcón, Spain

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8.1 Overview of Micromanufacturing Technologies

Micro-manufacturing technologies, capable of manufacturing devices with details in the typical range of 1–500 μm, started in the late 1950s mainly linked to the electronic industry, for producing circuits with improved performance, without a dramatic increase of final device size. Such beginning was very connected to the properties of silicon, which can be easily micro-machined using chemical attacks through specially designed patterns or masks.

The lecture "There's plenty of room at the bottom", given at Caltech in 1959 by Richard P. Feynman (reprinted 1992, revised 1993), focused on the possibility of improving and expanding the use of micro- and even nano-manufacturing technologies for obtaining more efficient, multifunctional and scalable systems, and made researchers aware of the related socio-economical importance.

The progressive adaptation of micro-techniques for micro-machining alternative materials, including metals, ceramics and polymers, and the introduction of novel manufacturing technologies, including laser micro-manufacturing, electron/ion beam milling, micro-replication tools and high-precision additive manufacturing, have since the 1960s greatly promoted final quality of the obtained microsystems, as well as the incorporation of additional features and the combination of materials, in many cases using thin-film technologies for special contact phenomena.

The applications of microsystems in the biomedical field are indeed remarkable and continuously evolving thanks to progresses in the aforementioned micro-technologies (Díaz Lantada 2012), as explained in detail in this chapter. As living organisms are made up with cells, whose dimensions typically range from 5–100 μm, micro-manufactured devices (with details precisely in that range) are very well-suited to interacting at a cellular level for promoting innovative diagnostic and therapeutic approaches.

Figure 8.1 provides an overview of micro-manufacturing technologies with special application to the development of micro-medical devices, technologies that will be covered in depth in the following sections. As a brief introduction we have divided here the field of micro-manufacturing in the following areas:

- Subtractive micromachining. Some of these technologies are based on processes similar to those used in conventional manufacturing (milling, drilling, lathing…) although with much more precise tools and capable of reaching detail levels in the range of a few microns. Other even more precise technologies also eliminate material from a substrate by using focused highly energetic beams from different sources, including lasers, electron-beams, ion-beams, X-rays or even water.
- Chemical micromachining. Initially linked to the electronic industry, although nowadays it has greatly evolved and can be applied to several materials including polymers, ceramics, metals and semi-metals, chemical micromachining is used to engrave substrates by chemical attacks (using acids or bases), after some parts have been protected by a mask.

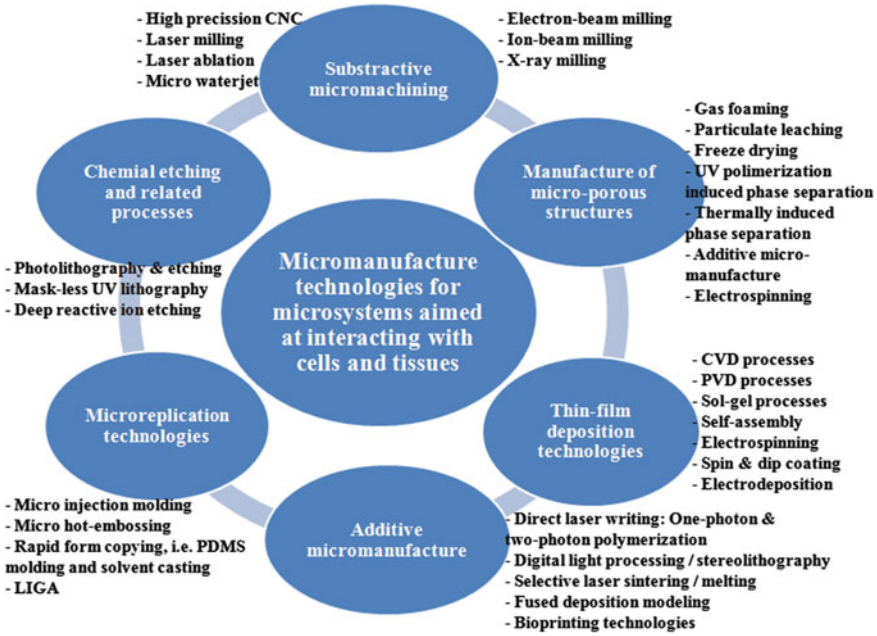


Fig. 8.1 Overview of micro-manufacturing technologies for microsystems aimed at interacting with cells and tissues

- Manufacture of micro-porous structures. Focused at the manufacture of solutions usually for the field of Tissue Engineering, these technologies are based on phase-separation process, foaming procedures or even additive processes.
- Micro-replication technologies. Aimed at the fabrication of large series of parts, normally using polymers, due to their plasticity, microstructured by hot-embossing, stamping or micro-injection molding.
- Thin-film deposition technologies. Allow the deposition of micrometric polymeric, metallic or ceramic films by means of chemical-vapour deposition, physical vapour deposition, sol-gel processes, electrospining, spin and dip coating and even self-assembly. Applications are normally aimed at providing special properties to a biodevice (biocidal properties, enhanced biocompatibility…) or as support for subsequent chemical micromachining processes.
- Additive micro-manufacturing. Uses similar principles as conventional additive manufacturing technologies and low-cost 3D printers, but with a higher precision due to the use of special materials and machines.

The following sections detail different micro-manufacturing technologies, which are especially effective for the rapid development of biomedical microdevices for interacting with cells. Some cases of study are also included.

8.2 Rapid Prototyping by Means of Micromanufacturing Technologies Derived from the Electronic Industry

Photo-lithography is a micro-manufacturing process used to selectively remove parts of a thin film or a substrate, for creating textures and channels and obtaining 2D½ micro-devices. It uses light to transfer a geometric pattern from a photomask to a light-sensitive chemical "photoresist" (normally photo-polymerizable resins), on the substrate. A series of chemical treatments then either engrave the exposure pattern into, or enable deposition of a new material in the desired pattern upon, the material underneath the photo resist (Maluf 2000; Madou 2002; Gad-el-Hak 2003).

These techniques share some fundamental principles with photography, because the pattern in the etching resist is created by exposing it to light, either directly or with a projected image using a physical mask, or even by using a mask-less process (being the mask obtained by software) as the Intelligent Micropatterning LLC SF-100 machine uses (see Fig. 8.2).

This procedure is comparable to a high precision version of the method used to make printed circuit boards. Subsequent stages in the process have more in common with etching than with lithographic printing. It is used because it can create extremely small patterns (down to hundreds of nanometers in size), it affords exact control over the shape and size of the objects it creates, and it can create patterns over an entire surface cost-effectively. Its main disadvantages are that it requires a flat substrate to start with, it is not very effective at creating non-planar or 3D objetcs, and it can require extremely clean operating conditions.

Fig. 8.2 Intelligent micropatterning LLC SF-100 machine

The 2D½ approach is, as already mentioned, widely used for the development of microsystems for microfluidic studies (capillarity, blood flow, studying biomimetic fluids), microsystems for cell motility and related studies (electrophoresis, dielectrophoresis,), "lab-on-a-chip" solutions and even "life-on-a-chip"/"body-on-a-chip" or micro-devices emulating the principles of living organisms (Sin et al. 2004; Shuler 2012).

Combinations of UV photolithography and chemical etching, have allowed us to obtain micro-textured devices for promoting interactions with cells and microorganisms, for obtaining microchannels for "lab-on-a-chip devices" or even for reducing friction in artificial joint prostheses, what can be highly relevant for promoting service life of final prostheses.

In our prior preliminary validations (De la Guerra et al. 2012) we have used copper plates and discs as substrate material due to its easy processability and the need of a lower etching time. The use of glass is also possible, but etching with HF acid, which requires special handling. For the manufacture of the micro-textures we have normally followed several steps including:

- Initial preparation of the copper discs by washing out the possible surface oxides in ultrasonic cube for around 30 min and subsequent drying.
- Coating of the discs using Dupont Riston PM-100 photoresin.
- Exposure of the photoresin to UV light by means of the SF-100 equipment from Intelligent Micro Patterning LLC. As previously mentioned, this process is known as mask-less photolithography, as the use of programmable light filters prevents from using a physical mask.
- Development, using a Na_2CO_3 0.85 % w. solution, for eliminating the uncured photoresin in those pattern zones that are going to be chemically etched.
- Chemical etching introducing the substrate in a $FeCl_3$ 40 % w. solution for attacking the uncoated pattern zones, hence obtaining the micro-texture.
- Stripping or elimination of the remaining photoresisn.
- Washing out debris and drying.
- Final dimensional verification.

Such obtained micro-textured and microchannelled surfaces can be also used for micro-replication activities, in a family of processed normally referred to as "soft lithography techniques" (see Sect. 8.4 for additional details). Soft stamps can be also obtained by casting PDMS or resin onto metallic or glass micro-textured substrates following "rapid form copying" procedures (as explained further on). Once the soft stamps are obtained, a solution or ink can be applied to the textured PDMS, which is subsequently used as a stamp for transferring the micro (or even nano) pattern to other substrates in an automatic replicating process. With this procedure, proteins can be patterned upon the surfaces of microsystems for helping cells to attach to desired positions of biodevices.

The mentioned processes are easy to industrialize for producing serial production of microsystems, as the electronic industry has widely shown. Once the process parameters are well-analyzed in the laboratory, the different solvents, etchers, concentration of solutions and time schedules for the steps involved are fixed for

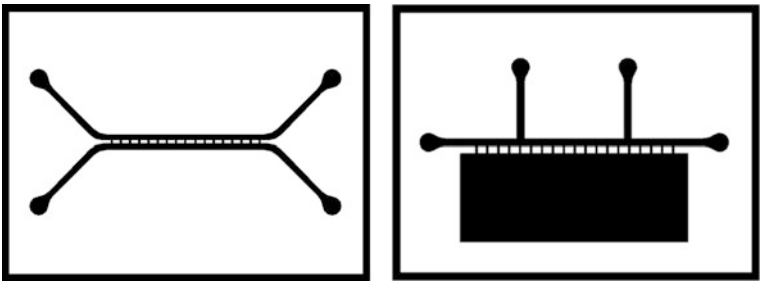


Fig. 8.3 Examples of masks for the manufacture by UV photolithography of a couple of labs-on-chips for cell co-culture and for studying cell behaviour

producing repetitive results and the operations can be automated. Clean-rooms with controlled level of pollution are needed for avoiding contamination of the substrates, as explained in ISO 14644.

The following Figs. 8.2, 8.3, 8.4, 8.5, 8.6, 8.7 and 8.8 help to illustrate the different development stages of microtextured and microchannelled substrate for interacting at a cellular level, from the design of masks (Figs. 8.2, 8.3, 8.4, 8.5 and 8.6) to the exposure for the final obtaining of a micropattern. In these cases, as we are just obtaining some master substrates for further replication in PDMS (as additionally detailed in Sect. 8.4), the etching process is not required.

In the case of Fig. 8.3, the masks for the manufacture by photolithography of two biomedical microfluidic systems are shown. In one case two parallel channels are connected by small gates or openings of 25 μm, so as to let the different cell types of the different channels interact with each other in a biomimetic way for imitating the physiological interactions present in some real tissues and organs, such as the interactions between differentiated cells or an organ an the irrigating vasculature (i.e. hepatocytes and endothelial cells). The second masks is designed for the manufacture of a microsystem including a chamber for static cell culture, typically oriented to cells that do not benefit from being cultured under controlled shear stresses, and with a channel for culturing endothelial cells and thus obtaining a simple vasculature interacting through the micrometric openings with the tisular cells statically cultured. The additional perpendicular channels allow for the use of additional cell types or for the incorporation of biochemical cues.

Therefore both examples are aimed at cell co-culture for studying physiological interactions under biomimetic in vitro conditions and linked to strategies for the straight-forward development of organs-on-chips, as further analyzed in Chap. 22.

The different steps of UV-photolithography, for the manufacture of both microsystems, are shown in Fig. 8.4 and include: incorporation of the mask to the SF-100 machine from Intelligent Micro-Patterning, UV exposure, development and final etching and cleaning or final use as mold insert for PDMS replication (see Sect. 8.4), depending on the desired final results. The results of the exposed

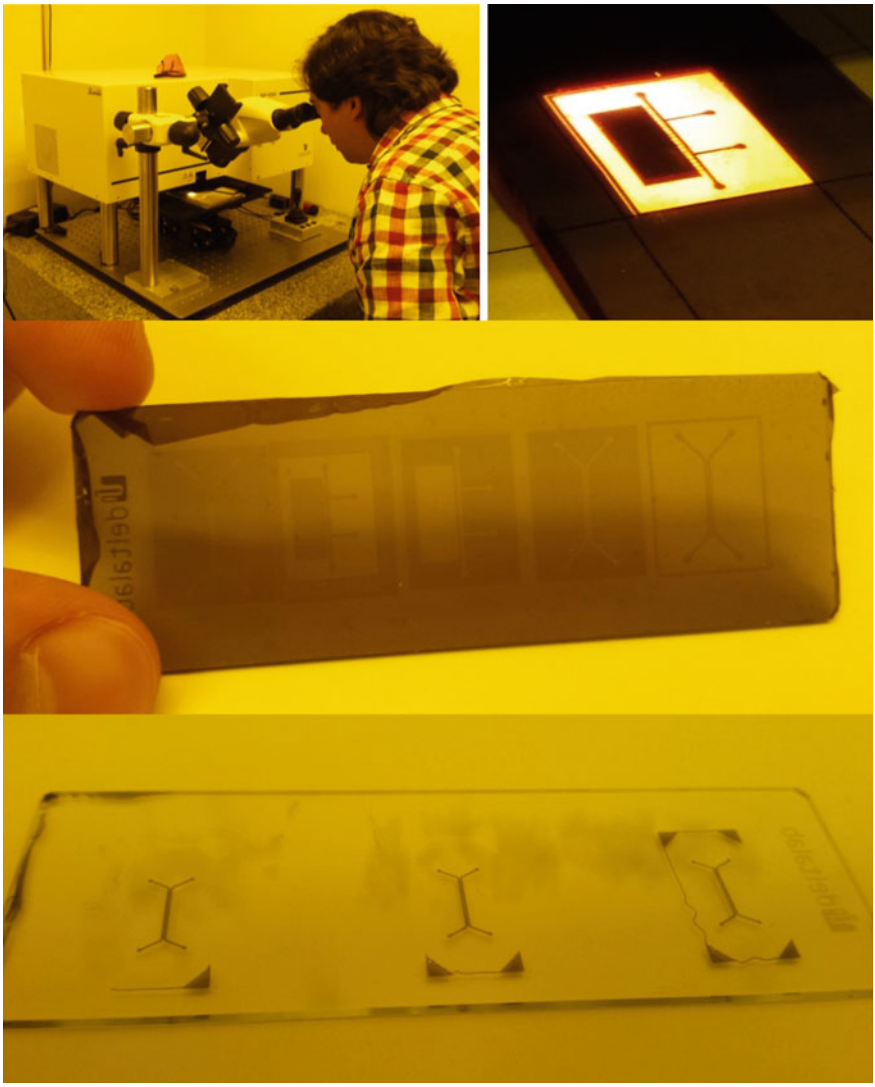


Fig. 8.4 The different steps of UV-photolithography: incorporation of the mask to the SF-100 machine from Intelligent Micro-Patterning, UV exposure, result showing the exposed photopolymer and microchannel structures obtained after developing the photopolymer and cleaning the unpolymerized zones

photopolymer and microchannel structures obtained, after developing the photopolymer and cleaning the unpolymerized zones, are also included.

Apart from using black and white masks for the development of 2D½ microdevices with homogeneous channel depths, it is also possible to control the surface topography of 2D½ microsystems by means of gray-scale masks, which allow for

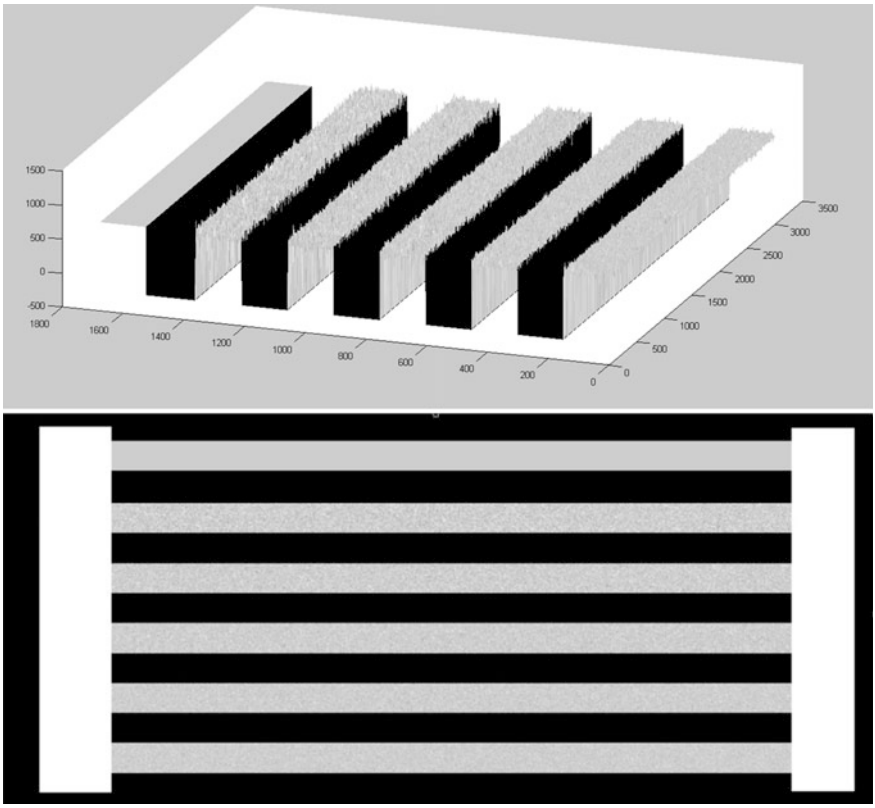


Fig. 8.5 Example of the 3D CAD design of a medical microsystem with microtextured channels and of the 2D image of a mask for its manufacture by photolithography. The *gray scale* used in the mask promotes the manufacture of three-dimensional features in just one exposure to UV light

the development of 3D textures, at least to some extent. Three-dimensional CAD models can be used for obtaining the masks in a very direct way. For instance, Fig. 8.5 shows an example of the 3D CAD design of a medical microsystem with microtextured channels, aimed at analyzing the impact of surface roughness on cell behaviour. The design has been carried out with the help of a Matlab (The Mathworks Inc.) programm included in the Annexes of the Handbook. The 2D image of a mask, for its manufacture by photolithography, is also presented. The gray scale used in the mask promotes the manufacture of three-dimensional features in just one exposure to UV light. The textures have been obtained applying fractal models, in a similar procedure as detailed in Chap. 6 (Sect. 6.5).

The number of points of the grid used for generating the height function Z(x,y) clearly affects the computational time employer for creating the design, but also the

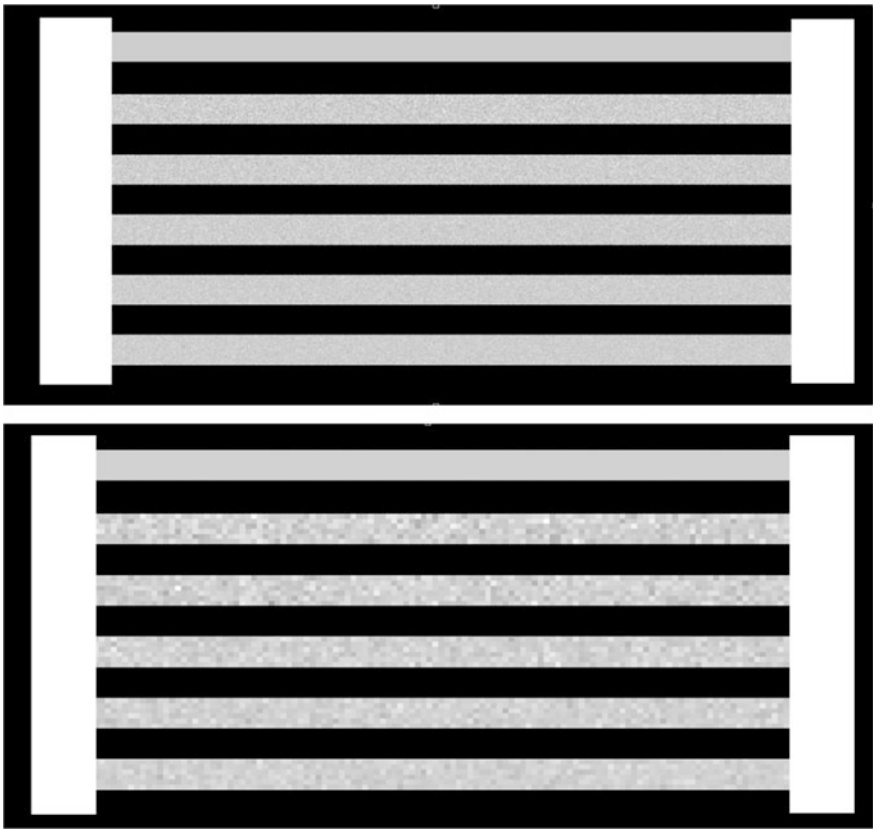
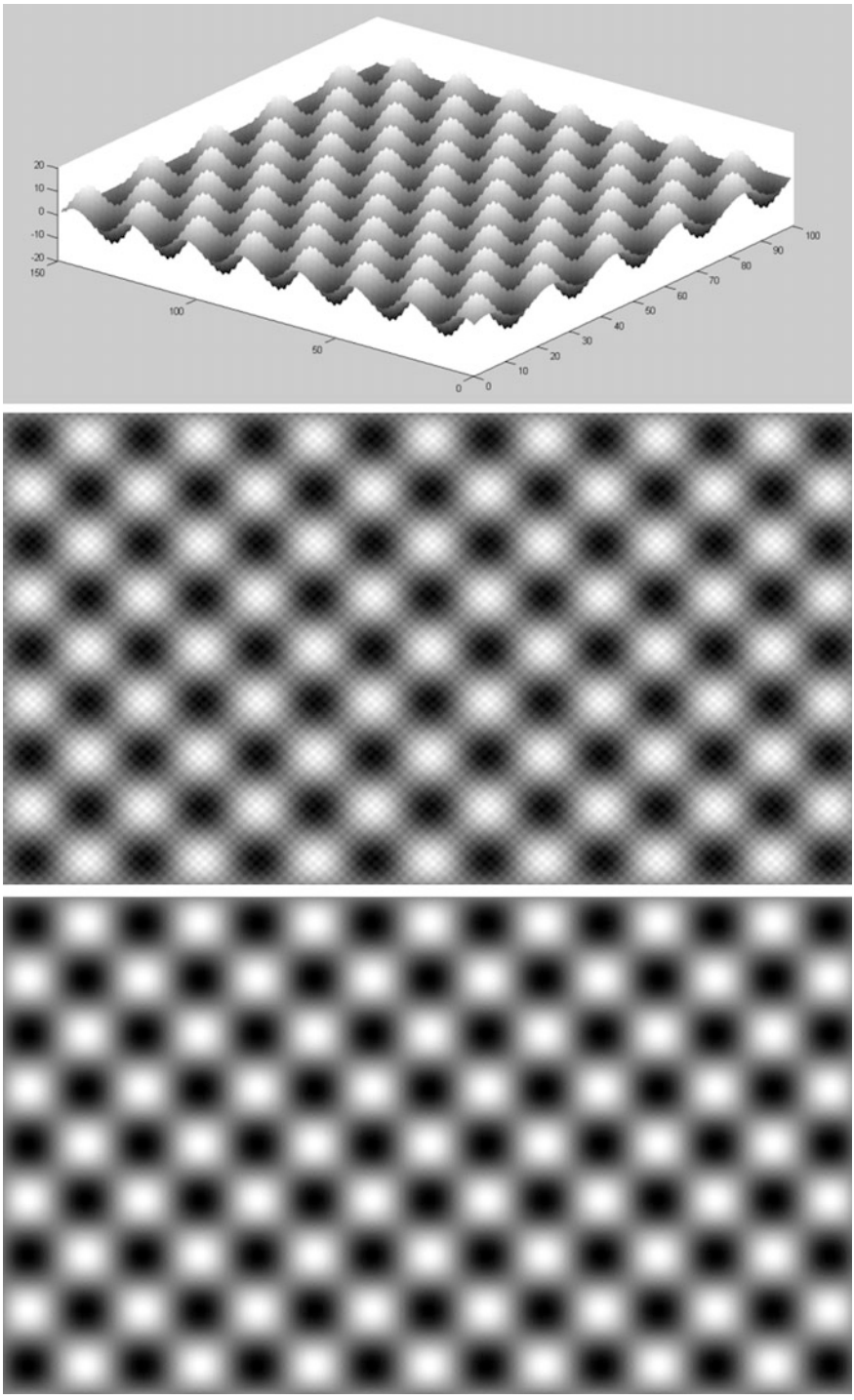


Fig. 8.6 Influence of the number of points employed, when programming the working grid, on the quality of the mask

quality of the mask and the expected final precision of the microsystems, as shown in Fig. 8.6. Ideally, the number of points defined should consider the number of pixels of the exposing system and the photopolymer voxel size, which is typically between 25 and 50 μm^3 for the more common resins used in photo-polymerization processes.

Figure 8.7 provides an additional example linked to the impact of the number of points selected for the grid and the quality of the mask, in this case aimed at the manufacture of a biomimetic super-hydrophobic surface inspired in the features of the lotus flower leaves (Barthlott and Neinhuis 1997).

Some preliminary manufacturing results of a microtextured microdevice with different textures in parallel channels can be appreciated in Fig. 8.8. It has been obtained by photo-lithography using a gray-scale mask. The process is interesting


20
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◀ **Fig. 8.7** Example of the 3D CAD design of a biomimetic self-cleaning surface for cell culture applications and of the 2D images of the masks for its manufacture by photolithography. The influence of the number of points employed, when programming the working grid, on the quality of the mask is also appreciated

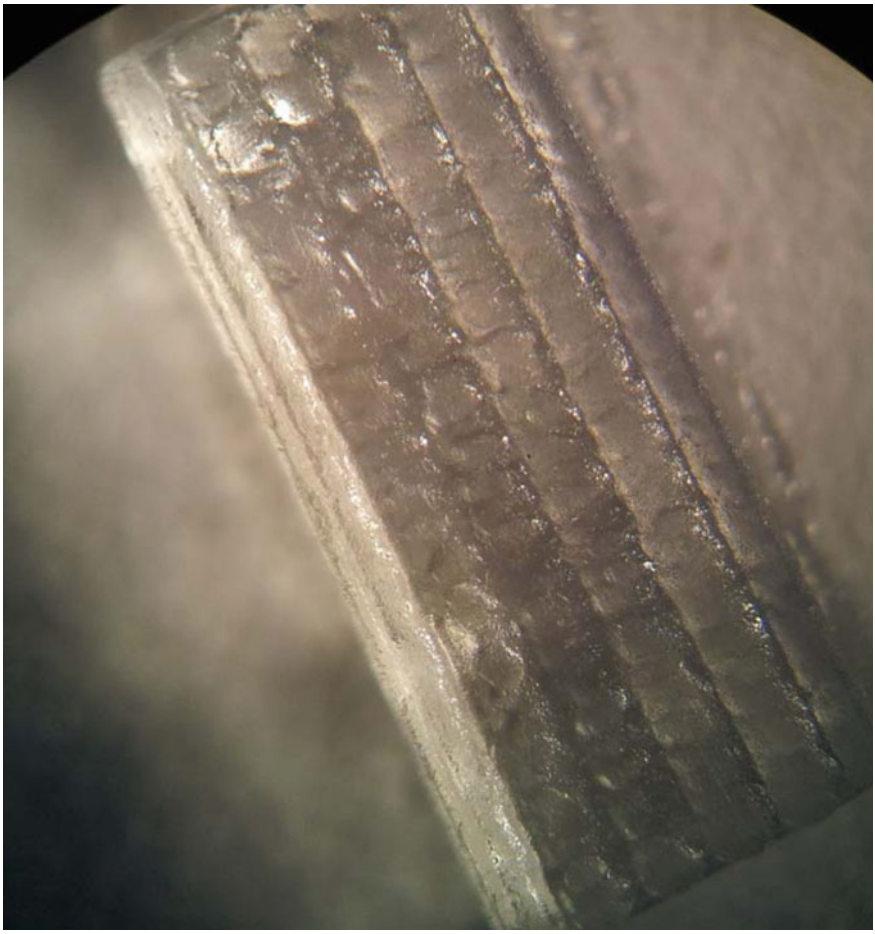

Fig. 8.8 3D microtextured channels manufactured by UV photolithography using a gray-scale mask for controlling the topographies

and can be a good complement or an alternative to other manufacturing options, such as high precision additive manufacturing, detailed in the following Sect. 8.3 and in Sect. 8.5, as well as more complex combinations between additive manufacturing and micro-injection molding for mass production, which are described in detail in Chap. 10.

8.3 Rapid Prototyping and Solid Free-Form Fabrication Strategies for Biomedical Microsystems

The progressive increase in precision of additive manufacturing technologies, together with their improved versatility thanks to a continuously increasing set of materials available for layer-by-layer processing, is greatly promoting applications linked to micro- and even nano-manufacturing of complex 3D geometries for very innovative medical solutions. The possibility of manufacturing in an additive way enables "freedom of design", as very complex geometries can be obtained. In consequence, these additive manufacturing technologies are sometimes referred to as "solid-freeform fabrication" resources.

Probably the first quantitative leap forward, towards working in the micro-scale and being able to interact at a cellular and even molecular level, came with the adaptation of conventional stereolithography to micro-stereolithography around a decade ago (Varadan et al. 2001), with which 3D details of around 15–20 μm began to be produced (what nowadays can be achieved with some multi-purpose systems, such as the Digital Light Processing from EnvisionTEC, among others). The process was initially based on combining a PC-controlled UV lamp with high-precision optics and positioning systems for enhanced polymerization (integrated harden polymer stereo-lithography, Ikuta and Hirowatari, validated 1992, published, 1993). The process progressively improved thanks to the incorporation of lasers, more sensible photopolymers and enhanced positioning systems.

Nowadays two-photon lithography or multiphoton lithography provides the most remarkable accuracy. Multiphoton lithography (also known as direct laser lithography, direct laser writing or 3D laser lithography) of polymer templates has been known for years by the photonic crystal community and is currently spreading to the biomedical field. Similar to standard UV-photolithography techniques, structuring is accomplished by illuminating negative or positive photoresists via light of a well-defined wavelength. The fundamental difference is, however, the avoidance of physical masks and the change from a 2D½ approach to a 3D layer-by-layer process. Instead, two-photon absorption is utilized to induce a dramatic change in the solubility of the resist for appropriate developers and high precision is attainable thanks to the use of femto-second lasers (Ostendorf and Chichkov 2006).

With these high-precision additive manufacturing technologies, not only complex 3D geometries (hollow structures, inner details…) can be achieved, but also extraordinary high aspect-ratio micro-structures, many of them with medical application as mentioned further on. Previously, such ultra-high aspect-ratio microstructures could only be manufactured through X-ray lithography, ion-beam lithography and some other micro-manufacturing technologies, whose possibilities of leading to complex 3D structures, with hollows and inner details, with application for instance in the development of novel metamaterials, were indeed limited.

Table 8.1 Comparison between high-precision rapid manufacturing processes

Technology	Precision	Materials	Working principle	Reference
Two-photon polymerization	Around 200 nm	Photo-polymers, biomaterials, ceramics	Additive	Inführ et al. (2007)
Micro-stereolithography	5–35 μm	Photo-polymers	Additive	Varadan et al. (2001)
Digital light processing	15–50 μm	Photo-polymers and ceramics	Additive	Ostendorf and Chichkov (2006)
Bioplotter and bioprinters*	150–350 μm	Biological materials	Additive	Mironov et al. (2009)
Laser micro-machining	5–50 μm	Organic and inorganic	Subtractive	Queste et al. (2010)
X-ray based microfabrication	100 nm–100 μm	Metals, plastics, glass, ceramics	Subtractive	Gad-el-Hak (2003)
Pressure-assisted microsyringe	10–600 μm	Biopolymers	Additive	Yeong et al. (2004)
Robocasting	100–1000 μm	Organic ink	Additive	Yeong et al. (2004)
Lithography-based ceramic manuf.	15–50 μm	(Bio-)ceramics, biopolymers	Additive	Schwentenwein and Homa (2015)
Conventional 3D printers	200–400 μm	Waxes, polymers	Additive	Wohlers (2010)
Conventional stereolithography	150–300 μm	Photo-polymers	Additive	Díaz Lantada (2009)
Conventional fused deposition modeling	200–400 μm	Waxes, thermoplastics	Additive	Masood et al. (2005)
Conventional CNC machining	50–150 μm	Mainly metals	Subtractive	Several manufacturers

Also includes some references to other conventional technologies. Adapted and updated from A. Díaz Lantada, P. Lafont Morgado: "Rapid prototyping for Biomedical Engineering: Current capabilities and challenges", 2012

The incorporation of bio-photopolymers to the range of materials available for 3D additive micro-manufacturing is also promoting final medical applications, especially in the fields of devices for drug delivery, "lab-on-a-chip" solutions for rapid diagnosis, scaffolds for tissue engineering and microsystems for interacting at a cellular level in general. Table 8.1 provides a brief comparison of several high-precision manufacturing technologies, including additive and subtractive ones, as well as some conventional procedures as additional reference.

Next pages provide some case studies linked to the rapid manufacture of complex 3D micro- and nano-biodevices, achieved thanks to stereolithography (Form1+ machine available at the Product Development Lab, TU Madrid) and to direct laser writing, (Photonic Professional by NanoScribe GmbH available at the Karlsruhe Institute of Technology), which currently offers the most versatile and precise additive manufacturing technology commercially available (Hengsbach and Díaz Lantada 2014a).

Among the most interesting biomedical microdevices aimed at interacting with cells and affecting their behavior and clearly promoted by means of manufacturing technologies working on additive approaches, it is necessary to highlight all types of scaffolds for tissue engineering and regenerative medicine. Tissue engineering is based on the combination of biological, physical and engineering knowledge to promote the artificial development of improved replacements for tissues and even organs linked to surgical repair strategies.

A very relevant component, involved in tissue engineering processes, is the extra cellular matrix (ECM) or tissue engineering scaffold which serves as framework for cell growth, aggregation, gene and phenotype expression and final tissue development (Langer and Vacanti 1993). According to biomimetic design and manufacturing principles, the biomaterials used as scaffolds should be porous, so as to allow cell migration during the colonization process, as well as the transport of nutrients and waste to and from cells. Such biomaterial constructs also have to be resistant enough to withstand mechanical demands, especially if the final implantation is desired.

In addition, as cells are capable of feeling their microenvironment and of responding to the substrate texture upon which they lie, by changing their overall morphology, cytoskeleton configuration, and intra- and extracellular signaling, an increasing number of studies are focusing on advanced design and manufacturing technologies, so as to generate and modify the structures and surfaces of biomaterial. Aspects such as porosity, pore distribution and size or surface micro- and nano-textures promote cell adherence, migration and proliferation within the scaffold, for subsequent gene expression and differentiation into relevant cell types. Hence, both tissue progenitor cells and the extra cellular matrices play a fundamental role in tissue engineering strategies. The controlled design and fabrication of biomaterials used as scaffold structures is becoming increasingly important for regenerative medicine (Thomas et al. 2010; Chen et al. 2010; Buxboim and Discher 2010).

Main alternatives, for improving the control of scaffolds' pore size and distribution, from the design stage, is the use of micro additive manufacturing technologies (AMT), normally working on layer-by-layer processes, following the geometries obtained with the help of computer-aided designs (Bartolo et al. 2009; Tan et al. 2010). Even though Chaps. 16–20 provide detailed explanations linked to main strategies for the development of advanced or "knowledge-based" tissue engineering scaffolds, we include here some interesting introductory examples, so as to highlight the relevance of micro-manufacturing technologies working on an additive way to develop adequate scaffolding structures.

Figure 8.9 shows several 3D CAD designs and the directly obtained porous prototypes of different porous and lattice structures with potential application as tissue engineering scaffolds. Prototyping has been accomplished with a Form1+ machine from Formlabs in epoxy resin, which is not adequate for in vitro or for in vivo trials, but helps to validate geometries to carry out some functional trials and to check if the manufacturing challenges can be overcome. Figure 8.9 shows the vectorial paths for guiding a 3D direct laser writing system towards the fabrication

Fig. 8.9 3D designs and prototypes of different porous and lattice structures with potential application as tissue engineering scaffolds. Prototyping has been accomplished with a Form1+ machine from Formlabs

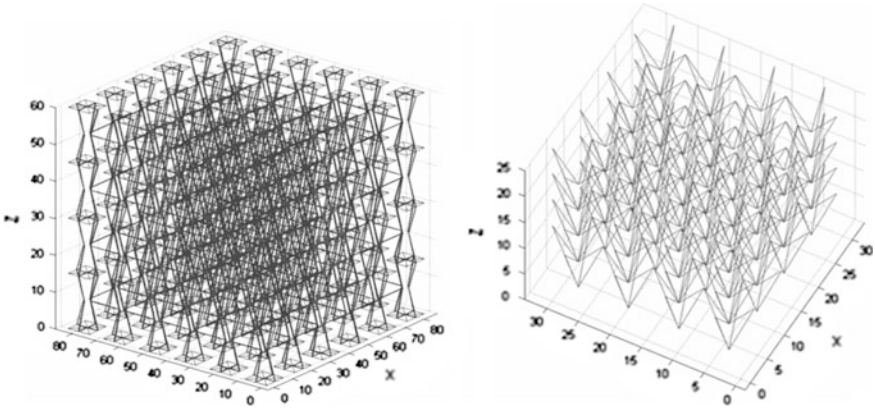

Fig. 8.10 Vectorial paths for guiding a 3D direct laser writing system towards the manufacture of tissue engineering scaffolds with auxetic properties

of even more precise tissue engineering scaffolds with auxetic properties for multi-axial excitation of cells during culture (following similar mechano-biological procedures to those explained in Chap. 14). The related prototypes obtained by direct laser writing are shown in Fig. 8.11 and additional details have been published elsewhere (Hengsbach and Díaz Lantada 2014a).

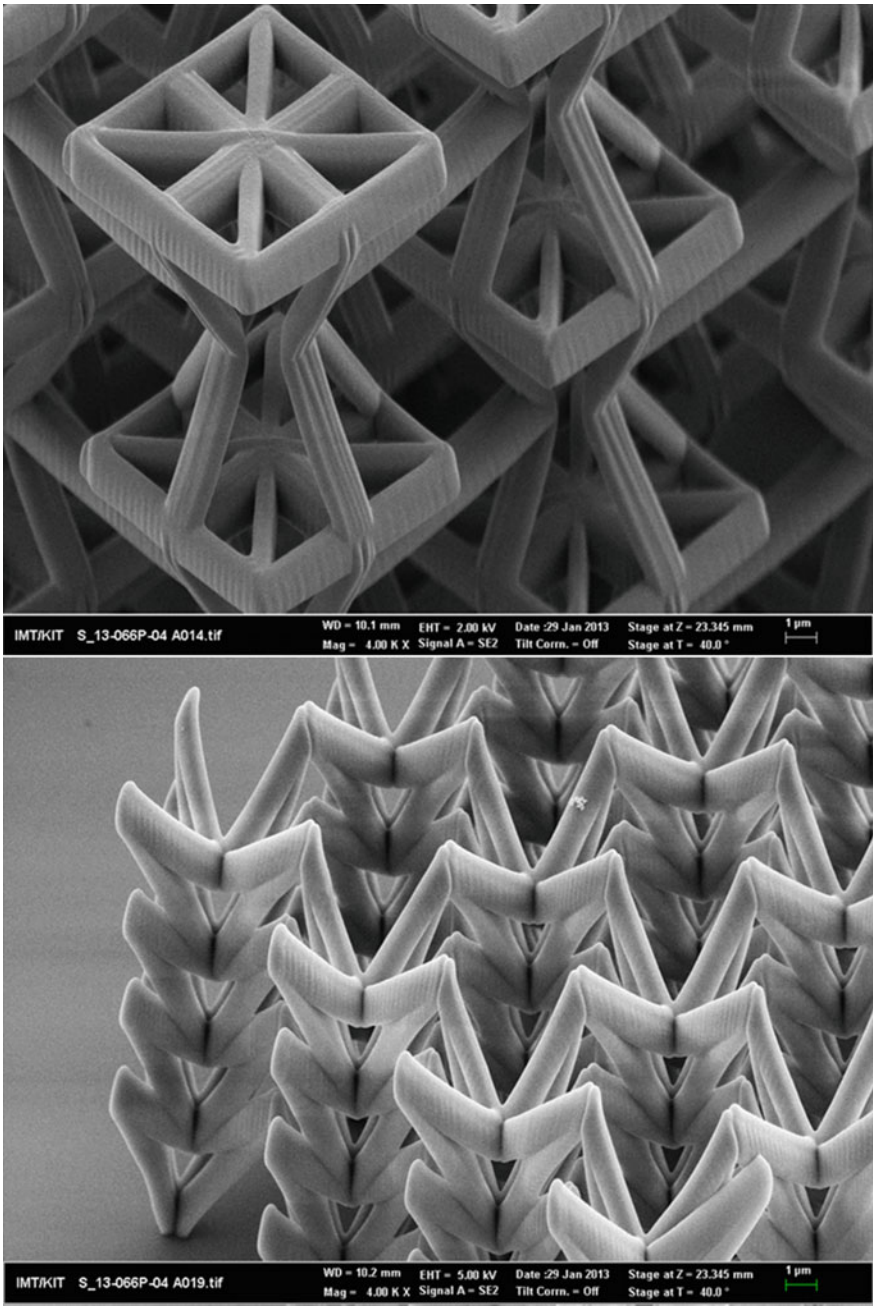

Fig. 8.11 Tissue engineering scaffolds with auxetic properties and micrometric features obtained by 3D direct laser writing based on two-photon polymerization. Prototypes manufactured with the support of the KNMF-KIT facilities

8.4 Rapid Form-Copying and Rapid-Tooling for Biomedical Microsystems

Regarding second phase prototyping processes, that is processes based on copying the geometries of initial masters typically obtained in an additive way or by means of other micro-manufacturing technologies, it is important to introduce some concepts widely spread in the manufacturing industry and progressively impacting the medical field. It is important to re-introduce some definitions, nowadays very common in the product development industry and progressively expanding into the biomedical field.

Rapid form/shape copying. Consists of using a master model or "green part" usually obtained by a rapid prototyping approach, based on additive manufacturing or on high-speed numerical control machining, as a pattern to create a replica very rapidly, by casting, vacuum casting, injection or even stamping or hot-embossing, in a more adequate material for the final purpose of the geometry being copied. In many cases the "green part" is designed with final geometry and the rapid form copying process leads to a mold (see "rapid tooling" below), in which pre-series can be obtained. In other cases the rapid copying process leads directly to a final part, in which case the master model has to be designed as the negative geometry of the desired part.

Rapid tooling. Is typically used to describe a process which either uses a rapid prototyping model as a pattern or master to create a mold easily, rapidly and in a very economic way, or uses a rapid prototyping process directly to fabricate a tool or mold cavity for a limited volume of prototypes. Tooling time is then much shorter (tools can be typically obtained in less than a week), cost is also much less than for conventional tools (i.e. in steel by electrodischarge machining), although tool life is considerably lower than for conventional tools and tolerances are also wider.

Many of the most used rapid tooling processes are based on rapid form/shape copying approaches. Using a rapid prototyped model the cavities of a mold can be obtained by placing the model in the middle of a case and filling the case with a pre-polymer, a pre-polymer with ceramic charge or a pre-polymer with metallic charge, so as to obtain a block of solid material. Final cutting and model extraction lead to the mold cavities (or tools) for further casting, vacuum casting or injection of more adequate materials for the second phase prototypes. In some cases, when the tooling material is especially hard, cutting is not an option and cavities are obtained by filling the case just to the partition line, although two steps are then needed.

In other cases, the mold cavity or tool can be directly obtained by using rapid prototyping facilities such as selective laser sintering, selective laser melting, among others typically working with metal powders for obtaining hard and highly resistant pieces.

For laboratory experiences even RP technologies working with polymers (normally thermoset polymers) can be useful for obtaining such rapid tools and subsequently stamping or embossing other thermoplastic polymers and low melting-point biomaterials. Depending also on the material's hardness and expected life of the rapid tool or cavity obtained, the rapid tooling processes can also be divided in some more groups commented below:

Soft tooling. Makes reference to silicone molds obtained by rapid form copying processes, which usually allow for the manufacture of short series of 25–50 polymeric parts by casting or vacuum casting, as the mold material progressively deteriorates due to thermal shock when the polymers are casted or thermally-cured.

Bridge tooling. Makes reference to harder molds obtained usually by rapid form copying using low melting-point alloys or pre-polymers with metallic or ceramic micro-/nano-charges used for increasing hardness, mechanical resistance and cavity service life. Normally short-medium series of 100–1000 parts can be obtained by conventional injection molding, once the cavities are arranged as a prototype mold. As the mold material has usually a polymeric matrix, temperature cycles produce continuous relevant mechanical deformations and stresses and the mold deteriorates rapidly.

Hard tooling. Makes reference to rapid molds obtained in metals aiming to provide the most possible similar results to those from final production injection molding using steel molds. Normally the cavities are obtained by easy machinable metals (aluminium alloys, brass…) by high-speed CNC machining or by additive manufacturing technologies capable of working with metals (normally starting from metallic power), such as selective laser sintering, selective laser melting, laser cusing and laser cutting, among others. Much larger series (in many cases for substitution of final production) even up to 100,000–500,000 parts can be obtained.

The aforementioned processes provide a step-by-step validation, increasing the level of detail of final prototypes and their resemblance to mass produced parts, helping to detect and correct possible defects before investing in final steel molds for mass production. Additional information on these processes and on the different tolerances, expected life of components and typical industrial applications can be found in the references (Lorenzo-Yustos 2008).

Even though every year novel additive technologies appear and the use of more and more polymeric, metallic, ceramic materials is possible (Wohlers 2010) and some recent advances are even aiming at the manufacture of 3D biodevices by deposition of biological materials, it is still true that many of the widely available technologies providing the best quality (precision)/cost relationship cannot directly work with materials adequate for in vitro, ex vivo or in vivo trials.

For instance, as already detailed, many technologies working on the basis of photopolymerization processes (laser stereolithography, digital light processing, polyjet…) usually work more properly with materials such as epoxy or acrylic resins, which are not adequate for final biodevices, due to their toxicity. In other cases, some technologies include a powder material (ceramic, polymeric, metallic

or even biological, including wood powder) and a second gluing material, as happens with conventional three-dimensional printing, what typically leads to non-biocompatible parts, due to the toxic effects of the gluing agent.

The use of alternative materials to those provided by the machine manufacturers can damage the prototyping machine and always goes at the researcher's own risk, as machine's guarantee does not cover such kind of "personalizations", being usually an expensive, although also a highly-interesting strategy.

Therefore rapid form-copying and rapid-tooling still provide several highly remarkable alternatives, for obtaining prototypes in more adequate materials for final purpose, especially if the use of biomaterials is required (as is usually the case in the development process of novel biodevices, when the different in vitro, ex vivo and in vivo trials are required), than most of the currently available rapid prototyping technologies.

In fact many biomaterials are difficult to be structured in an additive way but are indeed very apt to other conformation processes, such as casting, injection molding stamping or hot-embossing and the use of rapid tools and rapid molds proves to be very adequate for the rapid manufacture of prototypes for trials, as the case studies included in the following sections detail.

In this Section we concentrate on rapid-form coyping using PDMS (silicone) and gels and on rapid-tooling for obtaining PDMS molds (soft-tooling). The process of silicone (also polydimethylsiloxane or "PDMS") mold manufacture, based on a master model obtained normally through rapid prototyping, involves some steps described further on. We concentrate on the process aimed at obtaining a two-part mold, as the process for obtaining a single cavity is simpler.

First of all the partition line is defined, normally by attaching adhesive tape to the lateral part of the master model or by joining the different models together to a central entrance channel (in the case of a multicavity mold, as the following example shows). Then the model(s) is placed in the centre of a cubic cage, levitating thanks to some additional supports (tooth sticks, more adhesive tape…). The bi-component liquid mixture for producing the silicone is carried out and poured into the mold, until the whole model is covered, while polymerization is starting. It is advisable to carry out the polymerization process at low pressure atmosphere (into a vacuum chamber) so as to alow degasification of the silicone and avoid a final hollow structure. After polymerization the mold has to be cutted, following the partition line, for finally extracting the master model and leaving the two cavities ready for closure and casting of new formulations. Some interesting tutorials can also be found at www.makeyourownmolds.com including also references to mold making kits, materials suppliers and mold making suppliers and accessories.

Vacuum and open-air casting. By taking models manufactured using rapid prototyping technologies or additive manufacturing technologies, silicon molds can be made for rapid shape copying to reproduce the geometry of the original model with precision using polymer materials with different kinds of micro- and nanocharges or micro- and nanoforces. These "soft" molds can be used to make short runs of 25–50

units, usually to make concept samples and verify functions before proceeding to manufacture prototypes with the definitive process.

Vacuum casting makes it easier to fill the mold, improves the final precision and helps to eliminate the bubbles that form during the curing process of many of the dual component polymers usually used for this type of casting. During the mixing process, before casting, the micro- or nanocharges can also be added (like those in the image below) using mechanical and ultrasonic agitators to improve the dispersion of the particles and achieve more homogeneous properties.

Once the mold is obtained and the original models extracted, the mold is closed again, using metallic clips or adhesive tape, and places in the vacuum casting machine. The polymeric mixture to be casted (normally bi-component polyurethanes in conventional applications and several biopolymers in Biomedical Engineering) is prepared and subsequently introduced into the mold through a channel. At the beginning just gravity acts, but once the material begins to fill the mold, further assistance from vacuum in the mold chamber helps to fill the mold completely and to obtain a remarkable level of precision. After polymerization the mold is opened again and final prototypes are extracted for carrying out some last adjustments.

In some cases, open-air casting can be used for obtaining just a single cavity, which may be used as cell-culture substrate or as a part of a more complex biomedical microdevice, as in the examples provided in the following pages.

In any case it is very important to note the possibility of using rapid molds for casting several (bio)materials, even active or "intelligent" materials for including some additional functions to final biodevice. The possibility of using a mold in repeated occasions is also of great help for analyzing the influence of composition or additives in the performance of the different prototypes, as necessary part of the material selection process.

Such additives, especially some changing the optical properties of the base polymer (carbon nanotubes, graphite powder, carbon black, silver or gold nano-particles…) would dramatically affect a rapid prototyping process by photopolymerization, and probably prevent such comparative studies, while in the case of vacuum casting in rapid molds the influence of additives is minor and final prototypes with different properties can be easily obtained.

The similar strategy of rapid shape copying for obtaining rapid molds can be used for manufacturing final prototypes for trials. The process usually involves placing the master models in the bottom of a box or case and, subsequently, casting the desired final material (in a liquid state) upon them. Final solidification, polymerization or gelation leads to a stable replica that can be cut into small parts or probes.

We have used such an approach for obtaining micro-patterned and micro-textured biodevices in PDMS, several gels, beeswax, low melting-point alloys, among other materials susceptible of biomedical applications. The level of detail is remarkable and features of around 5–10 μm are simple to replicate if the process is carried out methodically.

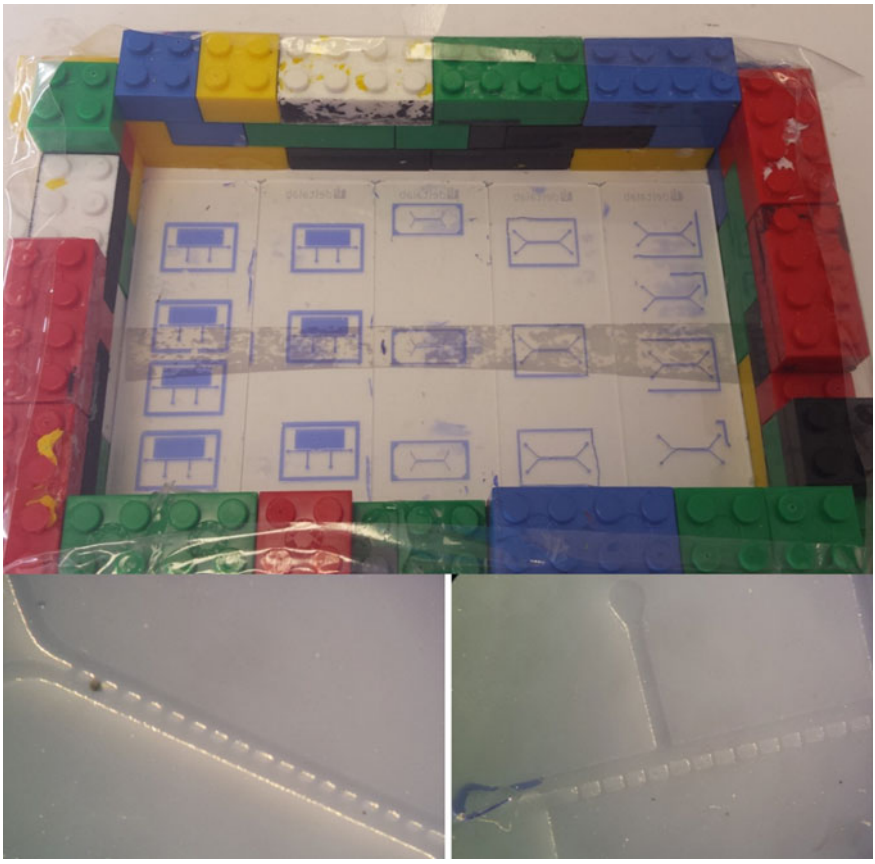

Fig. 8.12 Rapid mold with inserts obtained by UV photolithograpy and final PDMS replicas of the desired labs-on-chips for direct cell culture trials

The first example included in present Sect. 8.4 is linked to the microsystems detailed in Sect. 8.2 (Figs. 8.2 and 8.4). We use the master models obtained by UV-photolithography as rapid mold inserts for subsequent PDMS replication by casting in the rapid mold and final cell culture trials upon the adequate PDMS, as shown below in Fig. 8.12. The level of detail, with connecting openings between channels and between channels and chambers of around 25 μm, can be clearly appreciated.

Additional examples for putting forward the relevance of soft-lithography in the biomedical field, especially when carrying out tasks linked to the development of biomedical microsystems capable of interacting with cells, are shown in Figs. 8.13, 8.14 and 8.15. These microsystems correspond to different in vitro models for cell culture under biomimetic conditions and aimed at studying the cellular processes

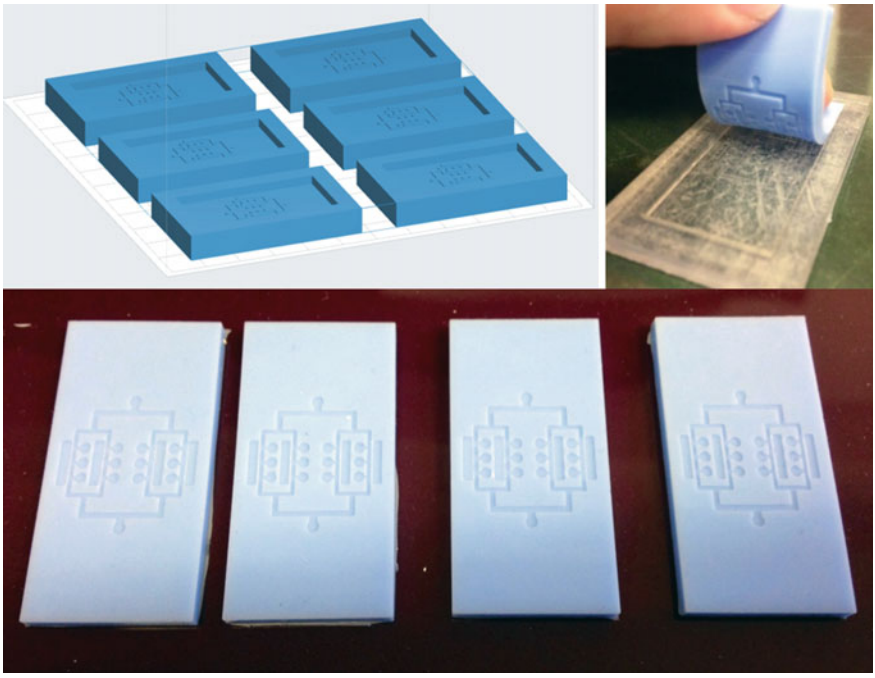

Fig. 8.13 Complete development process of a lab-on-a-chip with multiple channels and chambers aimed at cell co-culture for modeling the blood-brain barrier: 3D CAD models of a mold and PDMS replicas obtained by vacuum casting upon the epoxy masters for direct cell culture trials

and interactions between different cells typical from two relevant physiological structures. Figure 8.13 schematizes the complete development process of a lab-on-a-chip with multiple channels and chambers aimed at cell co-culture for modeling the blood-brain barrier. The images included show the 3D CAD models of a mold and the PDMS replicas obtained by vacuum casting upon the epoxy masters for direct cell culture trials. Figure 8.14 shows the complete development process of a cell culture platform for modeling bone growth in vitro, with the 3D CAD model of a mold and the PDMS replicas obtained by vacuum casting. Finally, Fig. 8.15 provides some cell culture results and details the colonization process of the biomedical microdevice. In other cases, the use of hydrogels instead of PDMS for replication tasks is also feasible and interesting, as shown in Fig. 8.16. These preliminary examples are connected with some of the organ-on-chips and related design and manufacturing strategies detailed in depth in Chap. 22.

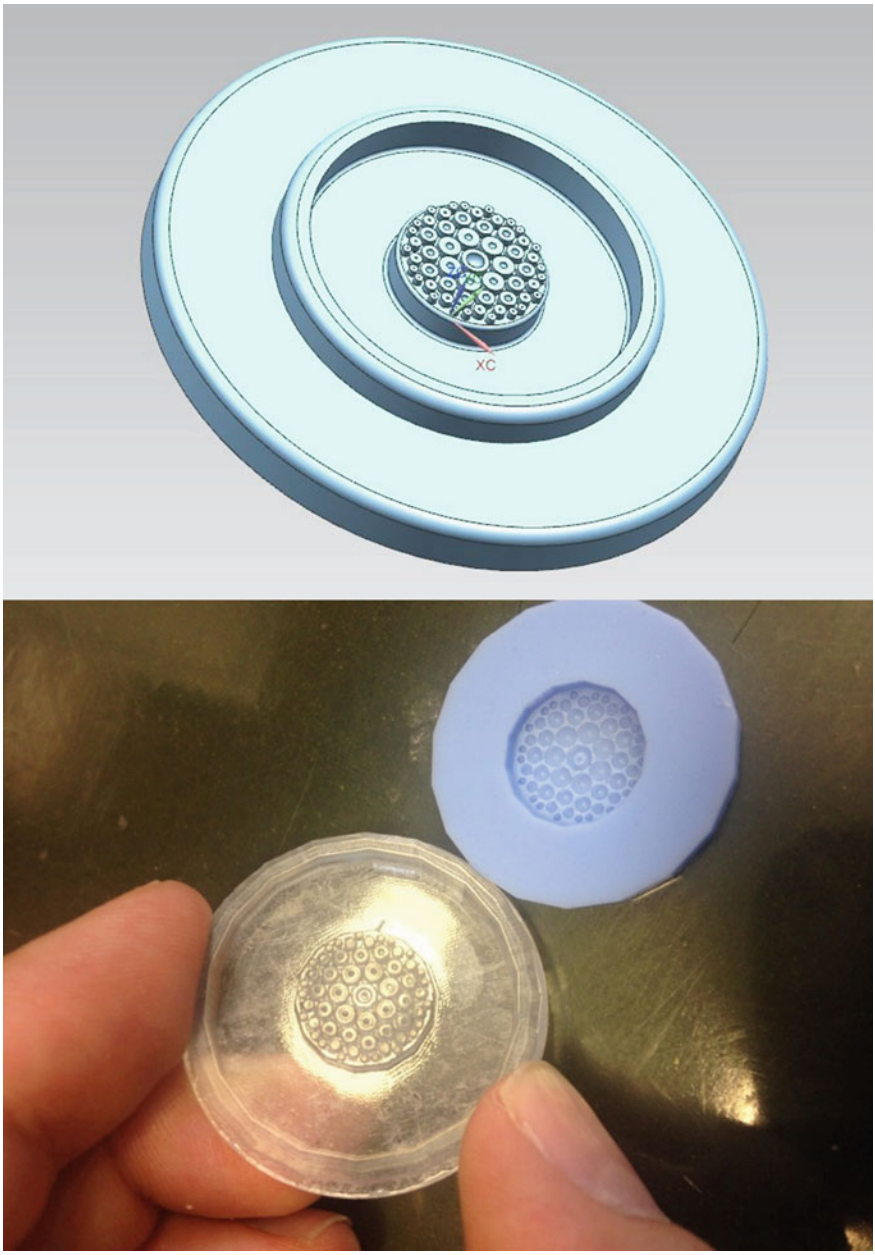

Fig. 8.14 Complete development process of a cell culture platform for modeling bone growth in vitro: 3D CAD model of a mold and PDMS replicas obtained by vacuum casting upon the epoxy master for direct cell culture trials

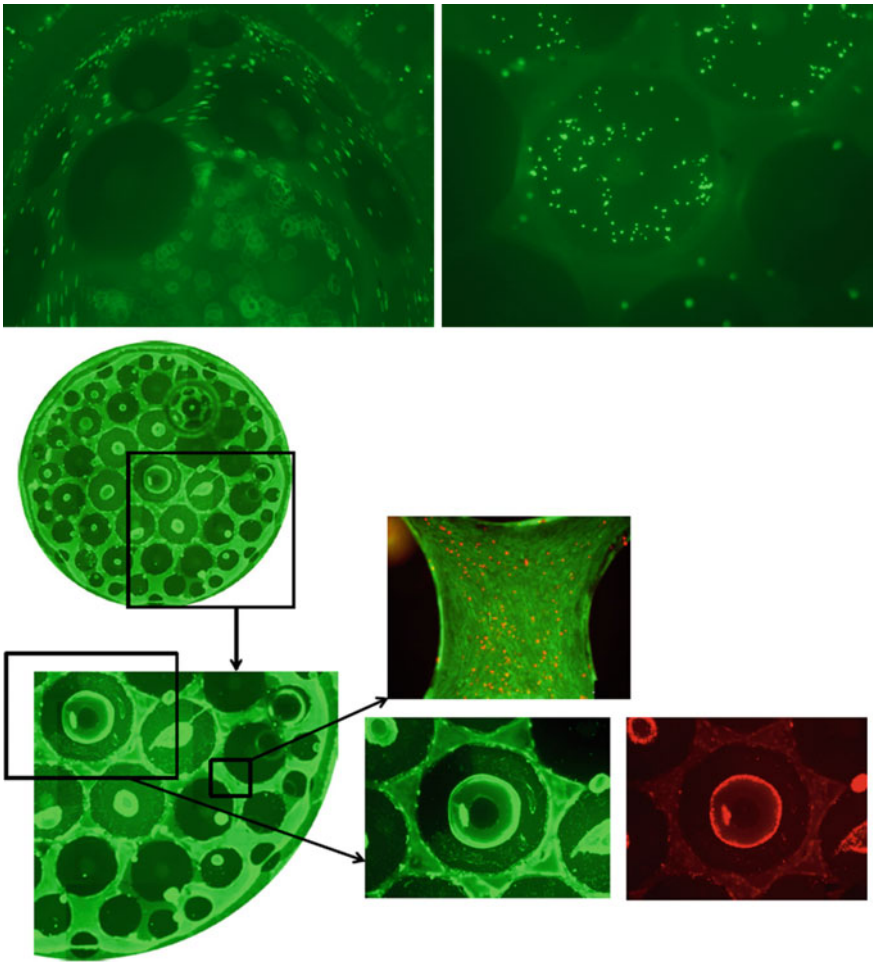

Fig. 8.15 MC3T3E1 cells (rat osteocytes) stained with calcein after 3 days (*upper* images) and after 1 week (*lower* images). The cells with *green* fluorescency are alive, while the in bright *red* are dead. A relevant proportion of living cells can be appreciated, clearly attached to the elevated features of the cell culture platform, while the valleys remain full of fluid without cells. The patterns formed by the cells, with larger empty *spheres* in the middle of the platform and more compact aggregations towards the periphere, show the potential for the development of an in vitro cell culture platform for modeling bone. Along the days, the cells colonize the platform and form aggregations. *Note* Some floating cells can be appreciated in the foreground of the microscopy. Cell culture and images courtesy of Prof. Milagros Ramos from the Center for Biomedical Technology at the Technical University of Madrid

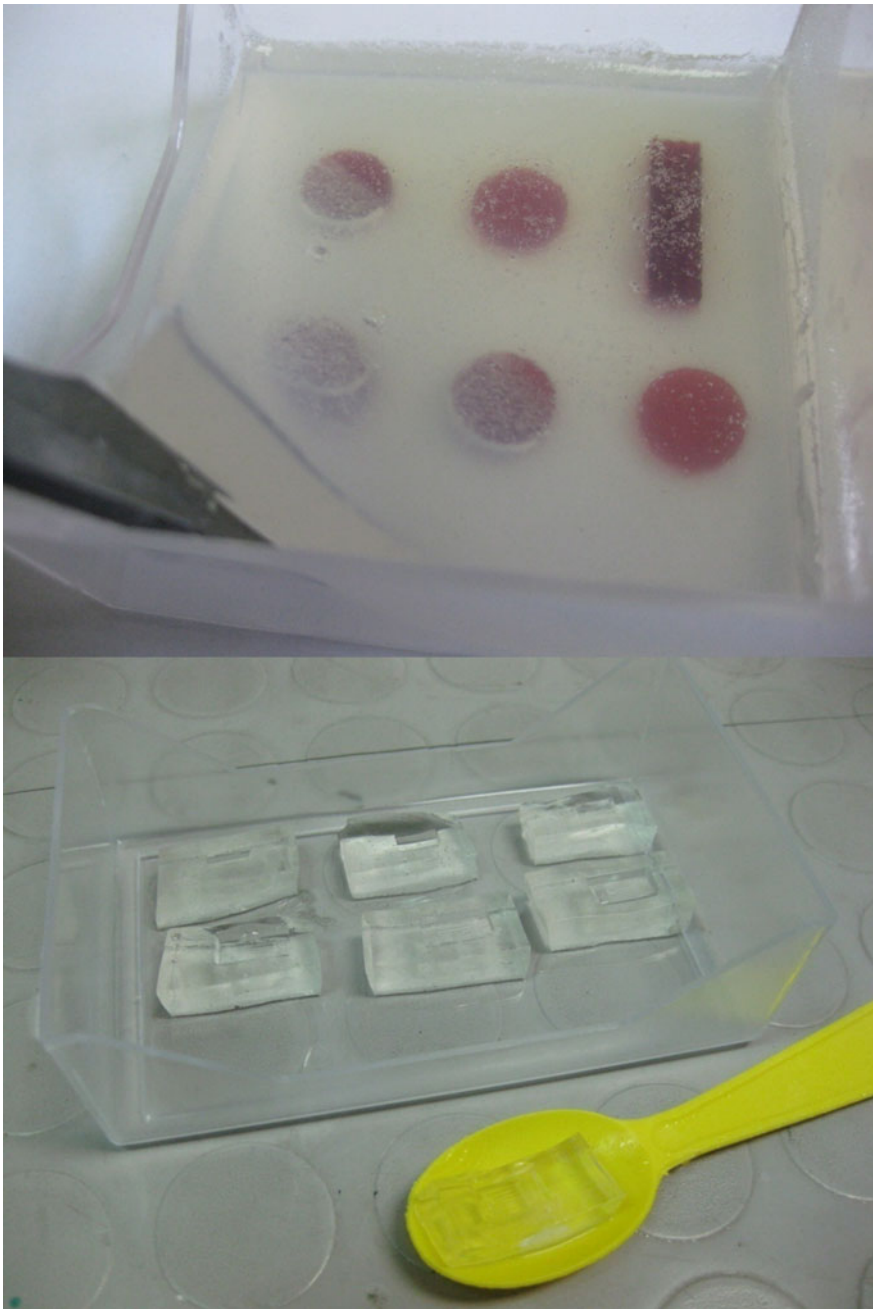

Fig. 8.16 Gel replicas of different microsystems aimed at studying cell dynamics obtained by sol gel upon a mold with rapid prototyped inserts with the negative geometries of the desired microdevices. Gel extraction and final parts

8.5 Combining Technologies for Multi-scale Biomedical Microdevices

Biomedical devices that include geometries and functions on multiple length scales and at different locations are able to interact with their environment and surrounding living systems in a more controlled and accurate way. Multi-scale biomedical devices help to promote biomimetic approaches, as living organisms also exhibit forms and functions at different scales (Place et al. 2009), thus helping to improve aspects such as biocompatibility and overall performance. Therefore, progressive research into design and manufacturing strategies that promote hierarchical materials and structures and their integration into complex appliances is helping to improve both the diagnostic and therapeutic results of several biodevices. In biomedical sciences, fields such as prosthetics (Ponche et al. 2010; Anselme et al. 2010), health-monitoring and diagnosis (Reljin and Reljin 2002), tissue engineering (Hosseinkhani et al. 2007, 2010) and even biofabrication (Borchers et al. 2012) are already starting to take advantage of multi-scale approaches, the applications of which are continuously evolving.

Combinations of top-down and bottom-up approaches are frequent and have usually focused on manufacturing the larger micrometric features by means of top-down processes (micromachining, etching, etc.). The smaller nanometric details, such as for the rapid prototyping of patterned functional nanostructures (Fan et al. 2000), are made using bottom-up techniques (like CVD, PVD, sol-gel, self-assembly, ink-jet printing). Normally these combinations are not aimed at obtaining 3D features at different scales, but at incorporating some surface patterns, 2D½ geometries or some sort of physical-chemical functionality, such as enhancing bio-compatibility and implementing special actuating-sensing functions.

Currently, advances in computer-aided design and in high-precision additive manufacturing technologies based on layer-by-layer deposition or construction are opening new horizons for controlling surface topography. They are being used from the design stage and can be applied in a manner that is very direct, rapid and simple. This is enabling the prototyping of multi-scale designs and hierarchical structures. Even though conventional computer-aided design packages are only capable of handling Euclidean geometries and mainly rely on simple operations (sketch based operations, extrusions, pads, holes, circular grooves, etc.) for obtaining "soft" solids and surfaces, recent approaches relying on the use of matrix-based programming have already proved to be useful for designing rough surfaces and textured objects adequately described by fractal geometries (Mandelbrot 1982; Falconer 2003). In parallel, the continued progress in additive manufacturing technologies (also called "solid free-form fabrication" due to the complex geometries attainable), especially during the last decade, has increased the range of materials capable of being additively processed and greatly promoted their precision, even down to nanometric features. This has implications in the development of advanced materials and metamaterials, many of which benefit from multi-scale approaches (Bückmann et al. 2012; Röhrig et al. 2012).

Ultra-high precision additive manufacturing technologies, however, mainly direct-laser writing based on two-photon polymerization, despite being capable of yielding nanometric details, are very slow and the attainable devices are normally smaller than 1 mm^3. Such tiny devices are normally aimed at very specific studies (i.e. single-cell mechanical-biological experiments). Obtaining successful implants, as well as easy-to-handle microsystems, is still challenging since most biodevices and medical appliances, either for diagnostic or for therapeutic tasks, are at least several mm^3. On the other hand, industrial rapid prototyping (i.e. laser stereolithography, digital-light processing and selective laser sintering), in spite of being fast and capable of yielding larger devices, is limited to manufacturing precisions typically in the 50–250 μm range.

It is thus still complex to produce biomedical microdevices with ad hoc features for interacting at the molecular or even cellular level. However multi-scale microsystems, for addressing the impact of surface topography on cell behavior, have been recently successfully obtained by combination of two additive manufacturing processes. First, a conventional laser writer is employed to manufacture the overall device structure. Secondly, a direct-laser writer, based on 2PP (two-photon polymerization), is used to yield the smallest details. The process stands out for its versatility, accuracy and manufacturing speed and allows for the manufacture of microsystems and implants with overall sizes up to several millimeters and with details down to sub-micrometric structures (Hengsbach and Díaz Lantada 2014a, b).

Figure 8.17 shows different designs of microtextured grids with diverse surface topographies for further computer-aided manufacture by means of 2PP (two-photon polymerization). The final biomedical device, for addressing the impact of topography on cell motility, showing the microtextured channels obtained upon an already structured microchannel framework is also shown.

For the initial stage, in which the overall structure of the microdevices is manufactured, we use SU-8 spin coated on a silicon wafer. SU-8 (MicroChem Corp.) is a commonly used epoxy-based negative photoresist. It is highly functional, optically transparent and photo imageable to near UV (365 nm) radiation. Cured films or microstructures are very resistant to solvents, acids and bases and have excellent thermal and mechanical stability. They are also important for the promotion of medical applications and studies in the field of tissue repair tissue engineering and biofabrication. For the detailed microtextures within the different channels, a resist with a much lower voxel size than that of the SU-8 is needed. In our case, the resist is also linked to the two-photon polymerization process employed. In this example the IP-Dip resist, a specially designed photoresist that guarantees ideal focusing and has the highest resolution of any NanoScribe IP-Photoresist (with feature sizes down to 150 nm and minimized shrinkage), is used.

Similar approaches could potentially be used to control the textures of several microsystems and implants, although issues including manipulation, assembly and integration strategies have to be also carefully considered, when designing multi-scale biomedical microdevices.

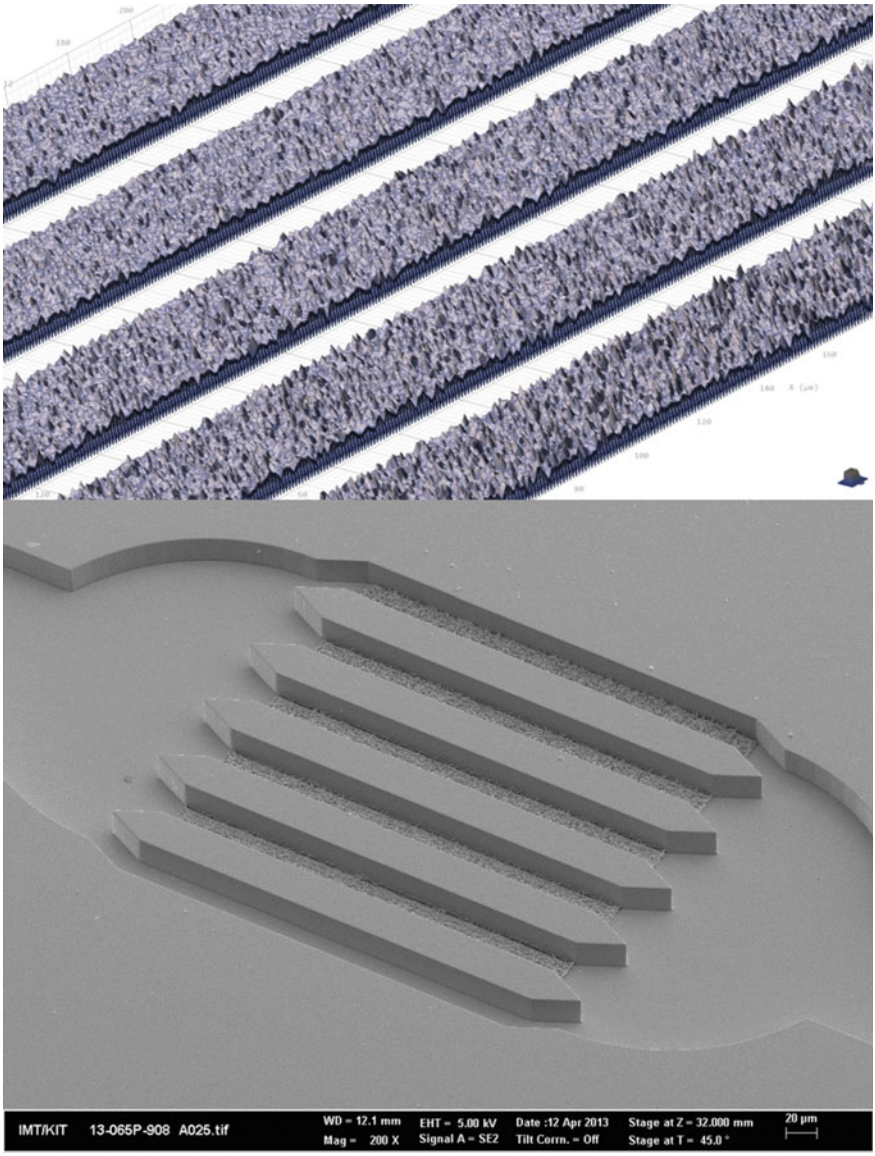

Fig. 8.17 Designs of microtextured grids with different surface topographies for further computer-aided manufacture by means of two-photon polymerization. Final biomedical microdevice for studying cell motility showing the microtextured channels obtained upon an already structured microchannel framework. Additional details: (Hengsbach and Díaz Lantada 2014b). Process developed in collaboration between Universidad Politécnica de Madrid and the Karlsruhe Institute of Technology, with support from the KNMF—Karlsruhe Nano-Micro Facility (http://www.knmf.kit.edu/)

8.6 Main Conclusions and Future Research

The applications of microsystems in the biomedical field are indeed remarkable and continuously evolving thanks to recent extraordinary progresses in the area of micromanufacturing technologies, capable of manufacturing devices with details in the typical range of 1–500 μm. As living organisms are made up with cells, whose overall dimensions typically range from 5 to 100 μm, micro-manufactured devices (with details precisely in that range) are very well-suited to interacting at a cellular level for promoting innovative diagnostic and therapeutic approaches.

This chapter has provided an overview of micromanufacturing technologies with special application to the development of micro-medical devices, as several of these manufacturing technologies are thoroughly applied along the Handbook for the development of different cases of study linked to fluidic microsystems for disease modeling, to cell culture platforms for understanding cell behavior, to labs-on-chips and organs-on-chips and to tissue engineering scaffolds and organ repair constructs.

The different technologies detailed in present chapter have been also illustrated by means of several application examples linked to the aforementioned types of biomedical microdevices aimed at interacting at a cellular level. The possibility of a combined use of technologies for the promotion of multi-scale and biomimetic approaches has been also analyzed in detail and some current research challenges has been also discussed.

Regarding future approaches, it is important to note that these technologies can be additionally complemented by the use of surface biofunctionalization nanotechnologies, which will be covered in Chap. 9. The challenge of mass-production, for the promotion of low-cost biomedical solutions, which micro-manufacturing technologies face, is covered in Chap. 10 and constitutes a key area of research for the universalization of advanced Healthcare.

Acknowledgements We gratefully acknowledge the support of the Karlsruhe Nano Micro Facility (KNMF, http://www.knmf.kit.edu/) a Helmholtz research infrastructure at the Karlsruhe Institute of Technology (KIT). Proposal KNMF-2013-010001542 (*muFractal: Microsystem for studying the influence of fractal dimension on cell behaviour*), linked to the rapid manufacture of microtextured microsystems, proposal KNMF-2013-010001541 (*NanoAUX: Additive nanomanufacture of 3D auxetic metamaterials*), linked to the nano-manufacture of auxetic metamaterials, and proposal KNMF-2012-009001145 (*Replic-AS: Replication of advanced scaffolds with biomimetric fractal features*), linked to replicating the presented multi-channelled microsystem with fractal channels, and the co-authors and their teams that made them possible are acknowledged. We acknowledge the support of the "Tomax: Tool-less manufacture of complex geometries" project, funded by the European Union Commission under grant nº: 633192 - H2020-FoF-2014-2015/H2020-FoF-2014 and led by Prof. Dr. Jürgen Stampfl from the Technical University of Vienna.

References

Anselme K, Ponche A, Bigerelle M (2010) Relative influence of surface topography and surface chemistry on ell response to bone implant materials. Part 2: biological aspects. Proc Inst Mech Eng [H]: J Eng Med 224(12):1487–1507

Barthlott W, Neinhuis C (1997) Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta 202:1–8

Bartolo PJS, Almeida H, Laoui T (2009) Rapid prototyping and manufacturing for tissue engineering scaffolds. Int J Comput Appl Technol 36:1

Borchers K, Bierwisch C, Cousteau J, Engelhard S, Graf C, Jaeger R, Klechowitz N, Kluger P, Krueger H, Meyer W, Novosel E, Refle O, Schuh C, Seiler N, Tovar G, Wegener M, Ziegler T (2012) New cytocompatible materials for additive manufacturing of bio-inspired blood vessels systems. In: International conference on biofabrication

Bückmann T, Stenger N, Kadic M, Kaschke J, Frölich A, Kennerknecht T, Eberl C, Thiel M, Wegener M (2012) Tailored 3D mechanical metamaterials made by dip-in direct-laser-writing optical lithography. Adv Mater 24:2710–2714

Buxboim A, Discher DE (2010) "Stem cells feel the difference. Nat Methods 7(9):695

Chen WL, Likhitpanichkul M, Ho A, Simmons CA (2010) Integration of statistical modeling and high-content microscopy to systematically investigate cell-substrate interactions. Biomaterials 31:2489

De la Guerra Ochoa E, Del Sordo Carrancio D, Echávarri Otero J, Chacón Tanarro E, Díaz Lantada A, Lafont Morgado P (2012) The influence of textured surfaces on the lubrication of artificial joint prostheses. In: Biodevices 2012—international conference on biomedical electronics and devices. IEEE engineering in medicine and biology society

Díaz Lantada A (2009) Metodología para el desarrollo de dispositivos médicos basados en polímeros activos como sensores y actuadores. PhD Thesis (Advisor: P. Lafont Morgado), Universidad Politécnica de Madrid

Díaz Lantada A (2012) Handbook of active materials for medical devices: advances and applications. PAN Stanford Publishing

Falconer K (2003) Fractal geometry: mathematical foundations and applications. Wiley

Fan H, Lu Y, Stump A, Reed ST, Baer T, Schunk R, Perez-Luna V, López GP, Brinker J (2000) Rapid prototyping of functional patterned nanostructures. Nature 405:56–60

Feynman RP (1992) There's plenty of room at the bottom (data storage). J Microelectromech Syst 1(1):60–66. doi:10.1109/84.128057

Feynman RP (1993) Infinitesimal machinery. J Microelectromech Syst 2(1):4–14. doi:10.1109/84.232589

Gad-el-Hak. (2003) The MEMS handbook. CRC Press, New York

Hengsbach S, Díaz Lantada A (2014a) Direct laser writing of auxetic structures: Present capabilities and challenges. Smart Mater Struct 23:085033

Hengsbach S, Díaz Lantada A (2014b) Rapid prototyping of multi-scale biomedical microdevices by combining additive manufacturing technologies. Biomed Microdevices 16(4):617–627

Hosseinkhani H, Hosseinkhani M, Tian F, Kobayashi H, Tabata Y (2007) Bone regeneration on a collagen sponge self-assembled peptide-amphiphile nanofiber hybrid scaffold. Tissue Eng 13 (1):11–19

Hosseinkhani H, Hosseinkhani M, Hattori S, Matsuoka R, Kawaguchi N (2010) Micro and nano-scale in vitro 3D culture system for cardiac stem cells. J Biomed Mater Res, Part A 94 (1):1–8

Ikuta K, Hirowatari K (1993) Real three dimensional microfabrication using stereo lithography and metal molding. In: Proceedings of the IEEE international workshop on micro electro mechanical system (MEMS 93), pp 42–47

Infür R, Pucher N, Heller C, Lichtenegger H, Liska R, Schmidt V, Kuna L, Haase A, Stampfl J (2007) Functional polymers by two-photon 3D lithography. Appl Surf Sci 254:836–840

Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920

Lorenzo-Yustos H (2008) Aplicación de nuevas tecnologías en la realización de herramientas para moldes de inyección de termoplásticos". PhD Thesis (Advisor: P. Lafont Morgado), Universidad Politécnica de Madrid

Madou MJ (2002) Fundamentals of microfabrication: the science of miniaturization, 2nd edn. CRC Press

Maluf N (2000) An introduction to micro-electromechanical systems engineering. Artech House

Mandelbrot B (1982) The fractal geometry of nature. W.H. Freeman, San Francisco

Masood SH, Singh JP, Morsi Y (2005) The design and manufacturing of porous scaffolds for tissue engineering using rapid prototyping. Int J Adv Manufact Technol 27:415–420

Mironov V, Trusk T, Kasyanov V, Little S, Swaja R, Markwald R (2009) Biofabrication: a 21st century manufacturing paradigm. Biofabrication 1(2):022001

Ostendorf A, Chichkov BN (2006) Two-photon polymerization: a new approach to micromachining. In: Photonics Spectra, October, 2006

Place ES, Evans N, Stevens M (2009) Complexity in biomaterials for tissue engineering. Nat Mater 8:457–469

Ponche A, Bigerelle M, Anselme K (2010) Relative influence of surface topography and surface chemistry on ell response to bone implant materials. Part 1: physico-chemical effects. Proc Inst Mech Eng [H]: J Eng Med 224(12):1471–1486

Queste S, Salut R, Clatot S, Rauch JY, Khan Malek CG (2010) Manufacture of microfluidic glass chips by deep plasma etching, femtosecond laser ablation, and anodic bonding. Microsyst Technol 16(8–9):1485–1493

Reljin IS, Reljin BD (2002) Fractal geometry and multifractals in analyzing and processing medical data and images. Arch Oncol 10(4):283–293

Röhrig M, Thiel M, Worgull M, Hölscher H (2012) Hierarchical structures: 3D direct laser writing of nano-microstructured hierarchical gecko-mimicking surface. Small 8(19):3009–3015

Schwentenwein M, Homa J (2015) Additive manufacture of dense alumina ceramics. Appl Ceram Technol 12(1):1–7

Shuler ML (2012) Modeling life. Ann Biomed Eng 40(7):1399–1407

Sin A, Chin KC, Jamil MF, Kostov Y, Rao G, Shuler ML (2004) The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors. Biotechnol Prog 20:338–345

Tan JY, Chua CK, Leong KF (2010) Indirect fabrication of gelatin scaffolds using rapid prototyping technology. Virtual Phys Prototyping 5(1):45

Thomas WE, Discher DE, Shastri VP (2010) Mechanical regulation of cells by materials and tissues. MRS Bull 35:578

Varadan VK, Jiang X, Varadan VV (2001) Microstereolithography and other fabrication techniques for 3D MEMS. Wiley

Wohlers T (2010) Wohlers' report: additive manufacturing state of the industry. Wohlers Associattes

Yeong WY, Chua CK, Leong KF, Chandrasekaran M (2004) Rapid prototyping in tissue engineering: challenges and potential. Trends Biotechnol 22(12):643–652

Chapter 9
Nanomanufacturing Technologies for Biomedical Microsystems Interacting at a Molecular Scale

Andrés Díaz Lantada and Jose Luis Endrino

Abstract Surface biofunctionalization techniques are essential resources for improving the biological and biochemical response of several biomedical devices and provide the opportunity of interacting with cells, even at a molecular level, by means of controlling matter in the range of nanometers. Applications of nanomanufacturing technologies, in many cases applied as post-processes, include: the improvement of biocompatibility, the promotion of wear resistance, the incorporation of special tribological (contact) phenomena linked to controlling adhesion, wettability or friction, the incorporation of anti-bacterial properties and the overall improvement of (bio)mechanical properties and aesthetics, among others. This chapter provides an overview of the more relevant nanomanufacturing technologies with special application to the development of advanced micro-medical devices with surface biofunctionalizations for optimal performance, as several of these manufacturing technologies will be applied thoroughly along the Handbook for the development of different cases of study. The different technologies detailed in present chapter are also illustrated by means of different application examples related to enhancing the biological response of different cell culture platforms and tissue engineering scaffolds aimed at interacting at a cellular level. The possibility of combining technologies for the promotion of multi-scale and biomimetic approaches is also analyzed in detail and some current research challenges are also discussed.

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory, Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

J.L. Endrino
School of Aerospace, Transport and Manufacturing, Cranfield University, Cranfield MK43 0AL, UK

A. Díaz Lantada (ed.), *Microsystems for Enhanced Control of Cell Behavior*, Studies in Mechanobiology, Tissue Engineering and Biomaterials 18,
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9.1 Overview of Nanomanufacturing Technologies

Nanotechnology may be defined as the science that studies the control of matter on an atomic and molecular scale. This means that structures of less than 100 nm, in at least in one of their dimensions, are normally used, which is also connected to the development of devices with feature at that scale level.

Inorganic, organic and biological nanostructured materials have been around in nature ever since the beginning of the evolutionary chain on Earth. This can be seen if the crystallised minerals forming the rocks, the component parts of micro-organisms or the particles in suspension in smoke or fog are examined in detail (Schwartz 2006). However, real progress in this discipline and the control of material on a nanoscale began to emerge at the beginning of the 1970s, and was closely linked to the developments in electronics and computing, which were continuously seeking circuits with ever tinier connections and sizes, so that more information could be stored in a smaller volume.

During the first decades of development, nanotechnology basically focused on the use of semi-conductor materials, particularly silicon, due to the ease and precision that could be attained when processing it with acid attacks, thanks to its peculiar crystallography. From the 1980s, however, attention began to focus on other materials, especially polymers. The intention was to obtain nanosystems that were cheaper to mass produce and that would be suited for other potential applications, particularly in Health Sciences, because of their good behaviour when in contact with human tissue.

According to previous reviews (Rocco 2006), the evolution of nanotechnology towards real industrial and social impact includes four steps: The first one, which began after 2000, involves the development of passive nanostructures. The second one, which began in 2005, focuses on active nanostructures that change their size, shape, conductivity or other properties during use. Starting around 2010, a third stage is focusing on systems of nanostructures, directing large numbers of intricate components to specified ends. The near future includes an expansion towards molecular nanosystems, conceived as heterogeneous networks in which molecules and supramolecular structures serve as distinct devices. These advances are already impacting areas such as transport, energy, health, communication, information technologies and, more specifically linked to the main topic of present Handbook: Biomedical micro-(and nano-)devices for interacting at a cellular level.

On these very tiny scales, the quantum effects between particles begin to take on considerable importance, and the typical physical, chemical and biological properties of the materials undergo a surprising alteration as their size gets smaller. This means that nanosystems and nanostructures can be obtained with very high mechanical strength, which makes amazingly fast chemical reactions possible, with extraordinary optical and chromic effects, among many other properties, and remarkable responses to external stimuli that can be managed (or controlled) by simple changes to size, shape or the relative geometric layout of the grain forming these materials.

Generally speaking, any material containing fibres, layers, particles or granules of a size less than 100 nm may be considered as a nanostructured material. If, moreover, the material is oriented to exploit some of the remarkable variations in the properties mentioned, depending on the different external stimuli, we can speak of the concept of "nanostructured active material".

Some manufacturing and processing technologies like "CVD" and "PVD" (chemical and physical vapor deposition) techniques, thin-film solution-deposition processes, self-assembly and related processes and even additive rapid manufacturing have multiple applications for the development of nanostructured materials with a growing impact in the biomedical field and, very specially, in areas linked to interacting at cellular and molecular levels.

In fact such capability of structuring materials at the nano-scale helps to improve the mechanical performance, corrosion resistance, long-term biocompatibility, and other relevant properties, of many medical devices and biomedical microsystems, which are already commercially available. This chapter provides a brief introduction to nano-manufacturing technologies and a discussion on main technologies currently being applied to promoting the performance of biomedical microdevices, especially those aimed at interacting with cells.

In most cases these nano-manufacturing technologies are based on the use of thin-film deposition processes, what involves a change of approach, from the conventional "top-down" manufacturing (either by subtractive machining processes or by chemical attacks), to a more versatile "bottom-up" or additive manufacturing approach. Actually this "bottom-up" manufacturing strategies can be even considered "biomimetic", as Nature itself also constructs its (bio)materials and (bio) structures usually using additive procedures.

It is hoped that the inclusion, in the references section, of various excellent texts and handbooks specifically devoted to nanotechnology and nanomaterials (Drexler 1986, 1991, among others) will be of use to any researchers wishing to examine these topics in greater detail than we have been able to do. Next paragraphs detail main already validated applications of these nano-manufacturing processes in the biomedical field, which are normally connected to improving the performance of passive biodevices (i.e. conventional implants) and microsystems (i.e. cell culture platforms). Even though enormous research efforts are currently focusing on the development of nano-biosensors, nano-biomarkers and nano-devices for improved drug delivery, among other innovative diagnostic and therapeutic approaches, we do not deal here with active implantable nanodevices, as most advances are currently in the conceptual validation stage.

In spite of the great potential of nanotechnology and nano-manufacturing for biomedical engineering (including enhanced cancer diagnosis, precise detection and location of infectious microorganisms, remote delivery of highly specific drugs and other research lines), we focus here on concrete already successful applications, commercially available and widely used in the manufacture of many kinds of implants and biomedical microdevices.

Nowadays, manufacturing technologies working on the nanoscale are being used, mainly as post-processes upon conventional implants and biomedical microdevices, for some of the following reasons:

- Improving mechanical performance. The use of multi-layers allows for a tailored adaptation of hardness and stiffness of implants, aiming in many cases at a more elastic bone-prosthesis contact for avoiding phenomena such as stress-shielding, by enhancing a more distributed loading. In other cases ultra-high hardness is desired, so as to limit wear ratio and improve implants' life.
- Improving corrosion resistance. The human organism is a corrosive environment and permanent implantable devices suffer its effects. The use of special coatings (i.e. titanium dioxide) limits corrosion and improves implants' life.
- Improving tribological properties. Multi-component implants include parts with relative motion and friction effects lead to progressive wear. By using specialized coatings for promoting very low friction coefficients wear ratio can be minimized, thus improving implants' life and patients' comfort.
- Enhancing biocompatibility of final biodevices. Some coatings and thin-films (i.e. diamond-like carbon, hydroxyapatite) are also used as a way of improving final device biocompatibility, either for final implantation, or for promoting in vitro or ex vivo trials.
- Obtaining biocidal and antimicrobial activity. The incorporation of some inclusions within already mentioned coatings (i.e. diamond-like carbon with Ag or Au nanoparticles) has also proven very useful for obtaining biocidal and antibacterial properties of implants.
- Enhancing aesthetics. In Odontology, implants normally require a tough metallic (titanium or titanium alloys) nucleus, as well as an outer aspect similar to that of remaining teeth, what can also be achieved by means of vapour deposition processes (i.e. zirconia).
- Including novel functionalities. Multi-layers can also promote the incorporation of some kind of active material to the surface of an implant, so as to achieve final active implantable devices, for improved diagnosis or even therapy (i.e. piezoelectric coating for enabling pressure monitoring).

Next sections deal with the most demanded nano-manufacturing technologies in the biomedical device industry, currently aimed at the deposition of thin-films for improving the aforementioned characteristics by means of nano-structured multi-layered materials. Some cases of study linked to the development of improved tissue engineering scaffolds and cell culture platforms are also included by means of example.

9.2 Physical Vapor Deposition Processes for Biomedical Microsystems

In physical vapour deposition (PVD) processes, the vapour to be deposited and condensed on the substrate to be coated is not the result of a chemical reaction, but is generated as a result of the different physical processes conducted on a solid sample of material to be deposited, without involving the chemical formation of novel species. Standard processes that might be mentioned are vapour deposition by evaporation, electron beam physical vapour deposition, plasma sputtering, cathodic arc deposition or laser ablation enhanced physical vapour deposition (Smith 1995; Albella 2006). Materials such as metal alloys, pure elements and composites like tungsten carbide, chrome nitrate or titanium nitrate are usually deposited by these PVD processes (Bunshah 1994; Glocker and Shah 2002).

In general, PVD processes lead to deposits of higher purity, than those obtained with chemical processes, since they start out from solid samples as a source of the material to be deposited and because there is no contamination from other reacting species, as is often the case in CVD techniques. Deposits can also usually be done at a lower temperature using PVD than with CVD, which is important for polymer or biological substrates. However, effects resulting from the directionality of PVD processes usually lead to somewhat less homogeneous depositions than by using CVD processes. The different processes and most suitable materials are dealt in the references mentioned at the end of the chapter and fall outside the scope of present Handbook. Using them depends on the pressures and temperatures admitted by the substrate during processing, as well as the end quality and coating thickness required, among others. However, being able to consult expert suppliers that can be subcontracted to do the coating of a particular device, is always very convenient for research teams that require a coating to enhance the properties of a biodevice, but who do not have access to the facilities and equipments required.

Most materials deposited using PVD or CVD are oriented towards obtaining very hard wear-resistant surfaces, on highly tough substrates that already show good performance towards vibrations and impacts. In other cases main objective is to minimize the friction coefficient, so as to obtain machine parts or implants with a longer useful life. Besides, these technologies can also be applied to produce active materials with optimised detection or actuation capabilities. Regarding the rapid manufacture of prototypes for the biomedical field, it is important to highlight the possibility of combining additively manufactured parts with a PVD or CVD post-processing for the promotion of a more adequate interaction of the part with surrounding cells and tissues. As several materials used in high-precision additive manufacturing technologies, i.e. acrylate-based and epoxy photopolymers, are not adequate for interaction with cells and tissues, the use of thin films can help to promote the biological response for in vitro trials. Examples of biomedical microdevices, for interacting at a cellular level, benefiting from the combined used of additive manufacturing or 3D printing resources and thin film deposition technologies have been previously published (Díaz Lantada et al. 2010; Díaz Lantada 2012).

By means of example, Fig. 9.1 shows a collection of microtextured cell culture platforms manufactured in epoxy resin by 3D laser stereolithography and biofunctionalized with a thin diamond-like carbon coating applied by cathodic arc deposition. The cell culture platforms are aimed at the assessment of the impact of

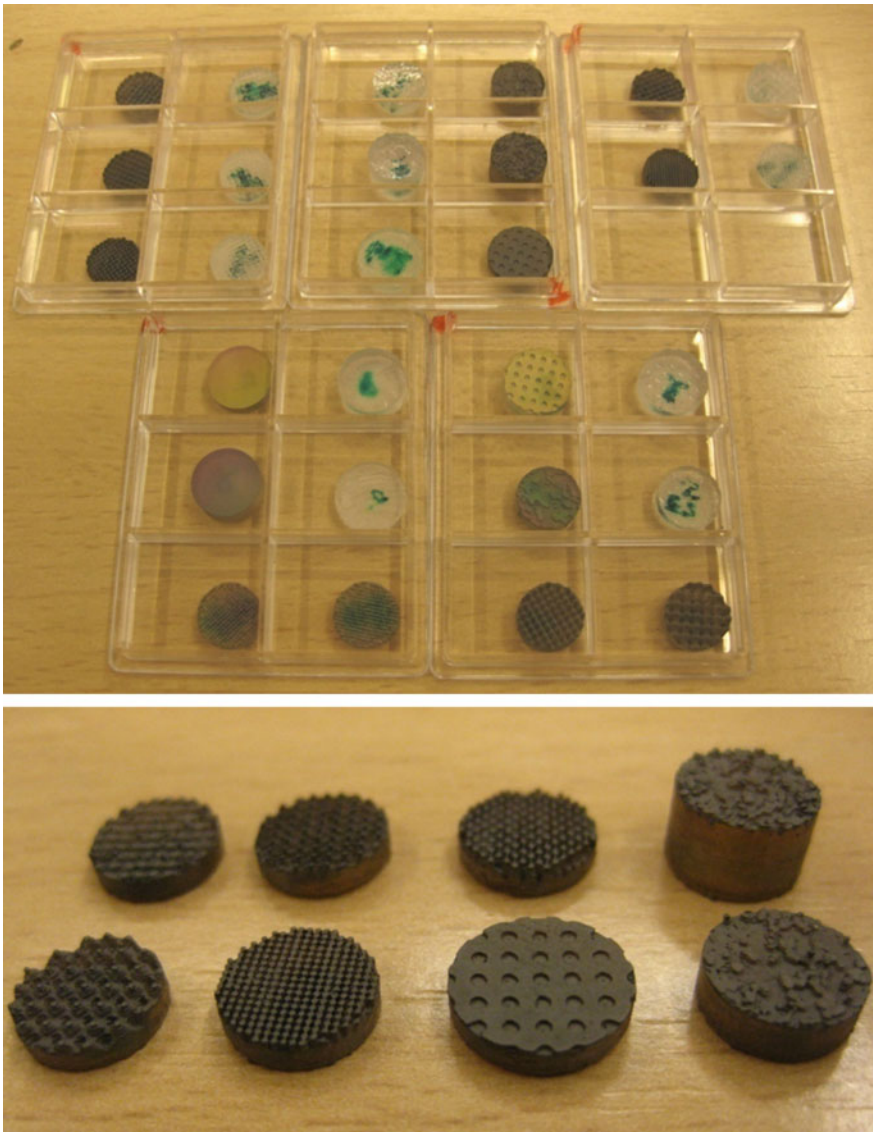

Fig. 9.1 Microtextured cell culture platforms manufactured in epoxy resin by 3D laser stereolithography and biofunctionalized with a thin diamond-like carbon coating applied by cathodic arc deposition

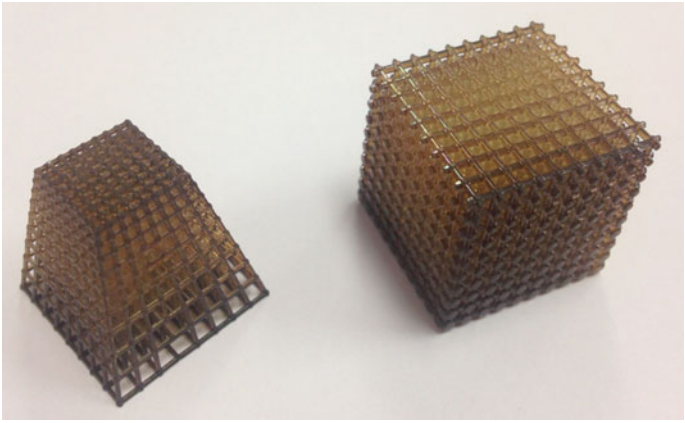

Fig. 9.2 Functionally graded "knowledge-based" tissue engineering scaffolds manufactured in epoxy resin by 3D laser stereolithography and biofunctionalized with a sputtered thin diamond-like carbon coating

microtextures on overall cell dynamics. The use of the coating allows for in vitro trials with cells, which otherwise would die if cultured upon the epoxy resin used for the additive manufacturing process.

Figure 9.2 includes an additional example of the potential of combining 3D printing and thin film deposition, linked to the development of a functionally graded "knowledge-based" tissue engineering scaffolds manufactured in epoxy resin by 3D laser stereolithography and biofunctionalized with a sputtered thin diamond-like carbon coating. It is important to mention that more complex porous and lattice geometries are harder to coat by using directional processes.

9.3 Chemical Vapor Deposition Processes for Biomedical Microsystems

A standard chemical vapour deposition process obtains a coating through the deposition on a substrate of a chemical product generated from a gaseous reaction. Other volatile by-products are usually produced in this reaction and are removed from the reaction chamber. On other occasions the chemical reaction between the gases introduced into the chamber and the substrate material is encouraged, so that the product of reaction can be then condensed on that surface (Smith 1995; Albella et al. 2003; Albella 2006).

By using these processes, thin layer deposits of micrometric thicknesses (and even nanometric) can be obtained, including details of microcrystalline, polycrystalline, amorphous and epitaxial structures. A wide range of materials is usually deposited in this way, such as silicon, fibres, nanofibres and carbon nanotubes,

silicon dioxide, tungsten carbide and various oxides and nitrates with a high surface hardness.

However, the consistency and adherence of the thin layer depends to a large extent on the compatibility of the substrate and the surface deposit. To encourage this compatibility, multilayer structures are often used in which the transition from substrate to the required final coating includes several intermediate layers for a better transition with fewer residual stresses that can cause the appearance of cracks in many thin coatings (Bunshah 1994; Glocker and Shah 2002).

Operating pressures for standard CVD equipment range from atmospheric (APCVD) to high vacuum (UHVCVD) and on occasions aerosols and plasmas are used, so as to favour the chemical reaction or to focus it on the substrate zone for a more efficient process. All this increases the complexity of the related systems whose installation and maintenance costs are usually high.

Although the CVD and PVD systems mentioned are expensive, there are many different suppliers who offer the chance to subcontract their thin-film deposition services for coating different materials, including metallic, ceramic and polymeric substrates. This can be very convenient in projects linked to the development of a novel implant or of a special material for medical applications, in case it might be interesting to compare the mechanical, chemical and biological behaviour of possible surface coatings, before choosing the most suitable substrate-coating combination for the end application.

Interesting suppliers of technology include: Oerlikon Balzers, Platit, ionbond, among others. A good association (and related website) for locating different services and information on technological supply and demand in the surface coating sector is "The Society of Vacuum Coaters" (www.svc.org) web site. There is also detailed information on teaching resources, seminars, and specific conferences and congresses, where specialised information on these tools can be found. It has free resources on vacuum generation techniques, surface and thin film characterisation and matters related to the preparation of substrate, deposition using different technologies and the major surface coating and multilayer structure applications.

9.4 Solution Deposition Processes for Biomedical Microsystems

Several surface functionalization techniques for biodevices, start from a solution or are carried out in a wet environment. These technologies stand out for their versatility, especially when trying to deposit biomaterials on a substrate, and the most relevant are detailed below.

Sol-gel. These sol-gel processes are characterized by their transition from a sol phase to a gel phase, usually by various hydrolysis and poly-condensation reactions, and are used for producing vitreous and ceramic materials.

The sol is made up of solid particles (usually around 0.1–1 μm in diameter) dispersed in a liquid, while gel comprises a solid network of macromolecules immersed in a solvent. The conventional stages of the process (Brinker and Scherer 1990; Hench 1998; Albella 2006) include:

(a) Dispersion of particles in a liquid to form the sol material comprising the initial chemical solution. Metal alkoxides are usually used as precursor particles (R–O–M, where R is a radical, O an oxygen atom and M a metal atom) and metal chlorides. Using particles of materials like SiO_2 as precursors and an organic additive, modified silica glass with multiple applications can be produced.
(b) Deposition of a thin layer of sol on the substrate to be coated, normally by centrifuging or spin-coating, immersion-extraction or "dip-coating" or by spraying. Different technologies, such as Langmuir–Blodgett deposition and other methods to prepare self-assembled monolayers are enabling even thinner thicknesses to be attained and more flexible films that can adapt to the geometries of more complex devices (see below).
(c) Polymerisation of sol particles by volatilisation of the stabilisers and the formation of the solid three-dimensional network that constitutes the gel. The alkoxides react rapidly in the presence of water (hydrolysis) to form R–OH and M–OH species molecules that can be then linked together by polycondensation to form OR–M–O–M–OR species three-dimensional networks with M–O–M bonds and remnants of H_2O and R–OH. The vaporisation of these H_2O and R–OH sub-products results in the required gel.
(d) Final thermal processing to obtain an amorphous or crystalline coating that is stable over time.

Of the different addition stages that can be achieved by sol-gel processes, depending on processing conditions and the end properties sought, we can cite the following:

- Xerogel. By gelation of the sol and conventional drying of the gel.
- Aerogel. By gelation of the sol and super-critical drying of the gel. Very porous solids are thus produced with an extremely high volume/mass ratio and are very useful for packaging.
- Dense ceramic. By sintering the dust from a milled xerogel or aerogel.
- Thin compact layer. By deposition of a thin layer of the sol on a substrate and subsequent polymerisation and drying.
- Fibres. By stretching the sol, followed by polymerisation and drying.

It should be mentioned that supercritical drying to obtain aerogels causes the fluid to reach supercritical conditions and a subsequent quasi-isothermal depressurisation is produced, thereby preventing the effects of contraction. It also prevents the effects of the surface tension produced on the surrounding solid structures by the appearance of liquid, which endows this supercritical process with numerous applications for the manufacture of MEMS (micro-electromechanical systems) and NEMS (nano-electromechanical systems).

Some of the many advantages of sol-gel processes for thin layer deposition are the wide ranges of attainable thicknesses (from tens of nm to several mm), the excellent adhesion between the substrate and the coating, low operating temperatures and the economical process. From a research point of view, what is exceptional is the ability to widely vary the end properties by making simple changes to parameters like initial concentration, precursor size, working temperature and initial sol viscosity.

Figure 9.3 shows, by means of example, a functionally graded "knowledge-based" tissue engineering scaffolds manufactured in epoxy resin by 3D laser stereolithography and biofunctionalized with a thin TiO_2 coating obtained by means of sol-gel deposition.

Spin coating. This process is used to deposit dissolved polymer thin-films (using solvents like chloroform or trichloroethylene) or even liquid state monomer layers, in order to then activate the polymerisation reaction using heat or UV light, for instance by using mass-less UV lithography. It can also be used to complement deposition in the sol phase of sol-gel processes as different substrates can be used.

If the process is repeatedly performed, homogeneous multilayers of different materials can be obtained. The process consists in supplying a liquid resin or dissolved polymer drop-by-drop on to the substrate that will be centrifuged. The centrifuge or spinner is then switched on and the material spreads over the substrate to form the film and is then left to dry.

It is sometimes also subjected to heating at different temperatures to evaporate the volatile elements and cure the film (soft bake and hard bake processes). The greater the viscosity of the liquid or resin supplied to the spinner, the greater the thicknesses attained. High rotation speeds lead to thinner layers. The process can be

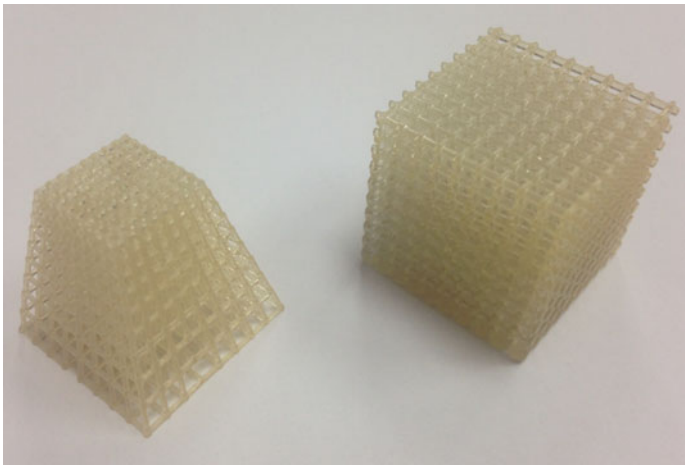

Fig. 9.3 Functionally graded "knowledge-based" tissue engineering scaffolds manufactured in epoxy resin by 3D laser stereolithography and biofunctionalized with a thin TiO_2 coating obtained by means of sol-gel deposition

used to obtain varied samples with very different properties in very short times, which for research is major added value. In the biomedical field simple planar geometries can benefit from this approach, such as "lab-on-a-chip" devices and scaffolds for tissue engineering.

Dip coating. The process consists in immersing a film vertically into a tank containing the polymer to be deposited in solution form, as liquid state monomers or in sol phase with dispersed particles. The film is then extracted at constant speed to achieve constant thickness (Newtonian fluid) and is left to dry. At the end, thermal processing can be applied to finally cure the polymer. Greater viscosities and higher extraction speeds (due to the short time the fluid has to become arranged and compact), give rise to thicker layers. It is mainly used to deposit dissolved polymer film or liquid state monomer layers to then activate the polymerisation reaction through heat or UV light, as is the case with spin-coating. The immersion or dip-coating process can also be used for sol-phase deposition in a sol-gel process and various metallic, ceramic or polymeric substrates can be used.

Electrolytic/electrochemical deposition. The fundamental principle of electrolytic or electrochemical deposition processes for obtaining coatings consists in converting the metal of the anode into metallic ions that are distributed in the solution. These ions are deposited on the cathode (device, part or substrate to be coated) forming a metal layer on its surface. The different coating properties, as well as the most suitable fields of application, depend on the type of crystalline structure of the metal deposited. The electrolytic coating of the parts is produced almost entirely through immersion in a bath. For this purpose the parts are immersed in the tanks of electrolyte, current is applied to a cathode and they are then coated and dried. When the parts are removed from the bath, part of the electrolyte becomes attached to the surface of the parts. This surface film is then eliminated by a washing process so that it will not interfere with any subsequent operations and will have the required finish.

Deposition using Langmuir-Blodgett technology. This is a very exact deposition technology (performed by the superposition of monolayers) using different organic coatings, such as fatty acids, phopholipids and polymers with long lateral hydrophobic chains. Nanostrucures can be produced with molecular alignment and controllable roughness and thickness and are widely used for producing biosensors. Their high precision means that the materials obtained can either be microstructured or nanostructured.

It can also be used for surface micro-engraving that can be combined with other CVD or PVD technologies to produce electrodes with special geometries or patterned biodevices (i.e. for cell motility studies).

New research proposes using them to produce multilayers with ferroelectric polymers for non-volatile RAM memories and mass storage devices. By means of this technology extremely thin layers of ferroelectric polymer have been deposited, PVDF as well as various copolymers, attaining thicknesses of 10 Å with properties similar to those of thicker multilayers but showing additional phase changes, what also promotes the development of self-sensing biodevices.

Functionalizations with extra cellular matrix components, growth factors and stem cells—conditioned media. Increasing data show that progenitor cell-niche formation is absolutely needed for tissue development and repair (Chan et al. 2009). Indeed, the niche composition and 3-D structure play an important role in stem cells state and fate. The niche is created by the specific combination of trophic factors produced by progenitor cells to maintain the capability for tissue repair and regeneration and by a specific extracellular matrix. Recent studies have helped to highlight the extreme relevance of the incorporation of adequate growth factors, within the scaffold, for promoting biological regulation, cell differentiation, angiogenesis and final tissue viability (Richardson et al. 2001; Perets et al. 2003; Laschke et al. 2008).

Such inclusion of biochemical effects, derived from the incorporation of growth factors, adds additional uncertainties to the already complex to understand interactions between scaffolds' structure, morphology and (bio)mechanical properties. In consequence, studies addressing the synergies between ECMs and growth factors and their impact on tissue viability are needed, in the quest for a general methodology for tissue engineering scaffold development.

The combined use of extra cellular matrix (ECM) and trophic factor (TFs) components, both shortened to TFs medium, obtained previously from the hMSCs culture supernatant, as coatings for cell culture platforms and tissue engineering scaffolds promote overall biocompatibility of the biodevices and enhance cell adhesion and motility, while also promoting differentiation into desired tissues (Díaz Lantada et al. 2014). These hMSCs-CM (conditioned media), if adequately applied to the scaffolds and cell culture platforms, can be the key for the development of 4D approaches.

Such next generation 4D scaffolds will not only promote adequate three-dimensional cell adhesion and proliferation, but will take into account that the process of musculoskeletal tissue regeneration needs the expression of different phenotypes in a dynamic environment, which must adapt to the requirements of the cells along the regeneration process and include dynamic response to disease.

The release of different growth or trophic factors at different rates, by using multi-coatings or by encapsulating some media within biodegradable polymers, can help to the aforementioned dynamic expression of phenotypes and optimize the therapeutic response of these types of microsystems.

9.5 Self-assembly and Related Processes for Biomedical Microsystems

Ionic self-assembled monolayer techniques. Developed by Decher and Hong (1991) and Decher et al. (1992) for polyelectrolytes and then extended to the production of multilayer structures with many electroactive polymers, fullerenes and other materials. Layers can be deposited on a substrate by consecutively alternating the adsorption of cationic and ionic species.

It is a relatively cheap and simple technology for controlling the molecular structure of materials and influencing their macroscopic properties. It has recently been used to obtain multilayer structures by bonding very different materials like polyelectrolytes, metal colloids, biological molecules, conductive polymers and light emitting polymers. The process begins by taking a clean substrate with a negative surface charge. This material is submerged in a solution with polymer molecules dissolved in it that have functional groups bonded to a polymer chain with a net positive charge. These molecules are attracted to the surface of the substrate, which is left coated with a layer that is neutral as a whole, but with a positive charge on its upper surface. When this cationic layer has been deposited, the external charge then incites the deposit of another anionic layer, and in this way a multilayer structure can be produced.

By adding appropriate functional groups to the substrate or using surface patterns, deposition can be encouraged in certain zones. By adjusting immersion times, solute concentration and dissolution temperature, 3D structures can be obtained by depositing material layers of controlled thickness that generally have more stable properties than those obtained by Langmuir-Blodgett technology (Madou 2002). It can then be subjected to chemical attack to eliminate zones of unwanted deposits.

This technology together with the production of multilayer deposits with electroactive polymers has succeeded in optimising the performance of light emitting diodes (LEDs), as well as enhancing the stability of luminescent organic pigments compared to films obtained by spin-coating (Bar-Cohen 2004). Medical applications based on active material substrates are also promising, as this process can also be used to functionalize substrates for biosensors and for improving the biocompatibility of active implantable devices (Saliterman 2006). In fact, ionic self-assembled monolayered techniques somehow resemble some processes carried out by Nature itself to produce its (bio)materials and (bio)structures. Self-assembly processes, by directing the deposition of molecules through charge-based mechanisms are common. Further research in the field and the use of biomimetic design principles will surely lead to more and more precise additive manufacturing machines for even constructing molecule-by-molecule or atom-by-atom.

Laser ablation. Laser ablation consists in eliminating the surface material of a substrate, usually solid, using a laser beam to produce evaporation, sublimation or to convert the zone exposed to the beam into a plasma. The process is performed by laser pulses (that last from milliseconds to femtoseconds), which means the elimination of material is so precisely focused that the rest of the substrate remains practically unaltered. It is therefore an extremely suitable technology for changing the surface of materials that cannot be subjected to high temperature processes (generally polymers and organic matter), as the heat-affected zone "HAZ" in conventionally extremely reduced. This process has also been used to produce carbon nanotubes and as a support for PVD processes in which a laser acts on the substance to be deposited and the plasma generated is projected on to the substrate to be coated (Phipps 2006).

Ion implantation. The process consists in coating a substrate with the ions of another material, in order to change the physical properties of the substrate. This

has numerous applications in the electronics industry for manufacturing semi-conductive devices but also in the biomedical field. For this process an ion source is required, together with an accelerator to project the ions into a chamber, where the substrate to be implanted has previously been placed (Rimini 1995).

With regard to active materials for medical devices, ion implantation is usually used to make the surface of different polymers become locally conductive. This avoids the deposition of electrodes that cover the whole surface of the substrate, as this is usually accompanied by an unwanted stiffening effect, which limits the capacity for deformation and the activation capabilities of microstructured polymer actuators (Díaz Lantada et al. 2012). This technology is an alternative to mask or mask-less photolithography techniques when micromanufacturing electrodes with complex patterns or geometric shapes.

Many additional nano-manufacturing technologies can be used or adapted to the performance enhancement of biodevices, although we believe to have provided here an overview of the most relevant ones. Additional combinations with 3D solid freeform fabrication approaches, by using the most precise additive manufacturing technologies currently available (3D laser writing based on one-photon or two-photon lithography), can be very useful for improved solutions in many areas, such as tissue engineering and micro-/nano-implants.

9.6 Main Conclusions and Future Research

Surface biofunctionalization techniques are essential resources for improving the biological and biochemical response of several biomedical devices and provide the opportunity of interacting with cells, even at a molecular level, by means of controlling matter in the range of nanometers.

Applications of nanomanufacturing technologies, in many cases applied as post-processes, include: the improvement of biocompatibility, the promotion of wear resistance, the incorporation of special tribological (contact) phenomena linked to controlling adhesion, wettability or friction, the incorporation of anti-bacterial properties and the overall improvement of (bio)mechanical properties and aesthetics, among others.

This chapter has provided an overview of some relevant nanomanufacturing technologies with particular application to the development of advanced micro-medical devices with surface biofunctionalizations for optimal performance, as several of these manufacturing technologies will be applied thoroughly along the Handbook for the development of different cases of study.

The different nanotechnologies detailed in present chapter have been also illustrated by means of application examples related to enhancing the biological or biomechanical response of different cell culture platforms and tissue engineering scaffolds aimed at interacting at a cellular level. The possibility of combining technologies for the promotion of multi-scale and biomimetic approaches has been

also analyzed in detail and some current relevant research challenges have been also discussed.

Some interesting related websites:

- http://e.drexler.com
- http://www.efds.org
- http://www.ceramed.pt
- http://www.nanoscribe.de
- http://www.springer.com/engineering/biomedical+engineering/journal/10544
- http://www.springer.com/materials/special+types/journal/10971
- http://www.springer.com/engineering/circuits+%26+systems/journal/12668
- http://www.svc.org

References

Albella JM (2006) Preparación y caracterización de recubrimientos y láminas delgadas. Programa de Doctorado en Ciencias Físicas de la Universidad Autónoma de Madrid – Instituto de Ciencia de los Materiales de Madrid (CSIC), Material Docente. Madrid

Albella JM, Gómez-Aleixandre C, Ojeda F, Martí FJ (2003) Síntesis de materiales cerámicos mediante técnicas químicas en fase de vapor (CVD). Boletín de la Sociedad Española de Cerámica y Vídrio 42(1):27–31

Bar-Cohen Y (2004) Electroactive polymer (EAP) actuators as artificial muscles: reality, potential and challenges, 2nd edn. SPIE Press, Washington DC

Brinker CJ, Scherer GW (1990) Sol-gel science: the physics and chemistry of sol-gel processing, 1st edn. Academic Press

Bunshah RF (1994) Handbook of deposition technologies for films and coatings: science, technology and applications, 2nd edn. Materials science and process technology series. Noyes Publications, Park Ridge

Chan CKF, Chen CC, Luppen CA, Kraft DL, Kim JB, De Boer A, Wei K, Helms JA, Kuo CJ, Weissman IL (2009) Endochondral ossification is required for hematopoietic stem cell niche formation. Nature 457(7228):490

Decher G, Hong JD (1991) Buildup of ultrathin multilayer films by a self-assembly process. II: Consecutive adsorption of anionic and cationic bipolar amphiphiles and polyelectrolytes on charged surfaces. Int J Phys Chem 95(11):1430–1434

Decher G, Hong JD, Schmitt J (1992) Buildup of ultrathin multilayer films by a self-assembly process. III: Consecutively alternating adsorption of anionic and cationic polyelectrolytes on charged surfaces. In: Thin solid films, international conference on Langmuir-Blodgett films

Díaz Lantada A, Endrino JL, Mosquera AA, Lafont P (2010) Design and rapid prototyping of DLC coated fractal surfaces for tissue engineering applications. J Phys: Conf Ser 252 (1):012003

Díaz Lantada A, Endrino JL, Sánchez Vaquero V, Mosquera AA, Lafont Morgado P, García-Ruíz JP (2012) Tissue engineering using novel rapid prototyped diamond-like carbon coated scaffolds. Plasma Process Polym (4 Oct. 2011) Online. doi:10.1002/ppap.201100094 (9(1): 98–107, 2012)

Díaz Lantada A (2012) Handbook on active materials for medical devices: advances and applications. PAN Stanford Publishing

Díaz Lantada A, Alarcón Iniesta H, Pareja Sánchez B, García-Ruíz JP (2014) Free-form rapid-prototyped PDMS scaffolds incorporating growth factors promote chondrogenesis. Adv Mater Sci Eng 2014, ID 612976

Drexler E (1990) Engines of creation: the coming era of nanotechnology (Anchor Books 1986). Oxford University Press
Drexler E (1991) Molecular machinery and manufacture with applications to computation. PhD thesis, MIT
Glocker DA, Shah SI (2002) Handbook of thin film process technology. Institute of Physics Pub., Bristol
Hench LL (1998) Sol-gel silica: properties, processing and technology transfer. William Andrew
Laschke MW, Rücker M, Jensen G, Carvalho C, Mülhaupt R, Gellrich NC, Menger MD (2008) Incorporation of growth factor containing Matrigel promotes vascularization of porous PLGA scaffolds. J Biomed Mater Res A 85(2):397
Madou M (2002) Fundamentals of microfabrication: the science of miniaturization. CRC Press, pp 62–63
Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld V, Cohen S (2003) Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J Biomed Mater Res A 65(4):489
Phips CR (2006) Laser ablation and its applications. Springer series in optical sciences, 1st edn
Richardson TP, Peters MC, Ennett AB, Mooney DJ (2001) Polymeric system for dual growth factor delivery. Nat Biotechnol 19:1029
Rimini E (1995) Ion implantation: basics to device fabrication. Kluwer Academic Publishers, Massachusetts
Rocco MC (2006) The four evolutionary stages of nanotechnology. Scientific American
Saliterman S (2006) Self-assembled monolayers (SAMs). Fundamentals of BioMEMS and medical microdevices. SPIE Press, pp 94–96
Schwartz M (2006) New materials, processes and methods technology. CRC Press, Taylor and Francis Group, Florida
Smith D (1995) Thin-film deposition: principles and practice. Mc Graw-Hill

Chapter 10
Issues Linked to the Mass-Production of Biomedical Microsystems

Andrés Díaz Lantada

Abstract The rapid prototyping of biomedical microsystems, which is usually based on additive manufacturing processes, is very well suited for single prototypes with complex geometries, but in many cases inadequate for mass production, due to the excessive cost and time involved, in comparison with replication technologies, such as injection molding and compression molding. In addition, the polymers used in the most precise additive manufacturing technologies, which are based on photo-polymerization processes, are typically toxic or inadequate for biomedical applications, what limits enormously the span of final applications. Exploring cooperative strategies, for taking advantage of the complexity of geometries attainable via rapid prototyping, while also benefiting from the possibility of manufacturing large low-cost series using mass replication techniques, is a relevant industrial need and can be a source of novel procedures for supporting research and innovation in several fields. The issue is of special relevance in biomedical applications, as mass production enables the democratization of Healthcare and helps researchers to carry out more systematic studies for addressing the problems of disease and for finding improved therapeutic solutions. This chapter provides an introduction to the more relevant mass-production technologies with application in the field of biomedical microdevices. Illustrative examples linked to the complete development and mass production of different cell culture platforms and biodevices for studying cell behavior are provided to further analyze the advantages and potentials of using this kind of manufacturing procedures. Main current research trends, linked to the progressive convergence between subtractive and additive manufacturing approaches and to the combined use of technologies for the promotion of multi-scale and biomimetic approaches, are also discussed.

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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10.1 The Relevance of Mass-Production

Revolutionary preventive, diagnostic and therapeutic approaches to global health concerns, in may cases based on the use of medical microdevices capable of interacting at a cellular level, cannot be a privilege and should be made available to all those needing the support of world-class medical technology. In the words of Mahatma K. Gandhi: "I hate privilege and monopoly. Whatever cannot be shared with the masses is taboo to me". Diseases and pathologies affecting millions of persons can only be globally and democratically handled, if the advantages of mass-production technologies are employed and if its potentials are achieved by further researching towards solving current challenges.

The success of mass-production technologies in the biomedical field is based on their capability of manufacturing very large series of effective microdevices in a rapid, cheap, sustainable, efficient and repetitive way. The fulfillment of such requirements is dependent on the collaborative work of experts in the biomedical and in the technological fields, especially as concerns the incorporation of design and manufacturing advisors in all kind of projects linked to the development of new biomedical microdevices. Finding novel synergies between existing mass-production resources and recently developed high-precision micro- and nano-manufacturing technologies, for enabling new ways of achieving the manufacture of millions of units of biodevices, is a key for the future of this industry.

The rapid prototyping of biomedical microsystems, which is usually based on additive manufacturing processes, is very well suited for single prototypes with complex geometries, but in many cases inadequate for mass production, due to the excessive cost and time involved, in comparison with replication technologies, such as injection molding and compression molding. In addition, the polymers used in the most precise additive manufacturing technologies, which are based on photo-polymerization processes, are typically toxic or inadequate for biomedical applications, what limits enormously the span of final applications.

Exploring cooperative strategies, for taking advantage of the complexity of geometries attainable via rapid prototyping, while also benefiting from the possibility of manufacturing large low-cost series using mass replication techniques, is a relevant industrial need and can be a source of novel procedures for supporting research and innovation in several fields. The issue is of special relevance in biomedical applications, as mass production technologies, as previously detailed, enables the democratization of Healthcare and helps researchers to carry out more systematic studies for addressing the problems of disease and for finding improved therapeutic solutions.

The following sections try to provide an introduction to the more relevant mass-production technologies with application in the field of biomedical microdevices. Illustrative examples linked to the complete development and mass production of different cell culture platforms and biodevices for studying cell behavior are provided to further analyze the advantages and potentials of using this kind of manufacturing procedures.

Main current research trends, linked to the progressive convergence between subtractive and additive manufacturing approaches and to the combined use of technologies for the promotion of multi-scale and biomimetic approaches, are also discussed and complement the technologies described in Chaps. 8 and 9.

10.2 Conventional Technologies for the Mass-Production of Biomedical Microsystems

Previous chapters have provided several examples of the advantages of using additive manufacturing technologies, either alone or combined with other post-processes, for the rapid development of biomedical microdevices capable of interacting at a cellular and even molecular level.

However, it is important to note that rapid prototyping (RP), based on additive manufacturing processes, is typically very well suited for single prototypes with complex geometries, but normally inadequate for mass production, due to the excessive cost and time involved, in comparison with replication technologies, such as injection molding and compression molding. In addition, the polymers used in the most precise rapid prototyping technologies, which are based on photo-polymerization processes, are typically toxic or inadequate for biomedical applications, what limits enormously the span of final applications. For instance, common thermoplastics used for the mass production of medical devices, including poly(methyl methacrylate) (PMMA) or polycarbonate (PC), cannot be processed using conventional additive manufacturing technologies.

Recent research has achieved groundbreaking improvements in the bio-compatibility of rapid prototyping materials (Baudis et al. 2009, 2010) and dramatically helped to increase the manufacture speed and the attainable precision of these technologies (Stampfl et al. 2008). Nevertheless, for efficient and economic mass production of polymeric microdevices, especially for the biomedical industry, mass replication technologies still have no rival. Other moldable thermoplastics can be of interest for further specific applications in mechanical engineering, aeronautics, electronics… taking advantage of engineering polymers with enhanced thermal, electrical or mechanical behaviors, which cannot be found among the typical properties of RP polymers.

Exploring cooperative strategies, for taking advantage of the complexity of geometries attainable via rapid prototyping, while also benefiting from the possibility of manufacturing large low-cost series using mass replication techniques, is a relevant industrial need and can be a source of novel procedures for supporting research and innovation in several fields.

Among mass production technologies, micro-injection molding provides a high efficiency concerning the replication of micro- or even nano-sized structures. Description of the so-called micro injection molding process and its advantages can be found in previous references (Piotter et al. 2008, 2010; Piotter and Prokop 2014),

which highlight the possibilities of obtaining multi-component and multi-material microsystems.

The use of micro-injection molding is important; not only for mass production, but also as a support to the development process of novel biodevices, when carrying out systematic in vitro and even in vivo trials may constitute the key to validating a novel diagnostic or therapeutic approach. Additive manufacturing technologies are also providing solutions for the testing of novel biodevices and may complement or substitute micro-injection molding in some cases, as discussed in Sect. 10.4.

The interesting work of Bissacco and colleagues (2014) describes different sequential processes, depending on the number of parts needed, for obtaining microinjection molding and hot-embossing tools. Typically such procedures include combinations of photolithography, etching, laser ablation, high-precision milling or EDM-milling upon soft surfaces, and subsequent electroforming or electrodeposition processes (by chemical or physical vapor deposition or electroplatting) for obtaining the mold insert.

Regarding precision, probably the most precise approach towards fabrication of microinjection molding tools is the LIGA process, whose high aspect ratio is also noteworthy (real 3D parts can be obtained, while processes based on surface micromachining by chemical etching typically lead to 2D½ features), but its use is limited due to the expensive hard X-ray radiation needed during the process (Gad-el-Hak 2003).

10.3 Micro-Injection Molding for the Mass-Production of Biomedical Microsystems

Recent collaboration between Universidad Politécnica de Madrid (TU Madrid) and the Karlsruhe Institute of Technology, has led to an original alternative procedure, for connecting rapid prototyping with micro injection molding, for the mass production of biomedical microdevices.

Two different microtextured microsystems linked to tissue engineering tasks (a textured cell culture platform and a textured microdevice for studying cell motility), using in this case different thermoplastics (PMMA and PC) as end materials, have been obtained and have helped to check the approach by using complex and multi-scale geometries.

Our preliminary in vitro trials with both microsystems were carried upon rapid prototypes adequately coated with diamond-like carbon, to avoid the toxic effects of the acrylic resin, and upon some rapid copies obtained using PDMS casting, as previously detailed (Díaz Lantada et al. 2011), and showed promising results regarding the beneficial effects of textures on cell culture. Some details regarding these key aspects are also provided in Sect. 13.4.

However, for additional systematic evaluations, taking into account several parameters of influence, the number of prototypes required increases dramatically

and the mentioned rapid prototyping processes, together with the post-processes needed for improving their biological interactions, result inefficient and alternative procedures are required.

In short, the procedure starts from additively manufactured rapid prototypes, continues with a thin-film (PVD) deposition technique for improving their surface conductivity, follows with an electroplating process for obtaining mold inserts and ends up with mold adjustment and with the mass production using micro injection molding. The proposed process stands out for the attainable degree of detail, for the versatility of final materials, for the manufacturing speed and for the possibility of obtaining final low-cost replicas (Díaz Lantada et al. 2015). The computer-aided design procedure followed for the incorporation of microtextures, aimed at controlling cell behaviour upon the different microsystems presented, has been described in Sect. 6.5 of present Handbook.

Figure 10.1 schematically shows the complete development process of these biomedical microsystems for studying the effect of microtextures on (stem) cell adhesion, motility, overall behaviour and fate. The images included show the steps of the procedure, from the CAD files, through the master prototypes, in this case manufactured in an additive way in toxic polymers, to the final replicas, obtained in PMMA by microinjection molding. Repeatability is outstanding and final parts are compact, without some typical injection molding problems such as the presence of pores or wrapping, in spite of the precise dimensions of interest. The accuracy is remarkable and even micro-metric details, such as the presence of succinct longitudinal lines consequence of the initial additive process and of the separation between layers in the original acrylic prototypes/masters, can be perfectly replicated. The replicas obtained present several advantages, when compared with the original acrylic rapid prototypes. They are made of bioinert polymers typically used in the medical industry (polycarbonate and poly(methyl methacrylate)), hence adequate for in vitro trials; they are transparent, what constitutes an enormous help for cell culture processes and related fluorescent microscopy tasks; and their manipulation is easier thanks to the presence of a supporting structure.

It is true that additive manufacturing technologies are solving the challenges derived from the toxicity of the materials traditionally structured using a solid freeform fabrication approach. These advances are not only based on bio-photo-polymers (Baudis et al. 2009, 2010), but also on the additive manufacture of metals and alloys by electron-beam melting or selective laser sintering/melting, and on the use of lithograpy-based additive manufacture of advanced ceramics (Schwentenwein and Homa 2015). However it is also true that the massive production of large series of biomedical microsystems cannot yet be obtained on the basis of current additive manufacturing resources.

Some advances and possibilities linked to the mass-production of medium-sized series (typically up to 1000–5000 parts) are discussed in Sects. 10.4 and 10.5. In addition, the potential of additive manufacturing for the mass production of biomedical microsystems is a very hot research topic, as can be perceived from the several calls from EU Horizon 2020 for funding, in 2016 and 2017, specific projects

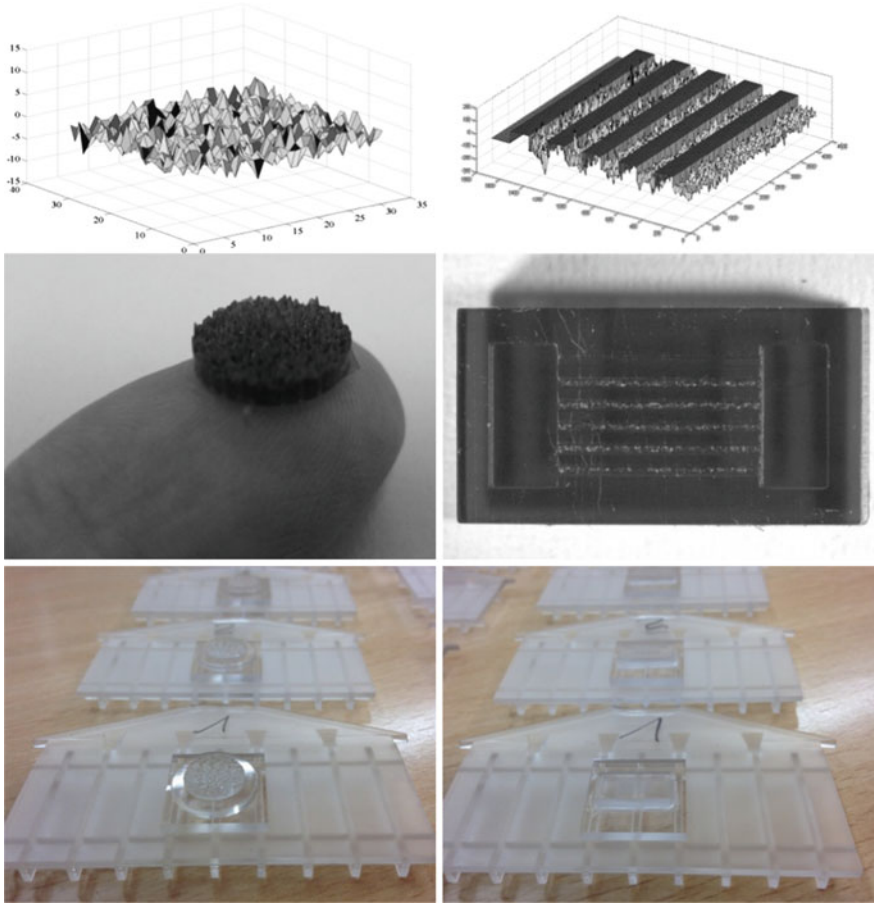

Fig. 10.1 Complete development process of biomedical microsystems for studying the effect of microtextures on cell behaviour. From the CAD files, through the master prototypes, manufactured in an additive way in toxic polymers, to the final replicas, obtained in PMMA by microinjection molding. Process developed in collaboration between Universidad Politécnica de Madrid and the Karlsruhe Institute of Technology, with support from the KNMF—Karlsruhe Nano-Micro Facility (http://www.knmf.kit.edu/) (Díaz Lantada et al. 2015)

linked to combining subtractive and additive resources and to the implementation of pilot lines for the mass production of polymeric and ceramic microdevices.

The proposed process stands out for the attainable degree of detail, for the very adequate biointeraction and versatility of final materials, for the manufacturing speed and for the possibility of obtaining final low-cost replicas of textured microsystems, which are quite complex to manufacture using conventional micromachining technologies.

The additive manufacturing process supplies geometrical complexity and high initial precision, while the micro injection molding allows for the rapid and

low-cost production of larger series of accurate replicas and provides the possibility of using several types of thermoplastics for a wider set of applications.

In the examples presented we have focused on biomedical microsystems and the PMMA and PC used are adequate for further in vitro trials. Other moldable thermoplastics can be of interest for other specific applications in mechanical engineering, aeronautics, electronics… taking advantage of engineering polymers with enhanced thermal, electrical or mechanical behaviors, which cannot be found among the typical properties of rapid prototyping polymers.

Regarding future studies we think it will be important to focus on exploring in depth the possible applications of design-controlled textured surfaces and related mass-produced devices. We foresee relevant implications for areas including: tribology, due to the potential promotion of adhesion using fractal textures; microfluidics, due to the possibility of controlling the hydrophobicity and hydrophilicity of surfaces by acting on their topography; optics, due to the option of changing surface reflection properties and overall aesthetic, and biomedical engineering, for the promotion of biomimetic designs.

The described process is also applied towards the end of the Handbook, for the mass-production of a "liver-on-a-chip" microsystem, as further detailed in Chap. 22, linked to "Strategies towards reliable organs-on-chips and humans-on-chips".

10.4 Mass-Production of Additively Manufactured Biomedical Microsystems

In some cases, additive manufacturing technologies, normally focused on the manufacture of rapid prototypes for design validation, can be also used for obtaining final parts and, if the adequate machine is used and part size is limited to some mm^3, even for mass production. They clearly constitute an alternative approach, for more directly linking the designed geometries with physical prototypes and even with production series.

The approach is typically based on the combination of computer-aided design (CAD) resources, rapid prototyping (RP) by means of additive manufacturing (AMT) procedures and rapid tooling processes for the development of low-cost tools for obtaining short and medium series for trials.

In this approach micro-manufacture can be accomplished, directly from the computer-aided design files with the three dimensional geometries of the different geometries of the biomedical microsystems under development, by means of laser stereolithography (SLA-3500 machine by 3D Systems). Such technology is able to construct vey complex and precise biodevices, working on a layer-by-layer (LMT) approach, and currently provides one of the best compromises between part size, manufacturing precision and productivity among all 3D printing resources.

It has been previously highlighted as a key resource for microfluidic systems, due to the possibility of manufacturing labs-on-chips with complex inner channels in a one-step process (Waldbaur et al. 2011).

Rapid prototypes obtained by laser stereolithography are usually employed for aesthetic and geometrical validations, for marketing purposes, for second stage processes towards manufacturing tools (normally "soft" PDMS molds) and as molds for the manufacture of final parts by subsequent casting. However their use for limited functional trials is also noteworthy.

The potential of laser stereolithography for the mass-production of these types of devices can be also perceived by taking a closer look at the upper image from Fig. 10.2. As can be seen, 40 cell culture platforms for in vitro studies have been manufactured using just a small portion of the building platform and using a process lasting for less than 1 h. With an adequate distribution of parts upon the building platform, more than 500 parts/h can be obtained using monolithic designs, which avoid the performance of joining processes and simplify mass-production. For sure, these resources may challenge the position of injection molding as reference production technology for biomedical microsystems in the near future.

The technology is also especially well suited for comparing alternative designs, as the manufacture of completely different geometries in a single building platform

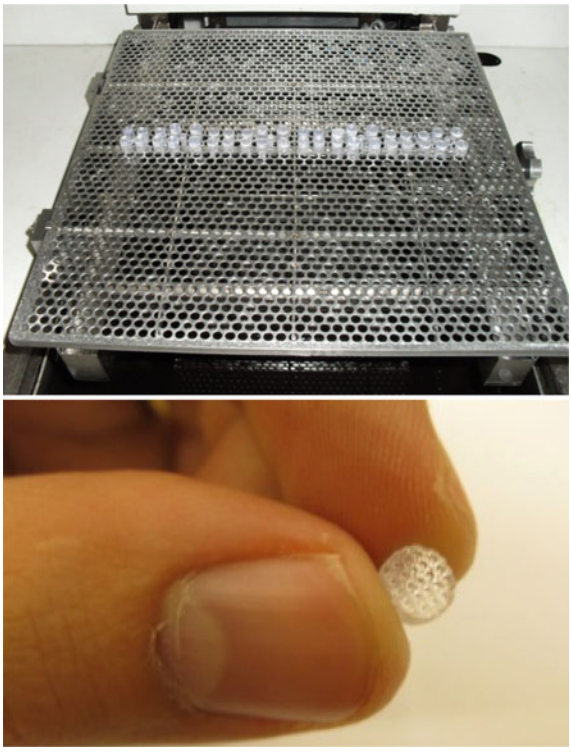

Fig. 10.2 Microtextured cell culture platforms obtained by means of laser stereolithography and detailed view of one of the designs manufactured. Forty platforms with a total of 10 different designs have been obtained in less than an hour. As can be seen from the dimensions of the vat, a production of almost 500 parts/h can be achieved

requires no additional tools or ad hoc designed work-benches, which in most cases impact final part cost is a relevant way.

Figure 10.2 shows also the detail of one of these cell culture platforms: a multi-well plate as simple "lab-on-chip" manufactured in epoxy via stereolithography, which has been adequate for obtaining 250 μm-diameter holes. Even though the toxicity of this material would not allow for in vitro or ex vivo trials, rapid form/shape coyping strategies, commented in Sect. 8.4 of present Handbook provide adequate solutions. A similar development is shown in Fig. 8.14, in this case linked to a wavy scaffold with potential as in vitro bone model, which is obtained by PDMS casting upon a rapid mold with the negative of the desired geometry manufactured in an additive way by stereolithography.

Apart from resorting to rapid-form coyping processes for validation trials, it is important to note again that additive manufacturing technologies are solving the challenges derived from the toxicity of the materials traditionally structured using a solid freeform fabrication approach. These advances are based on novel bio-photo-polymers (Baudis et al. 2009, 2010) and on the commerzialization of high-performance technologies capable of working with advanced ceramic materials (Schwentenwein and Homa 2015).

In addition, the use of surface bio-functionalization procedures, in many cases based on CVD and PVD technologies (see Chap. 9), also provides interesting solutions for improving the biological interaction of additively manufactured prototypes and pre-series. These post-processes can be industrialized for mass-production and can be easily incorporated to existing production lines.

10.5 Mass-Production of Biomedical Microsystems by Combination of Subtractive and Additive Processes

The application of additive manufacturing technologies (AMT) to producing final parts is growing exponentially; changing from the typical applications of AMT linked to rapid prototyping from the 1990s and 2000s, to the development of products with remarkable added values and integrated functionalities by means of complex geometries and multi-scale control of matter. The biomedical field is one of the principal sectors benefited from advances in these technologies, as well as in the supporting software resources and materials.

The possibilities of using medical imaging tools as design input, the synthesis of novel biomaterials capable of being additively processed, the improvements in manufacturing precision and speed of AMT, the development of new functional principles for AMT, including the use of multiple materials in a single machine, among other relevant advances of the last decade, are expanding horizons towards the generalized industrial use of additive manufacturing, especially for the

development of biomedical devices, including microsystems for interacting at a cellular level.

However, it is also true that even the most modern additive manufacturing tools are not capable of competing with the quantity and repeatability of parts produced in biomedical polymers by means of injection molding processes. In addition, the more precise additive resources, capable of producing micrometric details, are still very slow and cannot be used for production runs. On the other hand, current AMTs with the highest industrial impact, including laser stereolithography, digital light processing and selective laser sintering/melting, are capable of producing hundreds of parts per day, but cannot reach true micro- or nano-metric details.

In consequence, strategies towards the combined use of top-down and bottom-up approaches, linking traditional and modern manufacturing paradigms, can provide very interesting solutions for the field of biomedical microsystems, also including those aimed at interacting at cellular level thanks to the incorporation of special and multi-scale controlled geometries.

The potential of directly linking additive manufacturing with micro-injection molding has been detailed in Sect. 10.3, but it can be further researched towards the implementation of rapid mold inserts with features controlled at different scale levels by the use of several additive and substractive resources in a step-by-step manufacturing approach or even using novel machines combining additive and substractive procedures performed in a single chamber. For instance, a first master prototype can be obtained using additive photopolymerization procedures and layer-by-layer sintering processes, with which the main structure of the device can be obtained. Subsequently, surface patterns, microchannels and micro-wells, among other micro- and even nano-metric details can be carried out by means of ultra-high precision machining or laser ablation. Finally, a valid master model can be implemented by surface metallization of the master models and some closing dimensional adjustments.

These combinations are also analyzed and applied towards the end of the Handbook, for the systematic production of trial series for a “liver-on-a-chip” and for a “blood-brain barrier” microsystem, as further detailed in Chap. 22, linked to “Strategies towards reliable organs-on-chips and humans-on-chips”.

10.6 Main Conclusions and Future Research

The rapid prototyping of biomedical microsystems, which is usually based on additive manufacturing processes, is very well suited for single prototypes with complex geometries, but in many cases inadequate for mass production, due to the excessive cost and time involved, in comparison with replication technologies, such as injection molding and compression molding. In addition, the polymers used in the most precise additive manufacturing technologies, which are based on photo-polymerization processes, are typically toxic or inadequate for biomedical applications, what limits enormously the span of final applications.

Exploring cooperative strategies, for taking advantage of the complexity of geometries attainable via rapid prototyping, while also benefiting from the possibility of manufacturing large low-cost series using mass replication techniques, is a relevant industrial need and can be a source of novel procedures for supporting research and innovation in several fields. The issue is of special relevance in biomedical applications, as mass production enables the democratization of Healthcare and helps researchers to carry out more systematic studies for addressing the problems of disease and for finding improved therapeutic solutions.

This chapter has provided a brief introduction to the more relevant mass-production technologies with application in the field of biomedical microdevices. Illustrative examples linked to the complete development and mass production of different cell culture platforms and biodevices for studying cell behavior have been also provided to further analyze the advantages and potentials of using this kind of manufacturing procedures. Main current research trends, linked to the progressive convergence between subtractive and additive manufacturing approaches and to the combined use of technologies for the promotion of multi-scale and biomimetic approaches, have been discussed.

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References

Baudis S, Heller C, Liska R, Stampfl J, Bergmeister H, Weigel G (2009) (Meth)acrylate-based photoelastomers as tailored biomaterials for artificial vascular grafts. J Polym Sci Part A: Polym Chem 47(10):2664–2676

Baudis S, Steyrer B, Pulka T, Wilhelm H, Weigel G, Bergmeister H, Stampfl J, Liska R (2010) Photopolymerizable elastomers for vascular tissue regeneration. Macromol Symp 296(1):121–126

Bissacco G, Hansen HN, Tang PT, Fugl J (2014) Precision manufacturing of tools for injection molding of microfluidic systems. http://www.aspe.net/publications/Spring_2005/05SPAbs/Bissacco-1676.pdf. Accessed 26 Aug 2014

Díaz Lantada A, Endrino JL, Sánchez Vaquero V, Mosquera AA, Lafont Morgado P, García-Ruíz JP (2011) Tissue engineering using novel rapid prototyped diamond-like carbon coated scaffolds. Plasma Process Polym 9(1):98–107. doi: 10.1002/ppap.201100094

Díaz Lantada A, Piotter V, Plewa K, Barie N, Guttmann M, Wissmann M (2015) Towards mass production of biomedical microdevices: linking rapid prototyping with micro-injection molding. Int J Adv Manuf Technol 76:1011–1020

Gad-el-Hak M (2003) The MEMS handbook. CRC Press, New York

Piotter V, Bauer W, Hanemann T, Heckele M, Müller C (2008) Replication technologies for HARM devices—status and perspectives. J Microsyst Technol 14(9–11):1599–1605

Piotter V, Prokop J (2014) Galvanic deposition in partially conductive plastic molds for manufacture of finely detailed microcomponents. J Microsyst Technol 20:169–174

Piotter V, Prokop J, Ritzhaupt-Kleissl H-J, Ruh A, Haußelt J (2010) Multi-component micro injection moulding—trends and developments. Int J Adv Manuf Technol 47:63–71

Schwentenwein M, Homa J (2015) Additive manufacture of dense alumina ceramics. Appl Ceram Technol 12(1):1–7

Stampfl J, Baudis S, Heller C, Liska R, Neumeister A, Kling R, Ostendorf A, Spitzbart M (2008) Photopolymers with tunable mechanical properties processed by laser-based high-resolution stereolitoraphy. J Micromech Microeng 18:125014

Waldbaur A, Rapp H, Länge K, Rapp BE (2011) Let there be chip—towards rapid prototyping of microfluidic devices: one-step manufacturing processes. Anal Methods 3(12):2681–2716

Part III
Applications

Chapter 11
Biomedical Microsystems for Disease Management

Andrés Díaz Lantada, Pilar Lafont Morgado and Pedro Ortego García

Abstract The modern and integrated study of biomechanical and biochemical issues in disease is usually carried out with the fundamental support of fluidic microdevices and of microfluidic diagnostic platforms, as fluids allow for the transport of nutrients, gases, debris, pathogens and drugs to and from cells, help to control the movement of microorganisms in vitro and make the application of controlled stresses in culture systems possible. In consequence, biomimetic responses are promoted and in many cases results obtained in vitro are more accurate than those obtained from animal models. In fact the field of microfluidic systems for diagnosis has experienced an explosive growth in the last two decades, promoted by the convergence of clinical diagnostic techniques, computer-aided modeling and mature micro- and nano-fabrication technologies capable of producing submillimeter-size fluidic channels, reservoirs and nanometric features in several materials, structures and devices. This chapter provides and introduction to the field of biomedical devices for disease study and management, with examples of systems devoted to purposes such as: in vitro drug screening, disease modeling and diagnosis, disease modeling and prediction, and modeling of tumors, among the most important and already well-established applications. The application of computer-aided design and rapid prototyping resources to the complete development of a capillary actuated microfluidic platform, as versatile framework for the potential point-of-care testing of different diseases and their eventual response to different antibiotics, is detailed as additional case of study.

A. Díaz Lantada (✉) · P. Lafont Morgado · P. Ortego García
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2,
28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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11.1 Introduction to Modern Disease Management

The integrated study of biomechanical and biochemical issues in disease is usually carried out with the fundamental support of fluidic microdevices and microfluidic diagnostic platforms, as fluids enable the transport of nutrients, gases, debris, pathogens and drugs to and from cells, help to control the movement of microorganisms in vitro and make the application of controlled stresses in culture systems possible. In consequence, biomimetic responses are promoted and in many cases results obtained in vitro are more accurate than those obtained from animal models. In fact the field of microfluidic systems for diagnosis has experienced an explosive growth in the last two decades, promoted by the convergence of clinical diagnostic techniques, computer-aided modeling and mature micro- and nano-fabrication technologies capable of producing submillimeter-size fluidic channels, reservoirs and nanometric features in several materials, structures and devices (Jenkins and Mansfield 2013). According to the purpose of the microfluidic microsystems used for studying diseases, we can speak of different application fields: microsystems for in vitro drug screening, microsystems and labs-on-chips for disease modeling and diagnosis, cell culture models for disease modeling and prediction, and in vitro tumor models, among the most important and already well-established applications. This chapter covers such applications based on conventional microfluidic devices, while the more recent development of biomedical microdevices for studying cell behavior and interactions is detailed in Chaps. 12–14 and 21. The development of biomimetic cell culture scaffolds, which can be also applied to the study of diseases and the testing of new drugs, is explained in Chaps. 15–20. The more recent organ-on-chip and life-on-chip approaches are discussed in Chaps. 20 and 22 and biofabrication is introduced in Chap. 23.

11.2 Microfluidic Devices for in vitro Drug Screening

The process of finding a new effective drug against a chosen target for a particular disease involves high-throughput screening, wherein large libraries of chemicals are tested for their ability to modify the target, as opposed to the historical identifications of the active ingredients from traditional remedies and other sounded serendipitous drug discoveries. Microfluidic devices are capable of handling tiny amounts of different fluids, operate in a very rapid way due to the promotion of the surface/volume ratio, which accelerates chemical reactions, and enable multiplexing (performing multiple tests from an initial sample) and automation. In consequence, microfluidic platforms are very well-suited for drug screening and detection and for other high-throughput applications linked to disease study and management. Apart from several commercial drug screening platforms (multi-well plates) and drug test devices (typically saliva and urine colorimetric tests), we would like to highlight some novel developments in the fields of microsystems for in vitro drug screening.

Active drug screening devices are being developed that take advantage of cultured cells, for detecting drugs or studying their efficiency.

For instance, cell lines have been proposed as in vitro models for drug screening and toxicity studies. In fact, the use of tissue culture is a valuable tool to study problems of clinical relevance, especially those related to diseases, screening, and studies of cell toxicity mechanisms. Ready access to the cells provides the possibility for easy studies of cellular mechanisms that may suggest new potential drug targets and, in the case of pathological-derived tissue, it has an interesting application in the evaluation of therapeutic agents for diseases. However, the problems derived from primary culture, such as shortage of tissues and requirements of additional resources, including extra cellular matrices, growth factors and soluble media, have promoted the use of immortalized cell lines, which can provide unlimited tissue amounts, if their stability and viability challenges are overcome, as previously reviewed (Allen et al. 2005). More recently, cells have been cultured in continuous perfusion glass microchip systems for drug screening and the combination of such a microchip with a cell-based sensor has been proposed to monitor osteogenic differentiation, as well as for the development of a high-throughput drug screening assay for discovering osteogenic compounds (Jang et al. 2008).

11.3 Microfluidic Devices for Enhanced Disease Modeling

Lab-on-chip microfluidic devices or "labs-on-chips" are aimed at integrating the complex operations and procedures typical from biochemical and biological laboratories in just a few cm^2, by taking advantage of microfluidic operation, which promotes reaction speed, sustainability due to the use of low fluid and sample volumes, and repeatability, thanks to multiplexing and automation, as already mentioned. At present, most lab-on-chip devices are in fact "chip-on-lab" systems, as these complex microfluidic platforms still require from several support actuation and characterization technologies for a correct operation (Jenkins and Mansfield 2013). Fluid motion within the labs-on-chips is usually carried through capillary action, using external or internal pumps, by centrifugal force, by electromagnetic fields or resorting to the use of active materials. Among characterization technologies, optical laser-based systems and distributed sensors within the devices are common.

Even if further research in the field will promote additional miniaturization and integration of capabilities, lab-on-chip devices incorporating cells or tissue samples are already very interesting for disease modeling and for studying biomechanical and biochemical aspects of disease, either combined or decoupled, as several strategies included in Sect. 11.3 present in more detail. The typical components of a lab-on-chip for diagnosis include: gates for sample injection, on-chip reservoirs, droplet formation structures, fluidic pathways, mixing areas and optical detection sites on the same substrate (Srinivasan et al. 2004). The incorporation of microchannels in form of microvasculatures is also common, especially for modeling physiological

structures and derived problems, such as occlusions and thromboses that occur in hematological diseases (Tsai et al. 2010).

The use of microcapillaries is also interesting for modeling the transportation mechanism of red blood cells, for addressing hydrodynamic effects on their behavior, for analyzing red blood cell deformability and the consequences of pathological changes in their deformability on disease evolution (Chen et al. 2010). Platelet aggregation phenomena have been also analyzed using a microfluidic device incorporating contraction–expansion geometries that generate strain rate conditions mimicking the effects of pathological changes in blood vessel geometry (Tovar-Lopez et al. 2010). The effects of different drugs on blood rheology can be also compared using similar lab-on-chip and the related results are comparable to or even better than those obtained using animal models.

Other microfluidic models with microvasculatures, but with cells cultured within them, are also of great interest for modeling more complete and complex physiological structures and addressing the effect of drugs, toxins and other biochemicals, as well as the consequences of biomechanical phenomena, on disease appearance, evolution and management. There are already interesting examples linked to in vitro modeling the blood-brain barrier to monitor drug permeability into the central nervous system (Yeon et al. 2012). Considering the relatively short period of time needed for endothelial cell culture and ability to monitor the blood-brain barrier physiology continuously, authors proposed such system for its use as an invaluable first-line tool for CNS-related drug development.

11.4 Microfluidic Devices for Enhanced Disease Diagnosis

Some microfluidic devices, many of which can be also considered lab-on-chip devices, focus specifically on cell culture for disease modeling and for diagnosis and prediction. In these cases, the microsystems include additional features and components, such as cell cultivation chambers and a wider set of chemo- and biosensors for microfluidic and metabolic monitoring, as in the multiparametric microphysiometry system for the dynamic online monitoring of human cancer cell metabolism recently developed by Weltin and colleagues (2014). Such devices can be used, not only for studying disease evolution, but also for improved drug screening.

Advances in the field of microfluidic devices, labs-on-chips and cell culture models are in many cases aimed at obtaining more effective in vitro tumor models for improved diagnosis, drug screening and therapy. For instance, remarkable research collaboration between the Wyss Institute for Biologically Inspired Engineering at Harvard University and Children's Hospital Boston has created a microfluidic device that can harvest rare circulating tumor cells from blood to enable their expansion in culture for analysis. These cells, detached from a primary cancer site, hold an extraordinary amount of information regarding patient-specific drug sensitivity, cancer progression, and patient response to therapy. The capture is enabled thanks to a microfluidic-micromagnetic device (Kang et al. 2012).

Other cell trapping systems, based on infection-mimicking materials, are being also used to capture and activate tumoral cells, so as to enable more adequate assessment of cancer vaccines (Ali et al. 2009).

In vitro cancer research models can be also obtained by culturing cells on predefined extra cellular matrices with some geometrical, mechanical or biochemical features and components for promoting tumor growth and subsequently studying the effects of drugs and potential therapies, either in conventional well plates or by incorporation of the scaffolds into more complex microfluidic environments or labs-on-chips. Remarkable results have been obtained using commercially available 3D-Biotek® polystyrene scaffolds obtained by 3D-printing, which provide enhanced biomimetical responses, when compared with conventional monolayer 2D cell culture (Bergenstock et al. 2009; Caicedo-Carvajal et al. 2011).

11.5 Case Study: Capillary Microfluidic Platform for Point-of-Care Testing

As case of study we present in this section the complete development process of a capillary microfluidic platform for point-of-care-testing. The microsystem is aimed at the rapid and easy detection of urinary infections and at the adequate selection of successful antibiotics, without resorting to complex and expensive microbiological infrastructures. This lab-on-chip is well suited for developing countries and poor remote rural regions, where access to specialized equipment is difficult, where there are frequent cases of multi-resistances to antibiotics and where a response to the patient in the point of care (primary health service) is the best option. To sum up, the microsystem is aimed at performing a rapid and low-cost antibiogram and its development has been financed by the Government of Chile, via CORFO, to support a concept proposed by Diagnochip Spa.

Conceptual designs and prototypes of the lab-on-chip physical platform have been developed using different approaches and technologies, so as to validate the concept and to select the most adequate resources for subsequent steps aimed at the validation of the microbiological process with real biological samples and with larger prototype series. The conceptual design can be described as follows: the lab-on-chip should include a central chamber for placing the biological sample and several radial channels for driving the potentially infected urine, via capillary action, to a set of wells placed in the outer part of the lab-on-chip. Each well should include a different antibiotic and a chromophore, which can be synthesized by living bacteria and promote a colour change, so as to highlight the antibiotics that prove ineffective for treating the pathology. In consequence, the result is easy to read and the biodevice can be used by untrained personnel for a rapid diagnose and for avoiding the empirical use of antibiotics.

In this case, the use of a microfluidic system may be interesting, as it promotes a more sustainable use of biological samples and reactives, accelerates chemical

reactions towards a more rapid diagnose and can be easily industrialized and mass-produced for accelerating its potential social impact.

In addition, the use of capillary action for driving biological samples requires micro-metric features (channels) and manufacturing technologies capable of working in the micro-scale. In our case, different prototypes of the desired microsystem have been obtained using a wide set of rapid prototyping and rapid tooling resources, available at the Product Development Lab at UPM, which has helped us to compare the precision and potential for final mass-production of the different approaches, as further discussed.

Our first micro-manufacturing approach is based on UV-photolithography after the design of different masks with the desired geometry of the biomedical lab-on-chip for urinary infection diagnosis (see Fig. 11.1).

Combinations of UV photolithography and chemical etching, have allowed us to obtain micro-textured metallic plates, with the microchannels of the lab-on-chip, which can be further used for PDMS casting towards final prototypes for the validation of the proposal with real biological samples, antibiotics and chromophores. These micro-textured metallic plates can be also employed as mold inserts for the manufacture of larger series by micro-injection molding or by hot-embossing of thermoplastic polymers.

The manufacturing process has followed previous descriptions (De la Guerra et al. 2012) with some modifications. In short, we have used copper plates and discs as substrate material due to its easy processability and the need of a lower etching time. The use of glass is also possible, but etching with HF acid, which requires

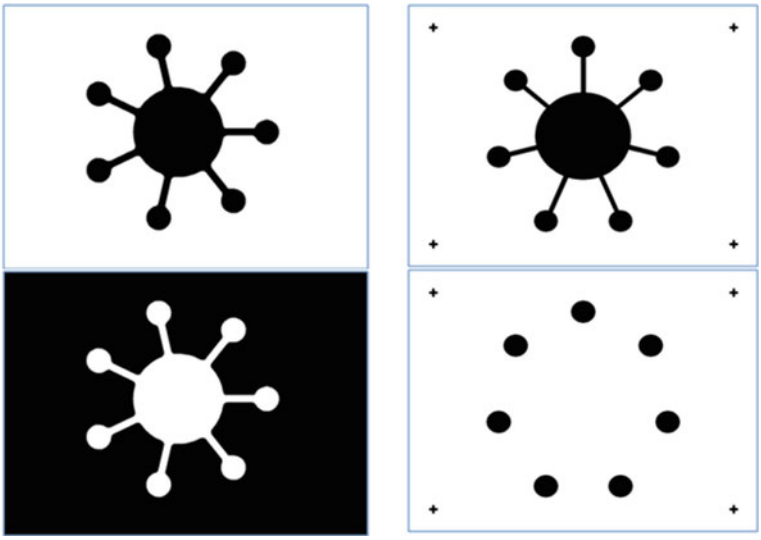

Fig. 11.1 Masks for the manufacture of a biomedical microsystem for disease diagnosis. Positive and negative masks for the use of different photoresist (*left images*) and masks for two subsequent micromachining steps aimed at obtaining the outer wells with an additional depth (*right images*)

special handling. For the manufacture of the microchannels we have followed several steps including:

- Initial preparation of the copper discs by washing out the possible surface oxides in ultrasonic cube for around 30 min and subsequent drying.
- Coating of the discs using Dupont Riston PM-100 photoresin.
- Exposure of the photoresin to UV light by means of the SF-100 equipment from Intelligent Micro Patterning LLC. As previously mentioned, this process is known as mask-less photolithography, as the employment of programmable light filters prevents from using a physical mask.
- Development, using a Na_2CO_3 0.85 % w. solution, for eliminating the uncured photoresin in those pattern zones that are going to be chemically etched.
- Chemical etching introducing the substrate in a $FeCl_3$ 40 % w. solution for attacking the uncoated pattern zones, hence obtaining the micro-texture.
- Stripping or elimination of the remaining photoresin.
- Washing out debris and drying.
- Final dimensional verification.

As already mentioned, such obtained microchannelled surfaces can be also used for micro-replication activities, in a family of processed normally referred to as "soft lithography techniques" (see Sect. 8.4 for additional details). Soft stamps can be also obtained by casting PDMS or resin onto metallic or glass micro-textured substrates following "rapid form copying" procedures.

The mentioned processes are easy to industrialize for producing serial production of microsystems, as the electronic industry has widely shown. In our case, it is important to note that the use of two masks (right images of Fig. 11.1) and of two subsequent UV-photolithography and chemical etching processes allows us to obtain the outer wells with an additional depth, which is useful for placing the reactives. The inclusion of different crosses in the masks is an advisable practice for helping with the always complex alignment processes, required when using multiple steps for manufacturing micro-devices.

Main results of this micro-manufacturing option towards the desired lab-on-chip are shown in Figs. 11.1, 11.2 and 11.3. Figure 11.2 includes the micro-textured metallic patterns manufactured by UV-photolithography and chemical etching, which can be used either as final parts or as mold inserts for the mass production of a biomedical microsystem for disease management via hot-embossing or micro-injection molding. Finally Fig. 11.3 shows a detailed view for putting forward that the use of a two-step process with different masks allows for the manufacture of textures with different depths.

In spite of the possibility of obtaining details down to 15–30 μm, which can clearly drive the biological samples via capillary action, if adequately closing the system with a lid, this process requires a remarkable degree of expertise and prototyping results depend on several parameters of influence (concentrations of reactives, exposure times, etching times…). Once adjusted, the process is easily industrializable towards mass-production.

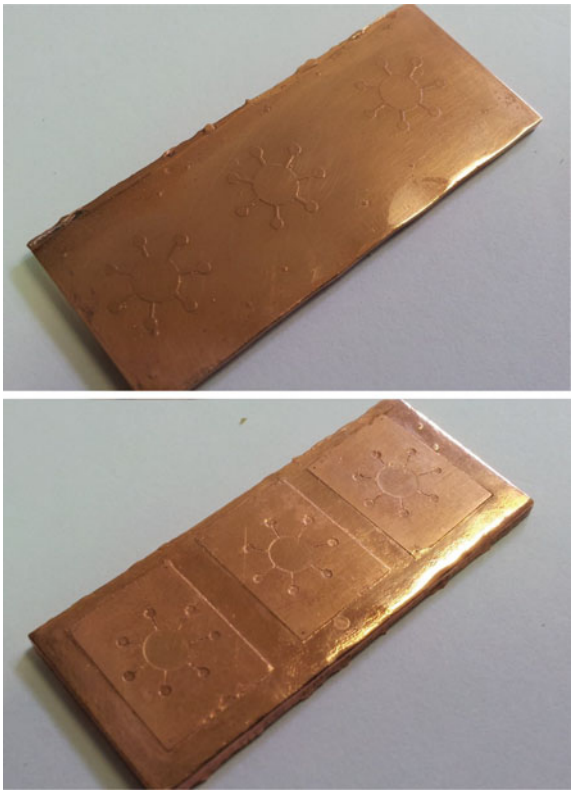

Fig. 11.2 Micro-textured metallic patterns manufactured by UV-photolithography and chemical etching, which can be used either as final parts or as mold inserts for the mass production of a biomedical microsystem for disease management via hot-embossing or micro-injection molding

An alternative approach, for more directly linking the designed geometries with the physical prototypes, is based on the combination of computer-aided design (CAD) resources, rapid prototyping technologies based on additive manufacturing procedures and rapid tooling processes for the development of low-cost tools for obtaining short and medium series for trials.

In this second approach micro-manufacture is accomplished, directly from the computer-aided design files with the three dimensional geometries of the different lab-on-chip geometries under development, by means of laser stereolithography (SLA-3500 machine by 3D Systems). Such additive manufacturing technology is able to construct complex biodevices, working on a layer-by-layer approach, and currently provides one of the best compromises between part size, manufacturing precision and productivity among all 3D printing resources. It has been previously highlighted as a key resource for microfluidic systems, due to the possibility of manufacturing labs-on-chips with complex inner channels in a one-step process (Waldbaur et al. 2011).

Figure 11.4 includes three different 3D CAD models of the desired biomedical microsystem for disease diagnosis actuated by capillary action. In these different

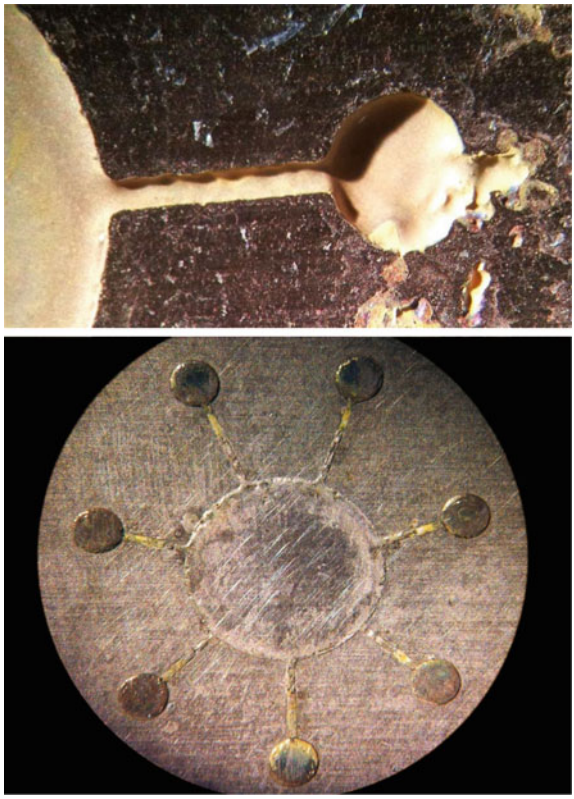

Fig. 11.3 Micro-textured metallic patterns manufactured by UV-photolithography and chemical etching, which can be used either as final parts or as mold inserts for the mass production of a biomedical microsystem for disease management via hot-embossing or micro-injection molding. Detail: The use of a two-step process with different masks allows for the micro-manufacture of textures with different depths

Fig. 11.4 Different 3D CAD models of a biomedical microsystem for disease diagnosis actuated by capillary action

designs, the microchannels are conformed by means of a patterned part and a lid or even integrated within the 3D structure of the device.

Rapid prototypes obtained by laser stereolithography are usually employed for aesthetic and geometrical validations, for marketing purposes, for second stage processes towards manufacturing tools (normally "soft" PDMS molds) and as molds for the manufacture of final parts by subsequent casting. However their use for limited functional trials is also noteworthy. For instance, Fig. 11.5 shows the rapid prototype of the biomedical microsystems for disease diagnosis and capillary actuation under development. The microchannels are aimed at driving the central sample radially to the outer wells for reaction. The additive manufacture by means of laser stereolithography allows for the monolithic production of such devices with inner channels capable of driving samples via capillary action.

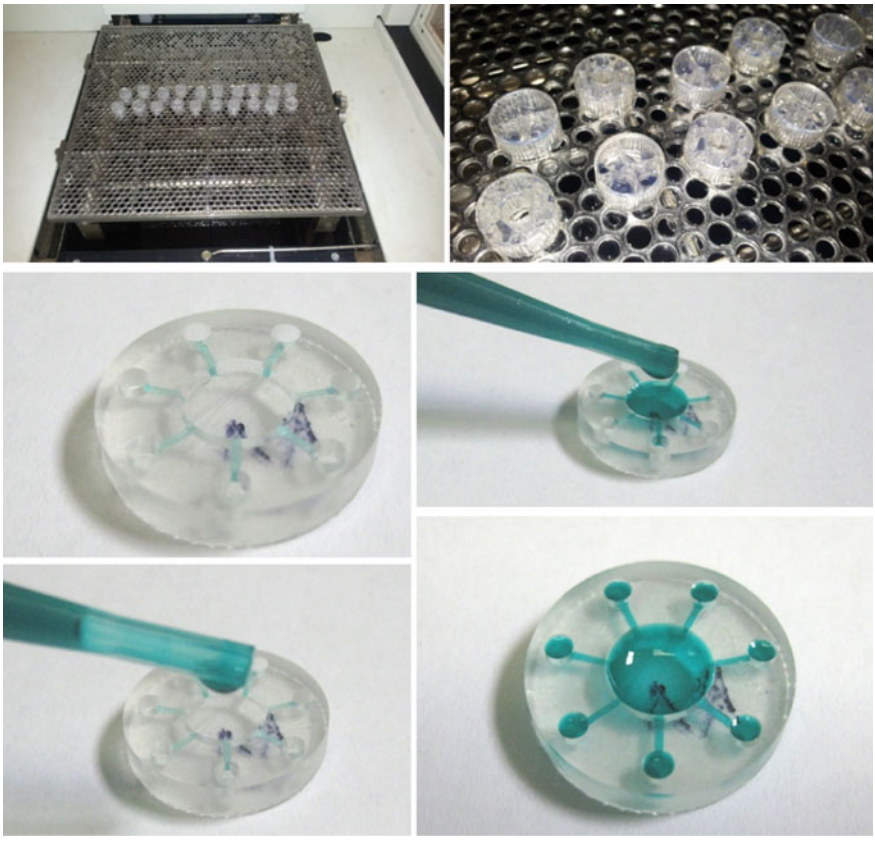

Fig. 11.5 Rapid prototyping of biomedical microsystems for disease diagnosis and capillary actuation aimed at driving the central sample radially to the outer wells for reaction. The additive manufacture by means of laser stereolithography allows for the monolithic production of devices with inner channels

The potential of laser stereolithography for the mass-production of these types of devices can be also perceived by taking a closer look at the upper images from Fig. 11.5. As can be seen, 20 labs-on-chips have been manufactured using just a small portion of the building platform and using a process lasting for less than 1 h. With some design optimizations and an adequate distribution of parts upon the building platform, more than 200 parts/h can be obtained using monolithic designs, which avoid the performance of joining processes and simplify mass-production. For sure, these resources may challenge the position of injection molding as reference production technology for biomedical microsystems in the near future, as already discussed in Chap. 10.

The technology is also especially well suited for comparing alternative designs, as the manufacture of completely different geometries in a single building platform requires no additional tools or ad hoc designed work-benches. In our case it has been a key for comparing the capillary action of different channel sections.

The epoxy prototypes obtained by laser stereolithography can be also employed as molds for PDMS casting or as master models for the manufacture of rapid PDMS molds for further casting other polymers with special properties, including biomedical grade silicones, softer or tougher materials, polymers with improved transparency or in different colours, among other possibilities.

Figure 11.6 includes by means of examples different PDMS and epoxy molds for obtaining larger series of the biomedical microsystem for diagnosis using an alternative design based on two layers. The adequate geometrical design of the channels for driving the biological samples by means of capillary action is also validated although, depending on the hydrophobicity of the polymers used, design modifications may be necessary when changing from epoxy resin to other final materials.

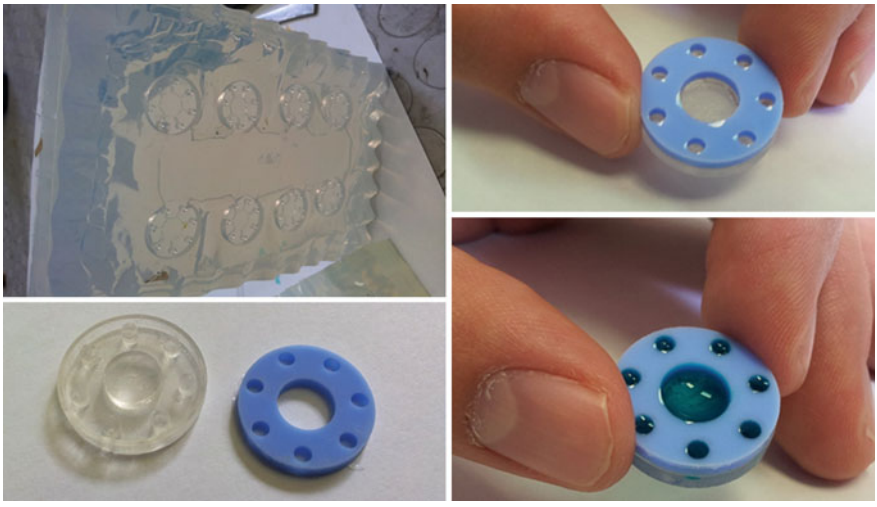

Fig. 11.6 PDMS and epoxy molds for obtaining larger series of the biomedical microsystem for diagnosis using an alternative design based on two layers

A final reflection regarding the proposed design of the diagnostic lab-on-chip is linked to the advantages and disadvantages of using capillary action for driving the fluid. It is true that capillary action simplifies micro-devices, as no additional internal or external pumping elements (i.e. piezoelectric resonators, micro-pumps, syringes, among others) are required. However, the design of channel geometries and the manufacturing tolerances must be considered carefully, so as to prevent asymmetric performances and to promote an adequate actuation. Regarding the fact that the different reaction wells keep connected to the central one, where the biological sample is placed, aspects linked to potential crossed influences between the reactions taking place in the different wells arise and must be considered for the in vitro validations.

11.6 Main Conclusions and Future Research

The modern and integrated study of biomechanical and biochemical issues in disease is usually carried out with the fundamental support of fluidic microdevices and of microfluidic diagnostic platforms, as fluids allow for the transport of nutrients, gases, debris, pathogens and drugs to and from cells, help to control the movement of microorganisms in vitro and make the application of controlled stresses in culture systems possible. In consequence, biomimetic responses are promoted and in many cases results obtained in vitro are more accurate than those obtained from animal models.

In fact the field of microfluidic systems for diagnosis has experienced an explosive growth in the last two decades, promoted by the convergence of clinical diagnostic techniques, computer-aided modeling and mature micro- and nano-fabrication technologies capable of producing submillimeter-size fluidic channels, reservoirs and nanometric features in several materials, structures and devices.

As for the future, the development of more versatile computer-aided design and engineering resources, capable of taking into account the impact of cell behavior on final performance, from the design stage, by means of multi-scale and multi-physical/chemical modeling approaches, will benefit researchers, designers and end users. Further developments linked to multi-material additive manufacturing resources, capable of rapidly obtaining these types of microdevices, with the cells, pathogens and toxins incorporated during the manufacturing process, will promote personalization and open new horizons for labs-on-chips and organs-on-chips, as well as for the emerging area of biofabrication.

This chapter provides and introduction to the field of biomedical devices for disease study and management, with examples of systems devoted to purposes such as: in vitro drug screening, disease modeling and diagnosis, disease modeling and prediction, and modeling of tumors, among the most important and already well-established applications. The application of computer-aided design and rapid prototyping resources to the complete development of a capillary actuated microfluidic platform, as versatile framework for the potential point-of-care testing of

different diseases and their eventual response to different antibiotics, is detailed as additional case of study.

Acknowledgements We acknowledge the support of Diagnochip Spa and of the Government of Chile, via CORFO, for the development of the "Diagnochip project" towards a rapid anti-biogram for urinary infections, which has been presented as case of study for explaining the complete development process of a point-of-care testing biomedical microdevice. We acknowledge Dr. Mario Soto and Dra. Sara Droguett for their confidence and friendship, developed along a highly motivating project aimed at providing solutions to relevant social demands.

References

Ali OA, Huebsch N, Cao L, Dranoff G, Mooney DJ (2009) Infection-mimicking materials to program dendritic cells in situ. Nat Mater 8:151–158

Allen DD, Caviedes R, Cárdenas AM, Shimahara T, Segura-Aguilar J, Caviedes PA (2005) Cell lines as in vitro models for drug screening and toxicity studies. Drug Dev Indus Farmacy 31(8):758–768

Bergenstock MK, Balaburski GM, Lau W, Sun W, Sun Q, Liu Q (2009) Polystyrene scaffolds: a novel tool for in vitro three-dimensional cancer models. Cancer Res 69(23):8–11

Caicedo-Carvajal CE, Liu Q, Remache Y, Goi A, Suh K (2011) Cancer tissue engineering: a novel 3D polystyrene scaffold for in vitro isolation and amplification of lymphoma cancer cells from heterogeneous cell mixtures. J Tissue Eng (online). doi:10.4061/2011/362326

Chen YC, Chen GY, Lin YC, Wang GJ (2010) A lab-on-a-chip capillary network for red blood cell hydrodynamics. Microfluid Nanofluid 9(2–3):585–591

De la Guerra Ochoa E, Del Sordo Carrancio D, Echávarri Otero J, Chacón Tanarro E, Díaz Lantada A, Lafont Morgado P (2012) The influence of textured surfaces on the lubrication of artificial joint prostheses. In: Biodevices 2012—international conference on biomedical electronics and devices. IEEE Engineering in Medicine and Biology Society

Jang K, Sato K, Igawa K, Chung UI, Kitamori T (2008) Development of an osteoblast-based 3D continuous-perfusion microfluidic system for drug screening. Anal Bioanal Chem 390(3): 825–832

Jenkins G, Mansfield CD (2013) Microfluidic diagnostics: methods and protocols. Springer, New York

Kang JH, Krause S, Tobin H, Mammoto A, Kanapathipillai M, Ingber DE (2012) A combined micromagnetic-microfluidic device for rapid capture and culture of rare circulating tumor cells. Lab Chip 12(12):2175–2181

Srinivasan V, Pamula VK, Fair RB (2004) An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids. Lab Chip 4:310–315

Tovar-López FJ, Rosengarten G, Westain E, Khoshmanesh K, Jackson SP, Mitchell A, Nesbitt WS (2010) A microfluidics device to monitor platelet aggregation dynamics in response to strain rate micro-gradients in flowing blood. Lab Chip 10:291–302

Tsai M, Kita A, Leach J, Rounsevell R, Huang JN, Moake J, Ware RE, Fletcher DA, Lam WA (2010) In vitro modeling of the microvascular occlusion and thrombosis that occur in hematologic diseases using microfluidic technology. J Clin Invest 122(1):408–418

Waldbaur A, Rapp H, Länge K, Rapp BE (2011) Let there be chip—towards rapid prototyping of microfluidic devices: one-step manufacturing processes. Anal Methods 3(12):2681–2716

Weltin A, Slotwinski K, Kieninger J, Moser I, Jobst G, Wego M, Ehret R, Urban GA (2014) Cell culture monitoring for drug screening and cancer research: a transparent, microfluidic, multi-sensor microsystem. Lab Chip 14:138–146

Yeon JH, Na D, Choi K, Ryu SW, Choi C, Park JK (2012) Reliable permeability assay system in a microfluidic device mimicking cerebral vasculatures. Biomed Microdevices 14(6):1141–1148

Chapter 12
Overview of Microsystems for Studying Cell Behavior Under Culture

Andrés Díaz Lantada, Alberto Bustamante, Alisa Morss Clyne, Rebecca Urbano, Adam C. Canver, Josefa Predestinación García Ruíz and Hernán Alarcón Iniesta

Abstract Understanding how cells behave and interact with surrounding cells, tissues, microorganisms and all types of biological, biochemical and biomechanical cues from their environment, constitutes a relevant research challenge and requires the support, not only of advanced manipulation and imaging technologies, but also of specifically designed biomedical microsystems with micrometric and even nanometric details for enabling interactions at a cellular and molecular level. These types of microsystems, together with the use of advanced design and manufacturing strategies for their efficient development, constitute the core topic of present Handbook. Biomedical microsystems aimed at interacting with and studying the behavior of cells, include: dishes for 2D culture, microsystems for studying cells under chemical gradients, electrophoretic microsystems, multi-culture platforms and devices for cell co-culture and dynamic bioreactors or cell culture platforms. This chapter provides an introduction to these different types of biomedical microdevices, illustrating them by means of different cases of study. Main current research trends are also outlined. Other emerging and possibly more complex microsystems for interacting with cells and controlling their behavior and fate, even with the potential of constructing whole tissues and organs from cultured cells, are covered in depth in Chaps. 13–23.

A. Díaz Lantada (✉) · A. Bustamante
Mechanical Engineering Department – Product Development Laboratory, Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

A. Morss Clyne · R. Urbano · A.C. Canver
Vascular Kynetics Lab – Mechanical Engineering and Mechanics, Drexel University, 3141 Chestnut Street, Philadelphia, PA 19104, USA

J.P. García Ruíz · H. Alarcón Iniesta
Department of Molecular Biology, Universidad Autónoma de Madrid, Madrid, Spain

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12.1 Introduction to Microsystems for Cell Culture Aimed at Studying Cell Behavior

Understanding how cells behave and interact with surrounding cells, tissues, microorganisms and all types of biological, biochemical and biomechanical cues from their environment, constitutes a relevant research challenge and requires the support, not only of advanced manipulation and imaging technologies, but also of specifically designed biomedical microsystems with micrometric and even nanometric details for enabling interactions at a cellular and molecular level.

Biomedical microsystems aimed at interacting with and studying the behavior of cells under culture, include: dishes for 2D culture, microsystems for studying cells under biochemical gradients, electrophoretic microsystems, multi-culture platforms, devices for cell co-culture and dynamic bioreactors or cell culture platforms. The following sections provide specific examples of different strategies, for developing desired biomechanical and biochemical phenomena, which can be combined, even in a personalized way, towards improved cell culture systems and biomedical microsystems for studying cell behavior, modeling disease and helping with adequate tissue formation. The strategies are grouped according to the objective pursued, including control of cell growth, interaction and motion, control of adequate tissue formation, co-culture of different cell types for more complex tissue engineering approaches and in vitro reproduction of disease conditions upon a cultured tissue.

Although more complex devices aimed at the promotion of 3D cell culture and at the in vitro development of tissues and small portions of organs will be covered in several chapters along the Handbook, present chapter deals with the basic types of cell culture devices, which have help to generate knowledge and understand the basic mechanisms of cell behaviors and interactions. The progressive shift to 3D cell culture systems, linked to the in vitro development of tissues and organs, is clearly an adequate and logical evolution of this field. However 2D cell culture has helped to set the foundations of disease modeling, of advanced drug screening, of tissue engineering and even of biofabrication. Therefore it is necessary indeed to devote an introductory chapter to explaining main types of cell culture systems, before focusing on more specific biomedical microsystems for interacting at a cellular level.

It is important to note that, even if more complex devices and strategies may promote biomimetic approaches and help to obtain more valuable information, the use of more simple models typically helps to reach interesting data more directly and with low-cost devices and more conventional equipment. For example, co-culturing several cell types in a single device may be perfect for developing more reliable physiological models, but the extra efforts required for maintaining alive different types of cells usually, under diverse culture conditions, will only be compensated if very specifical information or interactions are sought. In many cases the more simple devices will perform adequately and robustly as happens with most engineering challenges.

12.2 Petri Dishes and the Limitations of 2D Cell Culture

Among the most used environments for studying cells, tissues and pathogens and their mutual interactions, it is important to highlight cell-culture dishes or plates typically referred to as Petri dishes, which are named after the German scientist Julius Richard Petri and constitute one of the more common technologies present in any molecular biology and micro-biology laboratory, as detailed in some previous sections.

In short, Petri dishes are shallow cylindrical glass or plastic lidded dishes that biomedical professionals use to culture cells and pathogens. Modern Petri dishes usually incorporate rings or slots on their lids and bases so that they can be more easily stacked. Petri dishes are in many cases filled with liquid containing agar and a mixture of nutrients, blood, salts, carbohydrates, dyes, indicators, antibiotics and other bio-fluids to carry out microbiology studies. Once the agar cools and gelates, the dish can be inoculated with pathogens, such as bacteria and viruses, although studies with viruses are typically performed with bacteria used as hosts for the viral inoculum. Eukaryotic cells can be also studied upon Petri dishes, either expanding them in a liquid medium or in agar.

Many advanced Petri dish designs are based on incorporating or integrating several plates or wells into a single plastic part to create a "multi-well" plate, as happens with ELISA-devices (enzyme-linked immunosorbent assays) for carrying out detailed antibiograms. ELISA is also a popular format of micro-fluidic device that uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample (urine, blood, sweat, mucus, among other biofluids). The diagnostic process using ELISA devices includes the following steps: Antigens from the sample are attached to a surface (in form of solutions added to the micro-wells). Then, a further specific antibody is applied over the surface so it can bind to the antigen covering the well. This antibody is linked to an enzyme, and, in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change in the substrate for visual purposes. Typical ELISA multi-well plates include 96 wells for carrying out very systematic studies, although these must usually be performed by very well trained professionals. Recent point-of-care testing approaches, as has already been discussed in Chap. 11, are focusing on the development of rapid and easy diagnostic microsystems, as alternative to more complex diagnostic resources requiring relevant infrastructures and experienced professionals.

By means of example, Fig. 12.1 shows hMSCs cultured upon the gelatine placed in a Petri dish and used as control for comparison purposes with cells grown upon microtextured platforms.

However, conventional cell culture upon Petri dishes and related devices is not able to emulate the complex biochemical and biomechanical interactions present in living organisms that drive cell dynamics, differentiation and eventual tissue

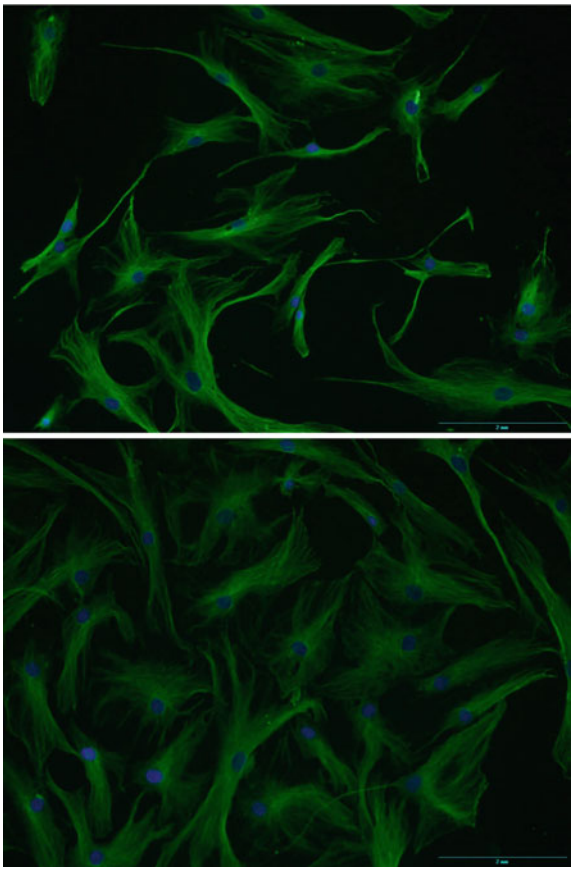

Fig. 12.1 hMSCs cultured upon the gelatine placed in a Petri dish and used as control for comparison purposes with cells grown upon microtextured platforms

formation and are, therefore, inadequate for precisely studying and modeling disease, as well as for evaluating the actual potential of novel drugs and therapies.

Cell performance is linked to their microenvironment, including the surrounding extra cellular matrix, other cells and soluble factors, such as growth factors and cytokines. Cells respond to biomechanical stimuli and parameters, such as topography of the surrounding material, stiffness of the substrate or environment, effects of vibrations, among others; but also interact biochemically according to surrounding materials' compositions, to the presence of other cell signals and to gradients of nutrients, drugs and pathogens (Yao et al. 2013).

As an alternative, much attention is being paid and great research efforts are being applied to the development of biomimetic platforms for human cell research (Vunjak-Novakovic and Scadden 2011), in many cases resorting to the concept of tissue engineering scaffolds and 3D cell-culture materials and environments or 3D cell-culture niches. Indeed, the niche composition and 3-D structure play an

important role in stem cells state and fate, as well as the incorporation of adequate growth factors and conditioned media (Chan et al. 2009).

These alternative scaffold-based solutions, especially focusing on the design and manufacturing strategies for the incorporation of the desired biomimetic and even personal properties for enhanced performance and response, will be further discussed in Chaps. 15–19.

In addition, microfluidic systems combined with or integrating advanced scaffolds and platforms for cell culture, are ideally suited for an optimized control of cell growth, interactions and motion, by means of adequately producing biomechanical and biochemical phenomena. Cell movement can be mechanically oriented, using channels, walls, holes, bridges, textures and patterns. The fluids in motion can be used to apply the desired shear stresses needed to promote certain cellular differentiations into relevant tissues. Including additional inlets and outlets to a microfluidic disease model can help to introduce nutrients, disease initiators and drugs for therapy. The use of support chambers can facilitate the establishment of biochemical gradients to induce cell motility. Producing mechanical and chemical modifications of certain zones of a biodevice, by changing surface topography or stiffness, by patterning surfaces with ligands…, can affect and help to control cell shape, cell size, cell-material adhesion, differentiation and other events necessary for implementing accurate disease models.

The following sections provide examples of biomimetic microfluidic devices specifically developed for the promotion of special effects upon the cells being cultured, so as to help them behave in a more natural way.

12.3 Microsystems for the Study of Cell Behavior Under Gradients of Biochemicals

Biomedical microsystems for cell culture incorporating microchannels for driving cell movements, by means of establishing gradients of biochemicals, such as nutrients, growth factors, extracellular matrix components and even pathogens, are providing interesting biomimetic solutions (Maher et al. 2010), especially due to the fact that 1D cell culture is more similar to 3D conditions than more conventional 2D cell culture on planar surfaces (Doyle et al. 2009). According to Doyle and colleagues, cell migration in both 1D and 3D is rapid, uniaxial and independent of extra cellular matrix ligand density, in contrast to 2D cultures, which brings out the need for alternative solutions aimed at a more adequate reproduction of the 3D environment (Doyle et al. 2009).

Recent developments have been linked to microsystems with cell-culture pools connected by channels to address the impact of gradients on cell behavior and fate (Díaz Lantada et al. 2015). In such devices, cells can move from one pool to another, driven by gradients of biochems, as already mentioned, but also due to the presence of other cell types in opposite pools and help to assess the impact on cell

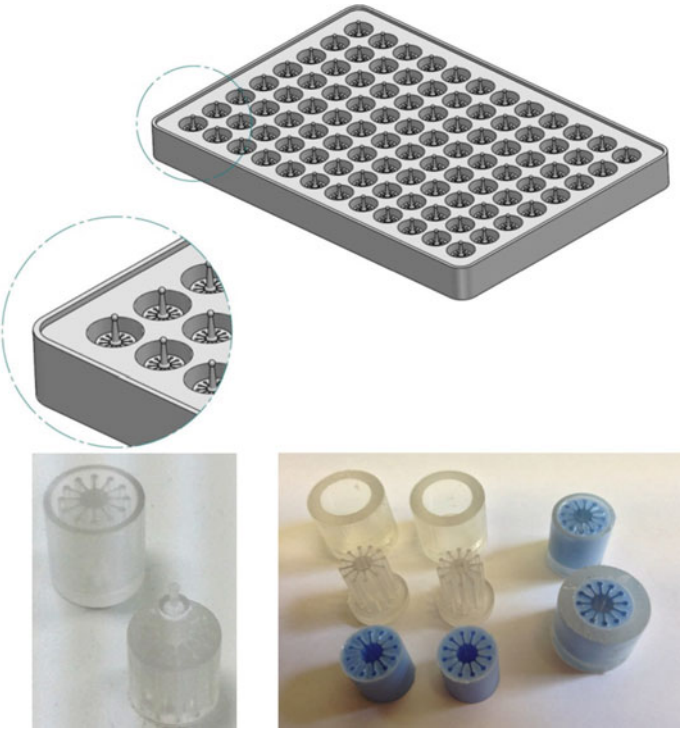

Fig. 12.2 CAD model, rapid master molds (in transparent epoxy) and final PDMS *blue replicas* of a microchannelled cell culture microsystem for addressing cell growth and motility. CAD model courtesy of M.Sc. Eng. Antonio Sillero (color online)

motility and fate, not just of biochemical gradients, but very specially of companion cells and a wide set of microorganisms.

Figure 12.2 shows, by means of example, the CAD model, the rapid master molds (in transparent epoxy) and the final PDMS blue replicas of a microchannelled cell culture microsystem for addressing cell growth and motility. The computer-aided design has been performed with the help of Solid Edge v.20 and the initial master molds in epoxy resin have been manufactured using the SLA-3500 3D Systems laser stereolithography machine, available at UPM the Product Development Lab.

The master molds are manufactured in two parts for helping with demolding tasks. They include thin walls of 300 μm, so as to help with the generation of 300 μm-wide microchannels for directing cell movement. As the epoxy resin is not adequate for cell culture trials, PDMS replicas are obtained. The final PDMS microsystems, placed upon microscope coverslips, can be used for culturing cells in the central chamber, for placing biochemical cues in the outer wells and for studying cell dynamics along the microchannels, depending on the different types and concentrations of biochemicals used. A hollow cylindrical micro-structure is

also developed, in order to prevent cells from escaping the central culture well, until the culture is well established and all cells are liberated at once.

Cell growth and cell expansion along the channels can be even automatically monitored and also assessed by means of real-time microscopy, cell labelling and automated cell-counting software (i.e. using Matlab files available at The Mathworks Inc. http://www.mathworks.com, or even commercial systems such as Cell Profiler, http://www.cellprofiler.org/) (Tholudur et al. 2006).

12.4 Electrophoretic Medical Microsystems for Studying Cell Behavior

Electrophoretic microsystems are state-of-the-art devices for the application of controlled electromagnetic fields to cell cultures. Electrophoresis is the motion of dispersed particles within a fluid typically due to a uniform electric field. Electrophoresis can be used to separate particles by size or by charge and hence is the basis of several analytical chemistries including Western blot. Electrophoretic procedures can be also performed using non-uniform fields (i.e., dielectrophoresis) and upon gels to study interactions and secreted proteins in dynamic cell cultures embedded within gels (Wimmer et al. 1994). These electrophoretic microsystems can be also used for cell micro-manipulation to separate cell mixtures into their component cell types or act as electrical grippers to transport cells and place them in specific locations (Rosenthal and Voldman 2005; Voldman 2006).

The preliminary CAD and FEM based simulations presented in this Sect. 12.4 are for a multiplexed dielectrophoretic biomedical microsystem for studying single adhered cell mechanical properties. A single dielectrophoretic device was designed, fabricated, and tested in the Vascular Kinetics Lab at Drexel University (Clyne and Urbano 2016).

Figure 12.3 includes an overall CAD model of the mentioned microsystem for cell dielectrophoresis, which includes a micropatterned gel (red) on which single cells are cultured in specific locations. A quadrupole electrode system is then assembled over each cell so that cell stiffness can be assessed. Since the multiplexed device is part of a microfluidic system, cell mechanics can be dynamically measured during application of a biochemical (e.g., cytokine or growth factor) or biomechanical (e.g., fluid flow) stimulus. Figure 12.4 shows a detailed view of the electrical connections for constructing the multiple quadrupoles on a single chip, which allows testing several conditions at the same time in a single experiment. Figure 12.5 shows a mask directly obtained from the 3D CAD model that would be used to create the microfluidic layer of the dielectrophoretic microsystem by means of UV photolithography (see Chap. 8 for additional details on micro-manufacturing

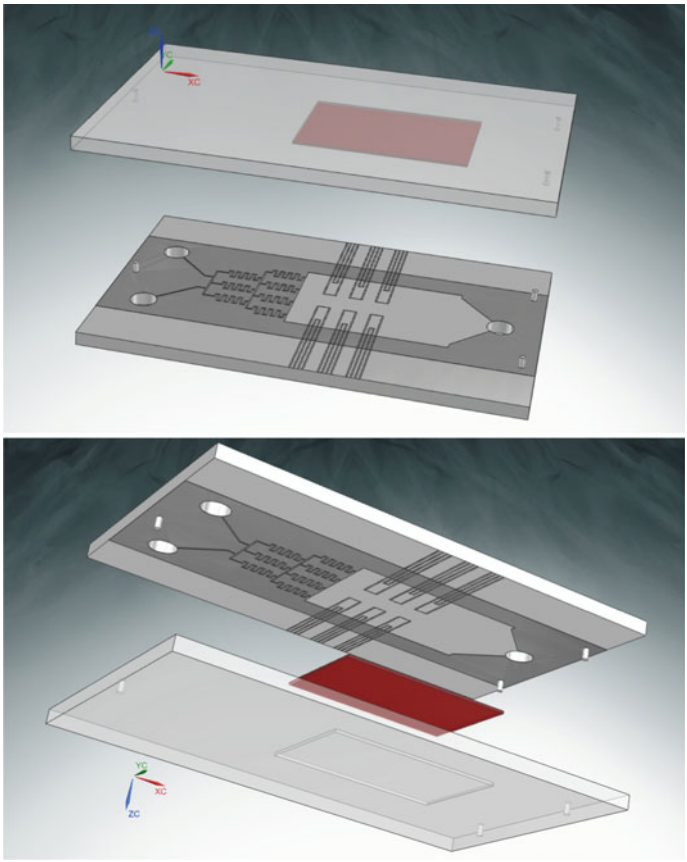

Fig. 12.3 CAD model of a microsystem for cell dielectrophoresis including a gel (red) on which cells are cultured, electrodes for applying dielectrophoretic forces, and a microfluidic system for assessing the impact of biochemical gradients or fluid flow on cell mechanical properties. Based on Clyne and Urbano, Lab Chip (2016). Designs created with NX-8.5 (Siemens PLM Solutions) (color online)

technologies in the biomedical field and Chaps. 20 and 22 for case studies of microfluidic devices for cell culture).

The preliminary FEM-based simulations of Figs. 12.6 and 12.7 assessed microsystem performance and the impact of fluid flow upon the cultured cells in silico. The results in Fig. 12.6 show the fluid velocity fields, highlighting the importance of culturing the cells far enough away from the fluid entrances so as to avoid flow disturbances. In Fig. 12.7, the slight influence of the extruded

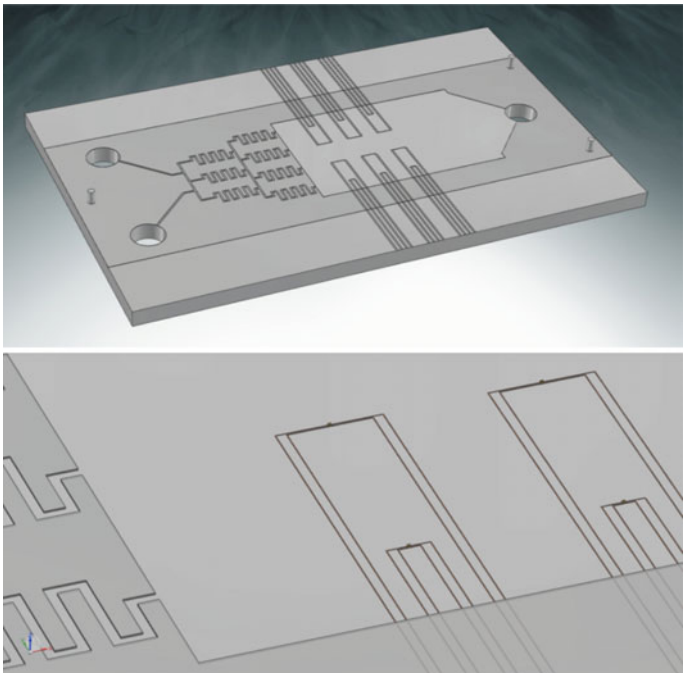

Fig. 12.4 Detailed view of the CAD model of a microsystem for cell dielectrophoresis showing the electrical connections required to place multiple quadrupole electrodes on a single device. Based on Clyne and Urbano, Lab Chip (2016). Designs created with NX-8.5 (Siemens PLM Solutions)

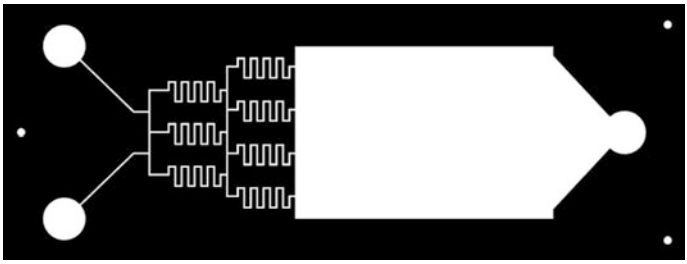

Fig. 12.5 Mask for the manufacture of an electrophoresis microsystem by means of UV photolithography

quadrupole electrodes on fluid flow can be perceived. These simulations were performed with the NX Nastran "Thermal-Flow" solver and with the "Flow" analyzer of NX-8.5 (Siemens PLM Solutions).

For the fluidic simulations, the meshing, material, loads and boundary conditions are not applied to the microsystem itself, but to the control volume of fluid moving

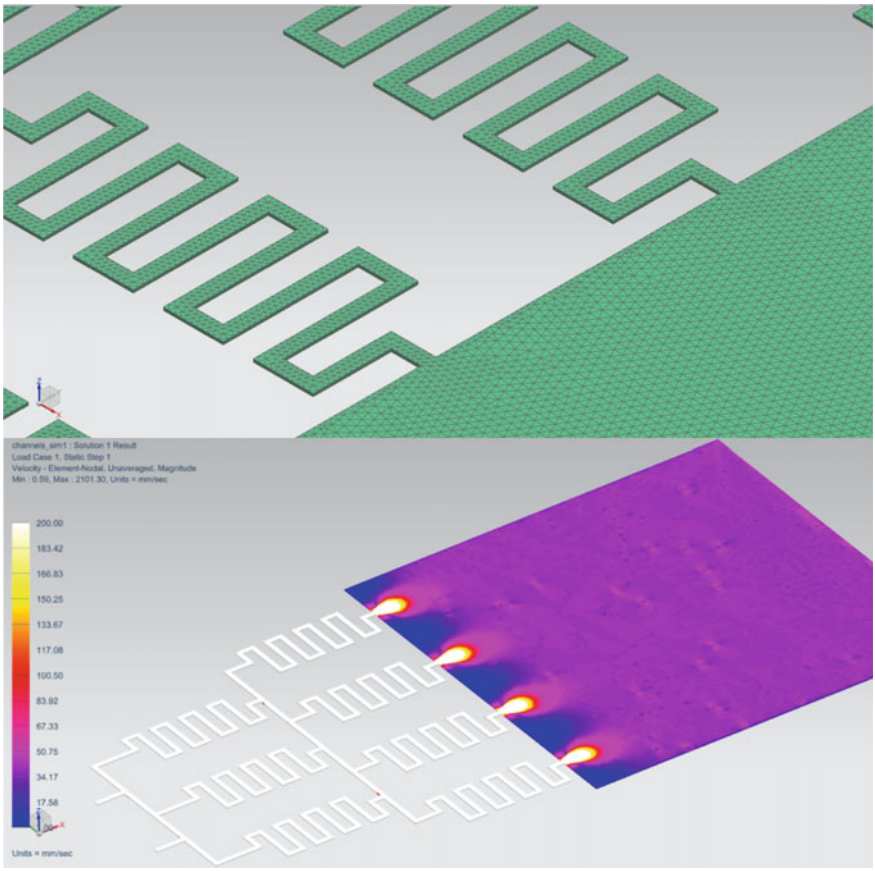

Fig. 12.6 Preliminary FEM based fluidic simulation of the performance of a microsystem for cell electrophoresis (mass flow: 0.1 g/s injected in each inlet). The results show the velocities field and the importance of culturing the cells sufficiently away from the entrances, so as to avoid turbulent phenomena. Simulations performed with NX-8.5 (Siemens PLM Solutions)

within the microsystem. As a consequence, water is used as a "material", and the inlet and outlet flow rates are applied as boundary – loading conditions. The performed FEM simulations help to assess the ideal flow rate to reach certain fluid velocities, which is directly connected with the desired shear stresses to be applied to cultured cells. These simulation results also evaluate the pressure losses along the microsystem, so as to select the pumping system and related operating conditions to meet the fluid flow needs.

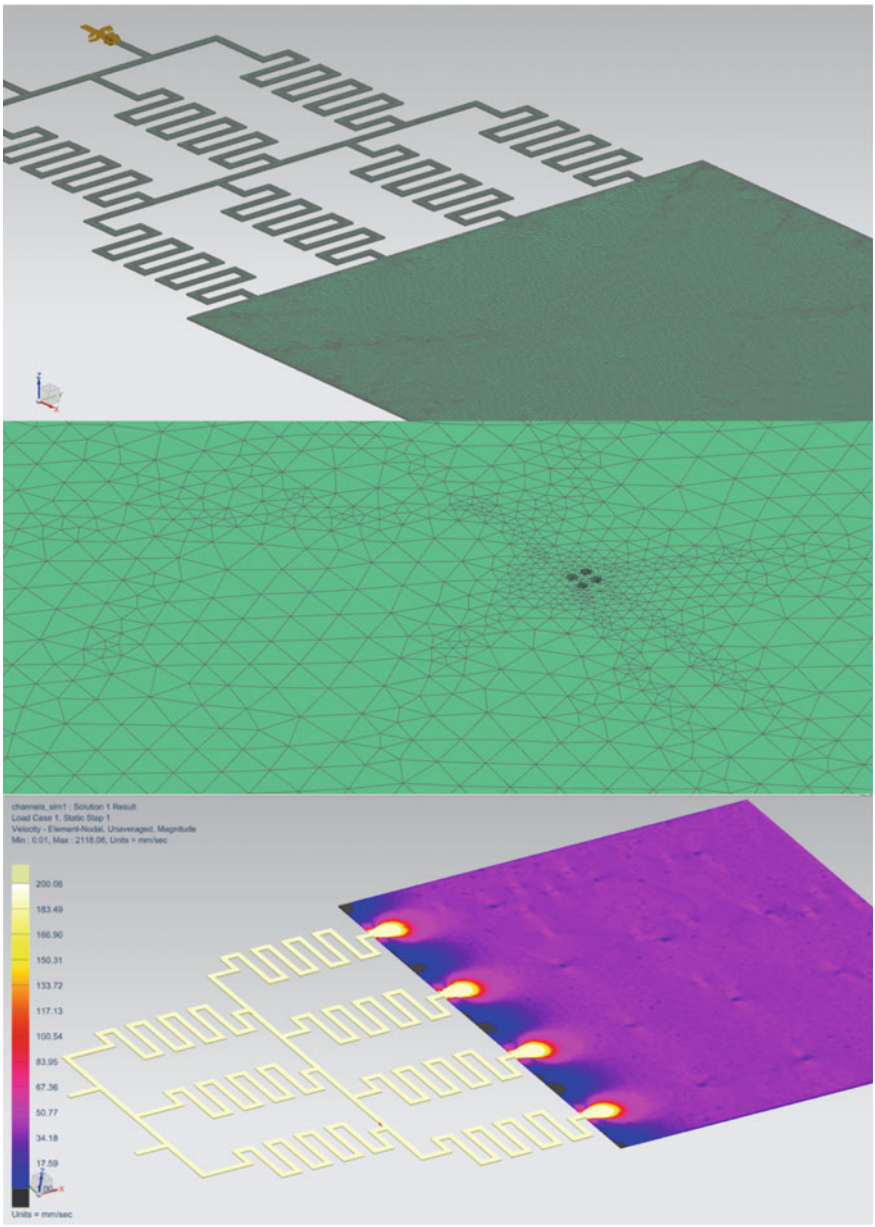

Fig. 12.7 FEM based fluidic simulation of the performance of a microsystem for cell electrophoresis considering the effect of the micropillars for fixing single cells (mass flow: 0.1 g/s injected in each inlet). The results show the velocities field and the importance of culturing the cells sufficiently away from the entrances, so as to avoid turbulent phenomena, as well as the slight influence of the micropillars and connecting elements. Designs and simulations performed with NX-8.5 (Siemens PLM Solutions)

12.5 Multi-culture Platforms for Studying the Interactions Between Different Cell Types

Additional requirements and difficulties appear when trying to obtain in vitro models of diseases affecting several types of cells and tissues or diseases linked to specific interactions between different cell types. Specific characteristics such as substrate stiffness and topography, surrounding fluid flow rate and related mechanical excitations, presence of growth factors and chemical gradients may be excellent for culturing some types of cells and tissues but may be harmful for others. In consequence, biomedical microsystems for modeling disease considering the interactions of different cell types are more complex to design, implement and operate, although some interesting solutions are already available, as discussed below, together with other remarkable potentials.

The most common commercial systems for co-culture are probably the Transwell® assays, normally comprised of a well with one type of cells and related cell culture medium at the bottom, upon which a permeable support (Transwell® support) is immersed with another cell type cultured upon the permeable support or micro-porous membrane. For instance, tumor-endothelium interactions have been studied using these assays (Khodarev et al. 2002). The membrane or permeable support can be also substituted by a filter, what has provided interesting results for co-culture of endothelial and neural cells (Abbott et al. 2012). Several microfluidic systems for co-culture have been also developed taking inspiration on these assays and co-culture strategies. They normally include a lower layer with fluidic channels for introducing nutrients and culturing one type of cells (i.e. endothelial, which benefit from shear stresses), an intermediate micro-porous membrane upon which another cell type is cultured and an upper closure chamber with additional biochemical agents (see Fig. 12.8). Other interesting devices (www.flocel.com), such as the Flocel Inc. in vitro model of the blood brain barrier, are comprised of micro-porous tubes immersed in a cylindrical chamber. Inside the tubes, endothelial cells are cultured under satisfactory flow-shear stress for simulating vasculature. Outside the tubes, astrocytes with adequate growth factors are cultured and interact with the endothelial cells through the tube pores.

In spite of the interesting results that can be obtained with the previously mentioned commercial co-culture micro-fluidic devices, the need of three layers in microsystems inspired on the Transwell® supports and the micro-porous tubes of the Flocel device, require complex manufacture technologies and can be simplified using just one functional bottom layer with the microfluidic structure and a simple lid (as in some of the examples provided further on, currently under development by our team, see Figs. 12.9, 12.10 and Chaps. 20–22). Instead of using intermediate porous membranes or porous fibers or tubes, an interesting possibility is to micromanufacture some gates or openings for connecting parallel adjacent channels and letting co-cultured cells interact.

In order to promote complexity and obtain even more biomimetic results, apart from co-culturing different cell types, mechanical and chemical stimuli can be also

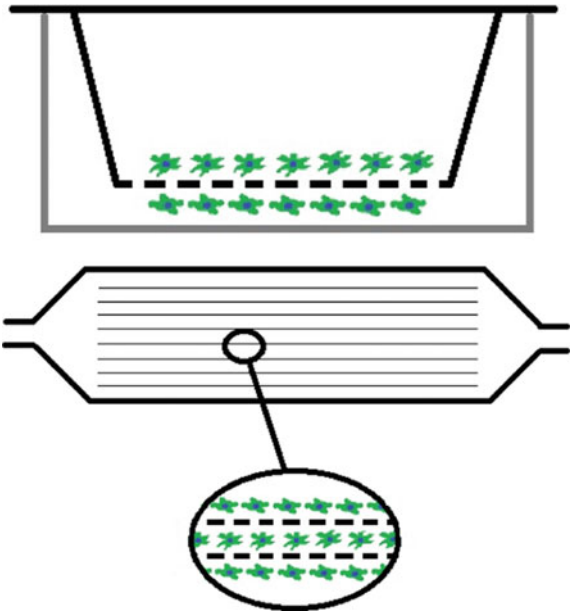

Fig. 12.8 Schematic description of Transwell® and Flocel devices. *Upper image* Transwell® assay, normally comprised of a well with one type of cells and related cell culture medium at the bottom, upon which a permeable support or micro-porous membrane is immersed with another cell type cultured atop. *Lower image* Flocel device, typically used for modeling the blood-brain barrier, comprised of micro-porous tubes immersed in a cylindrical chamber

applied and combined for reproducing disease conditions in vitro using aforementioned cell culture platforms, tissue engineering scaffolds and other biomedical microfluidic systems. Regarding the application of mechanical stimuli, it is a common practice to carry out scratches upon recently cultured tissues, so as to study wound healing processes and disease evolution (Kuo et al. 2012). In order to adequately model physiological conditions, as human tissues are continuously under different stresses, it is also common practice to introduce mechanical demands to cell culture platforms and microsystems for disease modeling including: mechanical vibrations, typically by means of piezoelectric resonators (Apicella and Aversa 2012); pulsatory fluid excitations, using peristaltic and diaphragm micropumps, which work using principles similar to those pumping principles in human organism (Voyvodic et al. 2012); and incorporation of artificially produced pressure losses, narrowings, blockages, leaks… to the microfluidic models, so as to mimic the effect of different pathologies (Wong et al. 2012).

The incorporation of biochemical stimuli is also a key factor for controlling the evolution of the cultured cells and the development of adequate disease models. The already mentioned use of growth factors, together with reprogramming factors, the generation of chemical gradients within the biodevices, the use of libraries of bacteria, fungi, parasites, prions and viruses, among other pathogens, and the

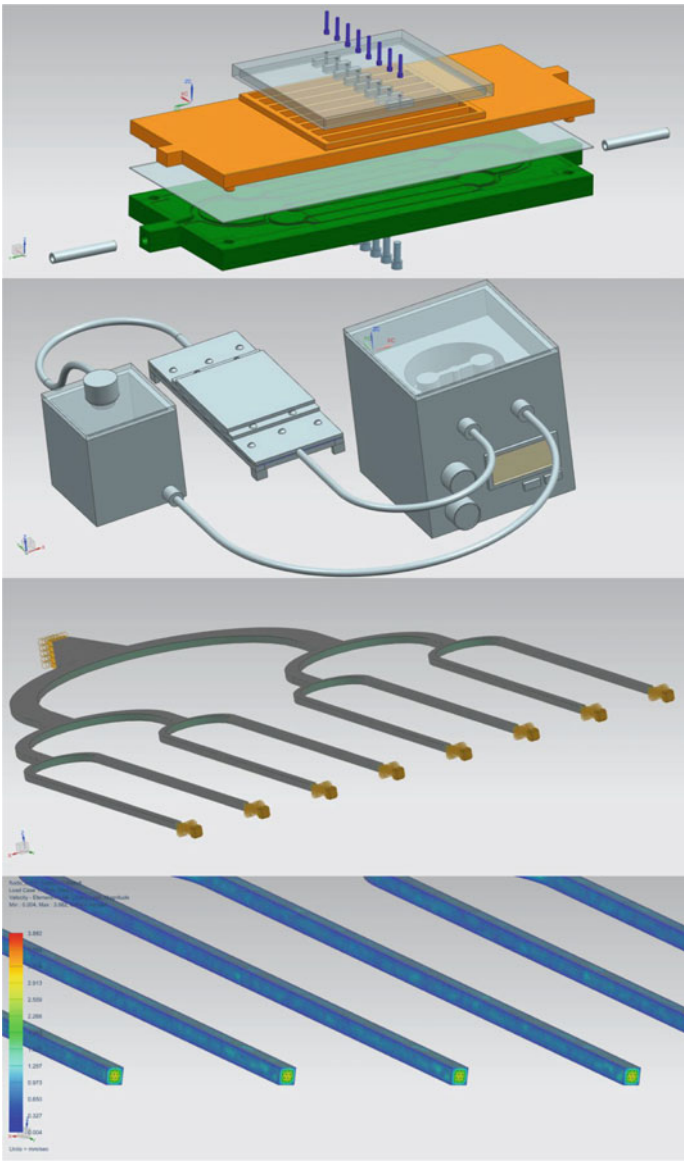

Fig. 12.9 Desing and FEM-based modeling of a complete dynamic cell culture platform for culturing different cell types under static and dynamic conditions

introduction of cancerous cells are just some possibilities of biochemically initiating a disease upon healthy cell cultures. In any case, very interesting possibilities arise when using microsystems capable of combining biomechanical and biochemical stimuli for controlled modulation. Some of the smallest and more functional

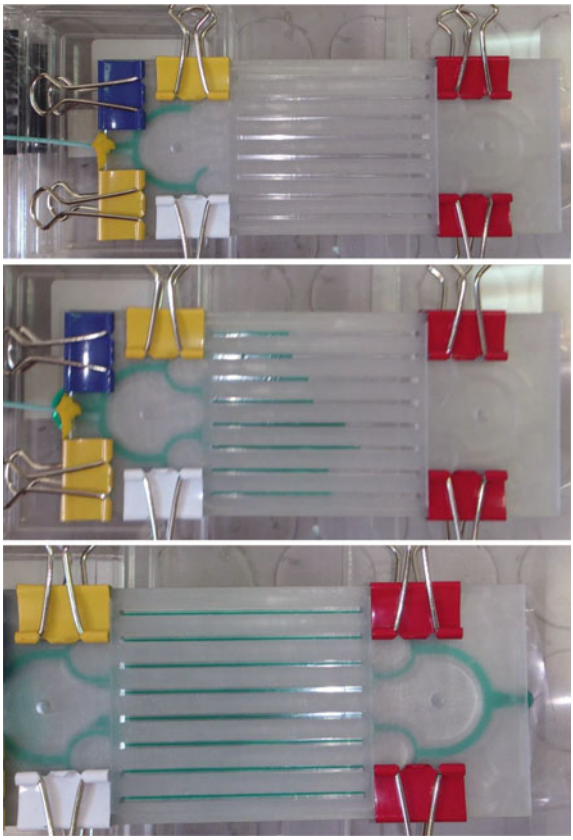

Fig. 12.10 Rapid prototype of the chambers of a dynamic cell culture platform and results from preliminary testing for validating the previously modelled flows

biomimetic models of physiological structures include both types of excitations, as the blood-brain barrier microfluidic model recently developed by a remarkably multidisciplinary team (Griep et al. 2013).

12.6 Case Study: Dynamic Cell Culture Platform

The combined use of computer-aided design resources and rapid prototyping facilities is also very useful for the development of ad hoc dynamic cell culture platforms, which can be adapted to the types of cells being cultured and to the physiological structures being studied. Simpler designs and more straight-forward manufacturing strategies, than those used for the dynamic cell culture system from previous Sect. 12.5, can be used, while also promoting an additional degree of versatility than using the more conventional commercial static culture systems.

In this Sect. 12.6 we present the complete development process of a dynamic cell culture system for studying interactions between the vasculature and the basic

tissular cells from different organs. The endothelial cells conforming the vascular networks irrigating our organs usually grow in a healthier biomimetic way if shear stresses from circulating fluids act upon them (Li et al. 2005). On the other hand, the cells of different organs, in spite of benefiting from the exchange of nutrients and debris with the surrounding vasculature, typically growth under more static culture conditions. In consequence, we propose a biomedical device, inspired on the Transwell device with slight modifications, consisting of a lower static cell culture chamber, an intermediate micro-porous membrane and an upper structure of channels for dynamic culture. The cells from different tissues can be cultured in the lower chamber and interact, through the micro-pores, with the endothelial cells cultured dynamically in the upper channelled structure.

The computer-aided design of the microsystem and the surrounding driving system (volumetric pump, connections and electrodes for monitoring the cell culture process) are shown in Fig. 12.9. The performed FEM simulations help to assess the most adequate flow rate for reaching certain fluid velocities directly linked with the most appropriate shear stresses for the dynamic cell culture. These simulation results help to evaluate the pressure losses along the microsystem, so as to select the more adequate pumping system. Figure 12.10 shows preliminary functional trials upon rapid prototypes obtained in epoxy resin by laser stereolithography.

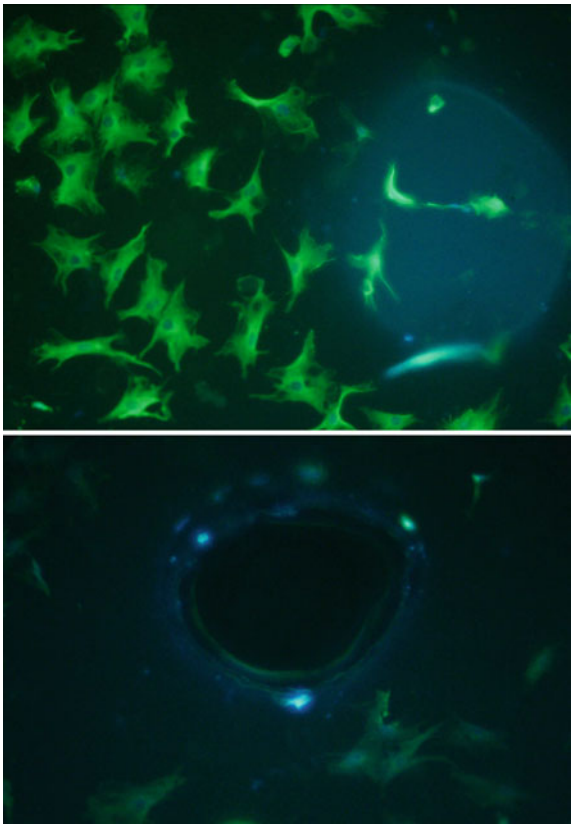

Fig. 12.11 hMSCs cultured upon the intermediate porous PDMS membrane of a dynamic cell culture platform showing the migration of cells towards the pores of the membrane and the capability of some cells of going through the membrane for eventually interacting with the cells cultured to the other side (Please consult Chap. 20 for details on the manufacture of micro-porous PDMS membranes.)

Figure 12.11 shows an in vitro validation of the system by culturing hMSCs upon the intermediate porous PDMS membrane of a dynamic cell culture platform and by highlighting the migration phenomena of cells towards the pores of the membrane, as well as showing some cells going through the membrane for eventually interacting with the cells cultured to the other side.

12.7 Main Conclusions and Future Research

Understanding how cells behave and interact with surrounding cells, tissues, microorganisms and all types of biological, biochemical and biomechanical cues from their environment, constitutes a relevant research challenge and requires the support, not only of advanced manipulation and imaging technologies, but also of specifically designed biomedical microsystems with micrometric and even nanometric details for enabling interactions at a cellular and molecular level.

These types of microsystems, together with the use of advanced design and manufacturing strategies for their efficient development, constitute the core topic of present Handbook. State-of-the-art microsystems aimed at interacting with and studying the behavior of cells, include: dishes for 2D culture, microsystems for studying cells under chemical gradients, electrophoretic microsystems and multi-culture platforms and devices for cell co-culture and dynamic bioreactors or cell culture platforms.

This chapter has provided an introduction to these types of biomedical microdevices, illustrating them by means of different cases of study, including details linked to the design, manufacturing and testing processes. Main current research trends have been also outlined. Other emerging and more complex microsystems for interacting with cells and controlling their behavior and fate, even with the potential of constructing whole tissues and organs from cultured cells, are covered in depth in Chaps. 13–23. However, their eventual success is still based on the knowledge already generated and the potentials detected by means of state-of-the-art microdevices, such as those covered in present chapter.

References

Abbott NJ, Dolman DE, Drndarski S, Fredriksson SM (2012) An improved in vitro blood-brain barrier model: rat brain endothelial cells co-cultured with astrocytes. Methods Mol Biol 814:415–430

Apicella A, Aversa A (2012) A biomimetic and biomechanical approach for tissue engineering: hybrid nanomaterials and a piezoelectric tunable bending apparatus for mechanically stimulated osteoblast cells growth. Biodevices 2012:280–285

Chan CKF, Chen CC, Luppen CA, Kraft DL, Kim JB, De Boer A, Wei K, Helms JA, Kuo CJ, Weissman IL (2009) Endochondral ossification is required for hematopoietic stem cell niche formation. Nature 457(7228):490

Clyne AM, Urbano R (2016) An inverted dielectrophoretic device for analysis of attached single cell mechanics. Lab chip 16:561–573

Díaz Lantada A, Alarcon Iniesta H, García-Ruíz JP (2015) Multi-channelled polymeric microsystem for studying the impact of surface topography on cell adhesion and motility. 7 (11):2371–2388

Doyle AD, Wang FW, Matsumoto K, Yamada K (2009) One-dimensional topography underlies three-dimensional fibrillar cell migration. J Cell Biol 184(4):481–490

Griep LM, Wolbers F, De Wagenaar B, Ter Braak PM, Weksler BB, Romero IA, Couraud PO, Vermes I, Van der Meer AD, Van den Berg A (2013) BBB on chip: microfluidic platform to mechanically and biochemically modulate blood-brain barrier function. Biomed Microdevices 15(1):205–230

Khodarev NN, Yu J, Labay E, Darga T, Brown CK, Mauceri HJ, Yassari R, Gupta N, Weichselbaum RR (2002) Tumor-endothelium interactions in co-culture: coordinated changes of gene expression profiles and phenotypic properties of endothelial cells. J Cell Sci 116:1013–1022

Kuo CT, Chiang CL, Huang RYJ, Lee H, Wo AM (2012) Configurable 2D and 3D spheroid tissue cultures on bioengineered surfaces with acquisition of epithelial–mesenchymal transition characteristics. NPG Asia Mater 4:e27

Li YS, Haga JH, Chien S (2005) Molecular basis of the effects of shear stress on vascular endothelial cells. J Biomech 38(10):1949–1971

Maher PS, Keatch RP, Donnelly K (2010) Characterisation of rapid prototyping techniques for studies in cell behaviour. Rapid Prototyping J 16(2):116–123

Morss Clyne A, Urbano R (2015) Dielectrophoretic devices for analysis of cell mechanics. US Patent, US 2015/0285760 A1

Rosenthal A, Voldman J (2005) Dielectrophoretic traps for single-particle patterning. Biophys J 88:2193–2205

Tholudur A, Giron L, Alam K, Thomas T, Garr E, Weatherly G, Kulowiec K, Quick M, Shepard S (2006) Comparing automated and manual cell counts for cell culture applications. Bioprocess Int 28–34

Voldman J (2006) Electrical forces for micro-scale cell manipulation. Annu Rev Biomed Eng 8:425–454

Voyvodic PL, Min D, Baker AB (2012) A multichannel dampened flow system for studies on shear-stress mediated mechanotransduction. Lab Chip 12(18):3322–3330

Vunjak-Novakovic C, Scadden DT (2011) Biomimetic platforms for human stem cell research. Cell Stem Cell 8(3):252–261

Wimmer K, Harant H, Reiter M, Blüml G, Gaida T, Katinger H (1994) Two-dimensional gel electrophoresis for controlling and comparing culture supernatants of mammalian cell culture productions systems. Cytotechnology 16(3):137–146

Wong KHK, Chan JM, Kamm RD, Tien J (2012) Microfluidic models of vascular function. Annu Rev Biomed Eng 14:205–230

Yao X, Peng R, Ding J (2013) Cell-material interactions revealed via material techniques of surface patterning. Mater Views 25:5257–5286

Chapter 13
Microstructured Devices for Studying Cell Adhesion, Dynamics and Overall Mechanobiology

Andrés Díaz Lantada, Adrián de Blas Romero, Josefa Predestinación García Ruíz, Hernán Alarcón Iniesta, Stefan Hengsbach and Volker Piotter

Abstract The fact that cellular development and fate is very dependent, not just of the biochemical signals of the environment and of their own genetic background, but also of the mechanical properties and mechanical stimuli acting within the extra cellular matrix, has promoted the birth of a new field of science and technology at the interface of biology and engineering, that of mechanobiology. Such novel field focuses on the way that physical forces, stresses and strains, and changes in cell or tissue mechanics contribute to tissue development, to the success of physiological interactions and even to the appearance of disease. A major challenge in the field is linked to understanding the complex mechanisms by which cells sense and respond to mechanical signals: the mechanotransduction properties of cells. Although completely understanding how cells respond to mechanical stimuli and learning about their mechano-sensitive properties and about the mechanical forces they can develop is a complex task, the use of biomedical microdevices with controlled microstructures and microtextures can be a useful strategy for the progressive comprehension of single and collective cell behavior. This chapter provides some introductory examples of microsystems developed for generating knowledge for the field of mechanobiology. The cases of study include cell culture platforms for obtaining different types of cell aggregations, biomedical microsystems for studying the impact of surface texture on cell behavior and some final textured surfaces with details reaching nanometric details.

A. Díaz Lantada (✉) · A. de Blas Romero
Mechanical Engineering Department – Product Development Laboratory, Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

J.P. García Ruíz · H. Alarcón Iniesta
Department of Molecular Biology, Universidad Autónoma de Madrid, Madrid, Spain

S. Hengsbach · V. Piotter
Institute of Microstructure Technology, Karlsruhe Institute of Technology, Hermann von Helmholtz Platz 1, Eggenstein–Leopoldshafen, Germany

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13.1 Introduction to Mechanobiology and Its Connections with Tribology

A key element involved in tissue engineering processes is the extra cellular matrix or scaffold which serves as substrate or framework for cell growth, aggregation and tissue development (Langer and Vacanti 1993). These scaffolds must be porous so as to allow cell migration during the colonization process as well as the transport of nutrients and waste to and from cells, but they have to be also resistant enough to withstand possible mechanical demands, especially if final scaffold (or device) implantation is desired.

Additionally, as cells are able to feel their microenvironment and substrate texture upon which they lie by changing their morphology, cytoskeleton configuration, and intra- and extracellular signaling, increasing efforts are continuously being focused on advanced design and manufacturing technologies, so as to generate and modify the structures and surfaces of biomaterials. Aspects such as porosity, pore size, and surface microtexture promote cell adherence, migration and proliferation within the scaffold, for subsequent differentiation into relevant cell types. Thus, tissue progenitor cells and the scaffold plays a fundamental role in most tissue engineering strategies as its properties can deeply influence the global success of new tissue formation and the controlled fabrication of the scaffold structures is becoming increasingly important for novel approaches within regenerative medicine (Thomas et al. 2010; Chen et al. 2010; Buxboim and Discher 2010).

The fact that cellular development and fate is very dependent, not just of the biochemical signals of the environment and of their own genetic background, but also of the mechanical properties and mechanical stimuli acting within the extra cellular matrix, has promoted the birth of a new field of science and technology at the interface of biology and engineering, that of mechanobiology.

Mechanobiology focuses on the way that physical forces, stresses and strains, and changes in cell or tissue mechanics contribute to cell and tissue development, to the success of physiological interactions and even to the appearance of disease. A major challenge in the field is linked to understanding the complex mechanisms by which cells sense and respond to mechanical signals: the mechanotransduction properties of cells (Jacobs et al. 2012).

Cells and tissues can be therefore considered “smart materials and structures” capable of sensing and actuating as a consequence of external stimuli. The possibility of controlling their response by means of adequate stimuli opens new horizons, even beyond mechanobiology, for the development of cell-based sensors and actuators, as discussed in Chap. 21.

Although completely understanding how cells respond to mechanical stimuli and learning about their mechano-sensitive properties and about the mechanical forces they can develop is a complex task, the use of biomedical microdevices with controlled microstructures and microtextures can be a useful strategy for the progressive comprehension of single and collective cell behavior.

For instance, devices with micropillars have been found useful for measuring cell forces during movement, implants with different microtextures have led to different biological responses and osseointegration rates and microsystems with textured channels have shown the impact of roughness on cell dynamics.

In consequence, mechanotransduction is profoundly connected with the field of tribology, the science aimed at studying contact phenomena, including adhesion, lubrication and wear. Fundamental aspects for an adequate tribological performance of materials and components, such as surface roughness, material hardness, material elasticity, among others, also affect cell behavior and should be taken into account as parameters of influence for mechanobiology, as well as for the design of microsystems aimed at studying cell-material interactions, cell dynamics and cell development into relevant tissues. Micro- and nano-manufacturing resources and surface functionalization techniques also affect the tribological properties of materials and structures and play a key role in the development of microdevices for studying, understanding and modeling cell behavior and fate.

The following sections provide some examples of microsystems developed for generating knowledge for the field of mechanobiology.

13.2 Microstructured and Microtextured Systems for Studying Cell Mechanobiology

As previously mentioned, devices with micropillars have been found useful for measuring cell forces during movements (Trichet et al. 2012). In addition, devices with micropillars can be also submitted to resonance for applying controlled stimuli upon the cells attached to them. These micro-pillar structures can be manufactured resorting to several procedures, including subtractive ones, such as the use of UV-photo-lithography and chemical etching, and additive ones, such as the employment of high precision stereolithography, among other options (see Chap. 8 for further information on micro-fabrication of biomedical microdevices).

To show the potential of these micro-structured devices, Fig. 13.1 includes the finite-element model and the simulation results of a single cell interacting in different ways with a micro-pillar platform used as culture substrate for studying cell mechanobiology. These FEM-assisted simulations show the deformations of the cell during its expansion and during its movement and the related micro-pillar deformations. The lower images compare the displacement and stress solutions for the same loading case and boundary conditions (the cell crawling). The designs, models and simulations are performed using NX-8.5 (Siemens PLM Solutions).

The most relevant aspect of these types of studies is that the displacements and deformations of pillars help to assess the forces performed by the cells (Sun and Fu 2013) during their development, growth, movement, interaction with the surrounding materials, gene expression, reproduction, evolution towards relevant

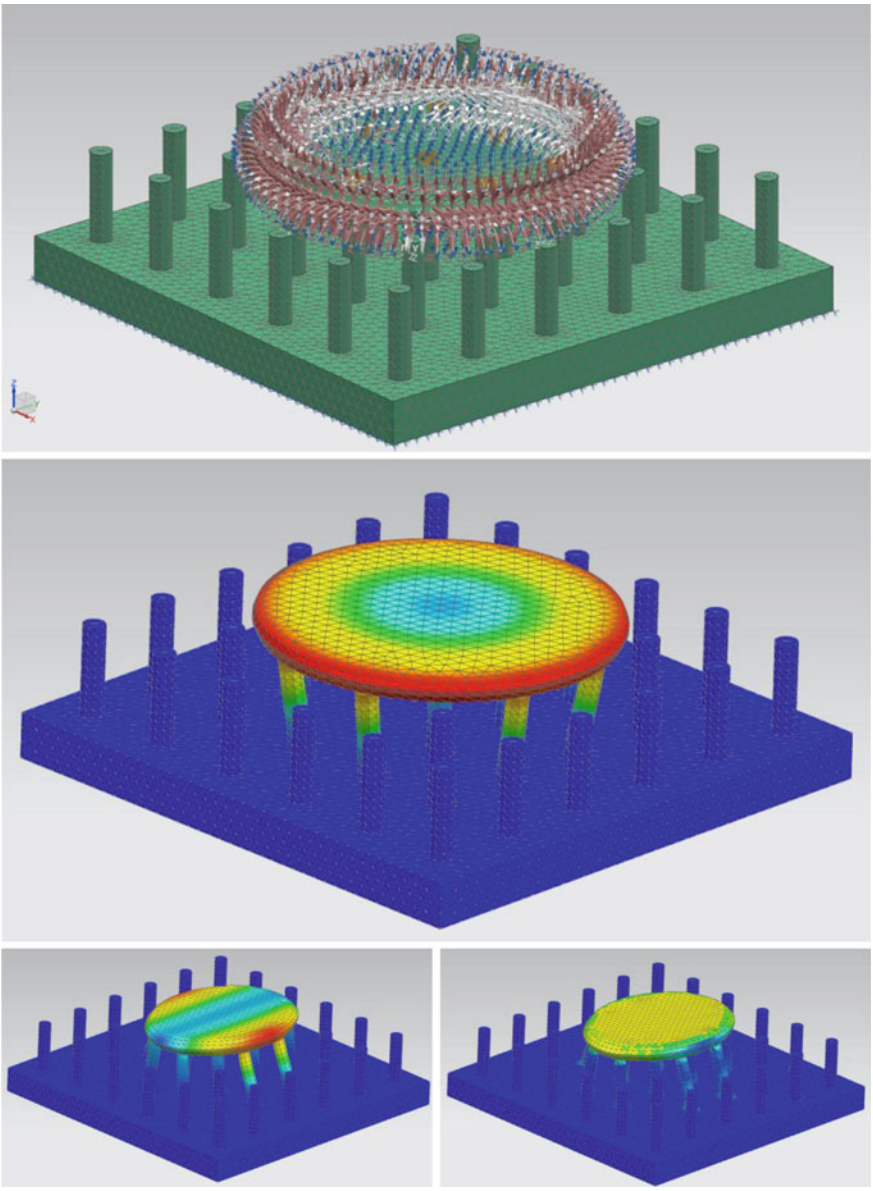

Fig. 13.1 Finite-element model and simulation results of cellular interactions with a micro-pillar platform for studying cell mechanobiology. Simulations show the deformations of a cell during expansion and movement and the related micro-pillar deformations. *Lower images* compare the displacement and stress solutions for the same loading case and boundary conditions (cell crawling). The deformation of pillars helps to assess the forces performed by the cells. Simulations performed with the help of NX-8.5 (Siemens PLM Solutions)

tissues and death. Using high-precision imaging systems and related software resources, the deformations and stresses can be monitored in real time and relevant information regarding cell behavior can be obtained.

13.3 Microtopographies and Their Impact on Cell Behavior

The use of microtopographies is not only useful from a mechanobiological point of view, for obtaining useful information on the stresses and strains that cells can develop and on the mechanical aspects of cell-material interactions, but also for helping cells behave in a desired way, for promoting interesting cell-cell interactions and even for controlling the final tissues formed. Several studies have focused on the importance of surface topography and microtexture for promoting positive effects in all kinds of biomedical devices (De la Guerra et al. 2012), from implantable prosthesis to extra cellular matrixes and scaffolds for cell growth and tissue engineering. These textures have a significant influence in osseointegration of prosthesis, cell proliferation and tissue growth; given that those cells and tissues seem to be more "comfortable" and spread more quickly when faced with biodevices with similar surface properties. In addition the use of biomimetic surfaces can help to introduce numerous desirable phenomena in machine, mechanical and structural elements, thus improving contact between parts, reducing wear or even obtaining self-cleaning objects (Barthlott and Neinhuis 1997; Groenendijk 2007).

Consequently, topography also plays a determinant role in material selection in engineering design, especially in the field of micro and nanosystem development for biomedical engineering, where the effects of topography on the incorporation of advanced properties are even more significant. More specifically, in the field of tissue engineering and regenerative medicine, surface topography plays a determinant role in several strategies, both in vitro and in vivo, as it influences the attachment of cells to substrates for in vitro studies and to extra cellular matrices and implants aimed at tissue repair. It also helps control cellular dynamics and serves to orient cellular proliferation, aggregation and differentiation and has an impact on final tissue formation (Francesco et al. 2010; Kumar et al. 2012). As already introduced, cells are able to feel the microenvironment and substrate texture on which they develop, by changing their morphology, cytoskeleton configuration, intra- and extracellular signaling, and gene expression, therefore increasing efforts are continuously being focused on advanced design and manufacturing technologies capable of generating and modifying the structures and surface topographies of biomaterials (Thomas et al. 2010; Chen et al. 2010; Buxboim and Discher 2010).

However, the process of introducing desired roughness on the surfaces of man-made objects is still mainly linked to carrying out machining operations, laser processing or chemical attacks. In all these cases, post-processing operations can be difficult to control and it would be very positive to directly impose special

topographies from the design stage. Fortunately, as detailed in Chap. 8, advances in computer-aided design and in high-precision additive manufacturing technologies, based on layer-by-layer deposition or construction, are opening new horizons for controlling the topographies of surfaces. They are being used from the design stage and can be applied in a manner that is very direct, rapid and simple. This is enabling the prototyping of multi-scale designs and hierarchical structures.

Even though conventional computer-aided design packages are only capable of handling Euclidean geometries and mainly rely on simple operations (sketch based operations, extrusions, pads, holes, circular grooves, etc.) for obtaining "soft" solids and surfaces, recent approaches relying on the use of matrix-based programming have already proved to be useful for designing rough surfaces and textured objects adequately described by fractal geometries (Mandelbrot 1982; Falconer 2003).

In parallel, the continued progress in additive manufacturing technologies, especially during the last decade, has increased the range of materials capable of being additively processed and greatly promoted their precision, even down to nanometric features. This has implications in the development of advanced materials and metamaterials, many of which benefit from multi-scale approaches (Bückmann et al. 2012; Röhrig et al. 2012).

Some development examples have been already provided in Chap. 8. In this section we provide additional cases of study, linked to the complete development of cell culture platforms, with controlled surface topography. They are aimed at obtaining different types of cell aggregations with potential for the development of different types of tissues.

The cell culture platforms with soft and wavy surfaces shown in Fig. 13.2 are designed with the help of conventional CAD software (Solid Edge v.18), while those with fractal features are obtained by the processes previously described in Chaps. 6 and 8. Prototyping is accomplished in two steps by means of additive photopolymerization combined with a final diamond-like carbon coating obtained by physical-vapour deposition, in order to promote biocompatibility. Additional details can be found elsewhere (Díaz Lantada et al. 2012).

In vitro assessment is carried out using h-MSCs (human mesenchymal stem cells) expanded, seeded upon the cell culture platforms and visualized in a fluorescence inverted microscope (Olympus IX81) coupled to a CCD camera, following previously described processes (Javed et al. 2000; Romero-Prado et al. 2006). Results, summarized in Fig. 13.2, show the clear impact of surface topography on cell behavior and aggregation. The more planar substrate leads to a more expanded growth, while substrates with micro-bumps lead to aligned cell aggregations, which may be of interest for mimicking 3D cell dynamics. Larger topographies promote isolated compact growth, in the valleys of the microstructure, forming spherical aggregations, with potential application for promoting differentiation into osteocytes for bone repair. Regarding the impact of the DLC coating, it is important to highlight that it dramatically promotes the cell-scaffold interaction, helping to resolve the typical problems of photopolymeric resins with acrylic-based chemistry for in vitro trials and medical applications.

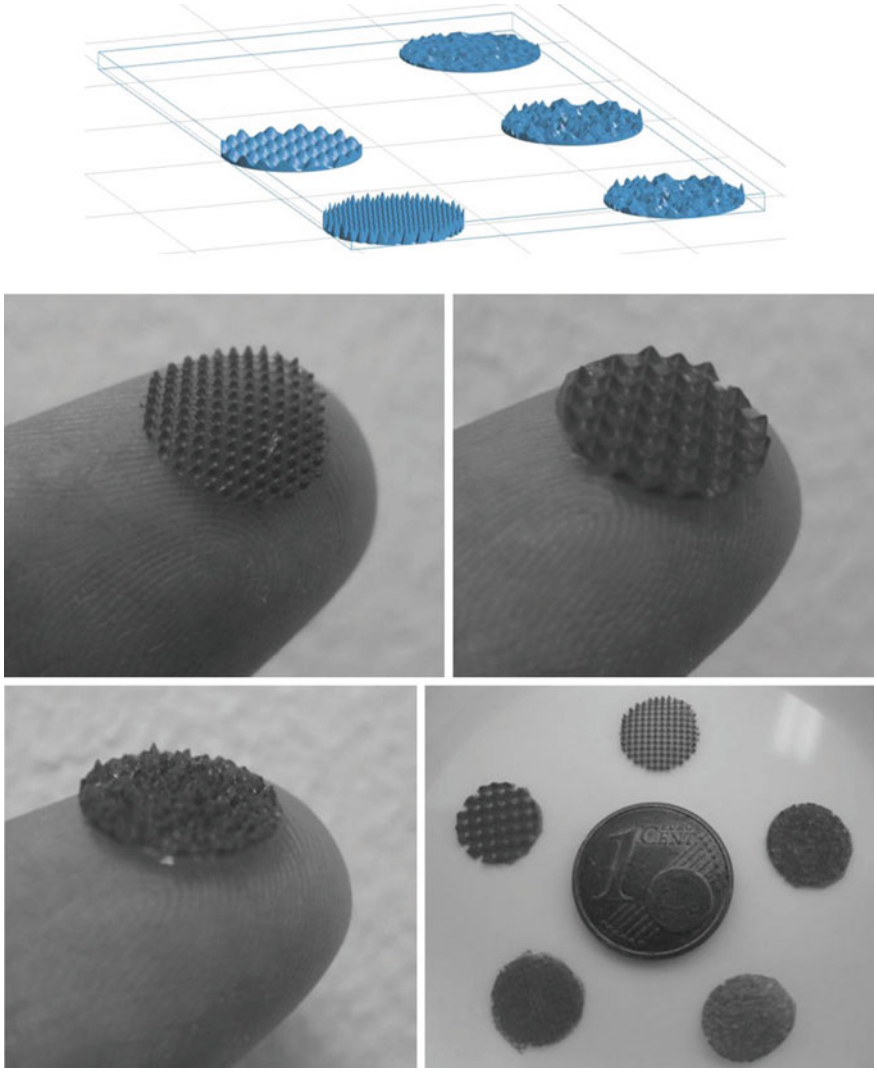

Fig. 13.2 Computer-aided designs and rapid prototypes of different cell culture platforms with predefined micro-topographies for studying cell behavior

Next sections further explore the topic, providing also an example of a microsystem for studying the impact of surface topography on cell motility and detailing current capabilities of high-precision additive manufacturing for the development of biomedical microdevices capable of mechanically interacting at a cellular and even molecular level (Fig. 13.3).

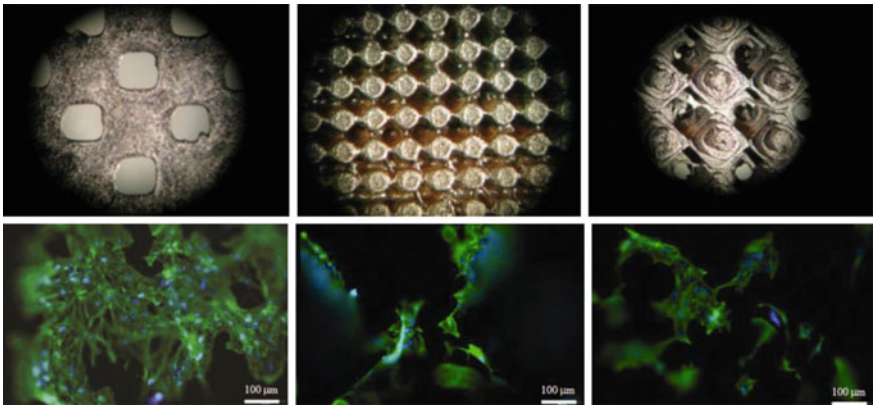

Fig. 13.3 Influence of surface topography on h-MSC adhesion, behaviour and aggregation as preliminary steps towards relevant tissues

13.4 Case Study: Microsystem for Studying the Impact of Microtextures on Cell Behavior

The proposed microsystem of present section includes two microchambers connected by several microchannels to guide cell movement, each with a different texture defined from the design stage at its bottom. The cell motility experiment should begin adding cells (with or without growth factors or nutrients) to one of the chambers and, eventually, adding growth factors, nutrients, chemicals, pathogens… to the other one, so as to promote or prevent cell movement from one chamber to another (details: Díaz Lantada et al. 2015).

The design presented here is obtained by means of design and manufacturing procedures detailed in Chaps. 6, 8–10. It is adapted to scales well suited to interacting at a cellular level and to enabling the cell culture processes without resorting to ultra-high precision manipulators.

In short, the overall structure, which mainly comprises the different walls of the two pools and the six microchannels, has been designed using conventional 3D computer-aided design methods. The CAD files can be converted into .stl (standard tessellation language) format, currently the most common file type used in 3D additive manufacturing. Different technologies, including digital light processing, conventional laser stereolithography, selective laser sintering, direct laser writing or melting and fused deposition modeling, allow .stl file as information input. The specific method chosen depends on the desired material and precision.

In our case we use an EnvisionTEC digital light processing machine with acrylic resin for the master prototypes. The 3D design can also be converted into a black-white mask for 2D½ manufacture of the overall structure using lithographic approaches typical of the electronic industry. Subsequently, to incorporate the desired microtextures (capable of interacting at a cellular level) into the channels,

additional design operations rely on the generation of fractal-based geometries via matrix-based approaches.

In such matrix-based designs the geometries are stored in the form of [X, Y, Z(x, y)] matrices, where X and Y are column vectors with the x and y components of the working grid, and Z(x, y) is a column vector whose components are the height values for each (x, y) couple (spherical and cylindrical coordinates can be used for the cases of spherical and cylindrical meshes). Then, fractal features can be introduced to incorporate controlled random textures into the initially regular meshes (z_0), as previously detailed in Chap. 6. We use a fractional Brownian fractal surface model for defining the microtextures and control them by changing the value of the typical parameter "alfa" from one channel to another.

The microtextures obtained following this strategy are more similar to the surfaces of biological materials than artificial textures based on pillars, struts, wood-pile structures or similar designs based on Euclidean geometry. An additional planar control channel is included for purposes of comparison.

The channels are 300 μm wide and around 7 mm long and the pools have a volume of $4 \times 2 \times 0.6$ mm^3. The intermediate walls between channels are 300 μm wide and 200 μm high, which in some cases promotes cell escape from some channels, but otherwise proves to be an adequate design. Surface roughness goes from 130 μm in the "softest" fractal channel to 250 μm in the roughest fractal channel. The planar channel can be just used as control if desired.

The master models are manufactured using digital light processing, a high precision rapid prototyping technology working on an additive approach that layer by layer projects on a photopolymer, images corresponding to the slices of the three-dimensional objects being built. For that purpose a Perfactory SXGA machine (EnvisionTec GmbH) is used, together with the R11 EnvisionTec acrylate based photo-resin. Figure 13.4 includes the master prototypes (red-orange resin) directly obtained from the three-dimensional geometries stored in the .stl computer-aided design files. As the acrylic resin used for obtaining the master models is not apt for cell culture processes, replication technologies are used for producing the final micro injected parts in poly(methyl methacrylate) (PMMA).

Using the master model, a mold insert for is manufactured. In short, the process includes gluing the masters to a thick copper substrate and evaporation for coating the master and substrate with layers of 7 nm chromium and 50 nm gold. This is followed by: (i) immersion in a galvanic bath for nickel electroplating until a thickness of 6 mm is reached, (ii) separation from the substrate and (iii) cutting and rinsing steps with ethyl acetate and acetone. The process leads to a stiff homogeneous metal block which can withstand the forces applied in the injection molding process. Additional details can be found elsewhere (Díaz Lantada et al. 2014, 2015).

The first action before starting the injection molding trials is to adjust the electroplated nickel mold inserts to a standard mold. Replication is accomplished on a Ferromatik Elektra 50S injection molding machine, which is equipped with the necessary features, such as tool evacuation and vario-thermal-temperization. This procedure allows for the replication of very fine structures with outstanding surface

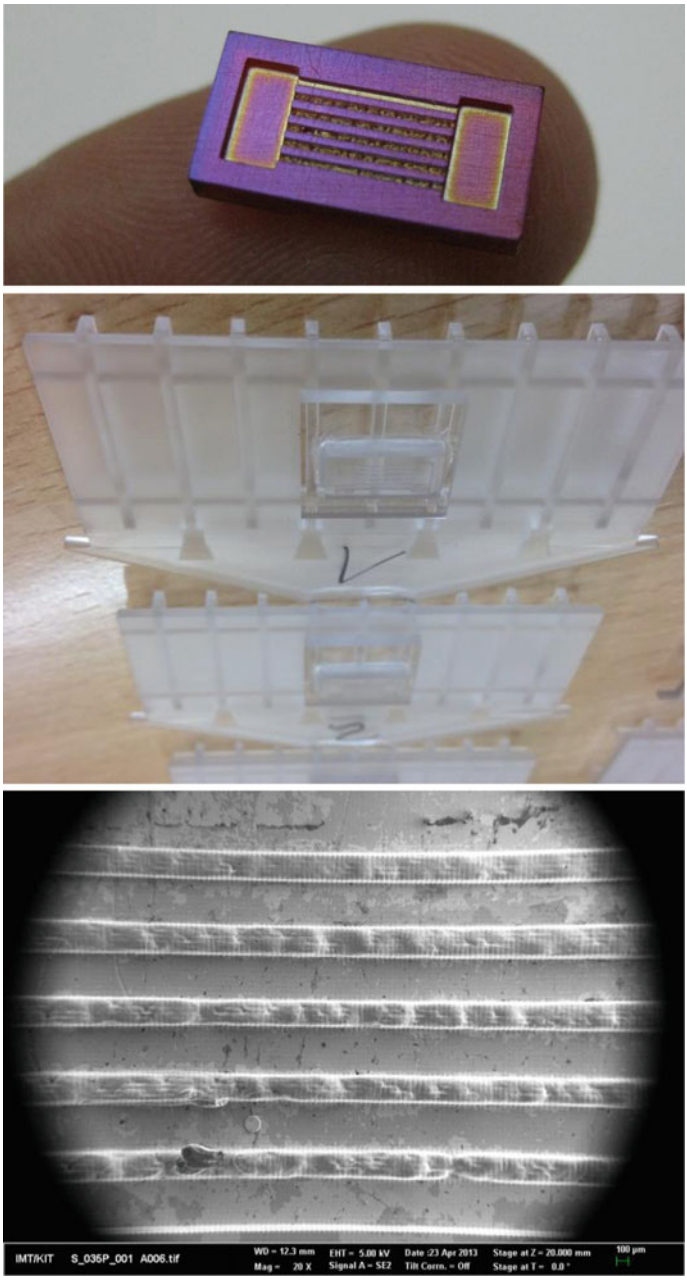

Fig. 13.4 Multi-channelled biomedical microsystem for studying the impact of surface texture on cell adhesion and motility. Master model, PMMA microinjected replica and microscopy of the microsystem before the cell culture trials

qualities. Figure 13.4 shows a replicated PMMA sample. Micro injection molding stands out for the degree of precision attainable and for the possibility of manufacturing large series of replicas for systematic trials. In our case, 200 copies of the PMMA microsystem were obtained and at least 10 of them were used to adjust the cell-culture processes.

The repeatability is outstanding and the final parts are compact, but without some of the typical injection molding problems, such as the presence of pores or wrapping, in spite of the precise dimensions of interest. The replicas obtained have several advantages when compared with the original masters. They are made of bioinert polymers typically used in the medical industry (polycarbonate and poly (methyl methacrylate)), and are therefore adequate for in vitro trials. They are also transparent, which constitutes an enormous help for cell culture processes and related fluorescent microscopy tasks, while it is easier to manipulate them thanks to a supporting structure.

In vitro assessment is carried out using h-MSCs (human mesenchymal stem cells) expanded, seeded upon the cell culture platforms and visualized in a fluorescence inverted microscope (Olympus IX81) coupled to a CCD camera, following previously described processes (Javed et al. 2000; Romero-Prado et al. 2006). Results are summarized in Figs. 13.5 and 13.6, which respectively show the impact of channel microtexture on cell adhesion and on cell motility.

According to the presented results, an increase on surface roughness promotes cell adhesion, with an almost linear tendency. This result is in accordance with the adherent nature of mesenchymal stem cells; an increase in the channel roughness allows them to extend the number of their focal adhesion points with the surface, thereby helping them maintain their energy balance by themselves.

Regarding cell motility, our interpretation is that the presence of an artificial surface roughness may promote opposing effects on the cells. On the one hand, the mere presence of obstacles and artificial textures may be sensed by the cells as something that must be avoided to maintain their energy metabolism. On the other

Fig. 13.5 Impact of channel microtexture on cell adhesion

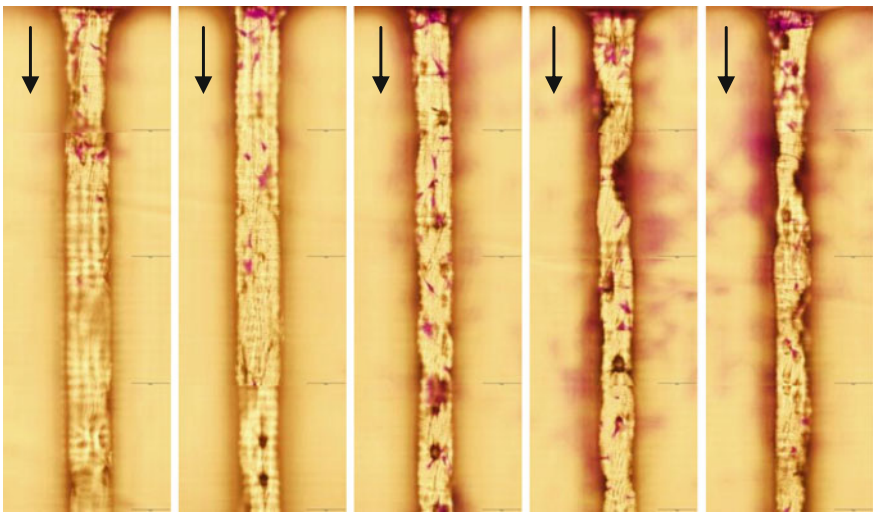

Fig. 13.6 Impact of channel microtexture on cell motility

hand, the increase in the surface to volume ratio and adhesion with the roughness and the fractal dimension may provide additional anchorage options for cell pseudopodia and help them crawl. At lower roughnesses the first effect seems to be prevalent, while at higher roughnesses the second effect stands out.

Taking into account that the cells have been cultured for 24 h in the motility study and that they have not reached the final pool, the proposed microsystem may also be useful for quantifying not only the impact of surface topography on cell adhesion, motility and behavior, but also for obtaining an estimation of cell velocity on different surfaces. In the microsystem, hMSCs reach a velocity value of 6000 μm/day, helping to show the positive impact of incorporating design-defined fractal microtextures to promote cell motility and eventual tissue growth.

In our opinion, the use of design-defined microtextured channels provides a more three-dimensional approach, in accordance with recent results, highlighting that 1D cell culture is more similar to 3D conditions than more conventional 2D cell culture on planar surfaces (Doyle et al. 2009). According to Doyle and colleagues, cell migration in both 1D and 3D is rapid, uniaxial and independent of extra cellular matrix ligand density, in contrast to 2D cultures, which brings out the need for alternative solutions aimed at a more adequate reproduction of the 3D environment (Doyle et al. 2009). We truly believe that our approach of providing 1D cell culture along channels, with inner three-dimensional features, can help with further studies linked to comparing 1D, 2D and 3D cell culture, as well as to take into account non-integer values, using a fractal-based definition of dimension. The noteworthy values of cell velocity obtained help to verify the potential of these kinds of design combinations.

The presented culture microsystem is aimed at the promotion of a real 3D cell culture environment and the microchannels may help cells to crawl in the adequate direction for a systematic cost- and time-efficient study. In fact, recent studies have put forward that 1D cell culture resembles the real behavior of cells in three-dimensional environments, than conventional 2D cell culture on dishes.

Combining manufacturing technologies in order to obtain similar microsystems to the one proposed here, but covering a wider range of surface topographies, from hundreds of nm to hundreds of μm, will be useful for increasing our knowledge of cell dynamics and response to surface topography. We expect to achieve this in forthcoming studies in collaboration with all colleagues that may find this approach of interest or, at least, of help as a complement to their current methods. Fractal-based design approaches, together with additive manufacture and replication techniques, may well indeed be useful for controlling such textures from the design stage, as detailed in the forthcoming section.

13.5 Case Study: Microtextured Platforms for Studying and Controlling Cell Behavior and Fate

Towards more precise studies, capable of addressing the mechanical behaviour of biological structures at sub-cellular and molecular levels, microsystems with nanometric features are required. Conventional additive manufacturing resources, commonly know as 3D printers, do not provide the required precision and novel high-precision processes are needed. Currently, two-photon polymerization is the most precise additive manufacturing technology and enables details down to nanometric scale. When combined with advanced computer-aided design and with additional processes for surface modification (see Chaps. 6, 8 and 9), very remarkable results can be obtained, regarding the development of biomedical microsystems for interacting at cellular and even molecular level.

The rough surfaces of Fig. 13.7 are designed using a fractional Brownian fractal surface model evaluated upon a working grid, in which points are separated 1 μm, as detailed in Chap. 6 and in previous microsystems with controlled roughnesses. The use of additional “mesh to solid” converters leads to final solid files, as those shown in Fig. 13.8, which can be used as normal CAD parts for further design, simulation and computer-aided manufacturing tasks.

The process can be adapted to the surfaces of any computer-aided designed implant and multi-scale designs are possible, normally using more conventional Euclidean surfaces for micrometric–millimetric features and the addition of the fractal term for the 100 nm–10 μm range. Hence, biomimetic approaches are promoted.

Even though converting the generated surfaces into solid .stl files is almost direct with CAD resources, subsequent geometry slicing (a typical operation of the software used for controlling layer by layer manufacturing machines) leads to very

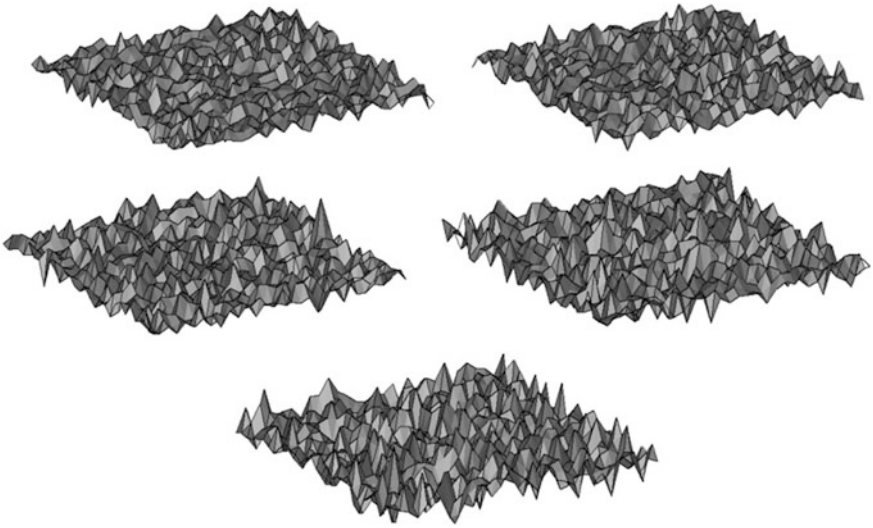

Fig. 13.7 Fractal surfaces based on fractional Brownian models. Surface topography can be controlled using a single parameter

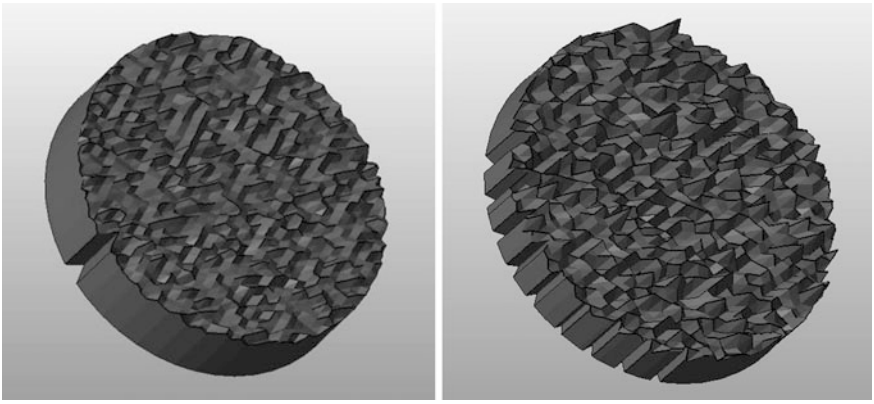

Fig. 13.8 Three-dimensional solid designs of microtextured scaffolds for the systematic assessment of the impact of surface topography on cell behavior

slow and expensive manufacturing processes. In our case, for speeding up the manufacturing process, the surfaces are obtained upon supporting pillars, as shown in the prototypes from Fig. 13.9. With this pillar-based design, a fractal surface of $40 \times 40\ \mu m^2$ can be manufactured in just 30 min, increasing production speed in more than an order of magnitude, when compared with the initial solid model, and reducing material and laser power consumption. The prototypes of the nano-textured surfaces are manufactured using the Photonic Professional System

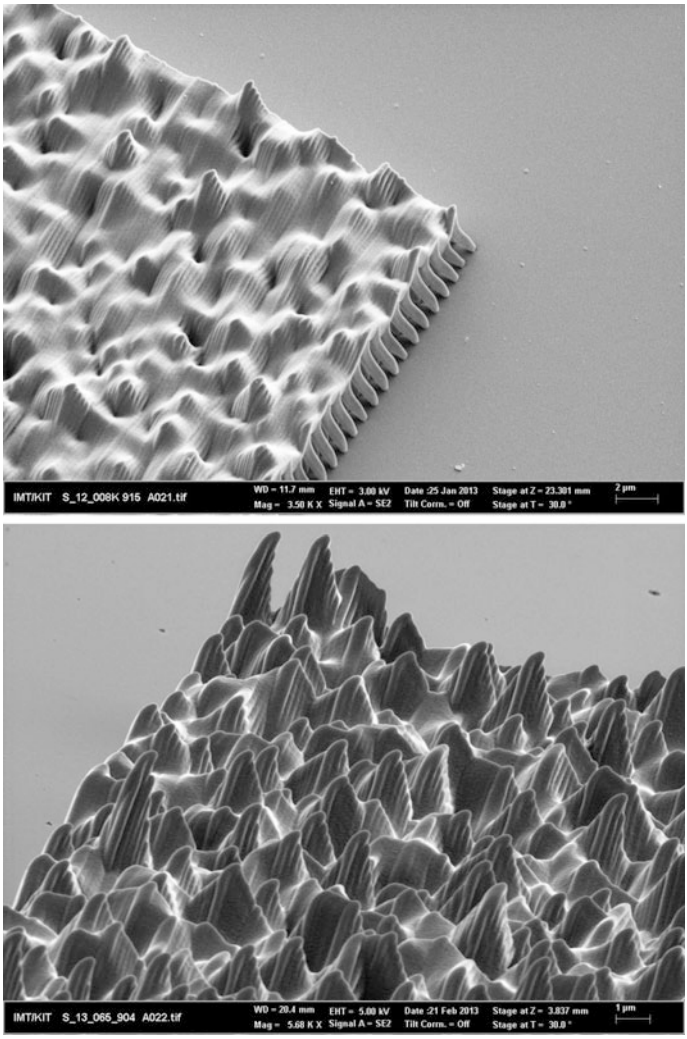

Fig. 13.9 Textured surfaces with nanometric details obtained by direct laser writing. Roughness can be controlled from the design stage and details are even smaller than single eukaryotic cells. Process developed in collaboration between Universidad Politécnica de Madrid and the Karlsruhe Institute of Technology, with support from the KNMF—Karlsruhe Nano-Micro Facility (http://www.knmf.kit.edu/) (additional details: Hengsbach and Díaz Lantada 2014)

from NanoScribe GmbH (www.nanoscribe.de), the first commercial direct laser writing system.

In this system, the structures are not written layer-by-layer, but following three-dimensional paths connected from the beginning to the end of the writing process, so additional programming for converting the original CAD files into

writable structures is usually needed. In any case, the attainable degree of precision is outstanding and opens new horizons in the field of biomimetic development of biomedical microsystems.

Multi-scale approaches are also possible, by combining different prototyping technologies, a more conventional 3D printer for the larger details and 2PP for the tiniest features (Hengsbach and Díaz Lantada 2014).

13.6 Main Conclusions and Future Research

Although completely understanding how cells respond to mechanical stimuli and learning about their mechano-sensitive properties and about the mechanical forces they can develop is a complex task, the use of biomedical microdevices with controlled microstructures and microtextures can be a useful strategy for the progressive comprehension of single and collective cell behavior.

This chapter has tried to provide some introductory examples of microsystems developed for generating knowledge for the field of mechanobiology. The cases of study detailed include cell culture platforms for obtaining different types of cell aggregations, biomedical microsystems for studying the impact of surface texture on cell behavior and some final textured surfaces with details reaching nanometric details.

As for the future, the use of the described procedures and technologies for the control of surface topography at micro- and nano-metric levels and its application to larger biomedical microdevices, with more complex geometries for studying cell-material and cell-cell interactions and for in vitro reproducing physiological phenomena is just a matter of time.

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References

Barthlott W, Neinhuis C (1997) Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta 202:1–8

Bückmann T, Stenger N, Kadic M, Kaschke J, Frölich A, Kennerknecht T, Eberl C, Thiel M, Wegener M (2012) Tailored 3D mechanical metamaterials made by dip-in direct-laser-writing optical lithography. Adv Mater 24:2710–2714

Buxboim A, Discher DE (2010) Stem cells feel the difference. Nat Methods 7(9):695

Chen WL, Likhitpanichkul M, Ho A, Simmons CA (2010) Integration of statistical modeling and high-content microscopy to systematically investigate cell-substrate interactions. Biomaterials 31:2489

De la Guerra Ochoa E, Del Sordo Carrancio D, Echávarri Otero J, Chacón Tanarro E, Díaz Lantada A, Lafont Morgado P (2012) The influence of textured surfaces on the lubrication of artificial joint prostheses. In: Biodevices 2012—international conference on biomedical electronics and devices. IEEE Engineering in Medicine and Biology Society

Díaz Lantada A, Endrino JL, Sánchez Vaquero V, Mosquera AA, Lafont Morgado P, García-Ruíz JP (2012) Tissue engineering using novel rapid prototyped diamond-like carbon coated scaffolds. Plasma Process Polym Online (4 Oct 2011). doi:10.1002/ppap.201100094; 9(1):98–107, 2012

Díaz Lantada A, Piotter V, Plewa K, Barié N, Guttmann M, Wissmann M (2014) Towards mass production of microtextured microdevices: Linking rapid prototyping with micro injection molding. Int J Adv Manuf Technol. doi:10.1007/s00170-014-6333-2

Díaz Lantada A, Alarcon Iniesta H, García-Ruíz JP (2015) Multi-channelled polymeric microsystem for studying the impact of surface topography on cell adhesion and motility. 7 (11):2371–2388

Doyle AD, Wang FW, Matsumoto K, Yamada K (2009) One-dimensional topography underlies three-dimensional fibrillar cell migration. J Cell Biol 184(4):481–490

Falconer K (2003) Fractal geometry: mathematical foundations and applications. Wiley

Francesco G, Tirinato L, Battista E, Causa F, Liberale C, Di Fabrizio EM, Decuzzi P (2010) Cells preferentially grow on rough substrates. Biomaterials 31(28):7205–7212

Groenendijk M (2007) Self cleaning Lotus leaf imitated in plastic by using a femtosecond laser. University of Twente. www.physorg.com

Hengsbach S, Díaz Lantada A (2014) Rapid prototyping of multi-scale biomedical microdevices by combining additive manufacturing technologies. Biomed Microdevices 16(4):617–627

Jacobs CR, Huang H, Kwon RY (2012) Introduction to cell mechanics and mechanobiology. Garland Science

Javed A, Guo B, Hiebert S, Choi JY, Green J, Zhao SC, Osborne MA, Stifani S, Stein JL, Lian JB, van Wijnen AJ, Stein GS (2000) Groucho/TLE/R-esp proteins associate with the nuclear matrix and repress RUNX (CBF(alpha)/AML/PEBP2(alpha)) dependent activation of tissue-specific gene transcription. J Cell Sci 113(12):2221

Kumar G, Waters MS, Farooque TM, Young MF, Simon CG Jr (2012) Freeform fabricated scaffolds with roughened struts that enhance both stem cell proliferation and differentiation by controlling cell shape. Biomaterials 33(16):4022–4030

Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920

Mandelbrot B (1982) The fractal geometry of nature. W.H. Freeman, San Francisco

Röhrig M, Thiel M, Worgull M, Hölscher H (2012) Hierarchical structures: 3D direct laser writing of nano-microstructured hierarchical gecko-mimicking surface. Small 8(19):3009–3015

Romero-Prado M, Blázquez C, Rodríguez-Navas C, Muñoz J, Guerrero I, Delgado-Baeza E, García-Ruíz JP (2006) Functional characterization of human mesenchymal stem cells that maintain osteochondral fates. J Cell Biochem 98:1457

Sun Y, Fu J (2013) Mechanobiology: a new frontier for human pluripotent stem cells. Integr Biol 5 (3):450–457

Thomas WE, Discher DE, Shastri VP (2010) Mechanical regulation of cells by materials and tissues. MRS Bull 35:578

Trichet L, Le Digabel J, Hawkins RJ, Vedula SK, Gupta M, Ribrault C, Hersen P, Voituriez R, Ladoux B (2012) Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness. PNAS 109(18):6933–6938

Chapter 14
Smart Microsystems for Active Cell Culture, Growth and Gene Expression Toward Relevant Tissues

Andrés Díaz Lantada, Enrique Colomer Mayola, María Consuelo Huerta Gómez de Merodio, Alban Muslija, Josefa Predestinación García Ruíz and Hernán Alarcón Iniesta

Abstract Cells and tissues respond to several mechanical properties and mechanical stimuli of their extra cellular matrix and surrounding environment, as well as to gradients of them, including Young's modulus, surface topography, hardness, presence of vibrations, among other external influences studied in the evolving field of cell mechanobiology. Interestingly, clear differences are perceived between cell culture processes carried out in static and dynamic conditions and even cell differentiation and fate can be controlled by means of such dynamic cultures. The use of fluid flows for the generation of dynamic culture conditions is common, as detailed in the chapters devoted to labs-on-chips and organs-on-chips. Here we focus on the use of mechanical vibrations for the promotion of dynamic cell culture processes and detail main challenges linked to producing such types of actuations upon devices with micrometric features. Issues linked to the design, modeling, manufacture and testing of microdevices for achieving resonant behaviors for dynamic cell cultures are detailed. The difficulties of using micro-resonators working at MHz frequencies, especially regarding experimental validation, are detailed and some proposals for successful results and further research are provided.

A. Díaz Lantada (✉) · E.C. Mayola · M.C. Huerta Gómez de Merodio
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

A. Muslija
Institute of Microstructure Technology, Karlsruhe Institute of Technology,
Hermann von Helmholtz Platz 1, Eggenstein–Leopoldshafen, Germany

J.P. García Ruíz · H. Alarcón Iniesta
Department of Molecular Biology, Universidad Autónoma de Madrid, Madrid, Spain

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14.1 From Cells to Tissues: Passive Versus Active Cell Culture

The global behavior and final fate of human stem cells is not just based on their genetic information, but is also regulated by other biochemical and mechanical signals (epigenetic cues), which come from their micro-environment. Such local micro-environment that provides physical and chemical support and signals for survival and regulation is commonly referred to as stem-cell niche. Therefore, for adequately replicating the 3D stem cell niche, biochemical components including cytokines, growth and trophic factors, generated by cells themselves or by their surrounding companions, and other chemical factors (i.e. presence of oxygen) have to be taken into account.

In addition, several physical forces from the surrounding environment, such as tensile and compressive stresses, vibratory excitations, fluid shear stresses and the presence of electro-magnetic fields and even of gravitational forces have to be considered. Other elements, such as the presence of companion stem cells and the differentiated cell types from the adjacent tissue, clearly affect stem cell dynamics, overall behavior and fate. Finally, the physical and biochemical properties of the extracellular matrix, such as stiffness, porosity, viscoelasticity, roughness, surface topography and composition, regulate stem cell function (Caplan 1991, 2008; Shamadikuchaksaraei et al. 2014; Lanza et al. 2014).

Passive cell culture is usually carried out upon Petri dishes to study disease, to analyze the impact of drugs, to assess interactions with other cell and pathogens, among other applications. However, being completely planar surfaces, it is clear that the cell-culture environment achieved by using a Petri dish is not a real 3D cell-culture system. In consequence, cells do not interact with their environment (pathogens, other cells, extra cellular matrix components…) as within the body and the quality of the information obtained is not as perfect as desired.

As an alternative, much attention is being paid and great research efforts are being applied to the development of biomimetic platforms for human cell research (Vunjak-Novakovic and Scadden 2011), in many cases resorting to the concept of tissue engineering scaffolds and 3D cell-culture materials and environments or 3D cell-culture niches. Indeed, the niche composition and 3-D structure play an important role in stem cells state and fate, as well as the incorporation of adequate growth factors and conditioned media (Chan et al. 2009). These materials, substrates, niches and scaffolds can be used for passive cell culture, by placing them in micro-wells loaded with buffer media, nutrients and cells, and monitoring how cells adhere to the culture platform, proliferate, carry out genetic expression and differentiate into relevant tissues.

Purely passive interactions between the stem cells and their micro-environment promote interesting phenomena. For instance, the surface micro-topography and elasticity of the cell culture platform, perceived by the cells as the extracellular matrix, is able to affect the differentiation of MSCs and to motivate them to choose different fates.

Interestingly, microtopographies promoting the adhesion of cells in rounded forms are more adipogenic, while those promoting sharper sub-cellular curvatures are more osteogenic. Increased cell culture densities promote adipogenesis and prevent osteogenesis. Substrates with elasticity values around 1 kPa (very soft) promote neuronal fates, while those with values around 10 kPa (still soft) promote differentiation into muscles and those more rigid substrates, typically above 100 kPa, promote bone formation. These and many additional interactions between cells and materials have been recently reviewed (Yao et al. 2013). Cases of study from Chaps. 12, 13 and 15–19 also provide a wide set of examples regarding h-MSCs cultured within inactive biomedical microdevices for Tissue Engineering, as well as discussions about their dynamics, behavior and potential destiny linked to the in vitro development of relevant tissues.

Nevertheless, the use of active culture systems, normally based on the forced circulation of fluids for shear stress application, on the application of electro-magnetic stimuli and on the stimulation by means of cyclic or vibratory strains and stresses, can provide an additional degree of control upon stem cell behavior and fate, as detailed in the following sections.

14.2 State-of-the-Art Devices for Active Cell Culture

The dynamic stimuli of active cell culture systems promote special responses and phenotype expressions, which can be extremely important for enhancing the effectivity, efficiency and sustainability of Tissue Engineering procedures. The fact that fluid shear stresses may promote endothelial (Wang et al. 2005) or osteogenic (Liu and Hu 2010; Liu et al. 2010) differentiations depending on the level of stress, on the presence of pulsatile flows and on the incorporation of adequate growth factors, among other parameters of influence, helps to put forward the versatility of active cell culture systems, but also provides an indication of the complexity of Cell Biology and Tissue Engineering.

The development of new active biomedical microdevices for active cell culture, with which the relevant parameters of influence can be controlled, to assess their impacts and mutual interactions in a systematic way, is importantly helping with the development of Tissue Engineering as field of study. Among existing devices for active cell culture, it is important to mention active cell culture platforms and scaffolds, typically obtained by incorporating networks of actuators and sensors for the introduction of dynamic stimuli and for the monitoring of the cell culture process (Apicella and Aversa 2012).

Special attention must be paid to bioreactors, engineering fluidic systems made of a vessel or reactor tank with inlets and outlets of fluid and gases controlled by pumps and thermal systems, which are designed to support biologically active processes, such as cell growth. Aspects including temperature, fluid flow rate, pH,

dissolved gases…, affect cell growth and their interactions with other cells, with portions of cultured tissues and with potential pathogens. Microfluidic devices used for active cell culture can be also considered bioreactors (see Chap. 20).

14.3 Case Study: Resonant Cantilevers and Bridges for Cell Culture Promotion and Monitoring

Microsystems with micrometric cantilevers and bridges, typically obtained by means of micro-manufacturing procedures, combining lithography and etching steps, commonly used in the electronic industry (see Chap. 8), are very useful for carrying out active cell culture processes. Actuated by means of piezoelectric ceramic or polymeric films and controlled by microprocessors, it is quite straight-forward to adjust (and control in real-time) the excitation intensity and frequency to the natural resonant frequencies of the micrometric cantilevers and bridges, so as to modulate the mechanical stresses and strains applied to the cells during their culture. By excitating different vibration modes and by changing the biological rhythm, cell-cell communication can be influenced and stem cell gene expression, potentiality and fate can be modulated (Muehsam and Ventura 2014).

Regarding the design, manufacture and testing of resonant microdevices for cell culture promotion and monitoring, it is important to note that the combined use of computer-aided design and engineering resources, including the application of finite element modeling, helps to develop these kinds of systems and to assess the impact of incorporating the ceramic actuators (Díaz Lantada 2011). Besides, the use of piezoelectric materials as the active part of these devices, also promotes self-sensing approaches, thanks to the possible use of piezoelectrics as actuators and sensors. During cell culture, expansion and tissue formation, the mass upon the cantilevers and bridges is dynamically changing (increasing) and consequently producing a decrease on the natural resonance frequencies of the micro-structures, which can be monitored, as a hi-tec control strategy for cell culture processes (Park et al. 2010).

Figure 14.1 shows the finite-element models and related simulation results of bridged and cantilever micro-structures, designed to carry out active cell culture processes and to actuate mechanically upon the cells (or tissues) attached to them, for potential control of gene expression and related tissue development in vitro.

These simulations have been performed with the help of NX-8.5 (Siemens PLM Solutions). These first simulation examples show the performance of both microsystems (overall displacements are shown), when a pressure is applied to the bridged or cantilever structure, being in consequence a simple static simulation for performance evaluation. These static evaluations are interesting to assess the stresses and strains within the structures and attached cells and to predict the limits of the structure, before concentrating on other more complex loading cases or on the dynamic performance of the structures. They also help to predict the required levels of deformation for excitating the cells and such information can be also of interest, when designing work benches to test these types of structures.

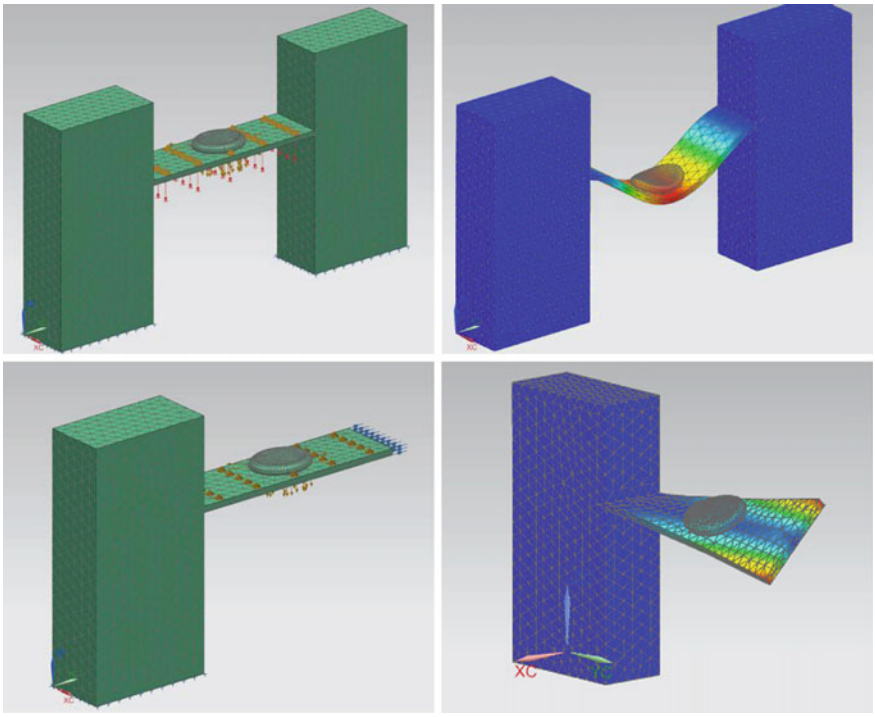

Fig. 14.1 Finite-element models and related simulation results of bridged and cantilever micro-structures designed to carry out active cell culture processes and to actuate mechanically upon the cells attached to them for potential control of gene expression and related tissue development in vitro. Displacements are shown. Simulations performed with the help of NX-8.5 (Siemens PLM Solutions)

Additionally focusing on the resonant cantilever, Fig. 14.2 shows the finite-element simulation results of a resonant cantilever micro-structure designed to carry out active cell culture processes. The shapes of four different vibration modes of the cantilever and the related displacements imposed to the cell, during the in vitro active culture process, are shown. The resonant excitations, for testing the micro-structure and for performing active in vitro cell culture experiments, can be obtained by means of piezo actuators attached to the structure of the cantilever or to the foundations of the workbench. Setups similar to the one described further on, in Sect. 14.5, can be implemented and the excitation frequencies can be tuned to those capable of promoting resonances according to the desired modes of vibration. Depending on the mode, the cells being cultured will be subject to very different excitations, from uni-axial to multi-axial, which can affect cell growth, gene expression and final tissue development. These FEM-based simulations help to calculate the excitation frequencies associated to the different modes and to predict the strains and stresses applied to the micro-structures and to the cells.

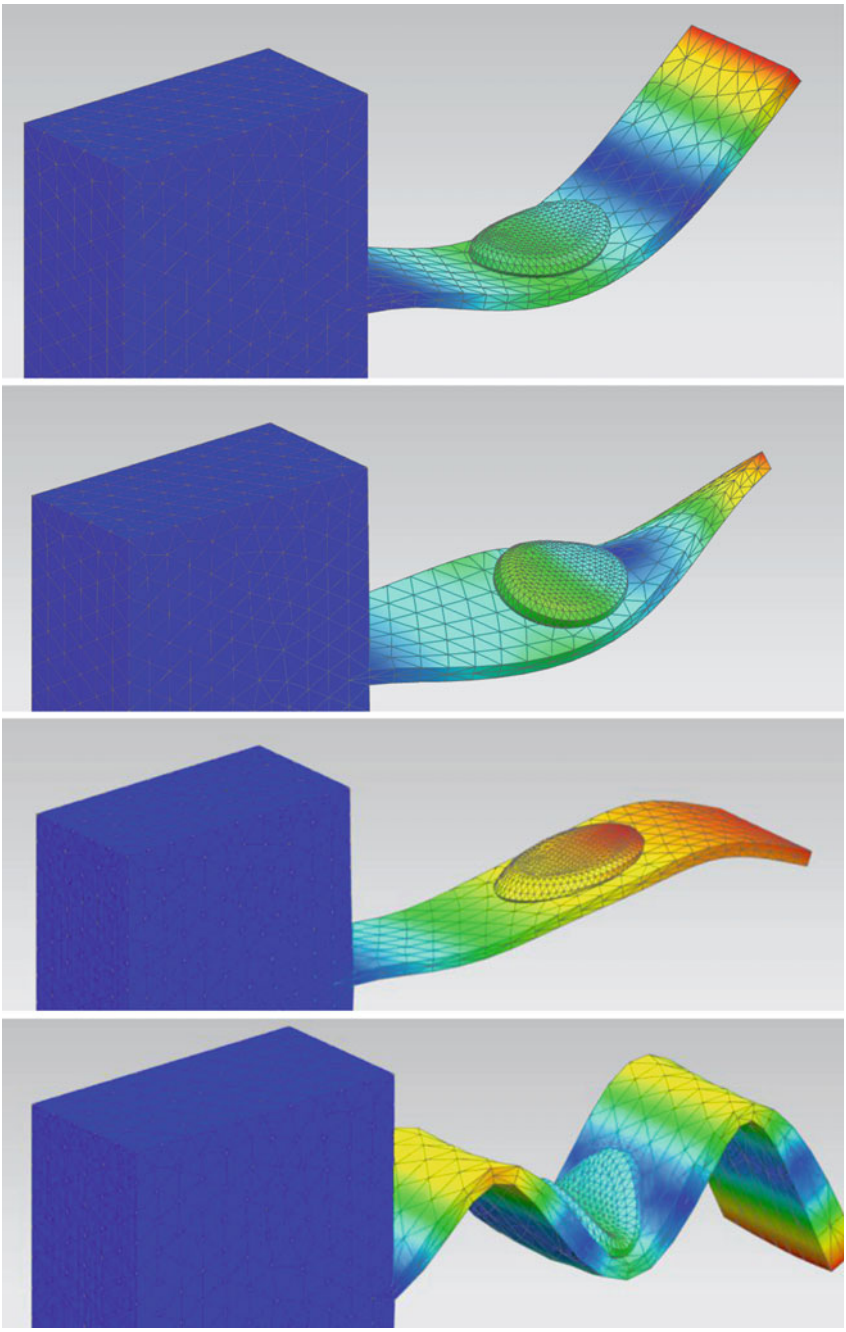

Fig. 14.2 Finite-element simulation results of a resonant cantilever micro-structure designed to carry out active cell culture processes. The shapes of four different vibration modes of the cantilever and the related displacements imposed to the cell, during the in vitro active culture process, are shown. Simulations performed with the help of NX-8.5 (Siemens PLM Solutions)

14.4 Case Study: Resonant 2D Lattices for Cell Culture Promotion and Monitoring

A typical limitation of resonant cantilever and bridges for dynamic cell culture is the fact that cells are normally uniaxially stretched. In many cases, cells from real tissues, as in the case of cardiac muscles, are bi-axially or multi-axially stretched and, in consequence, multi-axial excitation of stem cells may lead to very special types of gene expression and differentiation.

Auxetic materials and structures (or auxetic metamaterials) are those with a negative Poisson ratio and display the unexpected property of lateral expansion when stretched, as well as an equal and opposing densification when compressed (Lakes 1987; Evans et al. 1991; He et al. 2005; Liu and Hu 2010; Liu et al. 2010). Natural materials (some minerals, skins…) and synthetic ones (foams, Gore-Tex®…) showing auxetic properties have been described and special attention has been paid, since their discovery, to the search and development of auxetic structures designed and controlled on a molecular scale (Wojciechowski 1987; Evans et al. 1991 and Griffin et al. 2005). It is important to clarify that auxetics, understood as materials with negative Poisson's ratio, are not only consequence of their special geometries, but also of interactions with external conditions and constraints, such as negative pressure, proximity of certain phase transitions, specially woven materials, living tissues and their surroundings, polydispersions, among other possibilities described in the seminal papers of this field (Wojciechowski 1989, 2003; Hirotsu 1991; Narojczyk and Wojciechowski 2010).

Tissue engineering, with interactions at a cellular and even molecular level, can also benefit from the use of auxetic structures (Soman et al. 2012). During cell culture, uniaxial excitations of an auxetic scaffold lead to biaxial expansions and compressions of the tissue being grown, what promotes growth and can potentially control cell differentiation and tissue viability. However, using conventional photolithography or stereolithography for the manufacture of 2D auxetics, with typical distances between the lattices of the auxetic structure of around 50–100 μm, leaves important clearances between cells and prevents from interacting at single cellular level.

Not just mechanical applications benefiting from the special properties of auxetic metamaterials take advantage from micromanufacture, but also auxetic devices for optoelectronics and telecommunications require greater degrees of precision, than those attainable by traditional micromachining. Some additional remarkable proposals for obtaining real mechanical metamaterials, with the finest details reaching hundreds of microns, include both subtractive approaches, such as UV laser ablation (Alderson et al. 1999), and additive manufacturing procedures, such as stereolithography, digital light processing or direct laser writing based on single- or multi-photon polymerization for enhanced precision (Kadic et al. 2012). Normally

2D and 2D½ auxetic structures are obtained by means of surface micromachining, chemical etching, laser ablation and typical mass-production processes imported from electronics.

In this section, we use improved deep-reactive ion etching for improved level of detail. More complex 3D auxetics, with intricate geometries and inner details, require three-dimensional additive manufacturing or "layer-by-layer" processes, especially those support-less ones, such as selective laser sintering or selective laser melting.

Seminal papers in the field of auxetic metamaterials, linked to the assessment of their vibratory response and resonant properties, opened new paths towards the application of these geometries for the development of smart sensors and actuators. For instance, the modal analysis of auxetic butterfly cellular configurations has been already studied in depth (Scarpa and Tomlinson 2000) and applications have been also proposed in the aeronautic field (Lira et al. 2011). More recently, the vibration of structures with chiral lattices has been reported and applied to impact damage detection by using vibro-acoustic modulations (Klepka et al. 2013). Novel very comprehensive monographs on auxetic materials and structures have also paid attention to the vibratory behavior of auxetic rectangular plates similar to some of the designs used in our research (Lim 2015).

In this section we summarize a case study linked to an improved approach for the development of auxetics, based on the use of deep reactive ion etching after a high precision mask fabrication procedure, which constitutes an improvement of previous promising attempts (Muslija and Díaz Lantada 2014). The whole process stands out for its precision, reaching nanometric details, for the high aspect ratios attainable and for the possibility of promoting mass production (Díaz Lantada et al. 2015).

The computer-aided design is accomplished using NX-8.5 software (Siemens PLM Solutions), firstly by obtaining the auxetic unit cells and afterwards by using Boolean operations and two-dimensional matrix replications. The planar auxetics are designed in the XY plane for subsequent extrusion along z direction, so the expected auxetic behavior should lead to transversal contractions along the y axis, when compressed along the x direction, as well as expansions along the y axis, when tractions are applied along the x direction. In these examples we have used a simple re-entrant geometry (firstly introduced by Almgren 1985) and a chiral geometry, both of them previously included in our systematic library of auxetic geometries (Álvarez Elipe and Díaz Lantada 2012). Figure 14.3 shows multi-chamber microsystems with different designs of cantilever cell culture lattices with auxetic properties for multiaxial excitation of cells by the use of vibratory stimuli during the cell culture processes. The dimensions are aimed at obtaining unit cells well below the size of most eukaryotic cells for enabling new biomedical applications and at bridging the gap between state-of-the-art micromachined auxetics (with features down to a few micrometers) and molecular auxetics (with features in the tens of nanometers range).

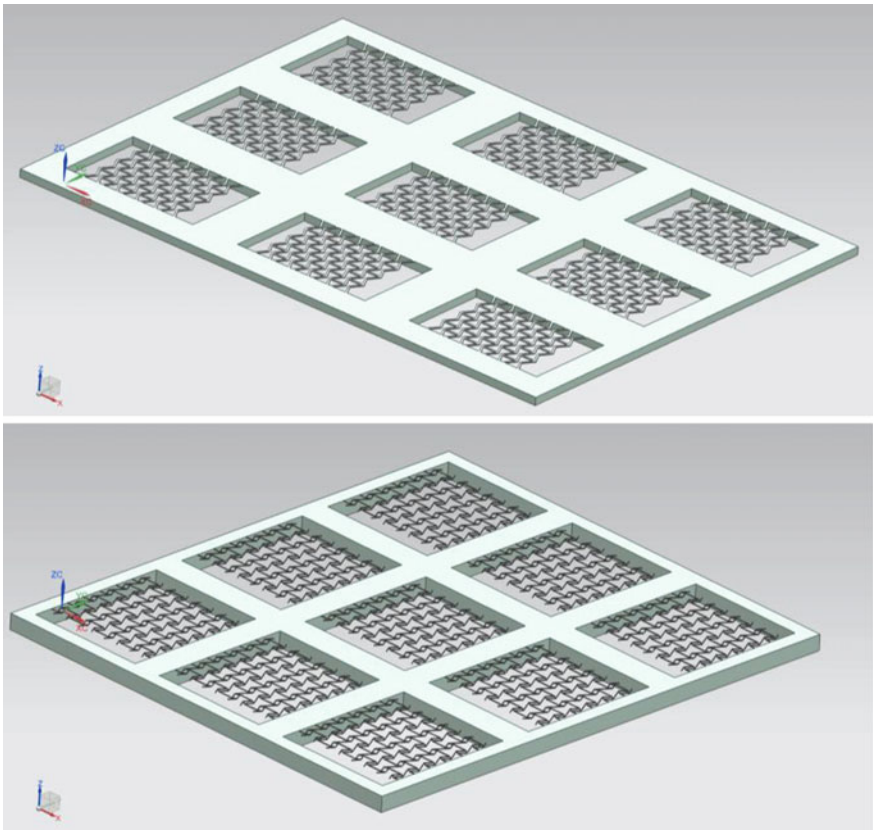

Fig. 14.3 Multi-chamber microsystems with different designs of cantilever cell culture lattices with auxetic properties for multiaxial excitation of cells by the use of vibratory stimuli during the cell culture processes

Figure 14.4 shows, as preliminary in silico assessment, the results from FEM based simulations for obtaining the resonant frequencies of the first vibration mode of the bridged cell culture lattices. Actuating by means of a piezo-resonator and applying such frequencies leads to the desired resonances.

Once the simulations are carried out, post-processing tools allow for an easy detection of the resonant frequencies at which the different modes of vibration of the structures appear, help to analyze the effect of the boundary conditions (of both the cantilever and the bridged structures) and enable discussions about the proposed potential application.

The manufacturing process is accomplished by means of deep-reactive ion etching (DRIE). The DRIE process used here is based on previous experiences (Muslija and Díaz Lantada 2014) with some modifications. In short, plasma phase

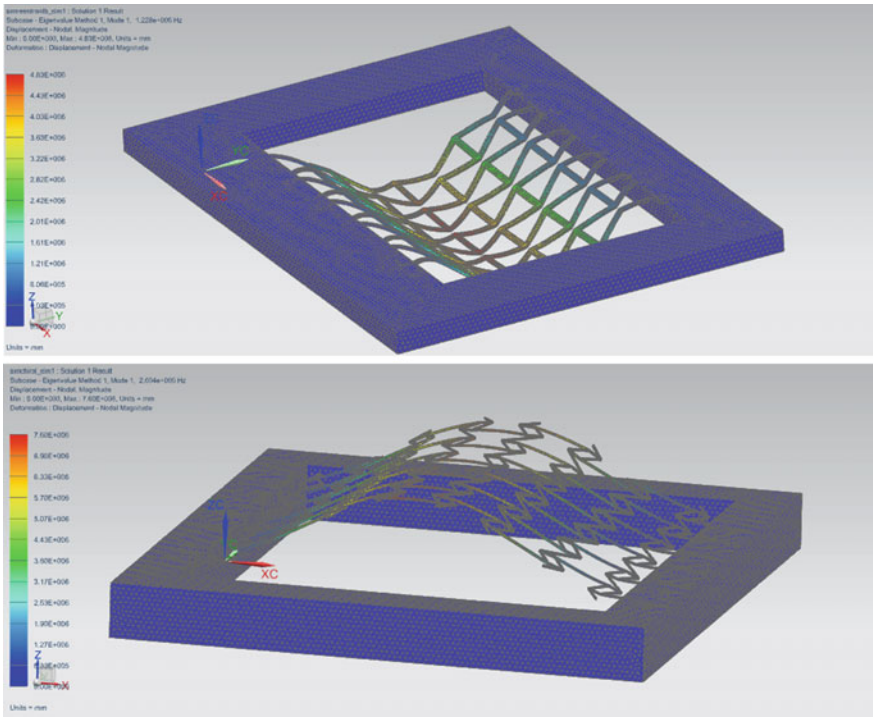

Fig. 14.4 FEM based simulations for obtaining the resonant frequencies of the first vibration mode of the cantilever cell culture lattices. Actuating by means of a piezo-resonator and applying such frequencies leads to the desired resonances

etching involves the generation of chemically reactive species accelerated under the effect of an electromagnetic field towards a target substrate, with protected and unprotected zones thanks to a physical mask. The reactive species are formed by the collision of molecules in a reactant gas with a cloud of energetic electrons excited by an RF field. Ion bombardment is used for higher aspect ratios. Finally, the etched silicon structures are under etched, removing the oxide layer with HF (wet etching) and thus generating freestanding bridges and cantilevers.

As a result from the manufacturing processes, Fig. 14.5 shows microscopies of the micromanufactured bridged cell culture lattices obtained by means of deep-reactive ion etching. The precision obtained, reaching nanometric details, is noteworthy and the stability of the bridges is remarkable. After manufacture, initial cell culture processes, although not yet taking benefit from the use of vibratory excitations, are carried out for preliminary validation.

By means of example, Fig. 14.6 shows hMSCs cultured upon bridged cell culture lattices for preliminary validation of the proposed microsystems.

The hMSCs used in this work were isolated in a Percoll gradient from 1 or 2 ml of human bone marrow samples from anonymous healthy donors and provided by

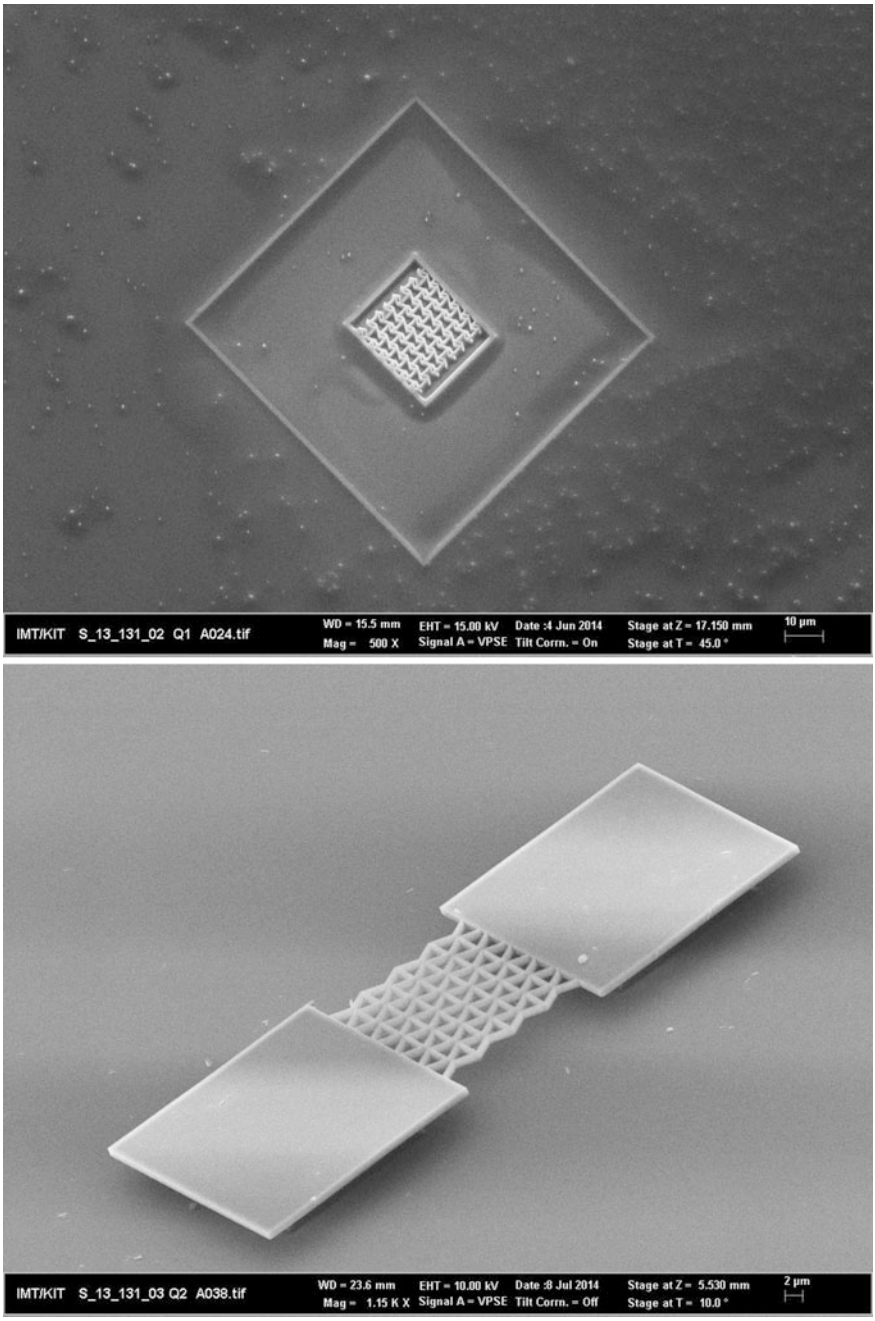

Fig. 14.5 Microscopies of the micromanufactured cantilever cell culture lattices obtained by means of deep-reactive ion etching. Additional details: Muslija and Diaz Lantada (2014), deep-reactive ion etching of auxetic structures. Prototypes manufactured with the support of the KNMF-KIT facilities

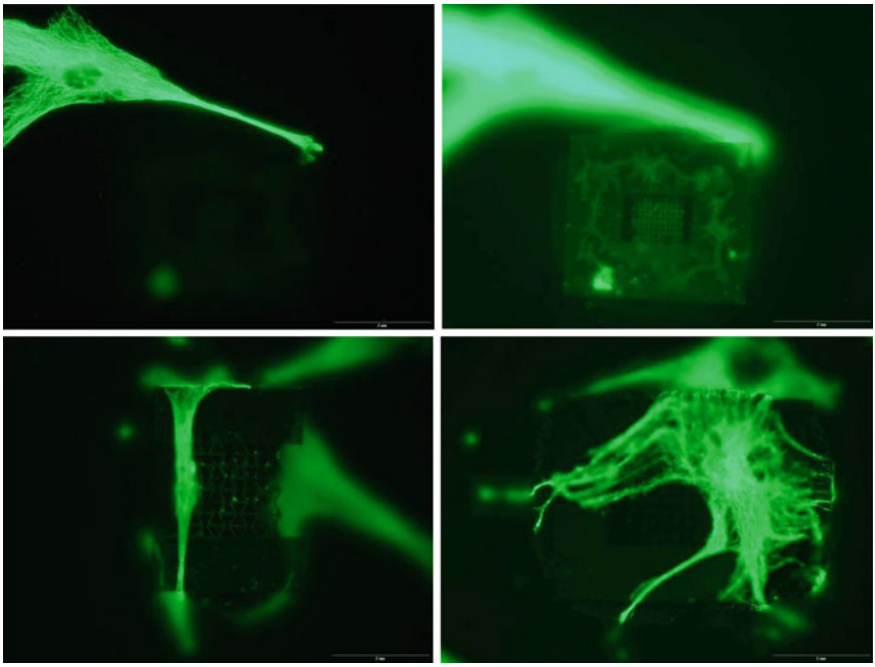

Fig. 14.6 hMSCs cultured upon cantilever cell culture lattices for preliminary validation of the resonant microsystems. Additional details: Diaz Lantada et al. (2015), auxetic tissue engineering scaffolds with nanometric features and resonances in the megahertz range. Prototypes: KIT-KNMF facilities

hematology services of the Hospital La Princesa, the Jiménez-Díaz Foundation and the University Biobank of Málaga, and expanded as described previously (Lennon et al. 2006; Ogueta et al. 2002). Cells were plated and incubated at 37 °C and 5 % CO_2 using DMEM-LG 10 % fetal bovine serum (FBS) of selected batches, and collected by treatment with 0.25 % trypsin-EDTA. Cell culture mediums were prepared by the research services of Molecular Biology Center "Severo Ochoa" (CSIC-UAM).

The auxetic scaffolds were UV irradiated, thoroughly washed using 2 ml of PBS three times and surface seeded with 60,000 hMSC per scaffold. As control 60,000 hMSCs were seeded on coverslips coated with 5 % gelatin. Cells were incubated in DMEM-LG-10 %FBS during 24 h at 37 °C and 95 % humidity. Then preparations were washed with PBS and fixed in 3.7 % formaldehyde in PBS during 30 min at room temperature (RT), washed again and kept until use with PBS at 4 °C.

The cell preparations described were permeated to analyze cytoskeleton morphology and nuclei as indicated early (Romero-Prado et al. 2006; Javed et al. 2000). Briefly, cells were permeated in 0.5 % Triton X-100 in C buffer containing 100 mM NaCl, 10 mM Pipes pH 6.8, 3 mM $MgCl_2$, 3 mM EGTA and 0.3 M sucrose for 30 min at RT. Then, cells were washed with PBS, fixed with formaldehyde and

blocked with 3 % BSA in PBS for 1 h at RT. Then, the samples were equilibrated to PBS with 0.5 % BSA used to dilute antibodies. We used mouse anti-alpha-Tubulin (1:2000, Sigma) in incubations of 1 h. The secondary antibody was a donkey anti-mouse labeled with Alexa 488 (1:500, Invitrogen) and nuclei were labeled with 4′,6-diamidino-2-phenylindole (DAPI) (1:5000, Calbiochem) in dark conditions for 45 min. After incubation, preparations were washed, dehydrated with absolute ethanol (Merck) and mounted with Mowiol/Dabco (Calbiochem).

Cells were visualized in a fluorescence inverted microscope (Olympus IX81) coupled to a CCD camera. The advantages of using two different fluorescent dyes for each microscopy are remarkable; one is used for the visualization of the cytoskeleton while the other one, as DNA marker (DAPI), is used for locating the cellular nucleus. Merging of the blue-labeled and green-labeled images has been accomplished as post-process for improved readability and results presentation.

We would like to mention that, in the whole culture, which included a matrix array of 16 microauxtetics manufactured upon a single silicon on insulator chip, the ratio of damaged cells or those showing apoptotic behavior was lower than 5 % and none of the cells found upon the auxetic microstructures showed symptoms of damage. The nuclei are perfectly rounded and, when placed upon micro-auxetic scaffolds, cells tend to develop an extended morphology, which can be observed when the cellular energetic state allows for the polymerization of tubulin in fibers conforming the cytoskeleton and reaching the cellular surface. Such state shows the very special affinity shown by cells to the micro-auxetics.

Even though the micro-auxetics count with sharp edges, cells seem to take benefit from such features, as they use them to crawl upon the micromanufactured lattice structures. The holes of the auxetic lattices do not affect cells in a negative way and no cells are found to fall through them, as the surface adhesion forces to the structures are much higher than the volume gravitational forces acting upon the cells. In addition, cells are relevantly larger than the unit cell sizes of the auxetic networks, as can be appreciated in the images.

It is also interesting to note that, when interacting with the auxetic structures, the cells lose their typical more rounded or star-like geometries, obtained in more conventional control cultures over gelatin-coated coverslips, and tend to migrate around the structure or to climb upon it. Cells stretching to dimensions of up to 50–60 μm and adopting linear or moon-like forms can be appreciated.

Our results show that the cells and the micro-auxetics are excellent companions for the artificial development of such complex networks and for potential tissue repair strategies. Towards future studies with similar studies, we would like to put forward some aspects of special relevance: MSCs are cells that remain attached to the vascular networks and constitute a set of co-workers for repairing the damages suffered by different tissues. In case of a tissue damage, the hMCSs-seeded microauxteic scaffolds may constitute a biocompatible flexible and porous support, in order to allow the permeation of nutrients and debris, to promote oxygenation, to enable adaptation and to provide cellular communication systems, even capable of locally inhibiting the immune system and of activating tissue repair. Last but not least, the hMCSs-seeded microauxteic scaffolds offer interesting possibilities to

study cellular mechanisms present in different types of tissues. Forthcoming trials using resonant systems will allow us to offer details about the impact of dynamic cultures on cell behaviour and fate.

14.5 Case Study: Resonant 3D Lattices for Cell Culture

Apart from using resonant cantilevers, bridges and 2D½ structures or layers for active cell culture, interesting results linked to the development of 3D artificial constructs for tissue repair and regeneration can be obtained by actively culturing stem cells upon 3D cell niches and scaffolds. In this section we present the design, modeling, manufacturing and testing of a 3D helical scaffold aimed at tendon and ligament repair, whose vibratory responses for active cell culture are analyzed.

In short, these scaffolds are designed with the help of NX-8.5 (Siemens PLM Solutions), mainly using combinations of parametric features and Boolean operations. Main bodies of the designed scaffolds for tendon or ligament repair are hollow tubular structures made of several interconnected helixes. The basic helix is designed in a parametric form, using its equations in Cartesian coordinates. Once the helical curve is designed in the working space, a sketch plane perpendicular to the curve is included. Then a circle with its centre coincident to the intersection between the helical curve and the sketch plane is drawn. The three-dimensional solid helix can be obtained by sweeping the circle along the helical curve. Finally, mirror operations, replicas, rotations and Boolean tools help to obtain the different scaffolds. The different helixes are connected, either using additional tubular elements or by intersections between the helixes. A complete library is presented in Chap. 18 and in Figs. 18.10–18.12.

Additional details regarding systematic design variations and their impact on scaffolds' mechanical properties, regarding the rapid manufacture using different resources and materials and regarding the behavior of cells cultured within these artificial constructs are provided in Chap. 18, when detailing the development of tissue engineering scaffolds for soft tissue repair and regeneration. Here we focus on the dynamic response and on the testing procedures to promote the appearance of different modes of vibration, controlling frequency and intensity, so as to apply the desired vibratory stimuli to the cells being cultured.

The use of FEM-based simulations can be used, not only to predict the basic mechanical features of the cell culture system, such as elasticity and porosity, but also to assess in silico its dynamic properties. Figure 14.7 shows the first five modes of vibration of a triple-helix scaffold for tendon and ligament repair. These simulation results provide a good indication of the frequency ranges at which the resonances may appear, which helps to select the adequate actuators for further trials. Manufacturing of preliminary prototypes for cell culture trials is carried out via selective laser sintering of polyamide powder.

In order to obtain the dynamic response of the construct, a set-up is prepared, including: Brüel & Kjaer 8011 and Brüel & Kjaer 4517C miniature accelerometers

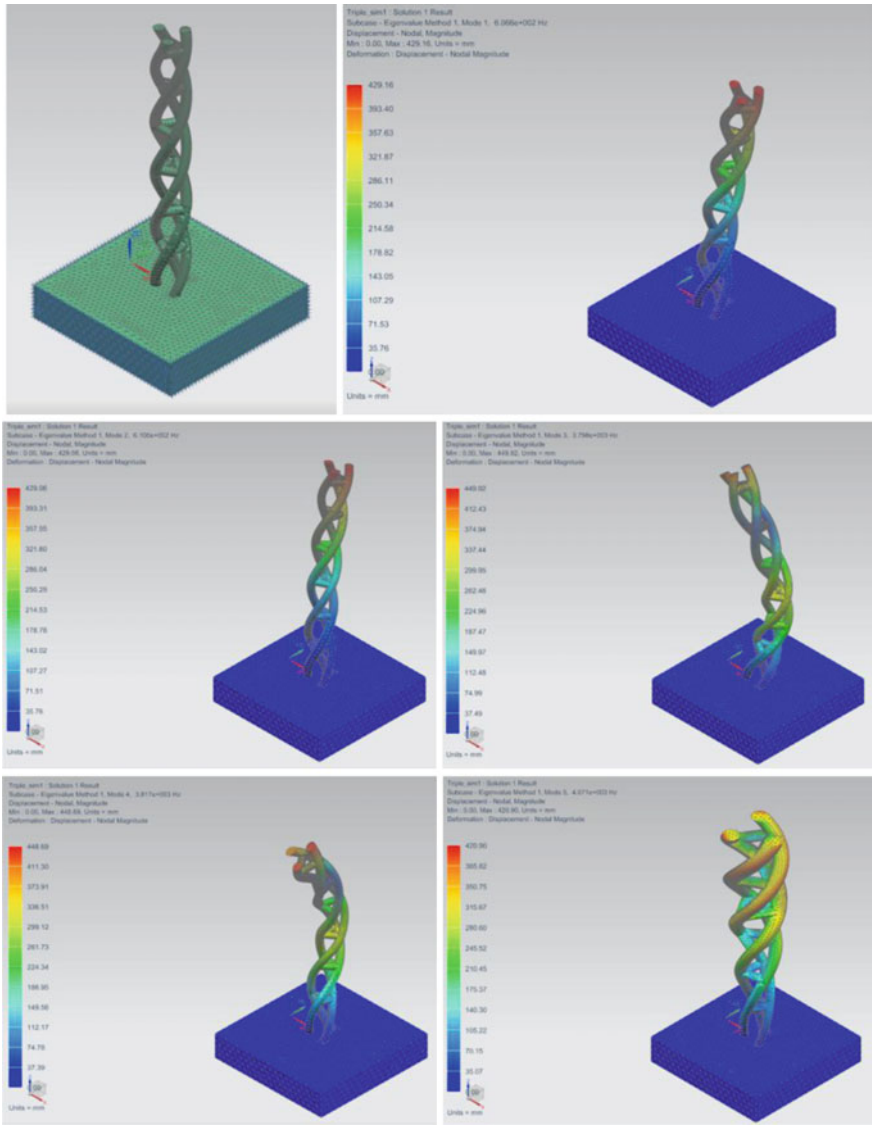

Fig. 14.7 FEM modeling of the dynamic properties of DNA-inspired triple-helix scaffolds for tendon and ligament repair

for monitoring purposes, a Brüel & Kjaer 2635 charge amplifier and a Brüel & Kjaer 4810 transducer for excitating the construct. The tested construct was placed in a work bench and tested in different orientations, to assess the effects of axial and normal excitations.

Fig. 14.8 Testing of the dynamic properties of polyamide protytpes of helical scaffolds for tendon and ligament repair obtained by selective laser sintering. Transversal excitation for the promotion of bending modes (*upper image*). Parallel excitation of the promotion of the axial modes (*lower image*)

The experiments were performed by carrying out frequency sweeps to find the resonances and with the support of stroboscopic lamps. Figures 14.8 and 14.9 show some images of the testing procedure of the dynamic properties of polyamide protytpes of helical scaffolds for tendon and ligament repair obtained by selective laser sintering. Transversal excitation is applied for the promotion of bending

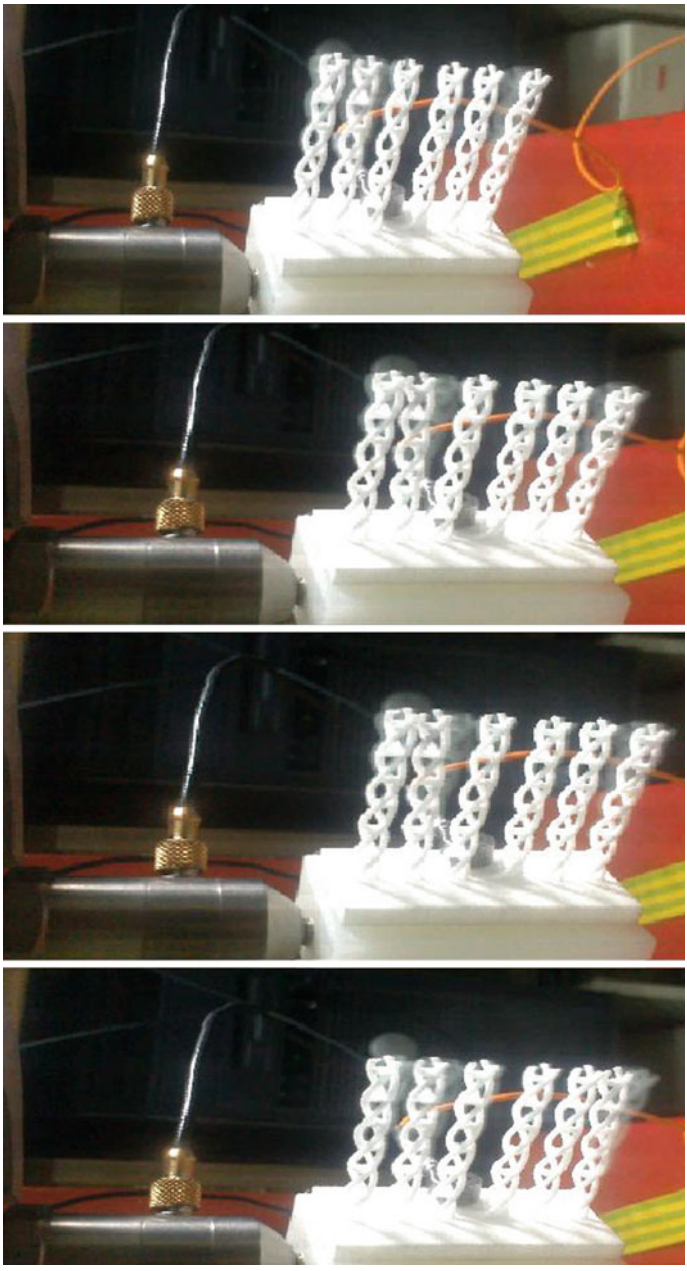

Fig. 14.9 Stroboscopic imaging of the bending modes during excitation

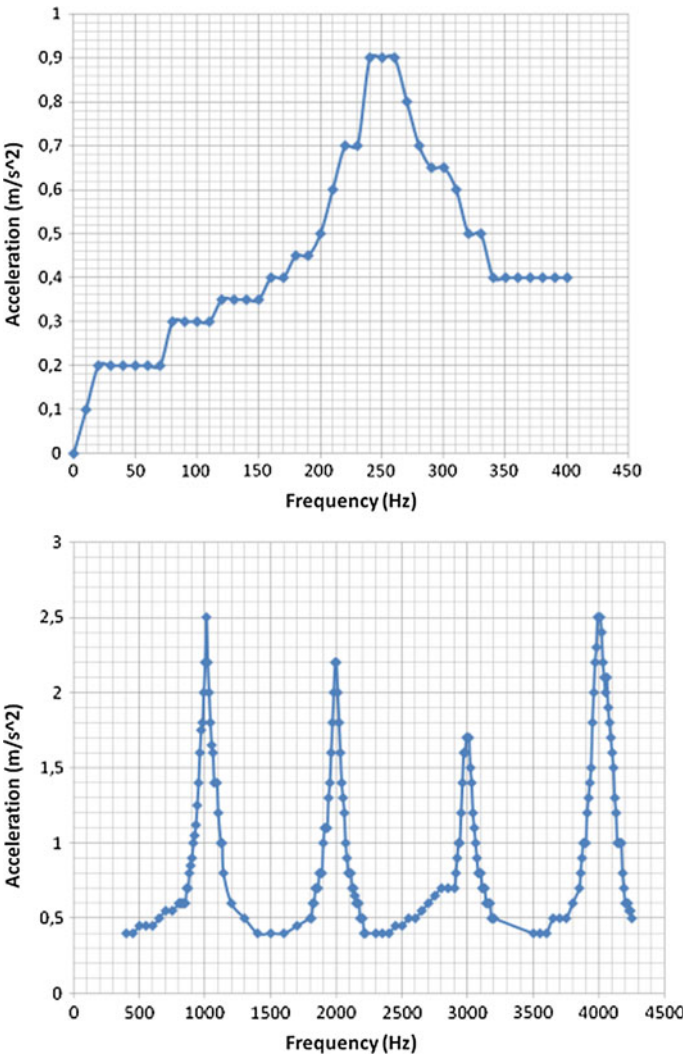

Fig. 14.10 Dynamic response (acceleration vs. frequency) curve showing the resonant frequency corresponding to the first bending mode (*upper image*). Dynamic response curve showing the axial vibration modes (*lower image*)

modes, while axial excitation is desired for the promotion of the axial modes. The dynamic responses are summarized in the images from Fig. 14.10. Future studies will focus on the impact of the different bending and axial modes of vibration on the phenotype expression and on the finally obtained tissues.

14.6 Main Conclusions and Future Research

The dynamic stimuli of active cell culture systems promote special responses and phenotype expressions, which can be extremely important for enhancing the effectivity, efficiency and sustainability of Tissue Engineering procedures. Hence, the use of active culture systems, normally based on the forced circulation of fluids for shear stress application, on the application of electro-magnetic stimuli and on the stimulation by means of cyclic or vibratory strains and stresses, can provide an additional degree of control upon stem cell behavior, dynamics and fate, as has been detailed in present chapter.

Issues linked to the design, modeling, manufacture and testing of microdevices for achieving resonant behaviors for dynamic cell cultures have been put forward and explained by means of particular cases of study, based on the employment of resonant structures for promoting cell growth, as the use of bioreactors and microfluidic systems for active cell culture is detailed in other chapters of the Handbook.

The development processes of auxetic cell culture bridges, for potential muscle regeneration and of helical scaffolds, for prospective tendon and ligament repair, has been explained, taking account of their vibratory responses for the use of actuators aimed at improved performance during the culture processes. The combined use of computer-aided design and engineering resources for carrying out in silico assessments for design optimization and the employment of high-precision rapid prototyping and micro-manufacturing technologies has been also highlighted, as similar technological combinations may prove very effective for future developments in the field.

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References

Alderson A, Rasburn J, Ameer-Beg S, Mullarkey PG, Perrie W, Evans KE (1999) An auxetic filter: a tuneable filter displaying enhanced size selectivity or defouling properties. Ind Eng Chem Res 39(3):654–665

Almgren RF (1985) An isotropic three-dimensional structure with Poisson ratio equal −1. J Elast 15:427–430

Álvarez Elipe JC, Díaz Lantada A (2012) Comparative study of auxetic geometries by means of computer-aided design and engineering. Smart Mater Struct 21(10):105004

Apicella A, Aversa R (2012) A biomimetic and biomechanical approach for tissue engineering—hybrid nanomaterials and a piezoelectric tunable bending apparatus for mechanically stimulated osteoblast cell growth. In: Proceedings of the international conference on biomedical electronics and devices, pp 280–285

Caplan AI (1991) Mesenchymal stem cells. J Orthop Res 9(5):641–650
Caplan AI (2008) All MSCs are pericytes? Cell Stem Cell 3(3):229–230
Chan CKF, Chen CC, Luppen CA, Kraft DL, Kim JB, De Boer A, Wei K, Helms JA, Kuo CJ, Weissman IL (2009) Endochondral ossification is required for hematopoietic stem cell niche formation. Nature 457(7228):490
Díaz Lantada A (2011) Handbook of active materials for medical devices: advances and applications. CRC Press–PAN Stanford Publishing
Diaz Lantada A, Muslija A, García-Ruíz JP (2015) Auxetic tissue engineering scaffolds with nanometric features and resonances in the megahertz range. Smart Mater Struct 24:055013
Evans KE (2004) Auxetic polymers: a new range of materials. Endeavour 15(4):170–174
Evans KE, Nkansah MA, Hutchinson IJ, Rogers SC (1991) Molecular network design. Nature 353:124–124
Griffin AC, Kumar S, Mc Mullan PJ (2005) Textile fibers engineered from molecular auxetic polymers. National textile center research briefs, materials competency, pp 1–2
He C, Liu P, McMullan PJ, Griffin AC (2005) Toward molecular auxetics: main chain liquid crystalline polymers consisting of laterally attached para-quaterphenyls. Phys Status Solidi B 242:576–584
Hirotsu S (1991) Softening of bulk modulus and negative Poisson's ratio near the volume phase transition of polymer gels. J Chem Phys 94:3949–3957
Javed A, Guo B, Hiebert S, Choi JY, Green J, Zhao SC, Osborne MA, Stifani S, Stein JL, Lian JB, van Wijnen AJ, Stein GS (2000) Groucho/TLE/R-esp proteins associate with the nuclear matrix and repress RUNX (CBF(alpha)/AML/PEBP2(alpha)) dependent activation of tissue-specific gene transcription. J Cell Sci 113(12):2221
Kadic M, Bückmann T, Stenger N, Thiel M, Wegener M (2012) On the practicability of pentamode mechanical metamaterials. Appl Phys Lett 100(19):191901
Klepka A, Staszewski WJ, Di Maio D, Scarpa F (2013) Impact damage detection in composite chiral sandwich panels using nonlinear vibro-acoustic modulations. Smart Mater Struct 22 (8):084011
Lakes RS (1987) Foam structures with a negative Poisson's ratio. Science 235(4792):1038–1040
Lanza R, Langer R, Vacanti J (2014) Principles of tissue engineering, 4th edn. Elsevier
Lennon DP, Haynesworth SE, Bruder SP, Jaiswal N, Caplan AI (2006) Human and animal mesenchymal progenitor cells from bone marrow: identification of serum for optimal selection and proliferation. In vitro Cell Dev Biol 32:602
Lim TC (2015) Auxetic materials and structures. Springer, Singapore
Lira C, Scarpa F, Rajasekaran R (2011) A gradient cellular core for aeroengine fan blades based on auxetic configurations. J Intell Mater Syst Struct 22(9):907–917
Liu Y, Hu H (2010) A review on auxetic structures and polymeric materials. Sci Res Essays 5 (10):1052–1063
Liu L, Yuan W, Wang J (2010) Mechanisms for osteogenic differentiation of human mesenchymal stem cells induced by fluid shear stress. Biomech Model Mechanobiol 9(6):659–671
Muehsam D, Ventura C (2014) Life rhythm as a symphony of oscillatory patterns: electromagnetic energy and sound vibration modulates gene expression for biological signaling and healing. Global Adv Health Med 3(2):40–55
Muslija A, Diaz Lantada A (2014) Deep-reactive ion etching of auxetic structures. Smart Mater Struct 23:087001
Narojczyk JW, Wojciechowski KW (2010) Elastic properties of degenerate f.c.c. crystal of polydisperse soft dimers at zero temperature. J Non-Cryst Solids 356:2026–2032
Ogueta S, Muñoz J, Obregon E, Delgado-Baeza E, García-Ruíz JP (2002) Prolactin is a component of the human sinovial liquid and modulates the growth and chondrogenic differentiation of bone marrow-derived mesenchymal stem cells. Mol Cell Endocrinol 190(1–2):51
Park K, Millet LJ, Kim N, Li H, Jin X, Popescu G, Aluru NR, Hsia J, Bashir R (2010) Measurement of adherent cell mass and growth. PNAS 107(48):20691–20696

Romero-Prado M, Blázquez C, Rodríguez-Navas C, Muñoz J, Guerrero I, Delgado-Baeza E, García-Ruíz JP (2006) Functional characterization of human mesenchymal stem cells that maintain osteochondral fates. J Cell Biochem 98:1457

Samadikuchaksaraei A, Lecht S, Lelkes PI, Mantalaris A, Polak JM (2014) Stem cells as building blocks. In: Lanza R, Langer R, Vacanti J (eds) Principles of tissue engineering, 4th edn. Elsevier

Scarpa F, Tomlinson G (2000) Theoretical characteristics of the vibration of sandwich plates with in-plane negative Poisson's ratio values. J Sound Vib 230(1):45–67

Soman P, Lee JW, Phadke A, Varghese S, Chen S (2012) Spatial tuning of negative and positive Poisson's ratio in a multilayer scaffold. Acta Biomater 8(7):2587–2594

Vunjak-Novakovic C, Scadden DT (2011) Biomimetic platforms for human stem cell research. Cell Stem Cell 8(3):252–261

Wang H, Riha GM, Yan S, Li M, Chai H, Yang H, Yao Q, Chen C (2005) Shear stress induces endothelial differentiation from a murine embryonic mesenchymal progenitor cell line. Arterioscler Thromb Vasc Biol 25:1817–1823

Wojciechowski KW (1987) Constant thermodynamic tension Monte Carlo studies of elastic properties of a two-dimensional system of hard cyclic hexamers. Mol Phys 61(5):1247–1258

Wojciechowski KW (1989) Two-dimensional isotropic system with a negative Poisson ratio. Phys Lett A 137:60–64

Wojciechowski KW (2003) Non-chiral, molecular model of negative Poisson ratio in two dimensions. J Phys A: Math Gen 36(47):11765

Yao X, Peng R, Ding J (2013) Cell-material interactions revealed via material techniques of surface patterning. Mater Views 25:5257–5286

Chapter 15
Tissue Engineering Scaffolds for 3D Cell Culture

Andrés Díaz Lantada, Diego Curras, Javier Mousa and Stefan Hengsbach

Abstract Even though pioneer studies in the field of tissue engineering, either for disease study or for tissue repair, were performed on flat 2D substrates (normally Petri dishes), more recent research has helped to highlight the relevance of three-dimensional systems in cell culture. In fact, even one-dimensional patterns upon have been found more adequate, for mimicking actual cell migration in three-dimensional environments, than conventional two-dimensional scaffolds for cell culture, what puts forward the need for alternative development procedures aiming at a more adequate reproduction of the 3D environment, taking account of both biochemical and biomechanical approaches. The combined employment of computer-aided design, engineering and manufacturing resources, together with rapid prototyping procedures, working on the basis of additive manufacturing approaches, allows for the efficient development of knowledge-based functionally graded scaffolds for effective and biomimetic three-dimensional cell culture in a wide range of materials. Applications of such tissue engineering scaffolds for cell culture include the repair, regeneration and even biofabrication of hard tissues, soft tissues and osteochondral constructs, as well as the modeling of disease development and management, as detailed in forthcoming chapters. In this chapter we present some design and manufacturing strategies for the development of knowledge-based functionally graded tissue engineering scaffolds aimed at different types of tissues. We also detail some prototyping approaches towards low-cost rapid prototyped scaffolds and tumor growth models, as cases of study for illustrating the complete development process of these types of medical devices.

A. Díaz Lantada (✉) · D. Curras · J. Mousa
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

S. Hengsbach
Institute of Microstructure Technology, Karlsruhe Institute of Technology,
Hermann von Helmholtz Platz 1, Eggenstein-Leopoldshafen, Germany

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15.1 Introduction: From 2D to 3D Cell Culture

Even though pioneer studies in the field of tissue engineering, either for disease study or for tissue repair, were performed on flat 2D substrates (Wald et al. 1993; Brem et al. 1993), more recent research has helped to highlight the relevance of three-dimensional systems in cell culture. In fact, even one-dimensional patterns upon biopolymers (i.e. polyvinyl alcohol) have been found more adequate, for mimicking actual cell migration in three-dimensional environments, than conventional two-dimensional scaffolds for cell culture (Doyle et al. 2009). Cell migration in both 1D and 3D is rapid, uniaxial and independent of extra cellular matrix ligand density, in contrast to 2D cultures, what puts forward the need for alternative solutions aiming at a more adequate reproduction of the 3D environment, taking account of both biochemical and biomechanical approaches. Novel high-precision additive manufacturing resources, such as direct laser writing, are helping to address the combined impact of material chemistry, structure and mechanical properties, on final cell culture results, by using a wide set of 3D scaffold designs as supporting structures (Ovsianikov et al. 2012). Several materials, both of biological and synthetic origins, are being processed additively for such purposes, including SU8 (MicroChem), acrylate- and methacrylate-based photoresins, sol-gel hybrids, Ti- and Zr-based sol-gels, polycaprolactone, poly (lactic acid), ORMOCER®, collagen and chitosan, among other successful biomaterials (Ovsianikov et al. 2012).

Attention-grabbing modifications of other additive technologies, such as electrospinning, are also of great help for generating knowledge in this field and for the production of biomimetic 3D scaffolds or cell culture platforms with controlled surfaces and structures in the micro- and nano-scales. Electrospun nanofiber sheets, comprised of composites of poly (glycolic acid) (PGA) and collagen, further modified by the incorporation of dextran-spermine-plasmid DNA nanoparticles, have been capable of genetically engineering mesenchymal stem cells (MSC) to express bone morphogenic protein-2 (Hosseinkhani et al. 2012). The use of collagen-PGA composites, thanks to its sponge-like micro- and nano-structure, has been found adequate for promoting cardiac stem cell culture (Hosseinkhani et al. 2010) and hybrid scaffolds have also performed adequately for bone regeneration (Hosseinkhani et al. 2007).

In addition, even if cell growth and expansion is obtained in adequate three-dimensional scaffolds, final tissue viability requires adequate vascularization, as diffusion can only provide the transport of nutrients and waste within hundreds of microns. However, vascularization induction within artificial tissues is still an unresolved challenge lying at the center of regenerative medicine research strategies (Boccaccini et al. 2012), and demands even more complexity of the geometries and materials of scaffolds and implants linked to tissue repair, so as to promote biomimetic responses. Some promising approaches, taking account of the decisive role that growth factors play, have also relied on the use of 3D scaffolds, formed by mixing of peptide-amphiphile (PA) aqueous solution with basic fibroblast growth factor (bFGF) suspension (Hosseinkhani et al. 2006).

Furthermore, in order to mimic in vivo the topography of the native tissue created by extracellular matrix (ECM) components, in tissue regeneration processes, the surface features of each biomaterial should be considered as a nanodimensional structure for taking account of all relevant cell-material interactions (Hosseinkhani et al. 2005). The use of biocoatings also proves to be an adequate strategy for introducing and modifying such surface nanostructures and improving cell culture processes (Lindström et al. 2010). All these studies have helped to verify the beneficial effects of complex multi-scale "fractal-like" materials for the promotion of biomimetic responses that in several cases may benefit from non integer fractal dimension (Díaz Lantada 2013).

Biomimetic three-dimensional cell culture niches, with ad hoc controlled outer geometry, mechanical properties and biochemical response are in fact not so complex and expensive to obtain, if resorting to phase separation processes for obtaining polymeric sponges, subsequently loaded with adequate growth factors (Díaz Lantada et al. 2014), even though the expectable geometries and results derived from phase-separation processes cannot be controlled from the design stage, as happens with more reproducible processes based on combinations of computer-aided design and additive prototyping facilities. It is important to note that the inclusion of biochemical effects, derived from the incorporation of the adequate growth factors, adds some additional uncertainties to the already complex to understand interactions between scaffolds' structure, morphology, topography and mechanical properties.

Consequently, studies addressing these interesting synergies between extracellular matrices and growth factors and their impact on final tissue viability are needed, in the quest for a universal methodology for versatile and successful tissue engineering scaffold development.

Present chapter focuses on the combined employment of advanced computer-aided design, engineering and manufacturing resources, together with rapid prototyping procedures, working on the basis of additive manufacturing approaches, for the efficient development of knowledge-based scaffolds for biomimetic three-dimensional cell culture in a wide range of materials. Alternatives based on the use of lattice and porous structures, as well as the potentials of functionally graded scaffolds, which can also be developed with the help of CAD-CAE-CAM tools and obtained by additive procedures, are discussed.

The impact of pore size and truss thickness on the mechanical properties of the cell culture scaffolds are also analyzed for the encouragement of knowledge-based solutions. Thus, some libraries of scaffolds and scaffold properties are obtained and provided, to help researchers in the field with geometrical and material selection tasks, towards biomimetic and biomechanical tissue engineering constructs, taking into consideration that human mesenchymal stem cells (h-MSCs) are influenced by the mechanical properties, porosities and surface topographies of the surrounding extracellular matrices (in this case scaffolds) during gene expression and the resulting generation of right tissues.

15.2 Design Strategies for Tissue Engineering Scaffolds

Computer-aided design, initially developed for architecture, the automobile industry and aeronautics, is currently an essential resource in any product development project and very valuable for the current advances in the medical device industry. The math-based curves and surfaces typical of CAD software (planes, cylinders, spheres, prisms, splines, Bezier curves and surfaces, among others) can be combined using Boolean operations for constructing complex geometries, even though sometimes it is challenging to model the complexity of biostructures and biological organisms in detail.

In most cases, CAD applications are used for designing the "soft" surfaces of conventional products, such as vehicles, toys, home devices, furniture, machines, buildings…, and surface roughness, textures and irregularities are a consequence of the manufacturing processes, so the control of texture and topography, so relevant in the field of microsystems for diagnosis is complex to control from the design stage.

Some recent approaches linked to the incorporation of fractal geometries to the computer-aided designs of biomedical microdevices (Díaz Lantada et al. 2010a, b; Díaz Lantada 2013) are enabling the incorporation of biomimetic features, such as controlled surface textures and topographies, which are providing interesting functionalities. In any case, as detailed throughout the Handbook, CAD resources are now widely used for the development of advanced biomedical microsystems for cell culture, tissue engineering and disease management, as they enhance communication among the research teams developing novel products, promote in silico validation, by means of multiphysical simulations, encourage automated manufacture approaches and help with production setup. Attention-grabbing designs with the incorporation of systematic variations for methodic assessments can be obtained, thanks to the use of pattern operations and Boolean tools, in just some hours.

These resources and operations have been used for the implementation of the scaffolds library depicted in Fig. 15.1 (using NX-8.5, Siemens PLM Solutions), which shows systematic variations to scaffolds' porosities and related mechanical properties, thanks to the use of parametric design procedures. Unit cells have been designed and replicated using pattern and Boolean operations for implementing the three-dimensional scaffolding geometries. The degrees of porosity of these structures can be also directly evaluated using measuring tools available in most CAD software. Figure 15.2 shows the influence of unit cell size and bar thickness on scaffolds' porosity, after carrying out such evaluations.

These graphical representations of relevant properties may help to select the most satisfactory scaffolding geometries for repairing or regenerating the different portions and tissues of interest. They are also helping to encourage the use of biomimetic design strategies and selection tasks.

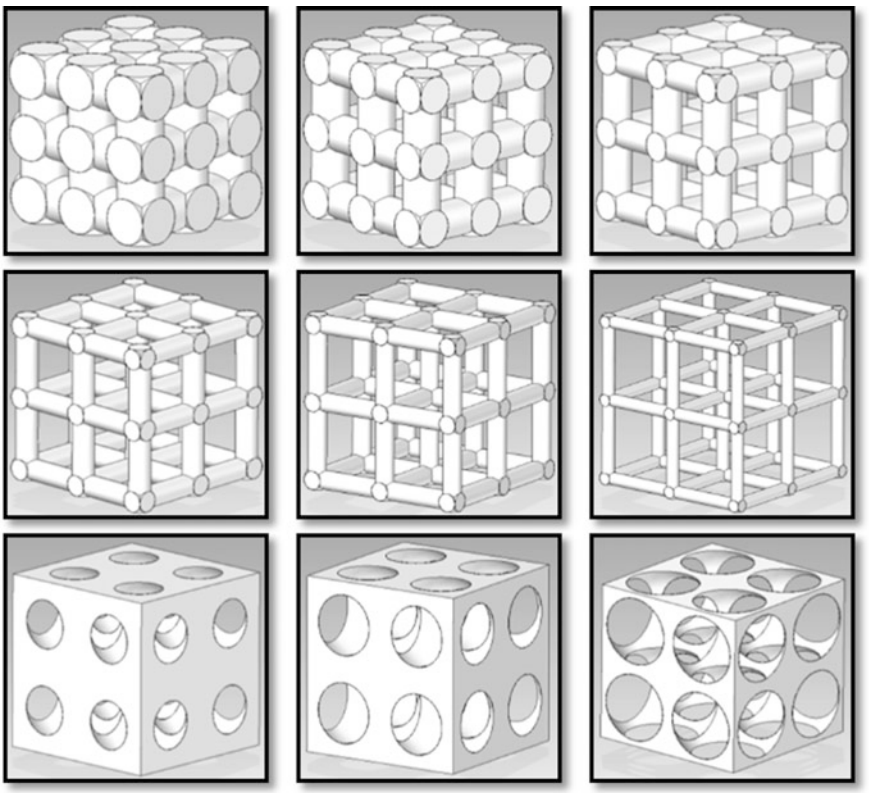

Fig. 15.1 CAD library of porous and lattice structures with potential use as tissue engineering scaffolds for culturing different types of cells and tissues

The use of FEM-based simulations, in our case resorting to the "advanced simulation" module available in the computer-aided design and engineering software used, can be also applied to analyzing the mechanical performance and properties of the tissue engineering constructs. After adequate evaluation, Fig. 15.3 shows the influence of unit cell size and bar thickness on scaffolds' elasticity. The values are obtained considering a biopolymer apt for additive manufacture with a Young's modulus in bulk state of around 3000 MPa. The incorporated porosities help to approximate the mechanical properties of the material to those of soft tissues. Results can be also presented in normalized form (Young's modulus of porous structure/Young's modulus of bulk material). These graphical summaries of relevant mechanical properties may help to select the most satisfactory scaffolding geometries, for repairing or regenerating the different portions and tissues of interest. These resources are also helping to promote, not only biomimetic, but also biomechanical design strategies and selection tasks.

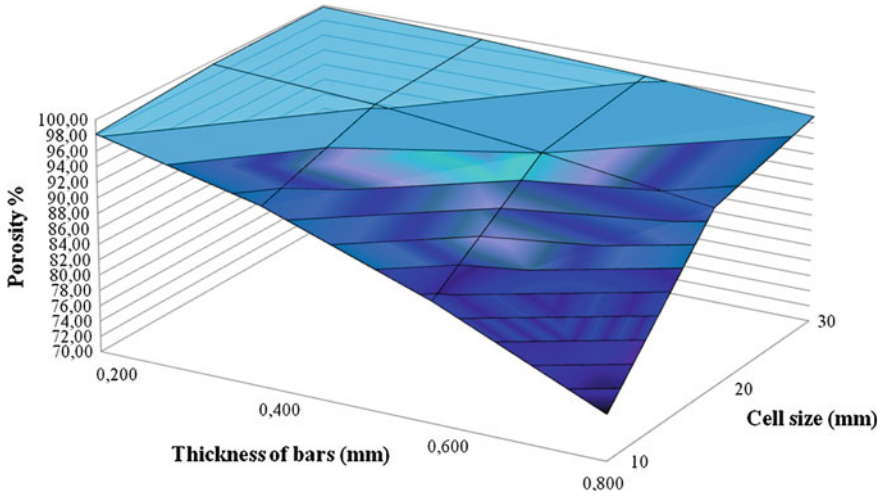

Fig. 15.2 Influence of cell size and bar thickness on scaffolds' porosity

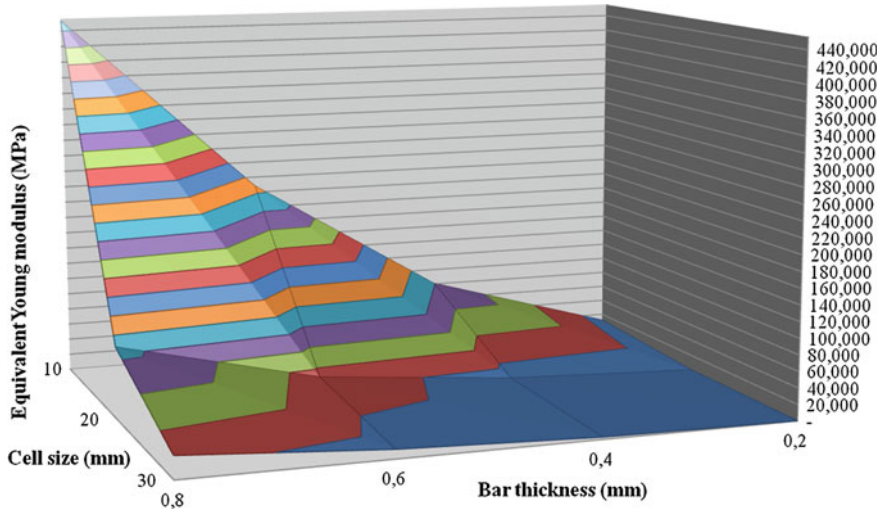

Fig. 15.3 Influence of cell size and bar thickness on scaffolds' elasticity

Additional details for adapting the mechanical properties of compact synthetic materials, to those of porous functionally-graded biomaterials, are presented in Chap. 16, when dealing with the development tissue engineering scaffolds for bone repair, and in Chap. 18, when dealing with the design and manufacture of tissue engineering scaffolds for the repair of softer tissues.

15.3 Manufacturing Strategies and Resources for Tissue Engineering Scaffolds

Most processes for manufacturing micro-porous structures and materials (or meta-materials) for tissue engineering (Díaz Lantada 2013) involve a combination of materials in some step of the process and a final phase separation or leaching process, for obtaining a solid part with distributed small pores. Among most extended processes, gas-assisted injection molding is an industrial method based on injecting a molten resin or thermoplastic into a mold cavity and then applying a quantity of pressurized gas into the resin, so as to help to fill out the mold cavity and to create hollows and pores in the polymer. The incorporation of foaming agents as additives to polymers also allows the manufacture of polymeric parts with pores. In many strategies involving the development of artificial tissues, to obtain 3D-porous structures is absolutely required to irrigate the tissue and maintain an adequate liquid dynamics. The use of porogens is also commonplace; normally, the process involves mixing a liquid prepolymer with solid particles (typically wax, sugar, salt…). Once polymerization is produced, normally by UV exposure or by heating, a solid structure, formed by a polymeric network with dispersed particles, is obtained. Final porous structure is obtained by dissolving such disperse particles in water, other solvents or by heating. The use of prepolymer-water emulsions is also typical for obtaining a polymerizable mixture that after thermal or UV-based polymerization provides a polymeric network with pores according to initial water content (i.e. polyHIPEs).

Main alternatives, for improving the control of scaffolds' pore size and distribution, from the design stage, is the use of micro additive manufacturing technologies (AMT), normally working on layer-by-layer processes, following the geometries obtained with the help of computer-aided designs (Bartolo et al. 2009; Tan et al. 2010). Electro-spinning can be also adapted to "layer-by-layer" fabrication and used for obtaining 3D porous structures (Ekaputra et al. 2009), even though the process is not as repetitive as the use of micro AMT. The progressive increase in precision of additive manufacturing technologies, together with their improved versatility thanks to a continuously increasing set of materials available for layer-by-layer processing, is greatly promoting applications linked to micro- and even nano-manufacturing of complex 3D geometries for very innovative medical solutions in several fields (Díaz Lantada 2013).

Scaffolds with design-controlled structures have been obtained by means of rapid prototyping technologies including: selective laser sintering (SLS) (Lohfeld et al. 2010), layered hydrospinning (Tzezana et al. 2008), laser stereolithography (SLA) (Díaz Lantada et al. 2010a, b), digital light projection (DLP) (Stampfl et al. 2004) or two-photon polymerization (2PP) (Infür et al. 2007), and different materials including hydrogels (Maher et al. 2009), gelatin (Tan et al. 2010), titanium alloys (Ryan et al. 2008; Warnke et al. 2009), (bio)photopolymers (Stampfl et al. 2008) and ceramics (Cox et al. 2015).

However in vitro validation of rapid-prototyped scaffolds is not so commonplace, as most combinations of processes and materials do not provide adequate biomaterials and in many cases generate toxic components.

Nevertheless some highly interesting research has already been published, including in vitro validation and systematic toxicity assessment (Schuster et al. 2007a, b). Advances in the field of biopolymers (Schuster et al. 2007a, b) together with the possibilities provided by thin coatings (Díaz Lantada et al. 2010a, b), are bringing new possibilities to this area, although access to such materials and technologies is not always easy, as some of them are currently under development or only available in large research centres.

In any case, it seems clear that a universal methodology for tissue engineering scaffold development is not yet available, first of all due to the complexity of biological materials and systems, but also due to all the possible design resources, manufacturing technologies and related materials available, whose results have not been systematically compared. For instance, additive manufacturing technologies allow precise control of final geometries from the design stage; however such designs are normally obtained by combining Euclidean based (simple) geometries and final result does not always adequately mimic the geometrical and mechanical complexity of biomaterials.

On the other hand, tissue engineering scaffolds obtained by phase separation, biomimetic templating and more "traditional" processes typically lead to more biomimetic sponges, even though their final outer form and repeatability are more difficult to control, than using computer-aided strategies linked to rapid prototyping using additive processes.

Therefore, further research is needed to address the advantages of combining different technologies (Tan et al. 2013) for manufacturing enhanced, even personalized, scaffolds for tissue engineering studies and extra cellular matrices with global (outer) geometries defined as implants for tissue repair. In addition, increasing data show that progenitor cell-niche formation is absolutely needed for tissue development and repair (Chan et al. 2009).

Indeed, the niche composition and 3D structure play an important role in stem cells state and fate. The niche is created by the specific combination of trophic factors produced by progenitor cells to maintain the capability for tissue repair and regeneration and by a specific extracellular matrix. Recent studies have helped to

highlight the extreme relevance of the incorporation of adequate growth factors, within the scaffold, for promoting biological regulation, cell differentiation, angiogenesis and final tissue viability (Richardson et al. 2001; Perets et al. 2003; Laschke et al. 2008).

Such inclusion of biochemical effects, derived from the incorporation of growth or trophic factors, adds uncertainties to the already complex interactions between scaffolds' material, structure, morphology, surface topography and mechanical properties. Only by systematic studies, capable of highlighting the more relevant factors of influence, will this field reach its potential.

Hence, studies addressing the synergies between ECMs and growth factors and their impact on tissue viability are needed, in the quest for a general methodology for tissue engineering scaffold development. In the following pages we present the development of a multi-scaffold cell culture platform for pursuing the generation of such knowledge.

Figure 15.4 shows, as example, the computer aided design of the multi-scaffold platform for cell culture, constructed using 20-μm diameter trusses. The lower row includes four culture zones with linear cantilevers for potentially promoting mesenchymal stem cell differentiation into tendon and ligament.

The different structures included in the four different culture zones differ in the distance between cantilevers. The central lower row includes some cylindrical scaffolds for orienting cell growth into the formation of tubular structures and fibrils, hence eventually promoting the formation of muscular soft tissues and even tendons and ligaments. The different structures of the central lower row differ in the diameters of the tubular scaffold. The central upper row includes four culture zones with some horizontal cantilevers crossing each other, trying to mimic the aspect and morphology of cardiac tissue. The different structures include cantilevers crossing at different angles, so as to address the most positive structures for obtaining cardiac tissue differentiation. The upper row includes four wood-pile structures with different distances between the struts and, therefore, with different porosities and elasticities, so as to address its effect on cell differentiation into bone and possibly adipose tissue.

The system stands out for its versatility and for enabling several cell culture experiments to be carried at once for performing systematic and rapid comparative studies. Ideally, this microsystem would promote cell differentiation into bone, cartilage, adipose tissue, tendon, ligament, muscle and cardiac tissue. However the final in vitro validation will be carried out after the manufacturing challenges are solved. The extreme precision required for this microsystem, aimed at interacting even with single cells, requires the most precise AMT resources available.

By means of example, Fig. 15.5 shows a micrometric tissue engineering scaffold manufactured by means of direct laser writing based on two-photon polymerization, as part of the designed multi-scaffold platform. This tubular structure is aimed at cellular differentiation into relevant tissues with cells aligned longitudinally. This

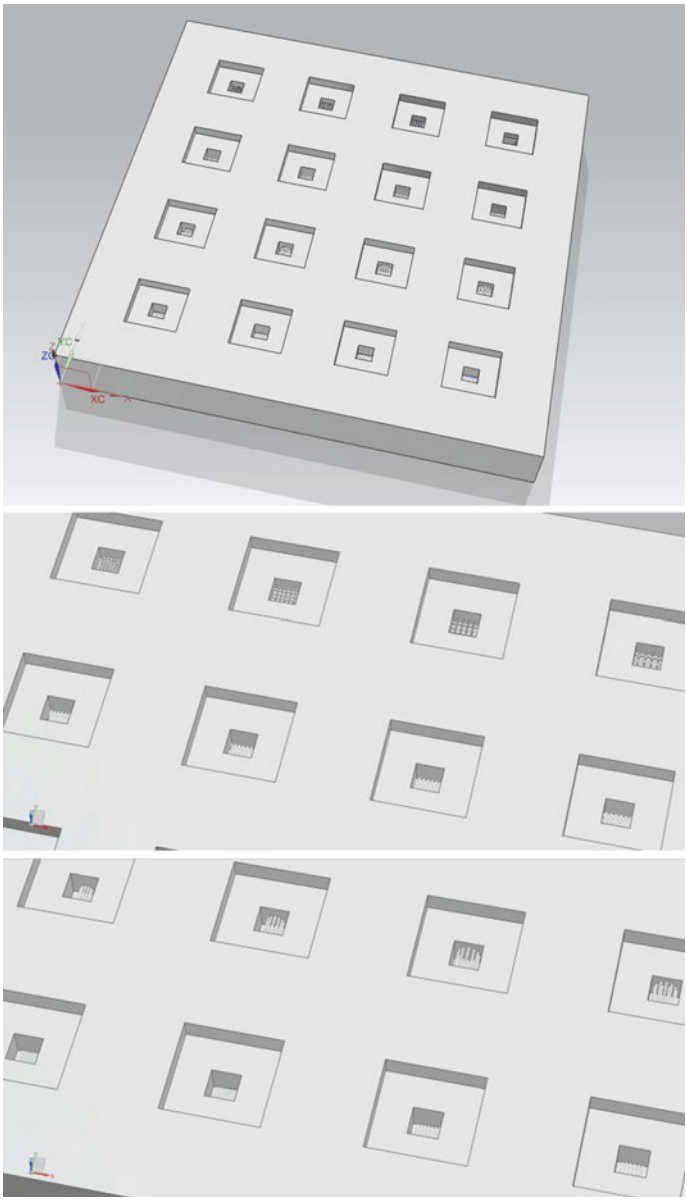

Fig. 15.4 Computer aided design of a multi-scaffold platform for cell culture. The different structures, included in the four different rows, are composed of 20-μm diameter struts and cantilevers (wood-pile structures, cylindrical scaffolds, bridges…) to promote cell differentiation into bone, cartilage, adipose tissue, tendon, ligament, muscle and cardiac tissue

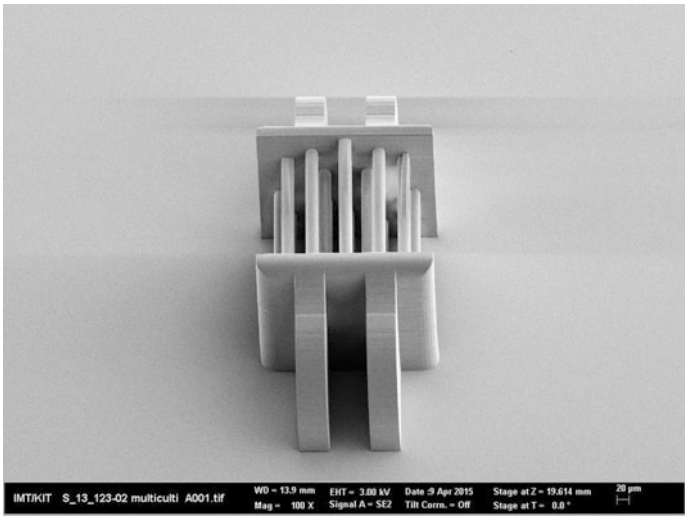

Fig. 15.5 Micrometric tissue engineering scaffold manufactured by means of direct laser writing based on two-photon polymerization, as part of the multi-scaffold platform from previous Fig. 15.4. The tubular structure is aimed at cell differentiation into tissues with cells aligned longitudinally. Prototype obtained with the support of the KNMF-KIT facilities

preliminary but promising result helps to put forward the potential of the proposed approach, towards biomimetic multi-culture platforms, with different scaffolds capable of interacting in a wide set of ways with the cells being cultured.

In any case, as already mentioned, even if cell growth is obtained in three-dimensional scaffolds, final tissue viability requires adequate vascularization, as diffusion can only provide the transport of nutrients within a few hundred microns. However, biomimetic vasculature within artificially developed tissues is not yet feasible, especially for larger biological structures and organs and lies at the center of regenerative medicine research strategies (Boccaccini et al. 2012). Some promising approaches, taking into consideration the decisive function that growth factors play, but also bearing in mind biomechanical aspects, as companions of the biochemical cues, have also relied on the use of 3D scaffolds, formed by mixing of peptide-amphiphile (PA) aqueous solution with basic fibroblast growth factor (bFGF) suspension (Hosseinkhani et al. 2006).

Other manufacturing options towards the promotion of vascularization in vitro and with potential for in vivo advances as well, are summarized in Fig. 15.6. In some cases 2D½ approaches may be very useful, for in vitro vascular models, which can be obtained by UV-photolithography and subsequent PDMS rapid form copying, following soft-lithography processes (Whitesides et al. 2001, see also Chap. 8 for additional details).

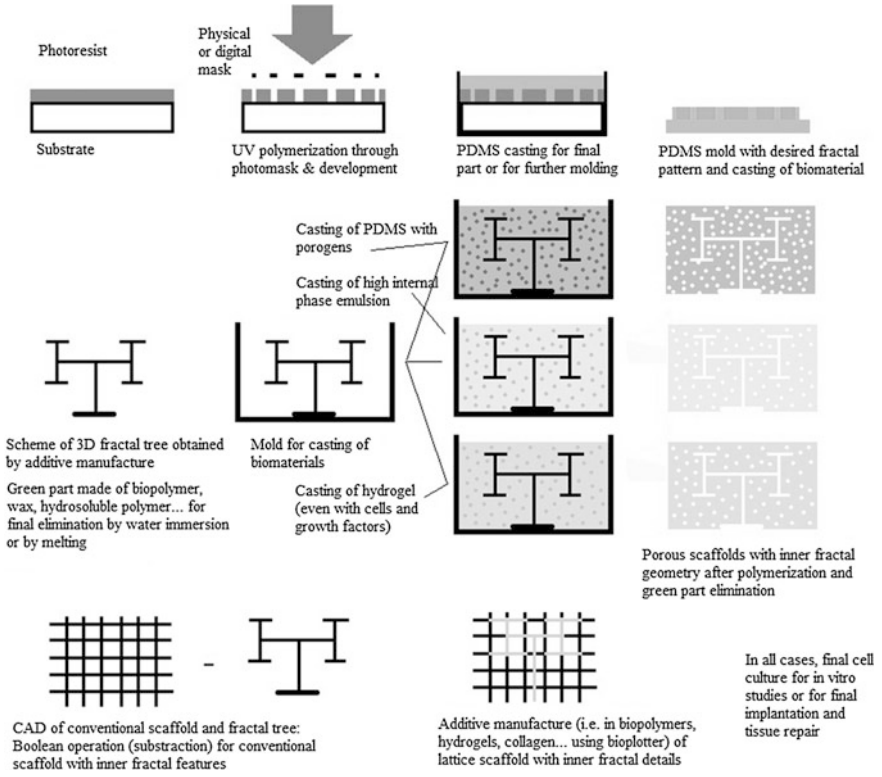

Fig. 15.6 Different manufacturing strategies towards the manufacture of cellculture platforms and scaffolds with biomimetic features and vascularization. **a** UV lithography for patterning and PDMS manufacture of mold for replication using biomaterials. **b** Alternatives for obtaining porous scaffolds with inner fractal features after green part elimination. **c** Direct additive manufacture of lattice scaffold with inner fractal-based vascular structure based on CAD design. Adapted from: Díaz Lantada et al. (2013), Fractals in tissue engineering

The use of soluble or meltable master models as mold inserts, for subsequently casting of biomaterials with porogens, and finally obtaining a porous scaffold with vascular structure is a remarkable option. The possibility of coating a rapid prototyped master or "green" part and subsequently solving the inner prototype structure, thus obtaining a scaffold formed by nanometric-thick hollow tubes (Schaedler et al. 2011), should also be explored due to its potential for helping in the development of vascularized tissues. Advances in the field of biophotopolymers are providing also adequate alternative solutions to the challenge of vascularization and are helping to obtain complex biomimetic scaffolds that combine precise structures, interesting mechanical properties and a satisfactory response for cell growth and tissue survival (Ovsianikov et al. 2012).

15.4 Case Study: Low Cost Rapid Prototyped Scaffolds

In the last years, additive manufacturing technologies have become much more accessible to small research teams and even to amateur designers, in part thanks to technological advances, but also to the expiration of several relevant patents in the field. One of the technologies that has helped to popularize and expand the impact of additive manufacturing is known as "fused-deposition modeling", but commonly referred to as conventional "3D printing".

In short, fused-deposition modeling (FDM) works on an "additive" principle by laying down material in layers and constructing the parts in a layer-by-layer way following the slices created after a CAD model. In the case of fused deposition modeling, a plastic filament (in some cases also metal wire) is unwound from a coil and supplies material to produce the desired parts. The more conventional FDM machines can be bought by components and mounted for less than 500 €, while some more professional models may cost up to 2,000–3,000 € for the desktop models and even 10,000 € for the industrial ones. In any case, these inversions in equipment are quite easy, when compared with industrial high-end additive manufacturing resources (laser stereolithography, selective laser sintering/melting, lithography-based ceramic manufacture, among others) with prices in the range 100,000–300,000 €.

An interesting feature of fused-deposition modeling for the biomedical industry is the fact that common polymers, typically used for biomedical microsystems for in vitro studies, can be incorporated to these machines and used for manufacturing biodevices. Even some biodegradable/bioabsorbable polymers can be employed (Hutmacher et al. 2001; Zein et al. 2002; Rosenzweig et al. 2015), with clear potentials for the tissue engineering and biofabrication fields, as well as for the promotion of drug delivery strategies. Combined materials can be used and even new machines, with several extruding systems, are being constructed for multi-material knowledge-based scaffold development.

A low-cost alternative for researchers in the field is linked to the employment of selective laser sintering of polyamide powder, which can be hired from several suppliers (i.e. i-Materialise) for the manufacture of in vitro models.

Figure 15.7 includes some examples of low-cost additive manufacture of biodevices for interacting at a cellular level: (1) a multi-layered honeycomb-like scaffold and (2) a functionally graded "knowledge-based" scaffold for tissue engineering and repair applications obtained by means of selective laser sintering of polyamide powder. On the other hand, Fig. 15.8 shows a "low-cost" FDM "Prusa Hephestos i3" machine for the production of rapid prototypes using thermoplastics such as PLA and Fig. 15.9 includes the computer-aided designs and the low-cost rapid prototypes (obtained by fused deposition modeling) of different geometries with potential use

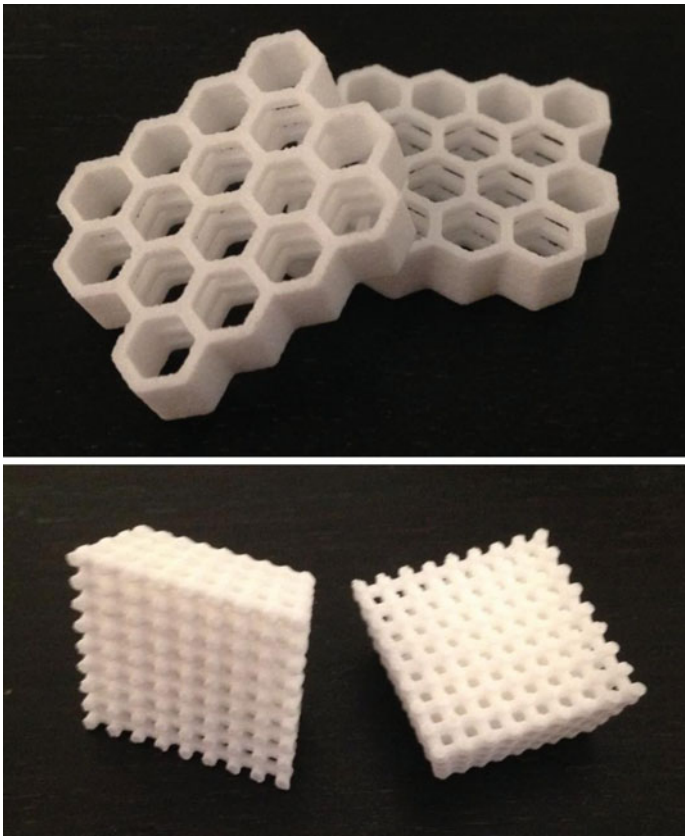

Fig. 15.7 Multi-layered honeycomb-like scaffold and functionally graded "knowledge-based" scaffold for tissue engineering applications obtained by means of selective laser sintering of polyamide powder

as tissue engineering scaffolds. The material used in this case is green PLA, although for cell culture purposes the use of coloured thermoplastics should normally be avoided, due to the potential release of toxic agents during culture.

A fundamental aspect of these low-cost alternatives is the common-place use of open-access software and the collaborative research environments that have burst out in the last years across internet. The RepRap project, aimed at the continued development of 3D printers, based on fused-deposition modeling and capable of printing their own parts for "self-replication", is one of the best examples of the impact of multidisciplinary collaboration for the setting the foundations of a new paradigm in manufacturing technology.

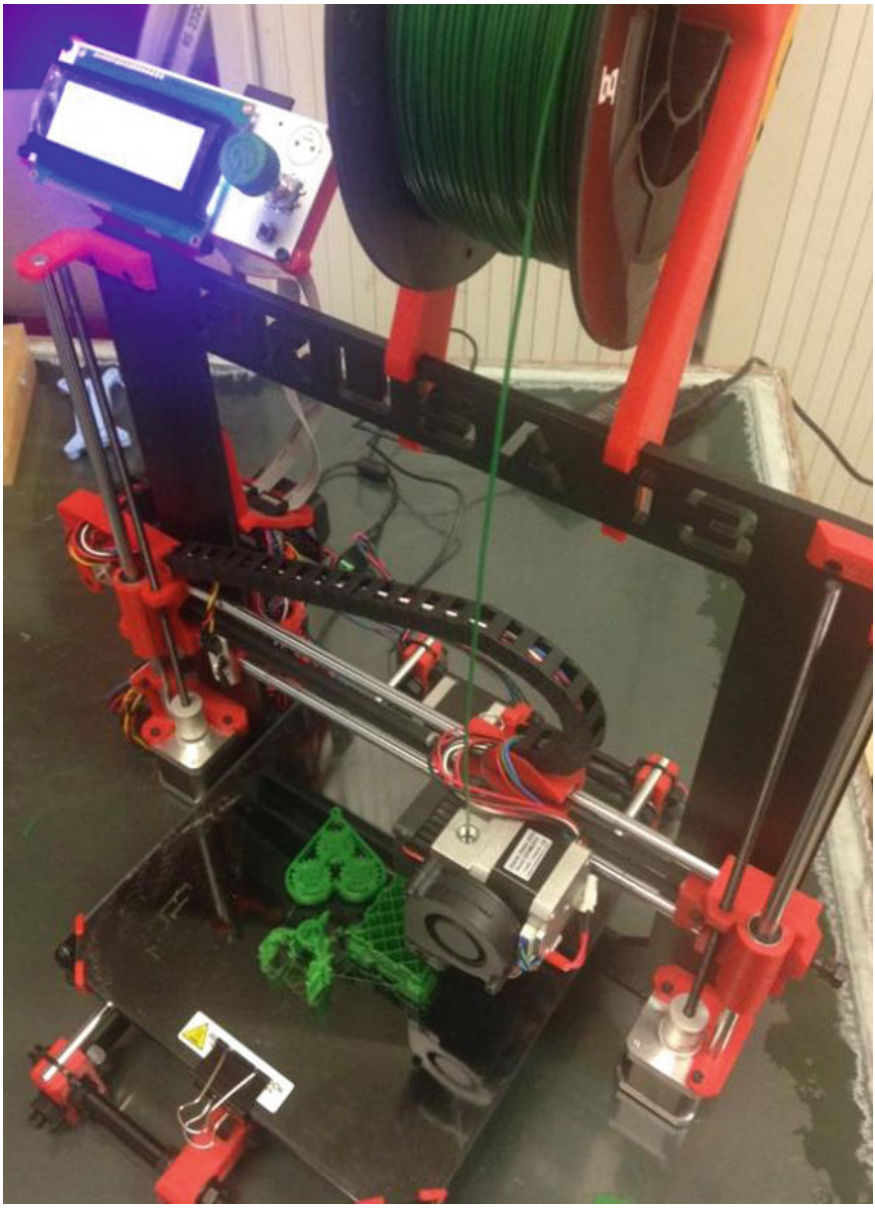

Fig. 15.8 Low-cost fused deposition modeling "Prusa Hephestos i3" machine for the production of rapid prototypes using thermoplastics such as PLA

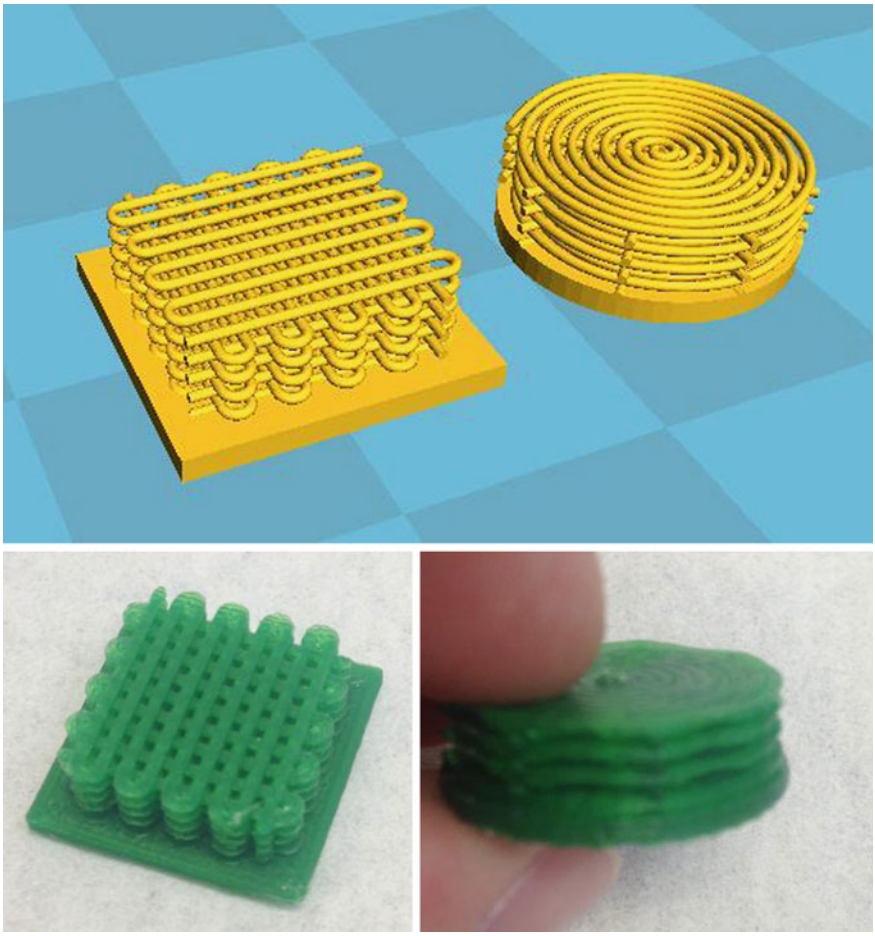

Fig. 15.9 Computer-aided designs and low-cost rapid prototypes (obtained by fused deposition modeling) of different geometries with potential use as tissue engineering scaffolds. Material: *green* PLA, although for cell culture purposes the use of coloured thermoplastics should normally be avoided (color online)

15.5 Case Study: Low Cost Models for Studying Tumor Growth

The complex microenvironmental conditions and interactions of the cell niche control cell behavior and fate, including tumorigenesis and tumor growth. Therefore, the development of biomimetic three-dimensional cell culture systems that allow for in vitro tumor modeling, by taking advantage of scaffolding

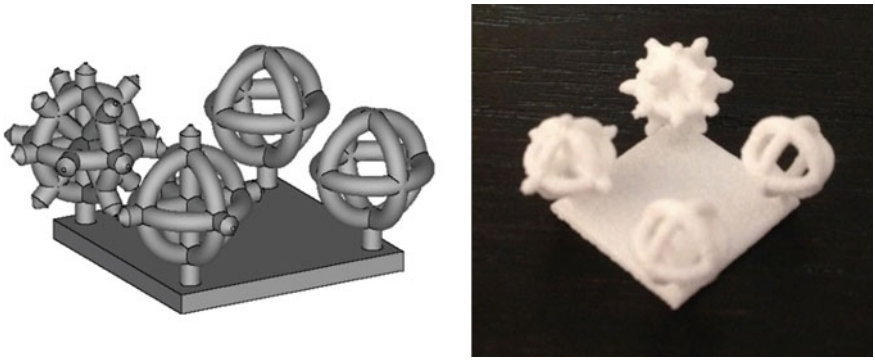

Fig. 15.10 Computer-aided design and low-cost rapid prototype, obtained by means of selective laser sintering of polyamide powder, of a multi-geometry cell culture microsystem for modeling tumoral growth

geometries for carrying out the culture and supporting cell growth, may greatly help studies of cancer cells' dependency on such microenvironmental conditions and interactions (Fischbach et al. 2007). The use of ex vivo approaches may even help with improved personalized evaluation of potential treatments for the benefit of patients.

In this direction, 3D porous polymeric scaffolds have been recently studied and compared to more common 2D tumor models and to other 3D culture models based on the encapsulation of cancerous cells within hydrogels. Clearly the three-dimensional approaches provide more biomimetic culture conditions, improved results and more effective information. The scaffolds-based solutions are easier to manipulate and provide better long-term performance, so it is interesting to further research in these directions (Zhang et al. 2013).

Figure 15.10 includes the computer-aided design and low-cost rapid prototype, obtained by means of selective laser sintering of polyamide powder, of a multi-geometry cell culture microsystem for modeling tumoral growth. The different spherical cages are aimed to obtain cellular spheroids under culture and the radial spikes, systematically introduced with variations in number and orientation, are thought to promote cell motility out of the spheroid for modeling the beginning of metastatic processes. The lattice spheres have an outer diameter of 6 mm and truss thickness, with a value of 600 μm, is near the current limits of the selective laser sintering technology applied to polymers. Forthcoming studies will be aimed to in vitro evaluation of these geometries, so as to qualitatively and quantitatively evaluate their potential as microsystems for modeling tumoral growth.

15.6 Main Conclusions and Future Research

Even though pioneer studies in the field of tissue engineering, either for disease study or for tissue repair, were performed on flat 2D substrates (normally Petri dishes), more recent research has helped to highlight the relevance of three-dimensional systems in cell culture. In fact, even one-dimensional patterns upon have been found more adequate, for mimicking actual cell migration in three-dimensional environments, than conventional two-dimensional scaffolds for cell culture, what puts forward the need for alternative development procedures aiming at a more adequate reproduction of the 3D environment, taking account of both biochemical and biomechanical approaches.

The combined employment of computer-aided design, engineering and manufacturing resources, together with rapid prototyping procedures, working on the basis of additive manufacturing approaches, allows for the efficient development of knowledge-based functionally graded scaffolds for effective and biomimetic three-dimensional cell culture in a wide range of materials. Applications of such tissue engineering scaffolds for cell culture include the repair, regeneration and even biofabrication of hard tissues, soft tissues and osteochondral constructs, as well as the modeling of disease development and management, as detailed in forthcoming chapters.

In this chapter we have presented some design and manufacturing strategies for the development of knowledge-based functionally graded tissue engineering scaffolds aimed at different types of tissues. We have also detailed the use of some prototyping approaches towards low-cost rapid prototyped scaffolds and tumor growth models, as cases of study for illustrating the complete development process of these types of medical devices.

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References

Bartolo PJS, Almeida H, Laoui T (2009) Rapid prototyping and manufacturing for tissue engineering scaffolds. Int J Comput Appl Technol 36:1

Boccaccini A, Kneser U, Arkudas A (2012) Scaffolds for vascularized bone regeneration: advances and challenges. Expert Rev Med Devices 9(5):457–460

Brem H, Walter K, Langer R (1993) Polymers as controlled drug delivery devices for the treatment of malignant brain tumors. Eur J Pharm Biopharm 39:2–7

Chan CKF, Chen CC, Luppen CA, Kraft DL, Kim JB, De Boer A, Wei K, Helms JA, Kuo CJ, Weissman IL (2009) Endochondral ossification is required for hematopoietic stem cell niche formation. Nature 457(7228):490

Cox SC, Thornby JA, Gibbons GJ, Williams MA, Mallick KK (2015) 3D printing of porous hydroxyapatite scaffolds intended for use in bone tissue engineering applications. Mater Sci Eng Part C 47:237–247

Díaz Lantada A (2013) Handbook of advanced design and manufacturing technologies for biodevices. Springer, Berlin

Díaz Lantada A, Endrino JL, Mosquera AA, Lafont P (2010a) Design and rapid prototyping of DLC coated fractal surfaces for tissue engineering applications. J Phys: Conf Ser 252 (1):012003

Díaz Lantada A, Mosquera A, Endrino JL, Lafont P (2010b) Design and rapid prototyping of DLC coated fractal surfaces for tissue engineering applications. J Appl Phys Conf Ser 252:012003

Díaz Lantada A, Pareja Sánchez B, Gómez Murillo C, Urbieta Sotillo J (2013) Fractals in tissue engineering: Towards biomimetic cell-culture matrices, microsystems and microstructured implants. Expert Rev Med Devices 10(5):629–648

Díaz Lantada A, Alarcón Iniesta E, Pareja Sánchez B, García-Ruíz JP (2014) Free-form rapid-prototyped PDMS scaffolds incorporating growth factors promote chondrogenesis. Adv Mater Sci Eng 2014:1–10. Article ID 612976. doi:10.1155/2014/612976

Doyle AD, Wang FW, Matsumoto K, Yamada K (2009) One-dimensional topography underlies three-dimensional fibrillar cell migration. J Cell Biol 184(4):481–490

Ekaputra AK, Zhou Y, Cool SMK, Hutmacher DM (2009) Composite electrospun scaffolds for engineering tubular bone grafts. Tissue Eng Part A 15(12):3779

Fischbach C, Chen R, Matsumoto T, Schmelze T, Brugge JS, Polverini PJ, Mooney DJ (2007) Engineering tumors with 3D scaffolds. Nat Methods 4:855–860

Hosseinkhani H, Hosseinkhani M, Kobayashi H (2005) Design of tissue-engineered nanoscaffold through self-assembly of peptide amphiphile. J Bioact Compos Polym 21(4):277–296

Hosseinkhani H, Hosseinkhani M, Khademhosseini A, Kobayashi H, Tabata Y (2006) Enhanced angiogenesis through controlled release of basic fibroblast growth factor from peptide amphiphile for tissue regeneration. Biomaterials 27(34):5836–5844

Hosseinkhani H, Hosseinkhani M, Tian F, Kobayashi H, Tabata Y (2007) Bone regeneration on a collagen sponge self-assembled peptide-amphiphile nanofiber hybrid scaffold. Tissue Eng 13 (1):11–19

Hosseinkhani H, Hosseinkhani M, Hattori S, Matsuoka R, Kawaguchi N (2010) Micro and nano-scale in vitro 3D culture system for cardiac stem cells. J Biomed Mater Res Part A 94 (1):1–8

Hosseinkhani H, Hong P-D, Yu D-S, Chen Y-R, Ickowicz D, Farber I-Y, Domb A-J (2012) Development of 3D in vitro platform technology to engineer mesenchymal stem cells. Int J Nanomed 7:3035–3043

Hutmacher DW, Schantz T, Zein I, Ng KW, Teoh SH, Tan KC (2001) Mechanical properties and cell cultural response of polycaprolactone scaffolds designed and fabricated via fused deposition modeling. J Biomed Mater Res 55(2):203–216

Infür R, Pucher N, Heller C, Lichtenegger H, Liska R, Schmidt V, Kuna L, Haase A, Stampfl J (2007) Functional polymers by two-photon 3D lithography. Appl Surf Sci 254:836

Laschke MW, Rücker M, Jensen G, Carvalho C, Mülhaupt R, Gellrich NC, Menger MD (2008) Incorporation of growth factor containing Matrigel promotes vascularization of porous PLGA scaffolds. J Biomed Mater Res A 85(2):397

Lindström S, Iles A, Persson J, Hosseinkhani H, Hosseinkhani M, Khademhosseini A, Lindström H, Svahn HA (2010) Nanoporous titania coating of microwell chips for stem cell culture and analysis. J Biomed Sci Eng 5:272–279

Lohfeld S, Tyndyk MA, Cahill S, Flaherty N, Barron V, Mc Hugh PE (2010) A method to fabricate small features on scaffolds for tissue engineering via selective laser sintering. J Biomed Sci Eng 3:138

Maher PS, Keatch RP, Donnelly K, Paxton Z (2009) Formed 3D bio-scaffolds via rapid prototyping technology. In: Presented at 4th European conference of the IFMBE, IFMBE proceedings, vol 22, issue 17, p 2200

Ovsianikov A, Mironov V, Stampfl J, Liska R (2012) Engineering 3D cell-culture matrices: multiphoton processing technologies for biological and tissue engineering applications. Expert Rev Med Devices 9(6):613–633

Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld V, Cohen S (2003) Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J Biomed Mater Res A 65(4):489

Richardson TP, Peters MC, Ennett AB, Mooney DJ (2001) Polymeric system for dual growth factor delivery. Nat Biotechnol 19:1029

Rosenzweig DH, Carelli E, Steffen T, Jarzem P, Haglund L (2015) 3D printed ABS and PLA scaffolds for cartilage and nucleus pulposus tissue regeneration. Int J Mol Sci 16(7):15118–15135

Ryan GE, Pandit AS, Apatsidis D (2008) Porous titanium scaffolds fabricated using a rapid prototyping and powder metallurgy technique. Biomaterials 29:3625

Schaedler TA, Jacobsen AJ, Torrents A, Sorensen AE, Lian J, Greer JR, Valdebilt L, Carter WB (2011) Ultralight metallic microlattices. Science 334(6058):962–965

Schuster M, Turecek C, Kaiser B, Stampfl J, Liska R, Varga F (2007a) Evaluation of biocompatible photopolymers I: photoreactivity and mechanical properties of reactive diluents. J Macromol Sci A 44:547

Schuster M, Turecek C, Kaiser B, Stampfl J, Liska R, Varga F (2007b) Evaluation of biocompatible photopolymers II: further reactive diluents. Monatsh Chem 138:261

Stampfl J, Fouad H, Seidler S, Liska R, Schwanger RF, Woesz A, Fratzl P (2004) Fabrication and moulding of cellular materials by rapid prototyping. Int J Mater Prod Technol 21(4):285

Stampfl J, Baudis S, Heller C, Liska R, Neumeister A, Kling R, Ostendorf A, Spitzbart M (2008) Photopolymers with tunable mechanical properties processed by laser-based high-resolution stereolitoraphy. J Micromech Microeng 18:125014

Tan JY, Chua CK, Leong KF (2010) Indirect fabrication of gelatin scaffolds using rapid prototyping technology. Virtual Phys Prototyping 5(1):45

Tan JY, Chua CK, Leong KF (2013) Fabrication of channeled scaffolds with ordered array of micro-pores through microsphere leaching and indirect rapid prototyping technique. Biomed Microdevices 15:83

Tzezana R, Zussman E, Levenberg SA (2008) Ultra-porous scaffold for tissue engineering, created via a hydrospinning method. Tissue Eng Part C 14(4):281

Wald HL, Sarakinos G, Mikos A, Vacanti J, Langer R (1993) Cell seeding in porous transplantation devices. Biomaterials 14:270–278

Warnke PH, Douglas T, Wollny P, Sherry E, Steiner M, Galonska S, Becker ST, Springer IN, Wiltfang J, Sivananthan S (2009) Rapid prototyping: porous titanium alloy scaffolds produced by selective laser melting for bone tissue engineering. Tissue Eng Part C: Methods 15(2):115

Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE (2001) Soft-lithography in biology and biochemistry. Annu Rev Biomed Eng 3:335–373

Zein I, Hutmacher DW, Tan KC, Teoh SH (2002) Fused deposition modeling of novel scaffold architectures for tissue engineering applications. Biomaterials 23:1169–1185

Zhang M, Boughton P, Rose B, Lee CS, Hong AM (2013) The use of porous scaffolds as tumor models. Int J Biomater ID 396056

Chapter 16
Tissue Engineering Scaffolds for Bone Repair: General Aspects

Andrés Díaz Lantada, Adrián de Blas Romero, Santiago Valido Moreno, Diego Curras, Miguel Téllez, Martin Schwentenwein, Christopher Jellinek and Johannes Homa

Abstract Hard tissue repair is a very relevant and challenging area for the emerging fields of tissue engineering and biofabrication due to the very complex three-dimensional structure of bones, which typically include important variations of porosities and related mechanical properties. The need of porous and rigid extra cellular matrices, of structural integrity, of functional gradients of mechanical properties and density, among other requirements, has led to the development of several families of biomaterials and scaffolds for the repair and regeneration of hard tissues, although a perfect solution has not yet been found. Further research is needed to address the advantages of different technologies and materials for manufacturing enhanced, even personalized, scaffolds for tissue engineering studies and extra cellular matrices with outer geometries defined as implants for tissue repair, as the niche composition and 3D structure play an important role in stem cells state and fate. The combined employment of computer-aided design, engineering and manufacturing (also CAD-CAE-CAM) resources, together with rapid prototyping procedures, working on the basis of additive manufacturing approaches, allows for the efficient development of knowledge-based functionally graded scaffolds for hard tissue repair in a wide range of materials and following biomimetic approaches. In this chapter we present some design and manufacturing strategies for the development of knowledge-based functionally graded tissue engineering scaffolds aimed at hard tissue repair. A complete case of study, linked to the development of a scaffold for tibial repair is also detailed to illustrate the proposed strategies.

A. Díaz Lantada (✉) · A. de Blas Romero · S. Valido Moreno · D. Curras · M. Téllez
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2,
28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

M. Schwentenwein · C. Jellinek · J. Homa
Lithoz GmbH, Mollardgasse 85a/2/64-69, 1060 Vienna, Austria

A. Díaz Lantada (ed.), *Microsystems for Enhanced Control of Cell Behavior*,
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16.1 Introduction: Passive Prostheses versus Active Scaffolds

Tissue engineering is based on the combination of biological, physical and engineering knowledge to promote the artificial development of improved replacements for tissues and organs linked to surgical repair strategies. A very relevant component, involved in tissue engineering processes, is the extra cellular matrix (ECM) or tissue engineering scaffold which serves as framework for cell growth, aggregation, phenotype expression and final tissue development (Langer and Vacanti 1993).

According to biomimetic design principles, the biomaterials used as scaffolds should be porous, so as to allow cell migration during the colonization process, as well as the transport of nutrients and waste to and from cells. Such biomaterial constructs also have to be resistant enough to withstand mechanical demands, especially if final implantation is desired. In addition, as cells are capable of feeling their microenvironment and of responding to the substrate texture upon which they lie, by changing their overall morphology, cytoskeleton configuration, and intra- and extracellular signaling, an increasing number of studies are focusing on advanced design and manufacturing technologies, so as to generate and modify the structures and surfaces of biomaterial. Aspects such as porosity, pore distribution and size or surface micro- and nano-textures promote cell adherence, migration and proliferation within the scaffold, for subsequent gene expression and differentiation into relevant cell types. Hence, both tissue progenitor cells and the extra cellular matrices play a fundamental role in tissue engineering strategies. The controlled design and fabrication of biomaterials used as scaffold structures is a key for regenerative medicine (Thomas et al. 2010; Chen et al. 2010; Buxboim and Discher 2010).

As previously detailed in Chap. 15, most processes for manufacturing micro-porous structures and materials (or metamaterials) for tissue engineering (Díaz Lantada 2013) involve a combination of materials in some step of the process and a final phase separation or leaching process, for obtaining a solid part with distributed small pores. Among most extended processes, gas-assisted injection molding is an industrial method based on injecting a molten resin or thermoplastic into a mold cavity and then applying a quantity of pressurized gas into the resin, so as to help to fill out the mold cavity and to create hollows and pores in the polymer. The incorporation of foaming agents as additives to polymers also allows the manufacture of polymeric parts with pores. In many strategies involving the development of artificial tissues, to obtain 3D-porous structures is absolutely required to irrigate the tissue and maintain an adequate liquid dynamics. The use of porogens is also commonplace; normally, the process involves mixing a liquid prepolymer with solid particles (typically wax, sugar, salt…). Once polymerization is produced, normally by UV exposure or by heating, a solid structure, formed by a polymeric network with dispersed particles, is obtained. Final porous structure is obtained by dissolving such disperse particles in water, other solvents or by heating. The use of prepolymer-water emulsions is also typical for obtaining a polymerizable mixture that after thermal or

UV-based polymerization provides a polymeric network with pores according to initial water content (i.e. polyHIPEs).

Main alternatives, for improving the control of scaffolds' pore size and distribution, from the design stage, is the use of micro additive manufacturing technologies (AMT), normally working on layer-by-layer processes, following the geometries obtained with the help of computer-aided designs (Bartolo et al. 2009; Tan et al. 2010). Electro-spinning can be also adapted to "layer-by-layer" fabrication and used for obtaining 3D porous structures (Ekaputra et al. 2009), even though the process is not as repetitive as the use of micro AMT. The progressive increase in precision of additive manufacturing technologies, together with their improved versatility thanks to a continuously increasing set of materials available for layer-by-layer processing, is greatly promoting applications linked to micro- and even nano-manufacturing of complex 3D geometries for very innovative medical solutions in several fields (Díaz Lantada 2013).

Scaffolds with design-controlled structures have been obtained by means of rapid prototyping technologies including: selective laser sintering (SLS) (Lohfeld et al. 2010), layered hydrospinning (Tzezana et al. 2008), laser stereolithography (SLA) (Díaz Lantada et al. 2010), digital light projection (DLP) (Stampfl et al. 2004) or two-photon polymerization (2PP) (Infür et al. 2007), and different materials including hydrogels (Maher et al. 2009), gelatin (Tan et al. 2010), titanium alloys (Ryan et al. 2008; Warnke et al. 2009), (bio)photopolymers (Stampfl et al. 2008) and ceramics (Cox et al. 2015). However in vitro validation of rapid-prototyped scaffolds is not so commonplace, as most combinations of processes and materials do not provide adequate biomaterials and in many cases generate toxic components.

Nevertheless some highly interesting research has already been published, including in vitro validation and systematic toxicity assessment (Schuster et al. 2007a, b). Advances in the field of biopolymers (Schuster et al. 2007a, b) together with the possibilities provided by thin coatings (Díaz Lantada et al. 2010), are bringing new possibilities to this area, although access to such materials and technologies is not always easy, as some of them are currently under development or only available in large research centres.

In any case, it seems clear that a universal methodology for tissue engineering scaffold development is not yet available, first of all due to the complexity of biological materials and systems, but also due to all the possible design resources, manufacturing technologies and related materials available, whose results have not been systematically compared. For instance, additive manufacturing technologies allow precise control of final geometries from the design stage; however such designs are normally obtained by combining Euclidean based (simple) geometries and final result does not always adequately mimic the geometrical and mechanical complexity of biomaterials.

On the other hand, tissue engineering scaffolds obtained by phase separation, biomimetic templating and more "traditional" processes typically lead to more biomimetic sponges, even though their final outer form and repeatability are more

difficult to control, than using computer-aided strategies linked to rapid prototyping using additive processes.

Therefore, further research is needed to address the advantages of combining different technologies (Tan et al. 2013) for manufacturing enhanced, even personalized, scaffolds for tissue engineering studies and extra cellular matrices with global (outer) geometries defined as implants for tissue repair. In addition, increasing data show that progenitor cell-niche formation is absolutely needed for tissue development and repair (Chan et al. 2009).

Indeed, the niche composition and 3D structure play an important role in stem cells state and fate. The niche is created by the specific combination of trofic factors produced by progenitor cells to maintain the capability for tissue repair and regeneration and by a specific extracellular matrix. Recent studies have helped to highlight the extreme relevance of the incorporation of adequate growth factors, within the scaffold, for promoting biological regulation, cell differentiation, angiogenesis and final tissue viability (Richardson et al. 2001; Perets et al. 2003; Laschke et al. 2008). Such inclusion of biochemical effects, derived from the incorporation of growth factors, adds additional uncertainties to the already complex to understand interactions between scaffolds' structure, morphology and mechanical properties. Consequently, studies addressing the synergies between ECMs and growth factors and their impact on tissue viability are needed, in the quest for a general methodology for tissue engineering scaffold development.

Regarding the in vitro development of hard tissues, biomechanical aspects have to be considered, as the typically cultured human-mesenchymal stem cells tend to differentiate into tissues according to the mechanical properties and structures of the scaffolds used as extracellular matrices. When pursuing the in vitro development of bone (as main representative of hard tissues), fundamental aspects such as: stiffness of the biomimetic construct, aiming at typical values of 5–20 GPa; degree of porosity, with values below 10 % for imitating the cortical bone and up to 80 % for imitating the trabecular parts; overall density around 1500–2000 kg/m^3; surface properties and microtopographies, hopefully to promote osseointegration by design; and aspects linked to bone vasculature, have to be adequately taken into account.

Considering such aspects and their mutual interactions requires the combined expertise of engineers and designers, materials scientists, biomedical professionals and manufacturing experts, so as to reach to the desired tissue engineering/repair/regeneration biomimetic and biomechanic constructs. Again the combined use of computer-aided design, of computer-aided engineering and of advanced (additive) manufacturing resources, for an effective, efficient, precise and sustainable control of matter in three-dimensional environments, lined to the geometry being repaired or regenerated, constitutes a remarkable good practice. The following sections provide some additional information regarding mechanical properties, biomimetic design strategies and industrially adequate manufacturing approaches. The use of some cases of study helps to put forward the versatility and adequacy of these synergies.

16.2 Mimicking Bone Properties with Synthetic Materials

Current solutions for bone repair typically rely on the use of compact metallic prostheses made of titanium, titanium alloys, Cr-Co and Cr-Co-Mo alloys, due to their mechanical endurance and long-term stability in the presence of biological fluids. However, the presence of mechanical mismatches between the common compact metallic prostheses and human bone leads in many cases to the stress-shielding phenomenon, which promotes non-uniform loading of the bone and loss of bone density (resorption) in the less-loaded parts. Consequently more flexible titanium alloys are being studied (Wang and Poh 2013). However other alternatives, which may lead to more adequate biomimetic and biomechanic performances and to the promotion of osseo-integration by design and elimination of bone-resorption by using functionally graded prostheses, are possible.

Having a look at Fig. 16.1, which shows Ashby-like diagram (Ashby 2005) aimed at helping researchers with materials selection tasks, it is evident a need for tuning the mechanical properties and density of synthetic materials for correctly mimicking the properties of bone. Both ceramics and alloys are more rigid than human bone, but currently constitute the more common materials families used for the development of (typically compact) prostheses. In order to adjust them to the characteristic properties of bone, novel design and manufacturing technologies pursuing porous, lattice or functionally graded geometries are needed, as described in the following Sect. 16.3.

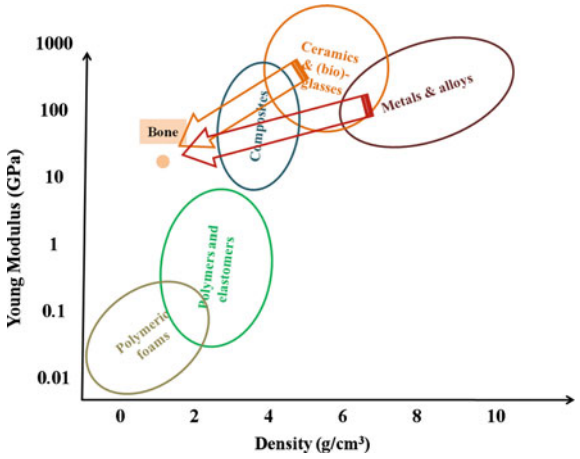

Fig. 16.1 Ashby diagram showing the need of tuning the mechanical properties and density of synthetic materials for mimicking the properties of bone

16.3 Design of Lattice Structures and Functionally Graded Materials

Lattice structures and functionally graded materials can be defined from the design stage, with the help of state-of-the-art computer-aided design resources, and directly obtained, even in one step, thanks to recent advances in the field of additive manufacturing. Several strategies can be applied to the generation of these biomimetic structures and present section details some cases of study.

In our case, computer-aided design of the different geometries are carried out with the help of NX-8.5 (Siemens PLM Solutions), mainly using combinations of parametric and matrix-based features and Boolean operations, as well as using convergent/divergent trusses for the incorporation of gradual variations to the values of density and mechanical properties. Figures 16.2, 16.3 and 16.4 show different lattices and porous structures of biomimetic lattice structures and functionally graded scaffolds for subsequent solid freeform fabrication for potential

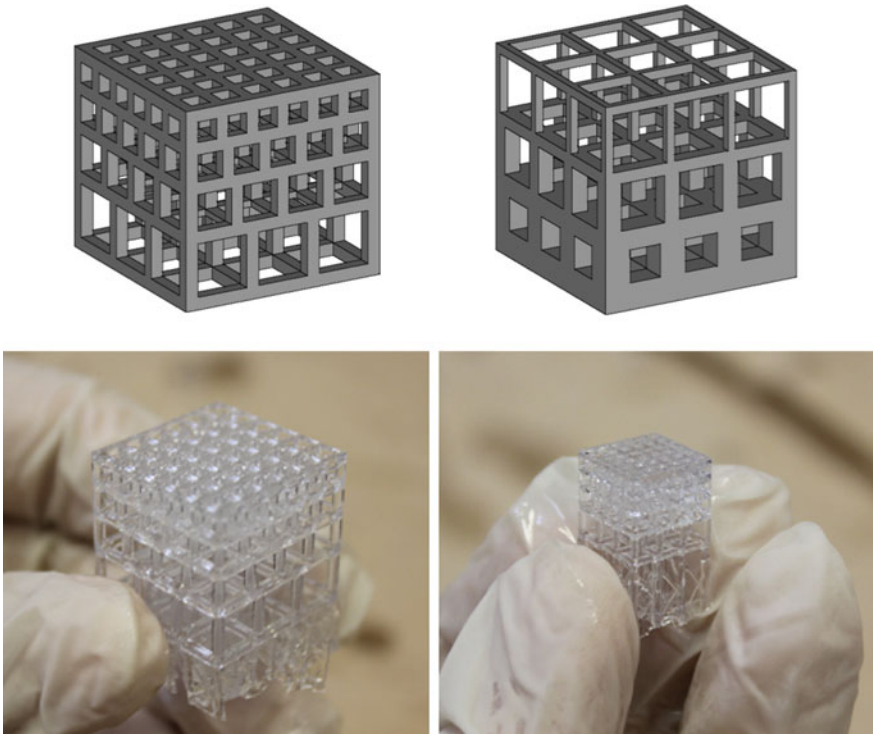

Fig. 16.2 Computer-aided designs and rapid prototypes obtained by laser stereolithography of biomimetic functionally graded scaffolds for bone repair

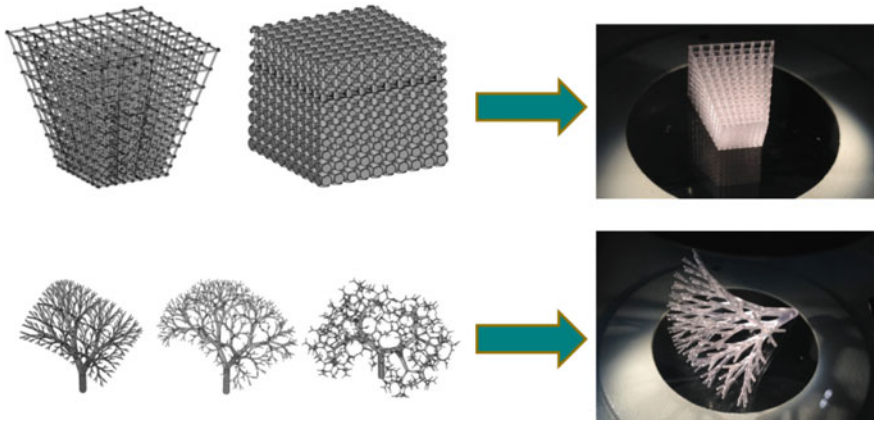

Fig. 16.3 Computer-aided designs and rapid prototypes obtained by laser stereolithography of biomimetic functionally graded scaffolds for bone repair and for modeling vascularization. The SLA-3500 machine from 3D Systems has been used for the manufacture of the epoxy prototypes

bone and even osteochondral repair strategies. In the different designs included, density and stiffness vary along their thickness, as happens in real living tissues.

The spatial control of scaffolds' properties is important, for making cells grow in a similar environment as can be found in real organisms and for artificially obtaining final tissues with a biomimetic structure. For instance, in advanced bone tissue engineering, the transition between the trabecular and cortical regions is very interesting indeed. Therefore, scaffolds designed in a similar fashion as those from Figs. 16.2, 16.3 and 16.4, may prove useful in articular repair strategies, especially for the bony phases.

The distances between pores and lattices are designed according and taking into account the manufacturing precision of state-of-the-art rapid prototyping facilities working with parts in the mm^3 range, which normally can achieve details in the range of 400–500 μm, as happens with the laser stereolithography technology used here to show the aspect of real prototypes in Figs. 16.2 and 16.3. Laser stereolithography has been used just for visualization purposes, as the toxicity of the epoxy resins normally used do not allow for the adequate development of in vitro trials.

Other state-of-the-art "3D printing" or additive manufacturing technologies, such as selective laser sintering, selective laser melting or electron beam melting, enable the manufacture of similar constructs, with details down to 400–500 μm for parts in the mm^3 range, using more adequate basis materials for in vitro and even in vivo trials, including Ti powder and other powders from different alloys. An additional degree of precision, even down to details of hundreds of nanometers, can be obtained by using two-photon lithography, as already detailed in Chap. 8, but only for parts in the μm^3 range. The promotion of final part size is challenging but

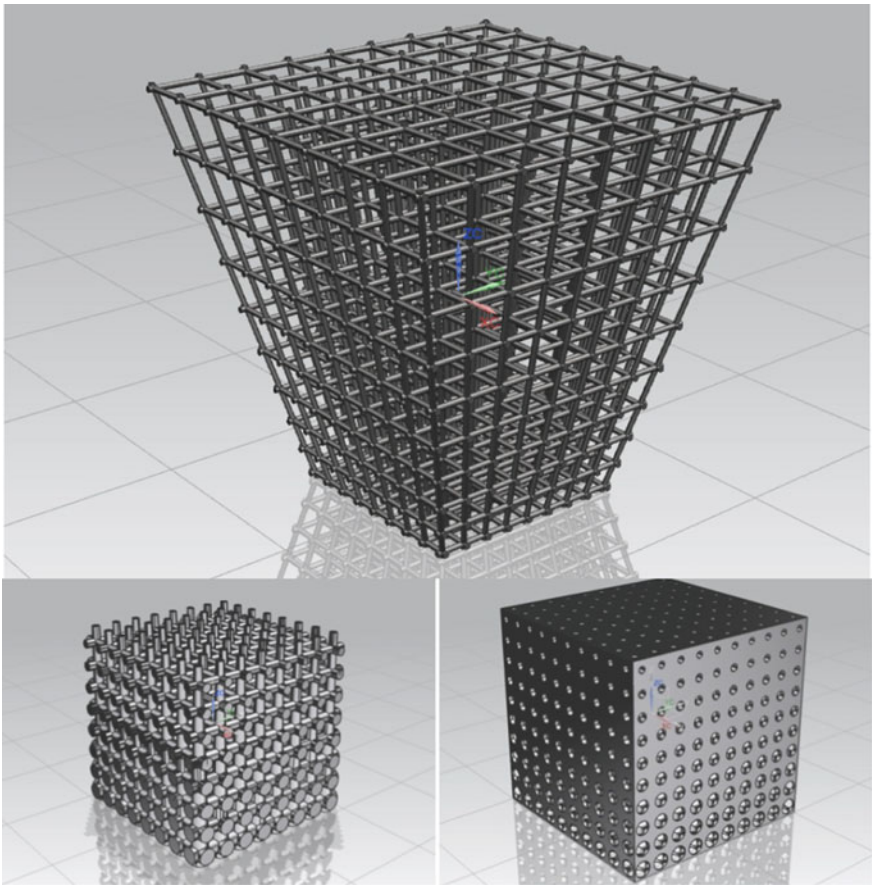

Fig. 16.4 Different computer-aided design approaches for the design of tissue engineering scaffolds with functional gradients of mechanical properties. CAD models courtesy of M.Sc. Eng. Antonio Sillero

necessary for applications interacting, not just at single cellular level, but with larger portions of tissues, whole organs and biological constructs.

Currently, a very thrilling compromise between achievable part size, manufacturing precision and use of ceramic and bioceramic materials, for the development of knowledge-based biomimetic tissue engineering scaffolds, is achieved by the technology called "lithography-based ceramic manufacture", developed under the lead of Prof. Stampfl and Prof. Robert Liska at the Technical University of Viena and commercialized by Lithoz GmbH (www.lithoz.com). Additional details are provided further on in Sect. 16.4.

16.4 Manufacture of Lattice Structures and Functionally Graded Materials

The manufacture of complex biomimetic and biomechanic lattice structures, porous geometries and functionally graded materials, whose applications in the biomedical field are notheworthy, can be directly accomplished by using additive manufacturing technologies, typically working on a layer-by-layer approach (even though new advances are enabling the manufacture of several layers at once) and following the information regarding the three-dimensional geometries of parts and constructs stored in form of CAD files.

As previously advanced in Sect. 16.3, state-of-the-art "3D printing" or additive manufacturing technologies, such as selective laser sintering, selective laser melting or electron beam melting, enable the manufacture of similar constructs, with details down to 400–500 μm for parts in the mm^3 range, using more adequate basis materials for in vitro and even in vivo trials, including Ti powder and other powders from different alloys, ceramics and even polymers, depending on final application. An additional degree of precision, even down to details of hundreds of nanometers, can be obtained by using two-photon lithography, but currently only for parts in the μm^3 range. The promotion of final part size is challenging but necessary for applications interacting, not just at single cellular level, but with larger portions of tissues, whole organs and biological constructs. Companies such as Stratasys, Realizer GmbH, SLM Solutions GmbH and Arcam provide some of the most interesting machines for metallic additive manufacturing based on different approaches and energy sources. Regarding two-photon polymerization, NanoScribe GmbH commercializes the most precise direct laser writing systems (the Photonic Professional series) currently available, although its medical applications linked to large implantable implants and tisular constructs would require larger manufacturing work-spaces (Fig. 16.5).

Concerning the manufacture of design-driven knowledge-based tissue engineering scaffolds, lattice structures and functionally graded structures, lithography-based ceramic manufacture provides the highest available degree of precision for the manufacture of highly complex geometries, using a wide set of (bio-)ceramic materials. The examples described further on and shown in Fig. 16.6 help to put forward the precision and quality of this approach. Precision is remarkable indeed and trusses with thicknesses down to 200 μm can be obtained, reaching the level of precision required for any biomimetic model of bone and with the possibility of promoting multi-scale and functional gradients of density, porosity and mechanical properties controlled from the design stage.

Once the functionally graded biomimetic tissue engineering scaffold designs are obtained and optimized for 3D printing, manufacture is accomplished by means of lithography-based ceramic manufacturing (LCM). The master models or green parts are manufactured, with previously prepared alumina slurries, by digital light processing (DLP) using the CeraFab 7500 machine, Lithoz GmbH.

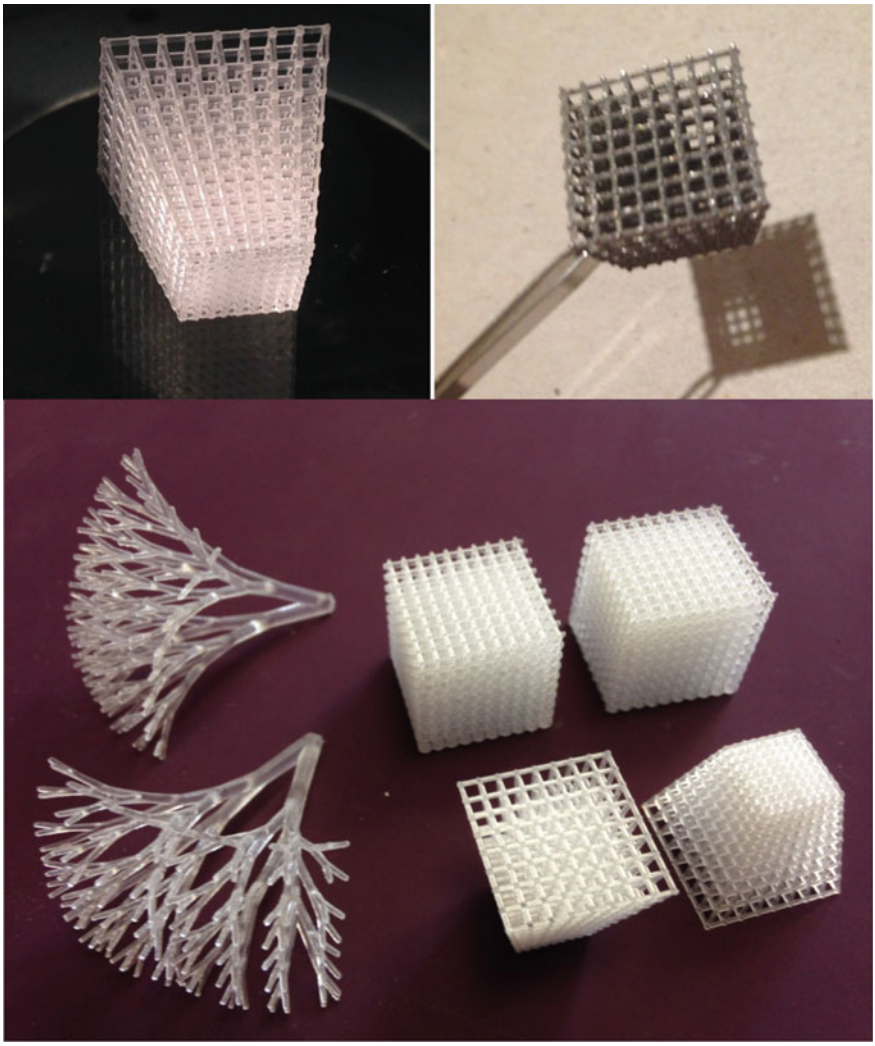

Fig. 16.5 Epoxy (*transparent-white*) and titanium (*grey*) functionally graded "knowledge-based" scaffolds for potential bone repair. The epoxy prototypes are obtained by laser stereolithography for ergonomic purposes, as the material is not adequate for cell cultures. The titanium prototype is obtained by selective laser sintering of titanium powder and aimed at in vitro cell culture studies. The SLA-3500 machine from 3D Systems has been used for the epoxy prototypes

The slurries are prepared with commercial Al_2O_3 powders. These powders are homogeneously dispersed, with the help of a dispersing agent, in a formulation containing reactive monomers and a solvent. In addition, the formulation contains a photo-initiator (in most cases less than 1 wt%). The photo-initiator reacts under an

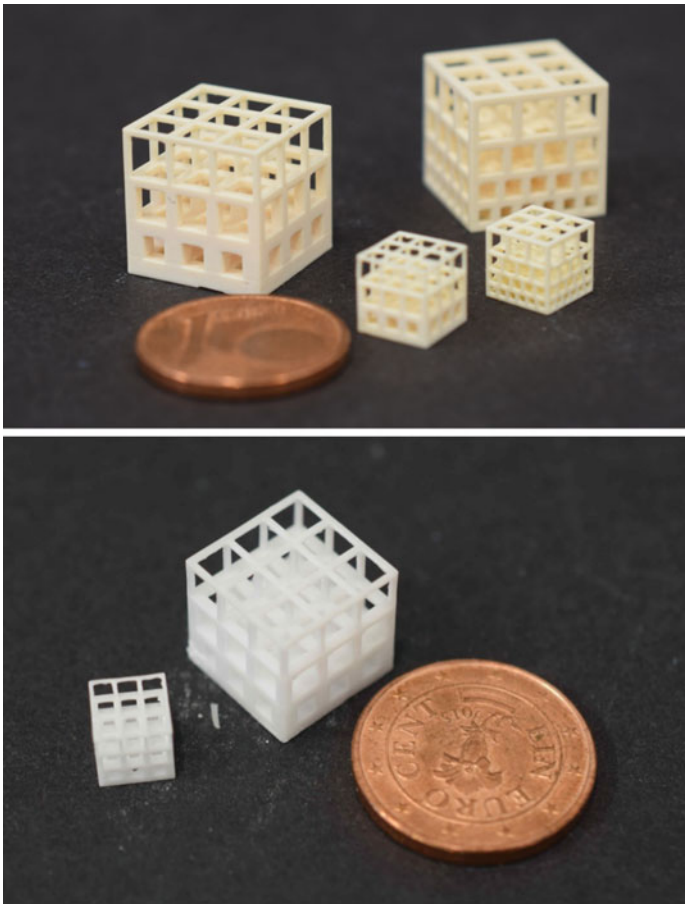

Fig. 16.6 Functionally graded "knowledge-based" scaffolds for potential bone repair manufacturing in ceramic material (alumina, among other options). The prototypes are obtained using lithography-based ceramic manufacture (LCM) technology commercialized by Lithoz GmbH, currently the most accurate AMT for the development of complex ceramic parts (www.lithoz.com). *Green* parts just after photopolymerization of a slurry with high content of ceramic power (*upper image*) and final sintered and compact ceramic parts (*lower image*). The manufacture of these prototypes was supported by the "Tomax: Tool-less manufacture of complex geometries" project, funded by the European Union Commission under grant n°: 633192—H2020-FoF-2014-2015/ H2020-FoF-2014

external energy source, in this case a LED emitting at 460 nm, which excites the initiator, creating radicals that chemically react with the monomers included in the mixture. The chain reaction forms the desired matrix of (meth)acrylate monomers that bind together the ceramic particles in the shape of the original part.

The reaction occurs in a brief lapse of time, while a determinate section of the part is being projected with specific intensity and exposure time parameters. DLP uses dynamic masks, which represent an individual cross section of the part being manufactured. The light engine uses high performance LEDs as light source and a DMD chip (digital mirror device) as dynamic mask with a resolution of 1920 × 1080 pixels and a pixel size of 40 × 40 μm (Schwentenwein and Homa 2015).

The fabrication of the parts is done in sequential layer-by-layer manner. For each individual layer fresh slurry is applied on the building envelope via a dosage system and subsequent rotation of the vat. Afterwards, the building platform is lowered into the dispersion to a distance of 25 μm to the bottom of the vat, which equals the thickness of an individual layer in the "green" bodies. Then the space-resolved exposure of the slurry is done by the projection of an image corresponding to the cross section of the current layer. After printing the desired parts, they are removed from the building platform. These "green" bodies are cleaned with a solvent for several minutes until the non-polymerized slurry is removed from the cavities of the part.

The polymeric-ceramic masters, once the parts are free of non-cured slurry, are subjected to a thermal treatment with the aim of obtaining final ceramic solid parts, free of any organic material which could be toxic for the cell development and for cell culture trials. The binder that makes up a large fraction of the "green" parts contains substances, which are not chemically compatible with the cells and must be eliminated for futher culture trials. In addition, as the ceramic particles are separated, green parts have lower density and mechanical properties than compact alumina.

The elimination of the organic components, for achieving final composition and properties, is carried out as described in previous research (Felzmann et al. 2012). In short, the thermal variation is controlled inside a high-temperature chamber furnace from 30 °C up to 400 °C. First of all, the solvent included within the slurry is evaporated. Subsequently, a slow temperature variation is provided, so as to get an adequate decomposition of the binder, without causing internal stresses due to the formation of gas at high temperature. In a second step, the alumina particles are sintered. The parts, already free of any toxic organic component surrounding the ceramic particles, are raised up to 1500 °C, hence achieving sintering and resulting in geometries made of a final compact material (although with a porous structure defined and controlled from the design stage).

16.5 Case Study: Design of a Scaffold for Tibial Repair

An area of biomedical engineering in which additive manufacturing resources are being frequently applied is the production of biological and anatomical models to support surgical training and planning tasks, as well as the development of

prototypes to simplify these surgical procedures. Alongside the development of such models for surgical training and planning, the direct manufacture of customised implantable medical devices has been, in the last two decades, the main bioengineering area to which additive manufacturing technologies have been implemented (Díaz Lantada and Lafont Morgado 2012). Major experiments have been performed to test their application in a number of surgical procedures, usually linked to bone and joint reconstruction (Gittard et al. 2009; Kocacikli et al. 2010), as well as cranioplasty or facial reconstruction, normally after traffic accidents (Goh et al. 2010; Probst et al. 2010). In these cases rapid prototyping technologies such as electro-beam melting or laser fusion are used to directly obtain meshes, structural supports or complete implants made of Ti or of Cr-Co alloys. Other technologies that work on ceramic materials, normally selective laser sintering and more recently lithography-based ceramic manufacture, are employed to produce prototypes with properties more similar to those of the bone to be replaced.

However, to optimize the personal approach it is important to continue making parallel advances in medical imaging technologies and supporting software for linking medical imaging with computer-aided design, and to influence aspects of policy and quality control so as to obtain completely safe products for patients. By means of example, we present a development of a tissue engineering scaffolds for repairing a portion of a damaged tibia. The tibia model is obtained from medical images and, using the NX-8.5 CAD software (Siemens PLM Solutions), a small conceptual cut for simulating an accident is performed. The "broken" portion of the tibia is used for performing an intersection with an already available lattice geometry from previously developed CAD libraries. FEM-based simulations (as shown in Chap. 15) help to select the adequate porosity for more adequately mimicking the mechanical properties of the bone being replaced. After the intersection is performed, the desired geometry can be manufactured using additive manufacturing resources.

Figure 16.7 helps to summarize the case of study, by showing the CAD model of a scaffold for tibial repair, obtained by means of Boolean operations, and the physical model of the repaired structure obtained by laser stereolithography (using the Form1 + machine from Formlabs and epoxy resin) for visual or pre-surgical studies. Final implantation would require the use of an additive manufacturing technology capable of working with metals or ceramics, which have been recently applied to the development of personalized implants for bone repair, usually in connection with plastic surgery and recovery from accidents (Wolfram 2014).

It is also important to note that the described porous, lattice or functionally graded geometries, developed as prosthetic elements towards hard tissue repair, may serve also as scaffolding elements for the incorporation of patients' cells, as well as of antibiotics and of analgesics, towards improved patient recovery based on the promotion of personalized approaches.

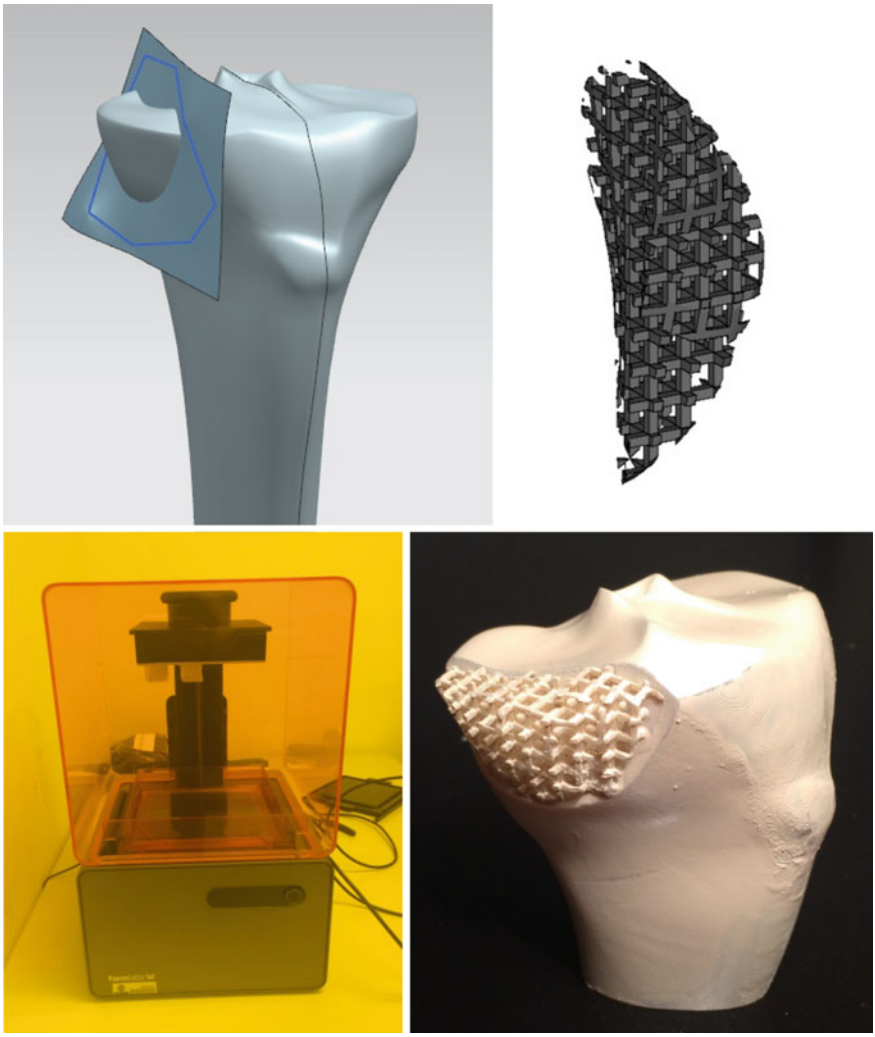

Fig. 16.7 CAD modeling of a scaffold for tibial repair by means of Boolean operations and physical model of the repaired structure obtained by laser stereolithography (Form1 + machine from Formlabs using epoxy resin)

16.6 Main Conclusions and Future Research

Hard tissue repair is a very relevant and challenging area for the emerging fields of tissue engineering and biofabrication due to the very complex three-dimensional structure of bones, which typically include important variations of porosities and related mechanical properties. The need of porous and rigid extra cellular matrices, of structural integrity, of functional gradients of mechanical properties and density,

among other requirements, has led to the development of several families of biomaterials and scaffolds for the repair and regeneration of hard tissues, although a perfect solution has not yet been found.

Further research is needed to address the advantages of different technologies and materials for manufacturing enhanced, even personalized, scaffolds for tissue engineering studies and extra cellular matrices with outer geometries defined as implants for tissue repair, as the niche composition and 3D structure play an important role in stem cells state and fate. The combined employment of computer-aided design, engineering and manufacturing (also CAD-CAE-CAM) resources, together with rapid prototyping procedures, working on the basis of additive manufacturing approaches, allows for the efficient development of knowledge-based functionally graded scaffolds for hard tissue repair in a wide range of materials and following biomimetic approaches.

In this chapter we have presented some design and manufacturing strategies for the development of knowledge-based functionally graded tissue engineering scaffolds aimed at hard tissue repair. A complete case of study, oriented towards the development of a scaffold for tibial repair has been also detailed to illustrate the proposed strategies. These strategies are also applied in Chap. 17, where the complete development of scaffolds for the very relevant field of dental repair is covered. The repair of soft tissues, also by means of knowledge-based scaffolds, is analyzed in Chap. 18, as an additional essential input for the more complex articular repair, which involves both hard and soft tissues, as detailed in Chap. 19.

Acknowledgments We acknowledge the support of the "Tomax: Tool-less manufacture of complex geometries" project, funded by the European Union Commission under grant nº: 633192 - H2020-FoF-2014-2015/H2020-FoF-2014 and led by Prof. Dr. Jürgen Stampfl from the Technical University of Vienna.

References

Ashby MF (2005) Materials selection in mechanical design, 3rd edn. Butterworth-Heinemann

Bartolo PJS, Almeida H, Laoui T (2009) Rapid prototyping and manufacturing for tissue engineering scaffolds. Int J Comput Appl Technol 36:1

Buxboim A, Discher DE (2010) Stem cells feel the difference. Nat Methods 7(9):695

Chan CKF, Chen CC, Luppen CA, Kraft DL, Kim JB, De Boer A, Wei K, Helms JA, Kuo CJ, Weissman IL (2009) Endochondral ossification is required for hematopoietic stem cell niche formation. Nature 457(7228):490

Chen WL, Likhitpanichkul M, Ho A, Simmons CA (2010) Integration of statistical modeling and high-content microscopy to systematically investigate cell-substrate interactions. Biomaterials 31:2489

Cox SC, Thornby JA, Gibbons GJ, Williams MA, Mallick KK (2015) 3D printing of porous hydroxyapatite scaffolds intended for use in bone tissue engineering applications. Mat Sci Eng: Part C 47:237–247

Díaz Lantada A (2013) Handbook of advanced design and manufacturing technologies for biodevices. Springer

Díaz Lantada A, Lafont Morgado P (2012) Rapid prototyping for biomedical engineering: Current capabilities and challenges. Annu Rev Biomed Eng 14:73–96

Díaz Lantada A, Mosquera A, Endrino JL, Lafont P (2010) Design and rapid prototyping of DLC coated fractal surfaces for tissue engineering applications. J Appl Phys Conf Ser 252:012003

Ekaputra AK, Zhou Y, Cool SMK, Hutmacher DM (2009) Composite electrospun scaffolds for engineering tubular bone grafts. Tissue Eng Part A 15(12):3779

Felzmann R, Gruber S, Mitteramskogler G, Tesavibul P, Boccaccini AR, Liska R, Stampfl J (2012) Lithography-based additive manufacturing of cellular ceramic structures. Adv Eng Mater 14(12):1052–1058

Gittard SD, Narayan RJ, Lusk J et al (2009) Rapid prototyping of scaphoid and lunate bones. Biotechnol J 4(1):129–134

Goh RCW, Chang CN, Lin CL et al (2010) Customised fabricated implants after previous failed cranioplasty. J Plast Reconstr Aesthet Surg 63(9):1479–1484

Infür R, Pucher N, Heller C, Lichtenegger H, Liska R, Schmidt V, Kuna L, Haase A, Stampfl J (2007) Functional polymers by two-photon 3D lithography. Appl Surf Sci 254:836

Kocacikli M, Korkmaz FM, Yazicioglu H et al (2010) Fabricating toe prostheses using 3D modeling technique: case report Turkiye Klinikleri Tip Bilimleri Dergisi 30(5):1750–1755

Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920

Laschke MW, Rücker M, Jensen G, Carvalho C, Mülhaupt R, Gellrich NC, Menger MD (2008) Incorporation of growth factor containing Matrigel promotes vascularization of porous PLGA scaffolds. J Biomed Mat Res A 85(2):397

Lohfeld S, Tyndyk MA, Cahill S, Flaherty N, Barron V, Mc Hugh PE (2010) A method to fabricate small features on scaffolds for tissue engineering via selective laser sintering. J Biomed Sci Eng 3:138

Maher PS, Keatch RP, Donnelly K, Paxton Z (2009) Formed 3D bio-scaffolds via rapid prototyping technology. In: Presented at 4th european conference of the IFMBE, IFMBE Proceedings 2009, vol 22, issue 17, p 2200

Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld V, Cohen S (2003) Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J Biomed Mat Res A 65(4):489

Probst FA, Hutmacher DW, Muller DF et al (2010) Calvarial reconstruction by customized bioactive implant Handchirurgie Mikrochirurgie Plastiche. Chirurgie 42(6):369–373

Richardson TP, Peters MC, Ennett AB, Mooney DJ (2001) Polymeric system for dual growth factor delivery. Nat Biotechnol 19:1029

Ryan GE, Pandit AS, Apatsidis D (2008) Porous titanium scaffolds fabricated using a rapid prototyping and powder metallurgy technique. Biomaterials 29:3625

Schuster M, Turecek C, Kaiser B, Stampfl J, Liska R, Varga F (2007a) Evaluation of biocompatible photopolymers I: photoreactivity and mechanical properties of reactive diluents. J Macromol Sci A 44:547

Schuster M, Turecek C, Kaiser B, Stampfl J, Liska R, Varga F (2007b) Evaluation of biocompatible photopolymers II: further reactive diluents. Monatsh Chem 138:261

Schwentenwein M, Homa J (2015) Additive manufacture of dense alumina ceramics. Appl Ceram Technol 12(1):1–7

Stampfl J, Fouad H, Seidler S, Liska R, Schwanger RF, Woesz A, Fratzl P (2004) Fabrication and moulding of cellular materials by rapid prototyping. Int J Mater Prod Technol 21(4):285

Stampfl J, Baudis S, Heller C, Liska R, Neumeister A, Kling R, Ostendorf A, Spitzbart M (2008) Photopolymers with tunable mechanical properties processed by laser-based high-resolution stereolitoraphy. J Micromech Microeng 18:125014

Tan JY, Chua CK, Leong KF (2010) Indirect fabrication of gelatin scaffolds using rapid prototyping technology. Virtual Phys Prototyping 5(1):45

Tan JY, Chua CK, Leong KF (2013) Fabrication of channeled scaffolds with ordered array of micro-pores through microsphere leaching and indirect rapid prototyping technique. Biomed Microdevices 15:83

Thomas WE, Discher DE, Shastri VP (2010) Mechanical regulation of cells by materials and tissues. MRS Bull 35:578

Tzezana R, Zussman E, Levenberg SA (2008) Ultra-porous scaffold for tissue engineering, created via a hydrospinning method. Tissue Eng Part C 14(4):281

Wang W, Poh CK (2013) Titanium alloys in orthopaedics. In Tech 1–20

Warnke PH, Douglas T, Wollny P, Sherry E, Steiner M, Galonska S, Becker ST, Springer IN, Wiltfang J, Sivananthan S (2009) Rapid prototyping: porous titanium alloy scaffolds produced by selective laser melting for bone tissue engineering. Tissue Eng Part C: Methods 15(2):115

Wolfram T (2014) Industrial development for resorbable CMF implants. In: KMM-VIN 2nd Industrial Workshop Current research and industrial issues in bone implant development. Fraunhofer IFAM Bremen, Germany

Chapter 17
Tissue Engineering Scaffolds for Bone Repair: Application to Dental Repair

Andrés Díaz Lantada and Axel Michel

Abstract Medical implants for bone repair are starting to benefit from advanced design and (micro-) manufacturing technologies that promote a precise control of final geometries and allow for the incorporation of design-controlled and in some cases personalized features for enhanced interaction at a cellular level. Recent advances in additive manufacturing technologies and available materials support solid free-form design and fabrication approaches, hence helping with device personalization and enabling a real 3D control of device geometry. Furthermore, the vast knowledge generated during last decades in the field of tissue engineering can be used as a source for redesigning all types of implants, pursuing improved biomechanical and biomimetic solutions, especially in the area of bone repair. Hybridizations between conventional compact bone implants and trabecular tissue engineering scaffolds can help to adjust the mechanical performance of bone repair solutions to that of real bone, thus promoting long-term stability and preventing bone resorption thanks to a more adequate stress distribution in service. The increased surface to volume ratio of such lattice or trabecular implants, based on the tissue engineering scaffold concept, helps with cellular attachment to the implant, improves cell motility due to the presence of irregularities that help them to "crawl", enhances osseointegration in the case of implants aimed at bone repair and promotes drug incorporation for disease prevention. The potential of dental scaffolds for the in vitro development of artificial teeth is also remarkable. The general aspects and main design and manufacturing strategies linked to these advanced scaffold-based implants for bone repair have been introduced in previous chapter. Here we focus on the area of dental implants, detailing novel concepts, describing the development process of a scaffold library for dental applications, modeling and discussing dental implant interactions with bone and also analyzing the more remarkable technologies capable of providing adequate results for the manufacture of high-precision dental solutions, when compared with other state-of-the-art manufacturing approaches.

A. Díaz Lantada (✉) · A. Michel
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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17.1 Introduction to Dental Implants and Prostheses

Dental implants are replacements for the root or roots of teeth. They are fixed to the jawbone or to the skull (normally screwed to the jaw or skull after an initial drilling) and are used to support dental prostheses (the visible parts of artificial teeth), including crowns, bridges, dentures, as well as facial prostheses. Usually dental implants are made of titanium and titanium alloys, with are: lightweight, very resistant to stresses and cyclic loadings, and provides a very interesting biological interaction, which normally leads to an adequate osseointegration. Some common types of dental implants and prostheses are included in Fig. 17.1. Due to the very adequate performance of titanium alloys, dental implants have the highest success rate of any implanted surgical device, according to the website: www.dentalimplants.com.

In spite of the well-known dental implant techniques and of the related commonly used good-practices, there are risks and possible complications related to dental implant therapy including: nerve injury and excessive bleeding during surgery and post-surgical problems due to a lack of osseointegration, to mechanical failures or to the appearance of chronical infection or inflammation.

Recent design, manufacturing, testing and implantation procedure, aimed at minimizing the required hands-on expertise of prosthetic technicians and maxillofacial surgeons, are helping to perform these interventions in a more systematic and automated way, as detailed in the next section. The use of novel materials and geometries for improved biological interactions and mechanical performances is also among the main research trends for dental substitutes with enhanced response and is clearly linked to synergistic advances in design technologies, modeling and simulation resource, synthesis, processing, manufacturing and characterization procedures and computer-aided surgical planning and support strategies.

In addition, hybrid concepts based on new combinations of traditional dental implants and tissue engineering scaffolds are gaining momentum and opening new horizons in this field, as will be also discussed along present chapter.

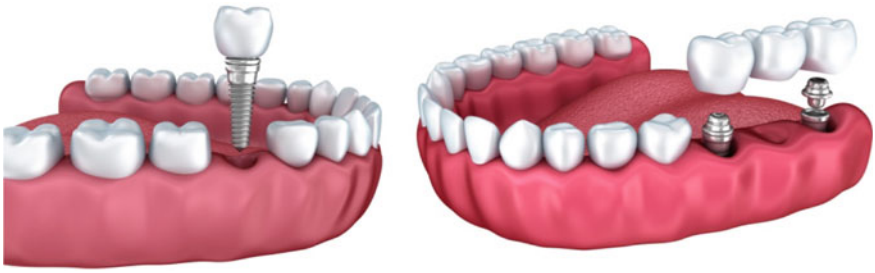

Fig. 17.1 Examples of dental implants and dental prostheses. *Left* Dental implant and crown. *Right* Dental implants and bridge. Purchased under standard license agreement: [alexmit]© www.123RF.com

17.2 Novel Concepts in the Field of Dental Implants

Medical implants for bone repair, including dental implants and prostheses, are starting to benefit from advanced design and (micro-) manufacturing technologies that promote a precise control of final geometries and allow for the incorporation of design-controlled and in some cases personalized features for enhanced interaction at a cellular level. Recent advances in additive manufacturing technologies and available materials support solid free-form design and fabrication approaches, hence helping with device personalization and enabling a real 3D control of device geometry.

Furthermore, the vast knowledge generated during last decades in the field of tissue engineering can be used as a source for redesigning all types of implants, pursuing improved biomechanical and biomimetic solutions, especially in the area of bone repair, and is also starting to have an impact in the fields of odontology and maxillofacial surgery. Hybridizations between conventional compact bone implants and trabecular tissue engineering scaffolds can help to adjust (in fact to define) the mechanical performance of bone repair solutions to that of real bone, thus promoting long-term stability and preventing bone resorption thanks to a more adequate stress distribution in service.

The increased surface to volume ratio of such lattice or trabecular implants, based on the tissue engineering scaffold concept, helps with cellular attachment to the implant, improves cell motility due to the presence of irregularities that help them to "crawl", enhances osseointegration in the case of implants aimed at bone repair and promotes drug incorporation for disease prevention. The potential of dental scaffolds for the in vitro development of artificial teeth is also remarkable: design strategies are covered in Sect. 17.3, in silico testing for design validation is covered in Sect. 17.4 and manufacturing procedures are finally analyzed in Sect. 17.5.

These technological breakthroughs act synergistically with improvements in the available biomaterials for the medical industry and with several technologies for micromanufacturing and for surface biofunctionalization detailed in Chaps. 8 and 9, as well as with the more specific resources cited here.

Although the general aspects and main design and manufacturing strategies linked to these advanced scaffold-based implants for bone repair have been introduced in previous chapter, particular details linked to device personalization must be taken into account and are covered in the following paragraphs.

It is necessary to mention that the advances seen in recent decades linked to all medical imaging systems (mainly, computed tomography (CT), Doppler-effect echo scans, nuclear magnetic resonance (NMR) or magnetic resonance imaging (MRI) and positron emission tomography (PET), as well as some more novel combinations PET/CT) have led to a very remarkable increase in the diagnostic capabilities of these tools, as well in the reliability of the diagnoses made based on this information and the therapeutic decisions consequently taken.

In parallel to the advances of medical imaging resources, during last couple of decades, biomedical device personalization has been greatly promoted by novel ways of combining medical imaging technologies and the related outer/inner-corporal information, with the capabilities of computer-aided design and engineering tools. In short, the information obtained using some of these medical imaging can be almost directly converted in three-dimensional objects (replicating the geometries and structures of the human body and of biological subsystems) and can subsequently be used as input in CAD programs, for designing personalized medical devices adapted to the morphologies of such biostructures (Osuna 2008; Ojeda Diaz 2009; Ojeda Diaz et al. 2009; Díaz Lantada et al. 2010; Díaz Lantada and Lafont Morgado 2011; Díaz Lantada 2013).

There are several software tools, for handling the information obtained from medical imaging technologies, and enabling computer-aided design, engineering and prototyping tasks. They are usually referred to as "MIMICS-like" programs (due to the relevance of MIMICS (Materialise NV)). Among such programs, due to their industrial impact and quality of results, it is important to mention at least:

- MIMICS (Materialise NV), for general purpose applications.
- Simplant (Materialise NV) especially oriented to Odontology.
- Surgiguide (Materialise NV) especially oriented to Odontology.
- 3D Doctor, for bone modeling from CT scan and soft tissue from MRI.
- Analyze (Mayo Clinic), for handling images from MR, CT and PET.
- MRIcro Software, for converting medical images to Analyze format.
- Biobuild, for converting volumetric imaging data to RP file formats.
- Volume Graphics, for general purpose applications.

Their applications to the dental field are noteworthy and the employment of medical imaging, combined with the adequate software, as input for the computer-aided design of personalized dental prostheses, mainly the personalized crowns and bridges, which will be supported by standard implanted screws, is now wide-spread. The use of symmetries, Boolean operations, patterns features, among other common operations available in CAD software, allows for a more systematic and rapid development of personalized crowns and bridges, especially as the CAD models can be used as input for CNC machining resources for direct manufacture of final parts in ceramic materials. Surgical planning is also possible and today constitutes one of the more relevant uses of rapid prototyping (Díaz Lantada and Lafont Morgado 2012).

In fact, not just surgical planning is possible, but also enhanced surgical procedures are being continuously developed thanks to the manufacture of guiding splints and other supporting prototypes, which help surgeons to carry out the more aggressive tasks with increased security. The use of stereolithographic templates for guiding the surgeons' tools and limiting their penetration, hence avoiding the potential damage to intramandibular nerve and helping to place the implants with the desired three-dimensional orientation for optimal performance, is note-worthy (Valente 2009).

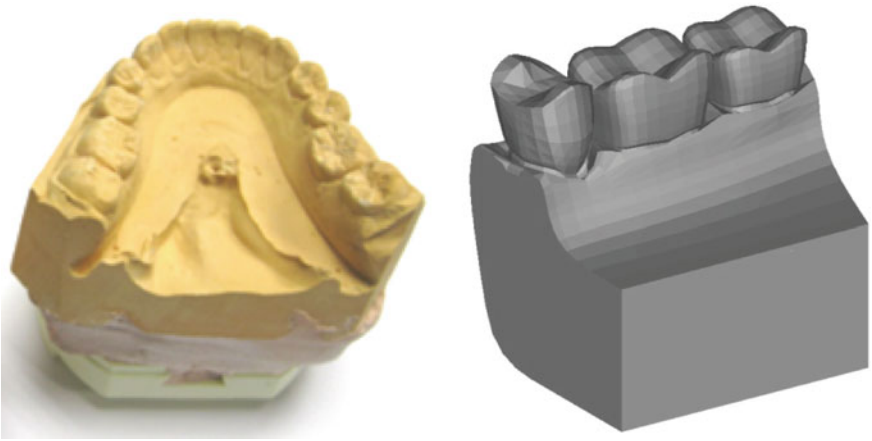

Fig. 17.2 Procedures for obtaining 3D CAD geometries of teeth for carrying out personalized prosthetics and implant design tasks

In order to carry out the aforementioned personalized approaches, it is first of all necessary to obtain a 3D CAD model of patient's jawbone and teeth. There are some alternative procedures, each with its own advantages and drawbacks. One possibility is to obtain a polymeric mold of patient's teeth by traditional processes and subsequently a ceramic or polymeric replica, as the one shown in Fig. 17.2 (left image).

The ceramic or polymeric replica can then be optically digitalized and further incorporated to a computer-aided design program for designing the crowns or bridges, for planning the optimal position and orientation of the different required implants, for designing surgical guides and even for in silico assessment of the mechanical performance of the desired reconstruction, as further detailed in the following Sect. 17.3.

Another option, possibly more precise, but more invasive for the patient, is the use of a medical imaging technology for obtaining a 3D model of the patient's jawbone and skull, which can be further processed with MIMICS-like programs and used as design input. In many cases the use of just a small portion of the teeth, as the example from Fig. 17.2 (right image) may be enough for some design tasks.

Apart from being a more dangerous procedure for the patients, due to being exposed to radiation during the medical imaging process, the difficulties related to the three-dimensional reconstruction of the softer tissues surrounding the bones and teeth, which are not always so clearly visualized and whose boundaries are sometimes fuzzy in the Hounsfield grayscale images typical from these imaging resources, must be also taken into account. Therefore, counting with well-trained professionals is a main key towards successful medical imaging-driven prosthetic designs.

17.3 Case Study: Development of a Scaffold Library for Dental Applications

The design of porous and lattice structures with the help of CAD resources is in fact simple and rapid, once a couple of operations are adequately combined. In some previous chapters a set of introductory examples have been provided and main design strategies have been also recently highlighted (Díaz Lantada and Lafont Morgado 2012; Díaz Lantada 2013).

In short, the process, for lattice structures, normally includes combination of solid operations (cylinders, piles…) for obtaining a unit cell and pattern or periodic replication of such solids and unit cells. Intersecting the obtained lattice with a solid implant leads to final device. In the case of porous structures, the process instead of additive is subtractive. It normally begins with a solid cube or cylinder, depending on the overall geometry of the desired implant, from which smaller spheres and cubes are usually subtracted. The porous structure (in fact a design-driven material, a knowledge-based material or a even metamaterial) obtained can additionally be intersected with the geometry of a solid prosthesis or biodevice, for finally obtaining a porous implant. Both processes are schematized in Fig. 17.3 included below.

Even though all conventional CAD programs already commented (Solid Edge, NX-8.5, Catia v.5, Solid Works, Autodesk-Inventor…) include several operations for designing unit cells and replicating them, for applying pores to solid objects and Boolean operations for applying an outer geometry to a lattice structure, novel CAD resources are being specifically developed for promoting the application of meta-materials to product development.

Among ad hoc CAD software oriented to the design of lattice and porous structures, for improved control of aspects such as density, stiffness and resistance of final geometries, we would like to cite "Within" (www.within-lab.com) and also "Netfabb" (www.netfabb.com), among the most advanced ones, and with direct application in Biomedical Engineering. Advances in topological optimization, a mathematical approach that optimizes material layout within a given design space, for a given set of loads and boundary conditions, are also helpful for deriving into lattice and porous structures and progressively being incorporated to conventional CAD resources (Bendsoe and Sigmund 2003; Schramm and Zhou 2006).

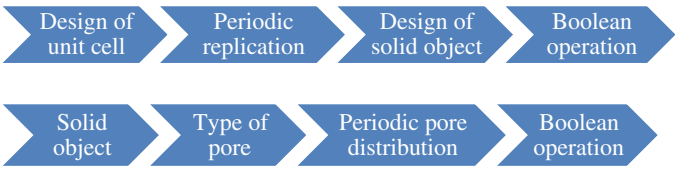

Fig. 17.3 Schematic processes for designing biodevices with lattice and porous structures

As application example, this section details the development of a CAD library of dental implants with porous, lattice and trabecular structures for a potentially improved osseointegration and mechanical performance, as the use of trabecular metallic implants helps to match the mechanical properties of bone, as described in previous chapters. Starting from four different common types of solid dental implants, which have been designed using the modeling resources of NX-8.5 (Siemens PLM Solutions), the final porous, lattice and trabecular structures are obtained with the help of "Within" (www.within-lab.com), thanks to its unique features and with the help of a trial version, which we acknowledge.

In more detail, "Within" is an engineering design software company that has created software and CAD designs with a degree of complexity and functionality oriented to the world of additive manufacturing. They have developed a new set of tools and design rules and implemented several software resources for optimizing component design with the help of tunable porous and lattice geometries to meet design specifications in an unmatched way. The optimized components can be manufactured using additive manufacturing machines to create products which perform beyond the state-of-the-art.

Within's technologies and software resources allow for the straight-forward development of lightweight materials and devices, flexible or robust structures depending on the desired displacement or loading requirements and truly functional bodies with functional gradients of properties. The osseointegration process may be even promoted from the design stage, thanks to the possibility of controlling main surface features.

Biomimetic approaches are additionally supported by Within's advanced processes for the generation of variable density lattices, which are ideally suited for mimicking the trabecular and compact structures of bones and the transitions among them. The process is much more direct than the use of Boolean operations with conventional CAD resources.

By means of example Fig. 17.4 shows the result of systematically applying different lattices of interest to develop a CAD library of trabecular dental implants based on the concept of the tissue engineering scaffold applied to conventional prostheses. Figure 17.5 shows the porosity distribution within the lattice core of an advanced porous dental implant.

After having analyzed different strategies for the personalized and biomimetic design of solutions for dental repair (and even regeneration, thanks to the potential of scaffold-based designs for incorporating patient's cells, growth factors and drugs for enhanced response), Sect. 17.4 focuses design validation by means of in silico assessment resorting to FEM-based modeling, considering the combined performance and the mutual interaction of implant and jawbone.

Fig. 17.4 CAD library of trabecular dental implants based on the concept of the tissue engineering scaffold applied to conventional prostheses (Within Lab software, trial version: http://www.withinlab.com/)

17.4 Case Study: Modeling the Interaction Between Dental Implants and Jaw Bone

Before investing in the manufacture of prototype series for mechanical testing and in vitro assessment of a potentially effective novel design of a dental implant, it is very advisable to carry out a set of systematic in silico evaluations, so as to predict the performance of the implant, to assess the influence of the main design parameters, to compare alternative possible solutions and to analyze the effects of the implant on the surrounding biological structures.

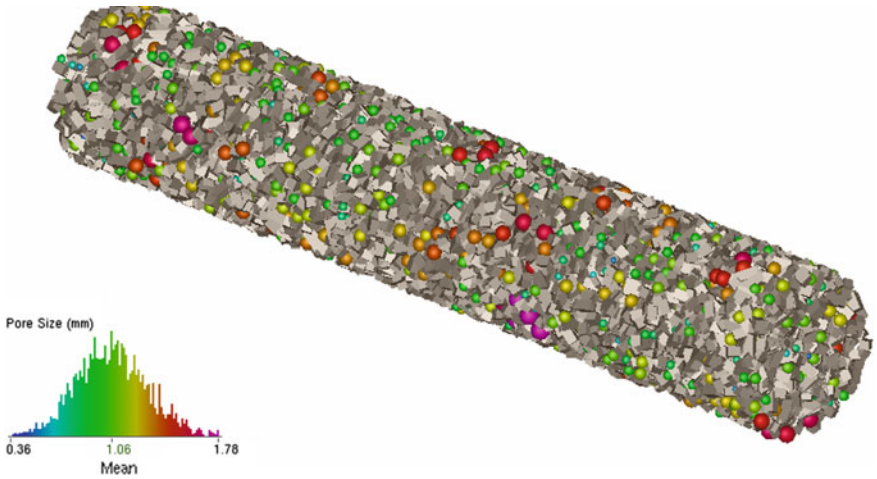

Fig. 17.5 Porosity distribution of the lattice core of an advanced porous dental implant (Within Lab software, trial version: http://www.withinlab.com/)

The complexity of geometries, the anisotropy of the materials involved (such as bone in the case of dental repair or other biological structures), the loading and boundary conditions, among other issues, prevent the use of analytical methods and promote the employment of numerical and computational approaches, such as FEM-based simulations.

In present case of study, we compare the performance of a solid titanium dental implant with that of an alternative implant with a lattice structure for biomimetic response. The implants are loaded axially with 1000 N and transversely with 200 N to simulate them in a very demanding situation. They are simulated screwed to a portion of a jawbone, reconstructed with the help of medical images, in which a trabecular bone nucleus and an outer cortical shell can be appreciated. Both of them are affected by the drilled implants. Bone properties have been applied using commonly used values and differentiating between the cortical and trabecular (or spongy) zones (Hasegawa et al. 2010).

Figure 17.6 helps to summarize the results from these FEM based simulations regarding the impact of using compact and porous implants on the stresses under loading and on the effects upon the jawbone.

Clearly, the lattice structure reaches higher stresses, up to 300 MPa and around a 30 % higher than the values obtained for the solid implant. However the implant is still well below the limit of the material and the presence of porosities may help to load cells from the patient for enhanced integration or drugs for preventing the appearance of disease and inflammation after implantation. Regarding the jaw, the use of a compact implant leads to lower maximal stresses, but also to some stress concentration phenomena in the cortical zone, related with stress-shielding, while the spongy part remains almost unloaded. The use of a lattice implant promotes compatibility of strains between implant and bone and leads to more areas of the

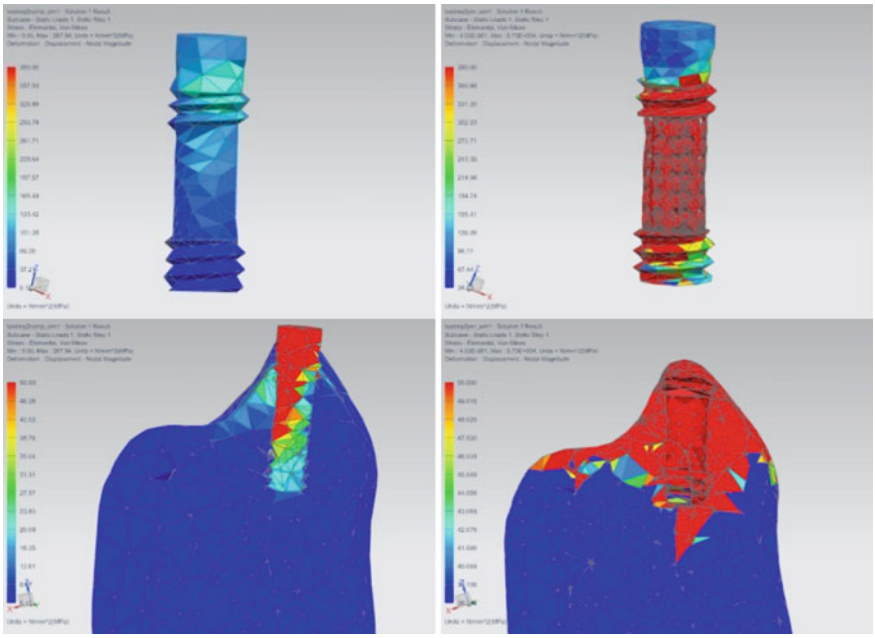

Fig. 17.6 FEM based simulation of the impact of compact and porous implants on the stresses under loading and on the effects upon the jaw

jawbone under acceptable stresses. The absence of stresses in the spongy bone, when using the compact implant, instead of having positive effects, may lead to bone resorption, loss of density and even implant failure. On the other hand, the lattice implant is more demanding for the jawbone, which may have very positive biomechanical effects for long-term durability of the union and for the mechanical stability of the implant, as bone is a living biomaterial, which regenerates itself taking into account the mechanical demands of the surrounding environment. The cells perceive them and respond accordingly, as the mechanical cues are part of the key aspects of the cell niche, together with other biochemical stimuli and with the presence of certain components within the extracellular matrix, towards gene expression and final tissue development.

17.5 Manufacturing Strategies for Dental Scaffolds and Trabecular Dental Implants

State-of-the-art CNC machining, being adequate for the direct manufacture of dental implants and crowns using the information from computer-aided designs, is inadequate for the manufacture of the very complex porous, lattice or trabecular

geometries of dental scaffolds, trabecular dental implants and similar hybrids for dental repair and even for dental regeneration (Zhang et al. 2013). Such more complex geometries must be obtained resorting to layer-by-layer manufacturing approaches or to real 3D printing technologies capable of producing detailed and complex parts with biomaterials including titanium, titanium alloys, (bio-)ceramics and, in the near future, composites, nano-composites, functionally graded materials and multi-layered materials, possibly resorting to combined processes (Park et al. 2010).

Computer-aided personalized dental implants, as well as more complex dental scaffolds and trabecular implants, can be obtained in titanium and titanium alloys, with adequate degrees of precision, using additive manufacturing resources such as selective laser sintering, selective laser melting and electron-beam melting. All these technologies start from a raw material in the form of metallic powder, which is heated with the help of different energy sources (laser-beam or electron-beam) until sintering or melting temperature. The processes are carried out in a layer-by-layer fashion and the final parts are obtained by the superposition of several layers following the desired geometry stored in the 3D CAD files, which drive the movements of the laser or electron beams. Companies such as Stratasys, Realizer GmbH, SLM Solutions GmbH and Arcam provide some of the most interesting machines for metallic additive manufacturing based on different approaches and energy sources.

Regarding the manufacture of design-driven dental scaffolds and trabecular dental implants in ceramic materials, which may prove even more biomimetic and biocompatible than titanium and related Ti-alloys, it is important to highlight the recent development of lithography-based ceramic manufacture (LCM), an additive manufacturing technology initially developed by the group of Prof. Jürgen Stampfl and Prof. Robert Liska at the Technical University of Vienna (TU Wien) and is currently commercialized by Lithoz GmbH (www.lithoz.com) (Felzmann et al. 2012; Schwentenwein and Homa 2015).

Lithography-based ceramic manufacture provides the highest available degree of precision for the manufacture of highly complex geometries, such as those from dental scaffolds and trabecular dental implants, using a wide set of (bio-)ceramic materials. Attainable precision, as already shown in the examples from Chap. 16, is remarkable indeed. Structural elements with thicknesses down to 200 μm can be obtained, reaching the level of precision required for any biomimetic model of bone and with the possibility of promoting multi-scale and functional gradients of density, porosity and mechanical properties controlled from the design stage.

17.6 Main Conclusions and Future Research

The vast knowledge generated during last decades in the field of biomedical materials and tissue engineering is starting to be applied as a source for designing novel types of implants for repair and regeneration tasks, pursuing improved

biomechanical and biomimetic solutions, especially in the area of bone repair and in the very relevant field of dental implants and prostheses.

Hybridizations between conventional compact bone implants and trabecular tissue engineering scaffolds are helping to adjust the mechanical performance of bone repair solutions to that of real bone, thus promoting long-term stability and preventing bone resorption thanks to more adequate distributions of stresses and strains. The increased surface to volume ratios of such porous, lattice or trabecular implants, based on the tissue engineering scaffold concept, help osseointegration thanks to enhanced cellular attachment to the implant, improve cell motility due to the presence of irregularities that help them to "crawl" and promote the use of incorporated drugs for disease prevention. The potential of dental scaffolds for the in vitro development of artificial teeth is also remarkable.

This chapter has focused on the area of advanced dental implants, detailing novel concepts, describing the development process of a scaffold library for dental applications, modeling and discussing dental implant interactions with bone (jaw-bone and skull are typically affected) and also analyzing the more remarkable technologies capable of providing adequate results for the manufacture of high-precision dental solutions.

Future challenges linked to optimizing the whole supply chain, from medical imaging and personalized implant design, to rapid manufacture of implantable components, taking account of the normative environment for the a combined promotion of personalized responses, optimal performance and patient security, still need to be addressed.

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References

Bendsoe MP, Sigmund O (2003) Topology optimization: theory, methods and applications. Springer

Díaz Lantada A (2013) Handbook of advanced design and manufacturing technologies for biodevices. Springer

Díaz Lantada A, Lafont Morgado P (2011) Enhancing product development through CT images, computer-aided design and rapid manufacturing: present capabilities, main applications and challenges. In: Theory and applications of CT imaging, PP 269–290. In Tech

Díaz Lantada A, Lafont Morgado P (2012) Rapid prototyping for biomedical engineering: current capabilities and challenges. Annu Rev Biomed Eng 14:73–96

Díaz Lantada A, Valle-Fernández R, Morgado PL, Muñoz-García J, Muñoz Sanz JL, Munoz-Guijosa JM, Otero JE (2010) Development of personalized annuloplasty rings: combination of CT and CAD-CAM tools. Ann Biomed Eng 36(1):l66–176

Felzmann R, Gruber S, Mitteramskogler G, Tesavibul P, Boccaccini AR, Liska R, Stampfl J (2012) Lithography-based additive manufacturing of cellular ceramic structures. Adv Eng Mater 14(12):1052–1058

Hasegawa A, Shinya A, Nakasone Y, Lassila LVJ, Vallittu PK, Shinya A (2010) Development of 3D CAD/FEM analysis system for natural teeth and jaw bone constructed from X-ray CT images. Int J Biomater 2010:1–7, ID 659802

Ojeda Diaz C (2009) Estudio de la influencia de estabilidad primaria en el diseño de vástagos de prótesis de cadera personalizadas. PhD thesis, Universidad Politécnica de Madrid

Ojeda Díaz C, Osuna López J, Lafont Morgado P, Díaz Lantada A (2009) Estudio de la estabilidad primaria, influencia en el diseño de vástagos de prótesis femorales personalizadas: Aplicación a paciente específico. 3º Congresso Nacional de Biomecânica. Sociedade Portuguesa de Biomecânica. 11–12 February 2009 in Braganza

Osuna JE (2008) Combinación de tecnologías para la optimización del desarrollo de prótesis. Master thesis, Universidad Politécnica de Madrid

Park CH, Rios HF, Jin Q, Bland ME, Flanagan CL, Hollister SJ, Giannobile WV (2010) Biomimetic hybrid scaffolds for engineering human tooth ligament interfaces. Biomaterials 31 (23):59455952

Schramm U, Zhou M (2006) Recent developments in the commercial implementation of topology optimization. Solid Mech Appl 137(6):238–249

Schwentenwein M, Homa J (2015) Additive manufacture of dense alumina ceramics. Appl Ceram Technol 12(1):1–7

Valente F (2009) Accuracy of computer-aided oral implant surgery: a clinical and radiographic study. Int J Oral Maxillofac Implants 24(2):234–242

Zhang L, Morsi Y, Wang Y, Li Y, Ramakrishna S (2013) Review scaffold design and stem cells for tooth regeneration. Jpn Dent Sci Rev 49(1):14–26

Chapter 18
Tissue Engineering Scaffolds for Repairing Soft Tissues

Andrés Díaz Lantada, Enrique Colomer Mayola, Sebastien Deschamps, Beatriz Pareja Sánchez, Josefa Predestinación García Ruíz and Hernán Alarcón Iniesta

Abstract Soft tissue repair is a very relevant and challenging area for the emerging fields of tissue engineering and biofabrication due to the complex three-dimensional structure in form of interwoven fibres and the relevant variations of mechanical properties present in these tissues. The need of elasticity, of structural integrity, of functional gradients of mechanical properties, among other requirements, has led to the development of several families of biomaterials and scaffolds for the repair and regeneration of soft tissues, although a perfect solution has not yet been found. Further research is needed to address the advantages of different technologies and materials for manufacturing enhanced, even personalized, scaffolds for tissue engineering studies and extra cellular matrices with outer geometries defined as implants for tissue repair, as the niche composition and 3D structure play an important role in stem cells state and fate. The combined use of computer-aided design, engineering and manufacturing resources together with rapid prototyping procedures, working on the basis of additive manufacturing approaches, allows for the efficient development of these types knowledge-based functionally graded scaffolds for soft tissue repair in a wide range of materials. In this chapter we present some design and manufacturing strategies for the development of knowledge-based tissue engineering scaffolds aimed at soft tissue repair. Complete cases of studies, linked to the development of several scaffolds for the repair of articular cartilage, tendons and muscles, with an example of a complete heart-valve scaffold and a set of scaffolds for artificial sphincters, are also detailed to illustrate the proposed strategies.

A. Díaz Lantada (✉) · E.C. Mayola · S. Deschamps · B. Pareja Sánchez
Mechanical Engineering Department – Product Development Laboratory, Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

J.P. García Ruíz · H. Alarcón Iniesta
Department of Molecular Biology, Universidad Autónoma de Madrid, Madrid, Spain

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18.1 Introduction: Tissue Engineering Soft Tissues

Tissue engineering, as detailed previously, is based on the combination of biological, physical and engineering knowledge to promote the artificial development of improved replacements for tissues and organs linked to surgical repair strategies. A very relevant component, involved in tissue engineering processes, is the extra cellular matrix (ECM) or tissue engineering scaffold which serves as framework for cell growth, aggregation, phenotype expression and final tissue development (Langer and Vacanti 1993). According to biomimetic design principles, the biomaterials used as scaffolds should be porous, so as to allow cell migration during the colonization process, as well as the transport of nutrients and waste to and from cells. Such biomaterial constructs also have to be resistant enough to withstand mechanical demands, especially if final implantation is desired. In addition, as cells are capable of feeling their microenvironment and of responding to the substrate texture upon which they lie, by changing their overall morphology, cytoskeleton configuration, and intra- and extracellular signaling, an increasing number of studies are focusing on advanced design and manufacturing technologies, so as to generate and modify the structures and surfaces of biomaterial. Aspects such as porosity, pore distribution and size or surface micro- and nano-textures promote cell adherence, migration and proliferation within the scaffold, for subsequent gene expression and differentiation into relevant cell types. Hence, both tissue progenitor cells and the extra cellular matrices play a fundamental role in tissue engineering strategies. The controlled design and fabrication of biomaterials used as scaffold structures is becoming increasingly important for regenerative medicine (Thomas et al. 2010; Chen et al. 2010; Buxboim and Discher 2010).

As previously detailed in Chap. 15, most processes for manufacturing micro-porous structures and materials (or metamaterials) for tissue engineering (Díaz Lantada 2013) involve a combination of materials in some step of the process and a final phase separation or leaching process, for obtaining a solid part with distributed small pores. Among most extended processes, gas-assisted injection molding is an industrial method based on injecting a molten resin or thermoplastic into a mold cavity and then applying a quantity of pressurized gas into the resin, so as to help to fill out the mold cavity and to create hollows and pores in the polymer. The incorporation of foaming agents as additives to polymers also allows the manufacture of polymeric parts with pores, which is absolutely required to irrigate the tissue and maintain an adequate liquid dynamics. The use of porogens is also commonplace; normally, the process involves mixing a liquid prepolymer with solid particles (typically wax, sugar, salt…). Once polymerization is produced, normally by UV exposure or by heating, a solid structure, formed by a polymeric network with dispersed particles, is obtained. Final porous structure is obtained by dissolving such disperse particles in water, other solvents or by heating. The use of prepolymer-water emulsions is also typical for obtaining a polymerizable mixture that after thermal or UV-based polymerization provides a polymeric network with pores according to initial water content (i.e. polyHIPEs).

Main alternatives, for improving the control of scaffolds' pore size and distribution, from the design stage, is the use of micro additive manufacturing technologies (AMT), normally working on layer-by-layer processes, following the geometries obtained with the help of computer-aided designs (Bartolo et al. 2009; Tan et al. 2010). Electro-spinning can be also adapted to "layer-by-layer" fabrication and used for obtaining 3D porous structures (Ekaputra et al. 2009), even though the process is not as repetitive as the use of micro AMT. The progressive increase in precision of additive manufacturing technologies, together with their improved versatility thanks to a continuously increasing set of materials available for layer-by-layer processing, is greatly promoting applications linked to micro- and even nano-manufacturing of complex 3D geometries for very innovative medical solutions in several fields (Díaz Lantada 2013).

Scaffolds with design-controlled structures have been obtained by means of rapid prototyping technologies including: selective laser sintering (SLS) (Lohfeld et al. 2010), layered hydrospinning (Tzezana et al. 2008), laser stereolithography (SLA) (Díaz Lantada et al. 2010), digital light projection (DLP) (Stampfl et al. 2004) or two-photon polymerization (2PP) (Infür et al. 2007), and different materials including hydrogels (Maher et al. 2009), gelatin (Tan et al. 2010), titanium alloys (Ryan et al. 2008; Warnke et al. 2009), (bio)photopolymers (Stampfl et al. 2008) and ceramics (Cox et al. 2015). However in vitro validation of rapid-prototyped scaffolds is not so commonplace, as most combinations of processes and materials do not provide adequate biomaterials and in many cases generate toxic components.

Nevertheless some highly interesting research has already been published, including in vitro validation and systematic toxicity assessment (Schuster et al. 2007a, b). Advances in the field of biopolymers (Schuster et al. 2007a, b) together with the possibilities provided by thin coatings (Díaz Lantada et al. 2010), are bringing new possibilities to this area, although access to such materials and technologies is not always easy, as some of them are currently under development or only available in large research centres.

In any case, it seems clear that a universal methodology for tissue engineering scaffold development is not yet available, first of all due to the complexity of biological materials and systems, but also due to all the possible design resources, manufacturing technologies and related materials available, whose results have not been systematically compared. For instance, additive manufacturing technologies allow precise control of final geometries from the design stage; however such designs are normally obtained by combining Euclidean based (simple) geometries and final result does not always adequately mimic the geometrical and mechanical complexity of biomaterials.

On the other hand, tissue engineering scaffolds obtained by phase separation, biomimetic templating and more "traditional" processes typically lead to more biomimetic sponges, even though their final outer form and repeatability are more difficult to control, than using computer-aided strategies linked to rapid prototyping using additive processes.

Therefore, further research is needed to address the advantages of combining different technologies (Tan et al. 2013) for manufacturing enhanced, even personalized, scaffolds for tissue engineering studies and extra cellular matrices with global (outer) geometries defined as implants for tissue repair. In addition, increasing data show that progenitor cell-niche formation is absolutely needed for tissue development and repair (Chan et al. 2009).

Indeed, the niche composition and 3D structure play an important role in stem cells state and fate. The niche is created by the specific combination of trophic factors produced by progenitor cells to maintain the capability for tissue repair and regeneration and by a specific extracellular matrix. Recent studies have helped to highlight the extreme relevance of the incorporation of adequate growth factors, within the scaffold, for promoting biological regulation, cell differentiation, angiogenesis and final tissue viability (Richardson 2001; Perets et al. 2003; Laschke et al. 2008). Such inclusion of biochemical effects, derived from the incorporation of growth factors, adds additional uncertainties to the already complex to understand interactions between scaffolds' structure, morphology and mechanical properties. Consequently, studies addressing the synergies between ECMs and growth factors and their impact on tissue viability are needed, in the quest for a general methodology for tissue engineering scaffold development.

Regarding the in vitro development of soft tissues, biomechanical aspects have to be considered, as the typically cultured human-mesenchymal stem cells tend to differentiate into tissues according to the mechanical properties and structures of the scaffolds used as extracellular matrices. When pursuing the in vitro development of muscle, cartilage, tendon, ligament, vasculatures, nervous tissues and skin (as main representatives of soft tissues), fundamental aspects such as: stiffness of the biomimetic construct, aiming at typical values of 10–1000 MPa; tissue micro-structure and the possible presence of inter-woven fibrils, with many different orientations depending on the organ or tissue type; overall density around 800–1200 kg/m^3; surface properties and microtopographies, hopefully to promote cell growth and conversion into relevant tissues; and all aspects linked to vasculature, have to be adequately taken into account.

Considering such aspects and their mutual interactions requires the combined expertise of engineers and designers, materials scientists, biomedical professionals and manufacturing experts, so as to reach to the desired tissue engineering/repair/regeneration biomimetic and biomechanic constructs. Again the combined use of computer-aided design, of computer-aided engineering and of advanced (additive) manufacturing resources, for an effective, efficient, precise and sustainable control of matter in three-dimensional environments, lined to the geometry being repaired or regenerated, constitutes a remarkable good practice. The following sections provide some additional information regarding mechanical properties, biomimetic design strategies and industrially adequate manufacturing approaches. The use of some cases of study helps to put forward the versatility and adequacy of these synergies.

18.2 Mimicking Soft Tissues with Synthetic Materials

Among soft tissues, artificial skin patches and artificial ligaments constitute the most common and successful synthetic solutions for repair and regeneration (Shores et al. 2007; Halim et al. 2010; Legnani et al. 2010). The success of such patches is connected with the simpler structure of skin and ligament, when compared to other soft tissues such as cartilage, tendon and muscle. In any case, all soft tissues will benefit in the forthcoming years of further advances and synergies among the fields of tissue repair and regeneration, biofabrication, materials science and advanced design and manufacturing technologies.

Having a look at Figs. 18.1 and 18.2, which show Ashby-like diagrams (Ashby 2005) aimed at helping researchers with materials selection tasks, it is evident a need, for tuning the mechanical properties and the density of synthetic materials, for correctly mimicking the properties of soft tissues. Both composites, polymers and elastomers are more rigid than most soft tissues, but currently constitute the more common materials families used for the development of (typically compact) prostheses. In order to adjust them to the distinctive properties of tissues such as tendon, ligament, cartilage and muscles, novel design and manufacturing technologies pursuing porous, lattice or functionally graded geometries are needed, as described in the following sections.

It is also important to note that the described porous, lattice or functionally graded geometries, developed as prosthetic elements towards soft tissue repair, may serve also as scaffolding elements for the incorporation of patients' cells, as well as of antibiotics and of analgesics, towards improved patient recovery based on the promotion of personalized approaches.

Considering that main design and manufacturing generic strategies towards the development of porous, lattice and functionally graded knowledge-based materials (and biodevices based on them) have been previously analyzed, the following pages

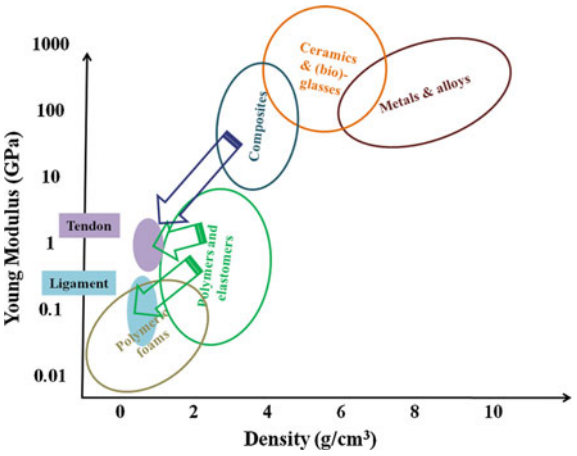

Fig. 18.1 Ashby diagram showing the need of tuning the mechanical properties and density of synthetic materials for mimicking the properties of tendons and ligaments

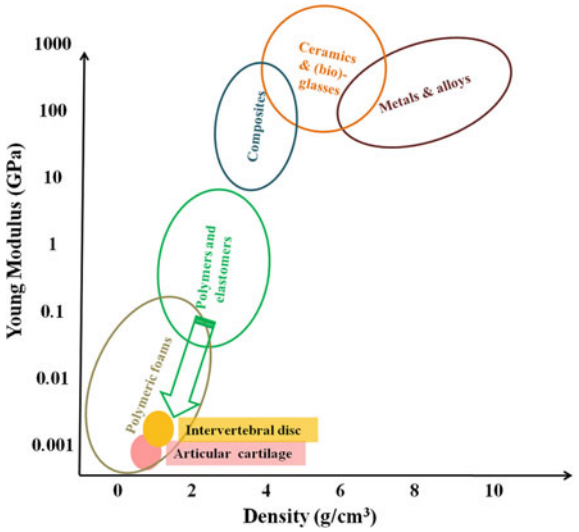

Fig. 18.2 Ashby diagram showing the need of tuning the mechanical properties and density of synthetic materials for mimicking the properties of cartilage

focus on different cases of study, which are linked to specific biodevices, with micro-metric features, developed for the repair and potential regeneration of cartilage, tendon, ligament and muscular cardiac tissue, as relevant representatives of soft tissues.

18.3 Case Study: Scaffolds for Cartilage Repair

Adult articular cartilage exhibits little capacity for self-renewal and repair, and thus even minor injuries or lesions, normally consequence of excessive exercise or accidents, may lead to progressive damage and osteoarthritic joint degeneration, resulting in significant pain and disability. There have been numerous attempts to develop tissue-engineered grafts or patches to repair chondral and osteochondral defects; however, success has been quite limited and significant challenges are still present, regarding the clinical application of cell-based therapies for cartilage repair. Some of the more promising solutions have been reviewed (Johnstone et al. 2013).

Apart from previously reviewed ideas, it is important to highlight that articular cartilage stands out for its elasticity, with a Young's modulus of 0.45–0.8 MPa (Mansour 2003), which is necessary for performing adequately as bone protector and impact dampener. Mimicking the properties of cartilage with synthetic elastomers seems adequate, although the needed elasticity may require the use of ad hoc developed porous elastomers. For instance, microporous PDMS has already been proposed as a functional material for cell culture where high degree of porosity is required to improve cell survival and functions. PDMS sponges have been obtained using precursor microemulsions (Chen et al. 2012; Peng et al. 2012) and other processes based on particle (usually salt or sugar) leaching (Yuen et al. 2011).

A recently proposed process by our team, which combines the adequate mechanical properties of porous PDMS obtained by particle leaching, with the great impact of using human-mesenchymal stem cells conditioned media for surface biofunctionalization, is also linked to potential solutions for cartilage repair.

Figure 18.3 helps to summarize the process used by our team for the development of biomimetic porous PDMS for mimicking cartilage. First of all, PDMS-sugar mixtures are casted into rapid molds; after polymerization, a final particle leaching by water immersion leads to the desired porous structures. The same process is also applied for the development of the scaffolds for osteochondral repair presented in

Fig. 18.3 Development of biomimetic porous PDMS for mimicking cartilage by means of casting PDMS-sugar mixtures into rapid molds and final particle leaching by water immersion after polymerization

Chap. 19. In vitro validation trials can be appreciated in Fig. 18.4, which shows hMSCs cultured in a portion of the developed PDMS porous scaffolds. Cell nuclei in healthy conditions are shown in blue. The generation of collagen type-2, connected to the potential of the scaffold for cartilage generation, is detected by the red stains.

Interestingly, scaffold's porosity, through capillary action, helps the growth factors to fill the three-dimensional interconnected porous structure, what proves to be positive for promoting cells to reach the inner cavities of the scaffold.

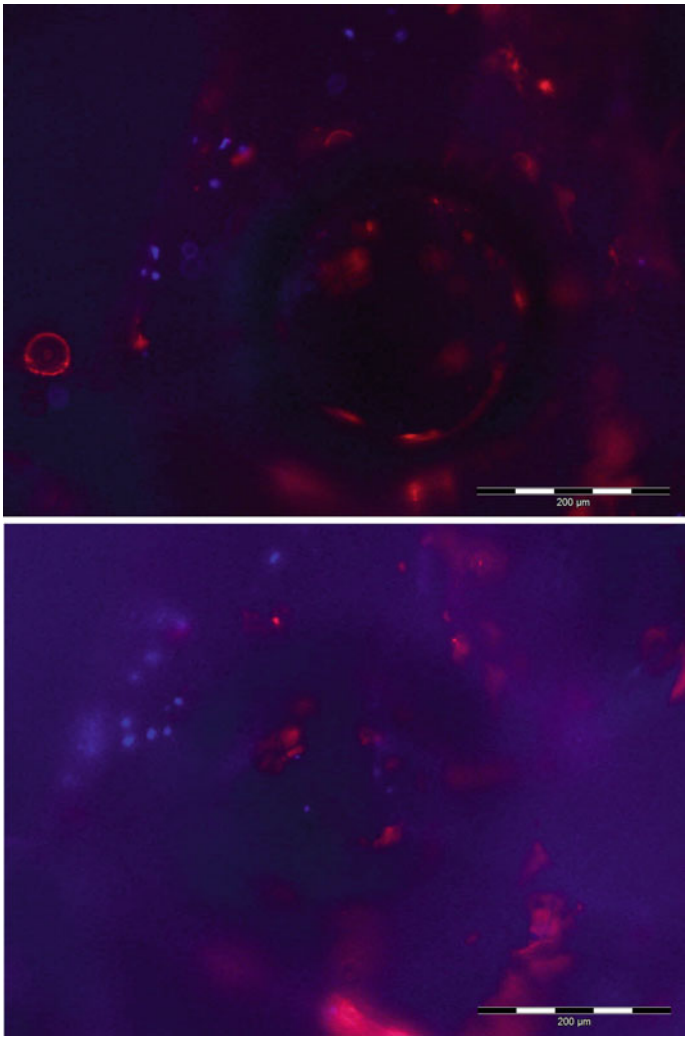

Fig. 18.4 hMSCs cultured in a PDMS porous scaffold. Cell nuclei in healthy conditions are shown in *blue*. The generation of collagen type-2, connected to the potential of the scaffold for cartilage generation, is detected by the *red* stains (color online)

The incorporation of trofic factors produced by hMSCs isolated from bone marrow is essential for cell adhesion and for final success of the PDMS-3D niche. In fact, the PDMS-3D niche, seeded with hMSCs and incubated in a chondrogenic medium during 3 weeks, realized chondrogenesis process expressing collagen type II validating a new method to obtain an excellent scaffold, at least, for cartilage and endochondral bone formation/repair strategies.

Therefore we believe that our scaffold's stiffness may have had a relevant impact on hMSCs growth and collagen generation, thus being potentially adequate for the promotion of chondrogenesis and for cartilage repair. Additional details can be found elsewhere (Díaz Lantada et al. 2014).

18.4 Case Study: Scaffolds for Muscle Repair

Muscular repair is also a relevant subject in tissue engineering and related strategies for tissue repair and regeneration, as well as in the recent research field of biofabrication. Even though the reconstruction of skeletal muscle tissue, either lost by injury, functional damage or surgical procedures, is complex and typically ends up with functional losses, some alternatives based on tissue engineering have been already reviewed (Bach et al. 2004). Regarding cardiac muscle, its damage typically leads to surgical interventions and to the use of prosthetic solutions, either biological (i.e. using porcine heart valves) or mechanically inert (such as artificial mechanical valves and annuloplasty rings).

The use of porous PDMS scaffolds, similar to the ones described in previous Sect. 18.3, have also shown potential for muscular repair, as their mechanical properties can be tuned to those of muscular tissues by controlling the quantity of porogens used for the leaching process (Díaz Lantada et al. 2014).

For instance our team has recently presented the use of circular porous PDMS rings, as possible reinforcements for tissues surrounding different sphincters, and proposed a couple of mitral valve annuloplasty rings (one closed and one open) for potential reinforcement of valvular tissues, as potential repair treatment for mitral valve insufficiency. Such designs have helped to verify the option of obtaining PDMS sponges and porous scaffolds, with controlled outer geometries, by casting into rapid prototyped molds of different emulsions and mixtures including PDMS, sugar and water.

However, such processes require additional improvements and can be changed or complemented by the use of computer-controlled designs of lattice and porous structures, capable of acting as muscular repair scaffolds. Different design and manufacturing strategies linked to scaffolds for tissue repair have been previously presented along the Handbook, here we provide some cases of study linked to the development of valvular constructs and of sphincter scaffolds, as some of the more remarkable possible applications of tissue repair to the muscular field, for complementing the more important advances already obtained in skeletal muscle repair.

By means of example, Fig. 18.5 presents solid and lattice computer-aided designs of biomimetic annuloplasty rings for potentially treating the problem of mitral valve insufficiency. Figure 18.6 includes a rapid prototype, obtained by 3D laser stereolithography, of a tissue engineering scaffold with the geometry of an annuloplasty ring for prospective treating of mitral valve insufficiency by using a porous prosthesis loaded with cells. Finally, Fig. 18.7 illustrates a conceptual computer-aided design and the related rapid prototype, obtained by laser

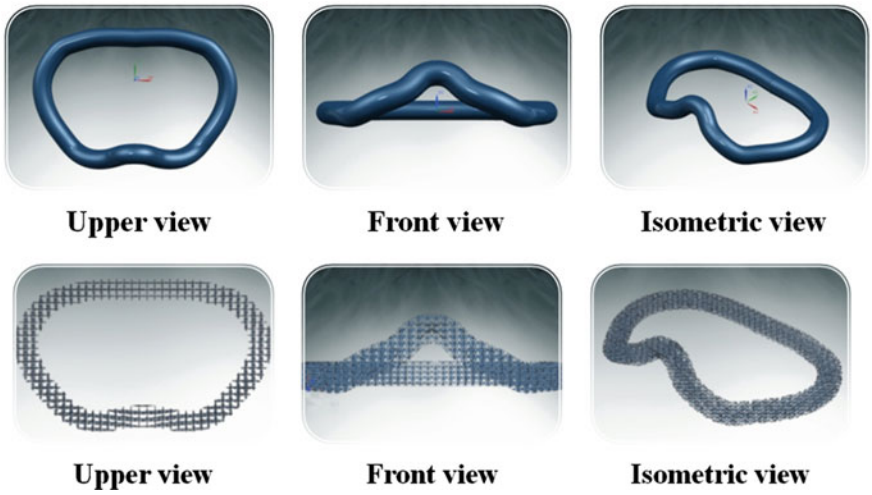

Fig. 18.5 Solid and lattice computer-aided designs of biomimetic annuloplasty rings for treating mitral valve insufficiency

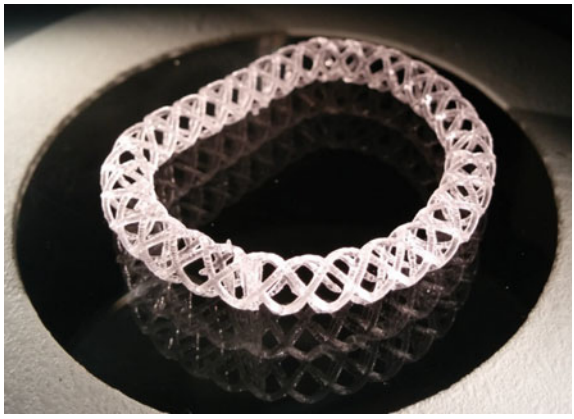

Fig. 18.6 Rapid prototype, obtained by 3D laser stereolithography, of a tissue engineering scaffold with the form of an annuloplasty ring for potentially treating mitral valve insufficiency by using a porous prosthesis loaded with cells

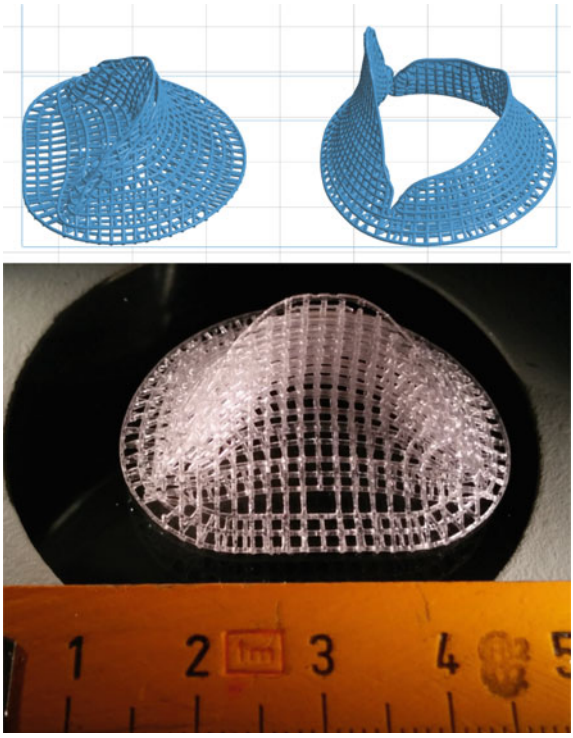

Fig. 18.7 Computer-aided design and rapid prototype, obtained by laser stereolithography, of a tissue engineering scaffold for the potential in vitro growth of complete heart valves. The geometry of a complete mitral valve with its leaflets has been used in this preliminary example

stereolithography, of a tissue engineering scaffold for the potential in vitro growth of complete heart valves. The geometry of a complete mitral valve with its leaflets has been used in this preliminary example as a basic geometry, upon which the different lattices are designed for obtaining a complex 3D construct (Fig. 18.8).

The concept of flexible and soft tissue engineering scaffolds can be also applied to the artificial development of sphincters, just by obtaining networked or lattice structures with the appropriate toroidal geometry. Here we present a novel strategy for the development of rapid prototyped (by additive manufacturing after computer-aided design models) toroidal scaffolds with enough space for letting the cells attach, grow, exchange oxygen, nutrients and debris with the surrounding tissues and hopefully express adequate genes towards the desired functionalization and tissue development.

In this case, only the design and manufacturing processes are presented, but we expect to provide additional details regarding in vitro and in vivo trials in the near future. The process described here is also applied as a complement to the tendon and ligament repair scaffolds detailed in Sect. 18.5, which may benefit from the incorporation of a toroidal extreme for helping with future surgical tasks, so as to better attach the artificial construct to the bone of the articulation being repaired.

The design process of the annular lattices or scaffolds for sphincter repair also benefits from the use of parametric features and Boolean operations available in

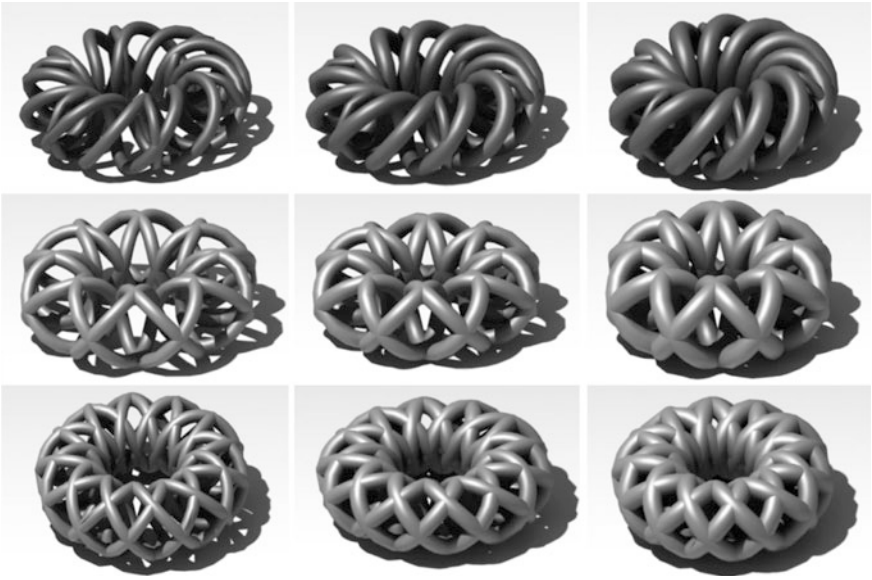

Fig. 18.8 Library of computer-aided designs of tissue engineering scaffolds with different porosities and mechanical properties aimed at the in vitro generation of sphincters and tissues arranged in circular patterns

most computer-aided design resources. In these cases, we use toroidal helixes based on the following parametric equations in Cartesian coordinates:

$$X = (B + b \cdot \cos(c \cdot t)) \cdot \cos(t); Y = (B + b \cdot \cos(c \cdot t)) \cdot \sin(t); Z = b \cdot \sin(t)$$

Being: "B" the major radius of the torus, "b" the minor radius of the torus, "*c*" the number of turns of the curve along the torus, and "t" = (0,2π) for a complete revolution. Once the toriodal helical curve is designed in the working space, we include a sketch plane perpendicular to the curve. Then we draw a circle with radius "a" and with its centre coincident to the intersection between the toroidal helical curve and the sketch plane. The three-dimensional solid toroidal helix can be obtained by sweeping the circle along the toroidal helical curve. Finally, mirror operations, replicas, rotations and Boolean tools help to obtain "m" solid toroidal helixes for obtaining the scaffolds' library shown in Fig. 18.12.

Preliminary rapid prototypes for geometrical validation are obtained using a "Form1+" laser stereolithography system from © Formlabs, after format conversion to .stl and batch preparation with the help of PreForm software (see Fig. 18.9). The material used for these preliminary prototypes is a photoreactive resin based on a mixture of methacrylic acid esters, acrylic acid esters and photoinitiators, under trade name "Clear-Form1". As previously detailed, laser stereolithography is an additive manufacturing process using a vat of liquid UV-curable photopolymer or "resin" and a UV laser to build parts layer by layer in an additive way. On each layer, the laser

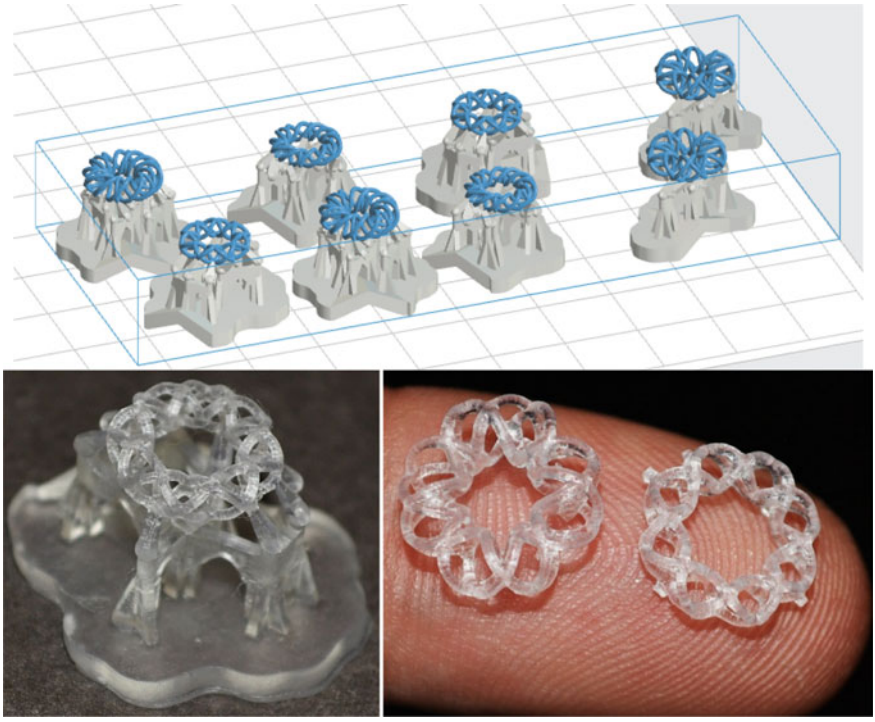

Fig. 18.9 Computer-aided manufacturing software (Preform by Formlabs) and rapid prototypes of tissue engineering scaffolds for sphincter repair. Manufactured using a Form1+ machine by Formlabs

beam traces a cross-section pattern on the surface of the resin. Exposure to the UV laser cures (and solidifies) the pattern traced on the resin, adhering it to the previous layer. After a pattern has been traced, the machine elevator platform moves vertically a single layer of thickness, typically 0.05–0.15 mm. Then, a resin-filled blade sweeps across the partial cross-section, re-coating it with fresh material. On this new liquid surface, the subsequent layer pattern is traced, adhering to the previous layer. A complete 3D part is formed by repeated iterations of this process. Additional details regarding post-processing and final results are explained in Sect. 18.5.

18.5 Case Study: 1D and 3D Scaffolds for Ligament and Tendon Repair

Tendon and ligament injuries are not just a major concern for young high-performance athletes, but also a chronic degenerative health problem of wear and tear tendons among elder people. A comprehensive study (Clayton and Court-Brown 2008)

on the epidemiology of tendinous and ligamentous injuries, described the injuries of the hand extensor tendons and the ruptures of the Achilles tendon and the ACL, among the most common traumatological problems, with incidences between 8/100,000 per year and 20/100,000 per year for each type of injure. The global incidence of tendinous and ligamentous injuries reached a figure of 166/100,000 per year for males and 52/100,000 per year for females. Such a relevant health problem is obviously being treated and studied following different strategies, supported by commercial products, experimental developments, synthetic materials and biological substitutes, which have been extensively reviewed recently (Chen et al. 2009).

In short, surgical treatment is reserved for patients who do not improve after a period of conservative treatment. If the tendon defect is large, autografts, xenografts or allografts may be used for repairing the native tissue. However, limited donor availability, donor site complications and increasing market demands have promoted the appearance of scaffold-based solutions, evolving from more traditional procedures using passive synthetic fibres (i.e. carbon fibres, PTFE, Gore-Tex, Dacron or polyester).

Scaffold-based options, for tendon and ligament surgical repair, clearly benefit from recent advances in the field of tissue engineering. Regarding synthetic scaffolds, there are already several commercially available options, most of them employing aforementioned synthetic fibres knitted in the form of chords or membranes for providing a better three-dimensional extra cellular matrix. Regarding biological scaffolds, decellularized mammalian tissues, which can be implanted with or without growth factors, are already being used as biomimetic extra cellular matrices.

In spite of the number of solutions available for tendon and ligament repair, both synthetic and biological scaffolds can cause adverse responses and additional research efforts, regarding potential more effective combinations of designs, materials, manufacture processes and biointegration strategies need to be implemented and assessed, both in vitro and in vivo, towards the ideal universal scaffold for tendon and ligament research and repair. The combined use of computer-aided engineering and additive manufacturing strategies, which already provides interesting results in the field of bone repair, may become again a remarkable alternative for these types of repairs aimed at softer tissues.

In consequence, we present in this section the systematic development of a digital library of scaffolds aimed at tendon and ligament repair. The designs developed are biomimetic tubular structures made of inter-connected helical threads with systematic variations.

Several control parameters are used for modifying the most relevant properties of scaffolds for potential tendon and ligament repair, including stiffness and porosity, which clearly affect the biomechanical response.

The mechanical performance of the different designs is analyzed, by means of computer-aided engineering. Simulation results help to validate the potential of the developed library for providing mechanically adequate solutions in the whole range of expected properties of tendons and ligaments. The rapid manufacture of preliminary prototypes, using laser stereolithography and selective laser sintering, and

the cell culture trials carried out provide information regarding the potential of the proposed approach. A human mesenchymal stem cell (h-MSC) conditioned medium (CM) is used for improving scaffold response. The following sub-section details the materials and methods used for this study, before describing and analyzing main results and proposing future steps towards the concept of the universal scaffold for tendon and ligament repair.

18.5.1 Materials, Design, Manufacturing and Testing Methods

Computer-aided design (CAD) of the different geometries of interest was carried out with the help of NX-8.5 (Siemens PLM Solutions), mainly using combinations of parametric features and Boolean operations. Main bodies of the designed scaffolds for tendon or ligament repair are hollow tubular structures made of several interconnected helixes. The basic helix is designed in a parametric form using the following equations in Cartesian coordinates:

$$X = R \cdot \cos(w \cdot t)$$
$$Y = R \cdot \cos(w \cdot t)$$
$$Z = k \cdot t$$

As the parameter "t" increases, the point (x(t), y(t), z(t)) traces a right-handed helix of pitch $2\pi \cdot k$ and radius "R" (also "D/2") about the z-axis, in a right-handed coordinate system. In consequence, the pitch of a helix is the width of one complete helix turn, measured parallel to the axis of the helix, and can be tuned by means of constant "k". Once the helical curve is designed in the working space, we include a sketch plane perpendicular to the curve. Then we draw a circle with radius "r" (also "d/2") and with its centre coincident to the intersection between the helical curve and the sketch plane. The three-dimensional solid helix can be obtained by sweeping the circle along the helical curve. Finally, mirror operations, replicas, rotations and Boolean tools help to obtain the different scaffolds shown in Figs. 18.10 and 18.11.

The different helixes are connected, either using additional tubular elements (only needed in the triple-helix scaffold) or by intersections between helixes. For the scaffolds with four, six and eight helixes we have included an additional design variation, depending on the use of "n" symmetric helixes starting in "n/2" points or on the use of "n" right-handed helixes starting in "n" different points, being "n" the number of helixes in each scaffold.

A set of annular lattices is also designed, following a procedure similar to that explained in Sect. 18.4, to be incorporated into the extremes of the tubular lattices, so as to promote the connecting and fixing options of the proposed scaffold library

Fig. 18.10 Systematic computer-aided design library of DNA-inspired helical tissue engineering scaffolds for tendon and ligament repair

for potential tendon and ligament repair. The design process of the annular lattices also benefits from the use of parametric features and Boolean operations. In these cases, we use toroidal helixes based on the following parametric equations in Cartesian coordinates:

$$X = (B + b \cdot \cos(c \cdot t)) \cdot \cos(t)$$
$$Y = (B + b \cdot \cos(c \cdot t)) \cdot \sin(t)$$
$$Z = b \cdot \sin(t)$$

Being: "B" the major radius of the torus, "b" the minor radius of the torus, "*c*" the number of turns of the curve along the torus, and "t" = (0,2π) for a complete revolution. Once the toroidal helical curve is designed in the working space, we include a sketch plane perpendicular to the curve. Then we draw a circle with radius "a" and with its centre coincident to the intersection between the toroidal helical

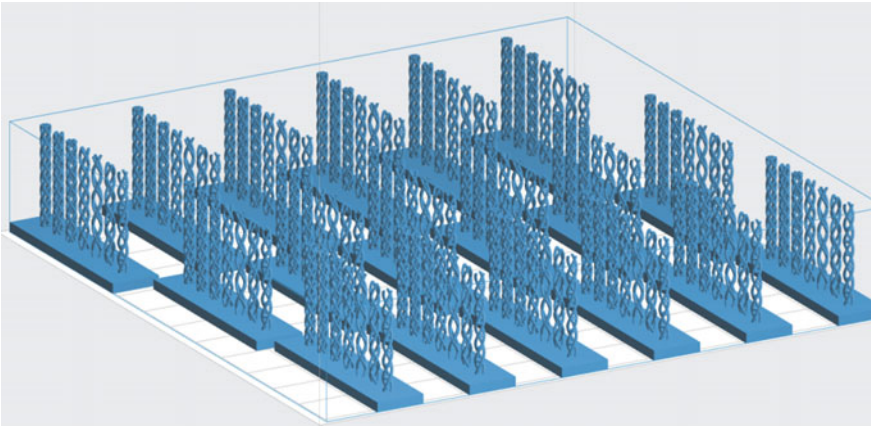

Fig. 18.11 Set of scaffolds for tendon and ligament repair prepared for additive manufacture using the Preform software by Formlabs

curve and the sketch plane. The three-dimensional solid toroidal helix can be obtained by sweeping the circle along the toroidal helical curve. Finally, mirror operations, replicas, rotations and Boolean tools help to obtain "m" solid toroidal helixes for obtaining the more complete tendon repair scaffolds shown in Fig. 18.12.

The different geometries are also simulated using the finite-element method capabilities of NX-8.5 for studying the mechanical performance of the different scaffolds designed and for analyzing potential applications in tissue repair. Main

Fig. 18.12 Different connecting distal elements for tendon and ligament tissue engineering scaffolds

details, regarding material, mesh, loads, boundary conditions, solver parameters used and post-processing analyses, are described under these lines.

Tetrahedral 10-node elements are used for meshing, which is carried out with help of the automatic meshing and refine tool from the software employed, what provides elements with sizes below 0.25 mm in almost all cases. Additionally, more than 90% of the elements obtained had a skewness value lower than 0.7, which provides adequate meshes for the purposes of the present study. A conventional polymeric material, with mechanical properties similar to the typical polyamides and epoxies used in additive "layer by layer" manufacturing technologies, has been defined and used for the simulations. Material bulk properties include a density of 1300 kg/m^3, a Young modulus of 3000 MPa, a Poisson ratio of 0.37 and yield strength of 27 MPa, properties similar to those of the polymers subsequently used for prototype manufacture.

Loads are applied as a group of punctual forces along "z" direction on the upper faces and edges of the different structures, trying to promote symmetry and homogeneous loading. Typically, a value of 1 N has been used for loading the different structures and the related stresses are below the material's yield strength, so we can assume linearity for the subsequent post-processing. As boundary conditions, the displacements of the lower faces and edges of the different structures are fixed.

From the different possibilities of NX-8.5, for carrying out FEM simulations, "NX-Nastran" solver and "structural analysis" type (solution type SESTATIC 101) are selected with the option of "element iterative solver" activated, as 3D elements are used for the simulations. Simulations are carried out at a default ambient temperature of 25 °C. It is important to remark the compatibility between the design and simulation programs, as geometries can be directly imported for simulation, without the typical limitations of universal format (.igs, .stl, .stp…) conversions and the information loss they normally involve. Once the simulations are carried out, post-processing tools allow for a straightforward measurement of displacements for subsequent calculation of the equivalent Young modulus of the different structures. Main simulations results are shown in Figs. 18.13, 18.14 and 18.15.

Rapid prototypes are obtained by means of additive manufacturing technologies, which allow for the manufacture of complex biomimetic geometries. Preliminary prototypes for geometrical validation are obtained using a "Form1+" laser stereolithography system from ©Formlabs, after format conversion to .stl and batch preparation with the help of PreForm software. The material used for these preliminary prototypes is a photoreactive resin based on a mixture of methacrylic acid esters, acrylic acid esters and photoinitiators, under trade name "Clear-Form1". The prototyping process by stereolithography can be briefly described as follows. As previously detailed, laser stereolithography is an additive manufacturing process using a vat of liquid UV-curable photopolymer or "resin" and a UV laser to build parts layer by layer in an additive way. On each layer, the laser beam traces a cross-section pattern on the surface of the resin. Exposure to the UV laser cures (and solidifies) the pattern traced on the resin, adhering it to the previous layer. After a pattern has been traced, the machine elevator platform moves vertically a

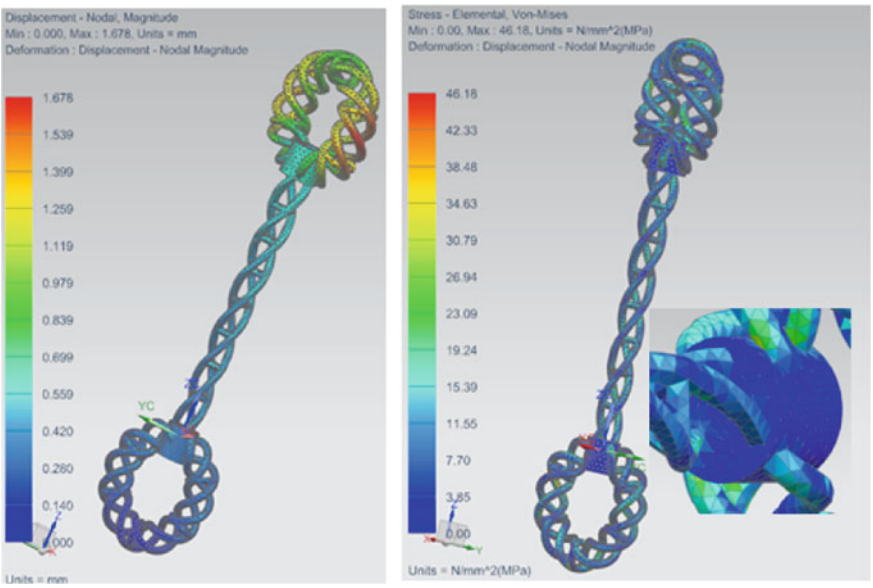

Fig. 18.13 FEM-based assessment of the mechanical performance of different solutions for tendon and ligament repair

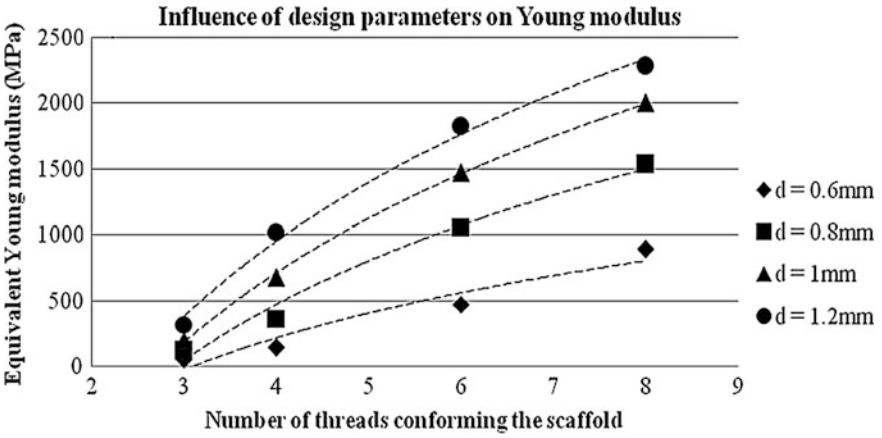

Fig. 18.14 Influence of design parameters on the elasticity of helical scaffolds with 10-mm pitch

single layer of thickness, typically 0.05–0.15 mm. Then, a resin-filled blade sweeps across the partial cross-section, re-coating it with fresh material. On this new liquid surface, the subsequent layer pattern is traced, adhering to the previous layer. A complete 3D part is formed by repeated iterations of this process. After manufacture, parts are cleaned by immersion in a chemical bath (normally 5–10 min in

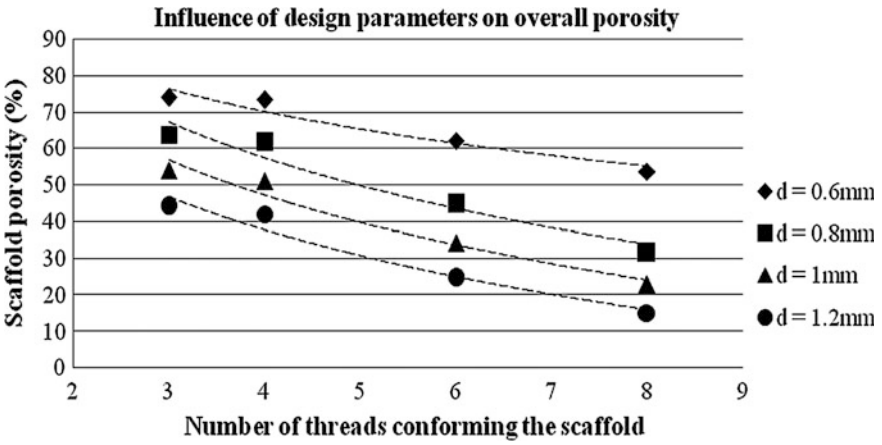

Fig. 18.15 Influence of design parameters on the porosity of helical scaffolds with 10-mm pitch

isopropylic alcohol or acetone) and finally post-cured to improve mechanical properties in a UV oven for around 10–20 min, depending on the resin's specifications. The "Form1+" machine stands out for the degree of precision attainable, even for large parts, as well as for being a low-cost system recently developed. In our case we select a 25 μm layer thickness for improved results.

The degree of precision and the quality of surface finish attainable by additive photo-polymerization processes is remarkable: i.e. layer thicknesses down to 25 μm and wall thicknesses down to 0.6 mm for parts with volumes in the mm^3 range and details even reaching 500 nm for parts in the μm^3 range. However, most resins available are not adequate for in vitro or in vivo tests in contact with cells or living organisms, as some debris may be toxic. In consequence, for the cell culture trials we obtain additional prototypes in polyamide by state-of-the-art selective laser sintering.

In selective laser sintering the models are also printed layer by layer, by a laser that draws thin lines upon polyamide powder. The laser melts and bonds the powder, so as to form a thin layer of the model. After a layer is printed, a new layer of fresh powder is spread over the surface by a roller. The printer has a print chamber that is heated to just below the melting point of the powder and the laser beam adds the extra energy to melt the powder, forming a solid model. Examples of the prototypes obtained are shown in Figs. 18.16 and 18.17.

Regarding cell culture processes, the hMSCs used in this work were isolated in a Percoll gradient from 1 or 2 ml of human bone marrow samples from anonymous healthy donors and provided by hematology services of the Hospital La Princesa, the Jiménez-Díaz Foundation and the University Biobank of Málaga, and expanded as described previously (Lennon et al. 2006; Ogueta et al. 2002). In addition, the cell lines used in present study we obtained before 2006 and are only used in vitro, never for research with patients. The Universidad Autonoma de Madrid Ethics Committee has granted written approval of research using the mentioned cell lines

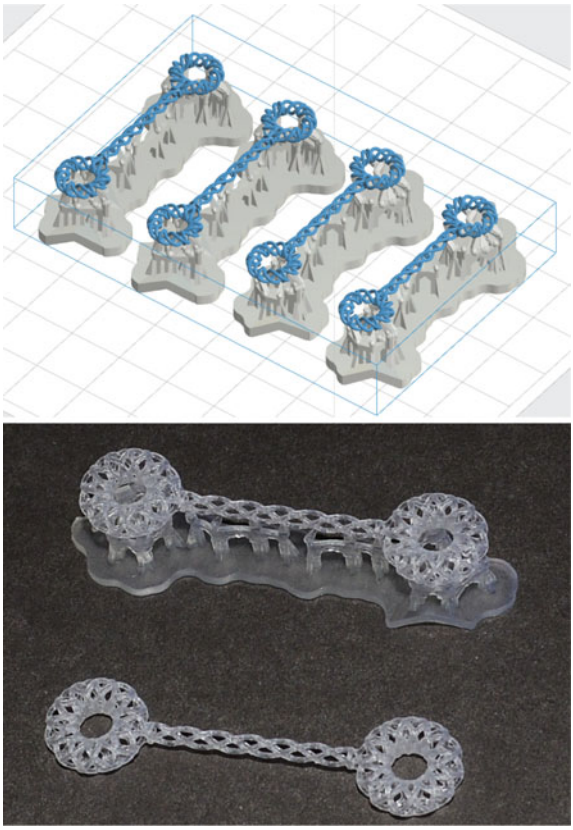

Fig. 18.16 Computer-aided manufacturing software (Preform by Formlabs) and rapid prototypes of tissue engineering scaffolds for tendon and ligament repair. Manufactured using a Form1 + machine by Formlabs

for in vitro material evaluation tasks. Reference to first studies using such lines for similar purposes is included in the reference section (Ogueta et al. 2002).

Cells were plated and incubated using DMEM-L plus 10 % FBS of selected batches. Cells were collected by treatment with 0.25 % trypsine-EDTA. Cell culture mediums were prepared by the research services of Molecular Biology Center "Severo Ochoa" (CSIC-UAM). For the preparation of hMSC-CM we used 8–10 p100 culture plates at 80 % confluence for each batch. Cells were washed with PBS and incubated in DMEM-L starved of FBS and supplemented with 2 mM pyruvate during 24 h. Afterwards culture medium was collected, cleaned of any floating cell by centrifugation at 1,500 rpm in a bench centrifuge during 5 min. The clean supernatant was cooled down on ice during 30 min, centrifuged in a Sorvall to remove salt precipitations and kept in 2 ml aliquots at −30 °C until use.

The lattice scaffolds were UV irradiated, individually placed in 25 ml Falcon tubes and received the following treatment: (i) thoroughly wash using 0.5 ml PBS and 5 min centrifugation; (ii) treatment with 2 M acetic acid during 20 min and then a rapid neutralization and PBS wash; (iii) treatment with hMSC-CM during 24 h or DMEM-LG as control; and (iv) scaffold seed with 150,000 hMSC and incubated in

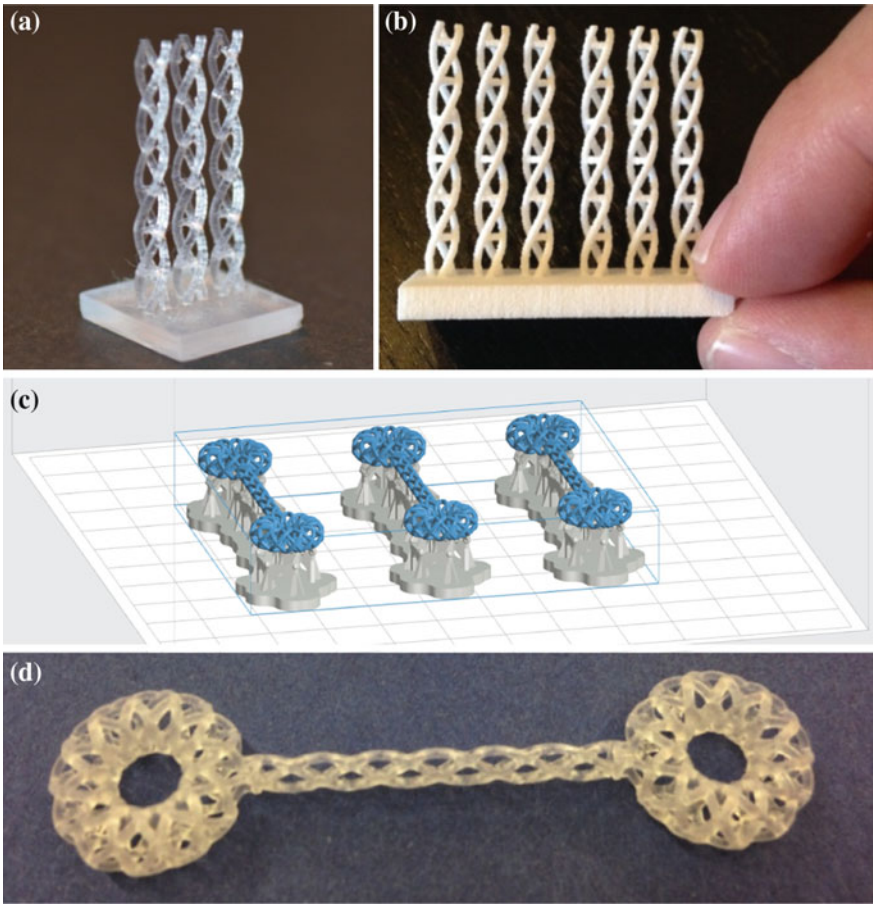

Fig. 18.17 **a** Preliminary resin prototype for geometrical validation. **b** Polyamide tubular scaffolds for subsequent cell culture trials. **c** Prototyping batch with three models, lying upon supporting structures, prepared for additive manufacture. **d** Prototype of a complete scaffold

DMEM-L-10 % FBS during 48 h at 37 °C in 5 % CO_2 as early indicated (Ogueta et al. 2002; Díaz Lantada et al. 2014). Then, samples were processed to analyze by using an immune fluorescence technique, as described previously (Díaz Lantada et al. 2014; Pittenger et al. 1999), with some modifications. Briefly, the tubular scaffolds were individually placed in M24 tissue dishes, cut into slices, rinsed with ice-cold PBS and fixed in 3.7 % formaldehyde in PBS during 30 min at RT and washed in PBS. Cells were visualized by their nuclei. To this end, cells were incubated with 0.5 % Triton in CSK buffer containing 10 mM pipes, pH 6.8, 3 mM $MgCl_2$, 100 mM NaCl, 1 mM EGTA, 0.3 M sucrose for 30 min on ice. After the treatment, samples were cleaned and fixed with 3.7 % formaldehyde and equilibrated in PBS. Nuclei were stained with DAPI (CALBIOCHEM) and observed using an inverted IX81 Olympus with a DP72 digital camera.

18.5.2 Main Results Regarding the Developed Library of Tendon Repair Scaffolds

A systematic library of tubular and annular lattice structures that, once connected, constitute complete scaffolds for potential tendon and ligament repair is obtained by methodically modifying the different design parameters. All combinations between the following parameter values are designed: D = (0.8 and 1 mm), d = (0.6, 0.8, 1 and 1.2 mm), n = (3, 4, 6 and 8), B = 3 mm, b = 1 mm, a = (0.6, 0.8, 1 and 1.2 mm), c = 3 and m = (3, 4 and 6). The length of the tubular scaffolds included in the library is l = 20 mm, which constitutes an adequate value for most in vitro studies to be performed and helps with the manufacture of prototypes with a size easy to manipulate and study. Results are shown in Figs. 18.10, 18.11 and 18.12.

The values selected for the different parameters are based on the typical dimensions of real tendons and ligaments, on the geometries and features of existing solutions for tendon and ligament repair. We also take into account the capabilities of state-of-the-art additive manufacturing technologies capable of obtaining the desired geometries with an adequate degree of precision.

Once the FEM-based simulations are performed, results post-processing allows us to obtain relevant information regarding the mechanical performance of the different scaffolds for tendon and ligament repair conforming the CAD library. First we concentrate on the tubular structures, as they constitute the most relevant part of the complete scaffolds. As previously mentioned, the annular lattices of the complete scaffolds are aimed at helping with the final integration of the scaffold into a trial bench or into animal models. We define the equivalent Young moduli of the tubular scaffolds for tendon and tissue repair as: $E_{eq} = \sigma_{eq}/\varepsilon_{eq} = \left[\sum F/(\pi\rho^2)\right]/[\Delta l/1]$, which is useful for comparing the elasticity of the designed lattices with that of the bulk material used for their manufacture. In former equation "ΣF" is the traction applied in the simulations (along z axis), "ρ" the radius of concentric cylinders tangent to the external surfaces of the tubular lattices, with ρ = R + r for each simulation, "l" the length of the scaffold and "Δl" the maximal displacement under load. After simulating the mechanical performance of the tubular lattices or scaffolds, the complete scaffolds, including the tubular and annular lattices, are also assessed by means of simulations, taking special account of the stress values attained in the connections, so as to select the most adequate ones. Figure 18.13 shows an example upon a complete scaffold.

Evaluating the Young moduli of scaffolds for tissue engineering is always interesting, as previous research has demonstrated the impact of scaffold elasticity on stem cell growth and differentiation into relevant tissues. Having an estimation of porosity values is also relevant, as it directly influences cell access to nutrients and elimination of debris, in a similar way as in native tissues. Tissue engineering scaffolds mimicking the elasticity and porosity properties of the extracellular matrices of real tissues tend to promote cell differentiation, into the tissues whose properties they mimic, provided that other biochemical stimuli, including material chemistry and adequate growth factors also help with cell viability.

In our case, thanks to systematic variations of the design parameters, the developed CAD library offers relevant ranges of variation for the Young moduli and for the porosity values of the tubular scaffolds aimed at potential tendon and ligament repair. Based on post-processing of simulation results, Figs. 18.14 and 18.5 include summaries of results for analyzing in depth the influence of design parameters on Young moduli and porosity values, respectively. Both Figures present the impacts of the number of helical threads ("n") configuring the scaffold and of thread diameter ("d"), which have been verified as main control parameters. Although the whole CAD library has been simulated, we just present the results for the scaffolds with pitch values of p = 10 mm and with R = 0.4 mm, for space reasons. The whole library is available for colleagues wishing to collaborate with us in forthcoming studies.

Among remarkable results, it must be noted that the variation range of Young moduli within the developed library, from 50 to 2250 MPa, is very similar to the variation range of Young moduli measured, both in vitro and in vivo, for real ligaments and tendons. Values of around 0.8–1.8 GPa have been reported for Young moduli of human tendons (Kurihara et al. 2012, Franzesi and Yannas 2006), while values of around 30–300 MPa have been reported for ligaments. The versatility of the proposed library is even greater if we consider that the proposed structures can be obtained, using additive technologies, in several polymers, ceramics, metals and alloys, whose bulk properties can be used for adapting the values obtained in our simulations.

In short, Young moduli and compactness increase with the number of threads and with thread thickness, as initially expected. Scaffold porosity variation range is also noteworthy, as values from 15 to 80 % can be obtained by adequately modifying the design parameters. The tendency is similar for helix-based scaffolds with pitches of 5 and 20 mm. For fixed values of porosity relevant variations of Young moduli can be obtained and for fixed values of Young modulus important variations of porosity can be also achieved, so both properties are not completely interdependent.

Additional freedom for independently tuning porosity and elasticity values can be obtained by modifying the connecting nodes of the lattices, as recently put forward by groundbreaking research in the field of mechanical metamaterials (Kadic et al. 2012; Bückmann et al. 2012; Kadic et al. 2014), which also benefit from advances in solid freeform fabrication technologies. In any case we believe that the developed library of lattice tubular structures, together with the mechanical assessment performed, is one of the most comprehensive CAD resources in the field of scaffolds for tendon and ligament repair.

The rapid manufacture of preliminary prototypes and the cell culture trials carried out have provided interesting information regarding the potential of the proposed approach. Regarding prototyping, it is important to note that the geometries can be controlled from the design stage with a remarkable repeatability. When with the complete geometry and using photopolymerization as manufacturing process, it is important to place the scaffold horizontally, so as to minimize the supporting structure and to accelerate the manufacturing process. The precision of the photopolymerization-based technologies is higher but the commercially-available

photo-polymers used typically prevent adequate cell culture, while the polyamide laser sintered ones can be directly used for cell culture. Some interesting comparisons between these technologies, taking account of precision, available materials, including examples of bio-photopolymers, and focusing on cell culture matrices have been published (Ovsianikov et al. 2012; Díaz Lantada 2013).

As for the cell culture trials, some results from h-MSCs interacting with a triple-helix polyamide scaffold are shown in the five images of Fig. 18.9. The nuclei are stained in blue (DAPI) and their rounded forms indicate that the cells are in healthy conditions and well attached to the scaffold, as more than 100 cells/mm^2 can be appreciated. The four upper images, taken forming an angle of 30º with scaffold axis, show the cells conforming helixes, according to scaffold structure. The lower image shows a more panoramic lateral view, in which aligned nuclei adopting sinusoidal patterns can be appreciated, starting to resemble the fibrillar structure needed for tendon and ligament repair strategies.

Our results show that the cells and the tubular helix-based scaffolds are excellent companions for potential tissue repair strategies. In case of tissue damage, the hMCSs-seeded tubular scaffolds may constitute a flexible support, in order to allow the permeation of nutrients and debris, to promote oxygenation, to enable adaptation and to provide cellular communication systems, capable of locally inhibiting the immune system and of activating tissue repair, although additional assessments, both in vitro and in vivo, need to be performed.

Last but not least, the hMCSs-seeded tubular scaffolds offer interesting possibilities to study cellular mechanisms present in different types of tissues, specially tendons and ligaments, although they may well be useful for studying other types of fibrillar tissues. The annular lattices may be also useful, not just as connecting elements, but also as scaffolds for muscle repair, with applications including cardiac tissue engineering (i.e. advanced annuloplasty ring) and artificial sphincters, in which we will also focus our attention in forthcoming studies. The cell-material interactions may be extended to a triad composed by hMSCs—tubular scaffolds—endoderm/exoderm derived cells for studies linked to complex tissues and organs.

Future studies will deal with more systematic in vitro and in vivo analyses for assessing the performance, not only of the tubular scaffolds, but also of the annular lattices, initially designed as connecting elements, but which may also provide interesting solutions for the field of tissue repair. It would be also interesting to further study cell behavior using biomimetic constructs with functional gradients of properties.

The computer-aided designs presented here may be improved, in terms of bone biomimicry, by using recently proposed strategies based on the use of multi-morphology transition hybridization CAD design of minimal surface porous structures (Yang et al. 2014). The irregular features of living tissues can be also taken into account and modeled by means of multi-scale approaches (Yang and Zhou 2014) and by resorting to stochastic design procedures (Yang et al. 2015). We would like to express that the developed library is at the disposal of colleagues who may find it useful for further collaborative research in the fields of tissue engineering and biofabrication (Figs. 18.18 and 18.19).

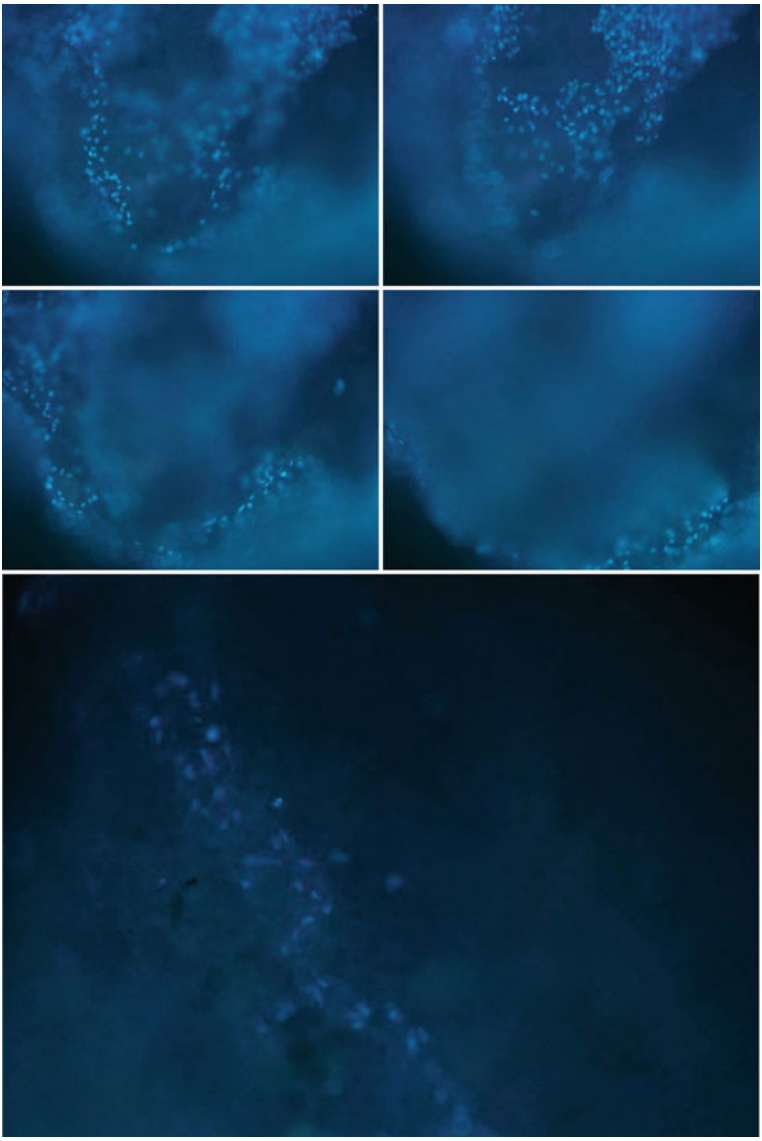

Fig. 18.18 Cell culture results showing h-MSC upon a tubular scaffold. The four *upper* images, taken forming an angle of 30º with scaffold axis, show the cells conforming helixes, according to scaffold structure. The *lower* image shows a more panoramic lateral view, in which aligned nuclei adopting sinusoidal patterns can be appreciated. DAPI (*blue*) for the nuclei (color online)

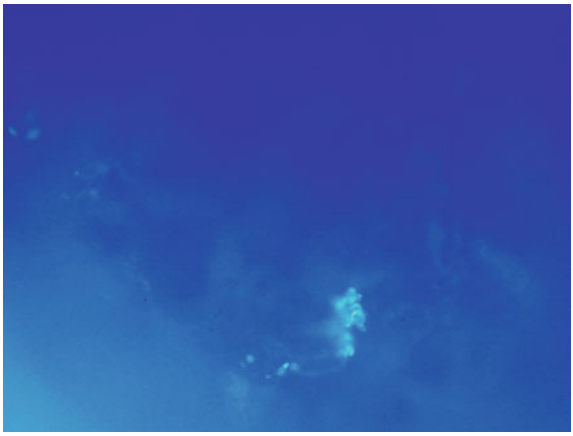

Fig. 18.19 Additional detail: Helical aggregations of cells can be appreciated as preliminary step towards adequate tissue formation. DAPI (*blue*) for the nuclei (color online)

18.6 Main Conclusions and Future Research

Soft tissue repair is a very relevant and challenging area for the emerging fields of tissue engineering and biofabrication due to the complex three-dimensional structure in form of interwoven fibres and the relevant variations of mechanical properties present in these tissues. The need of elasticity, of structural integrity, of functional gradients of mechanical properties, among other requirements, has led to the development of several families of biomaterials and scaffolds for the repair and regeneration of soft tissues, although a perfect solution has not yet been found.

Further research is needed to address the advantages of different technologies and materials for manufacturing enhanced, even personalized, scaffolds for tissue engineering studies and extra cellular matrices with outer geometries defined as implants for tissue repair, as the niche composition and 3D structure play an important role in stem cells state and fate. The combined use of computer-aided design, engineering and manufacturing resources together with rapid prototyping procedures, working on the basis of additive manufacturing approaches, allows for the efficient development of these types knowledge-based functionally graded scaffolds for soft tissue repair in a wide range of materials.

In this chapter we have presented some design and manufacturing strategies for the development of knowledge-based tissue engineering scaffolds aimed at soft tissue repair. Complete cases of studies, linked to the development of several scaffolds for the repair of articular cartilage, tendons and muscles, with an example of a complete heart-valve scaffold and a set of scaffolds for artificial sphincters, have been also detailed to illustrate the proposed strategies.

References

Ashby MF (2005) Materials selection in mechanical design. Butterworth-Heinemann, 3rd edn
Bach AD, Beier JP, Stern-Staeter J, Horch RE (2004) Skeletal tissue engineering. J Cell Mol Med 8(4):413–422
Bartolo PJS, Almeida H, Laoui T (2009) Rapid prototyping and manufacturing for tissue engineering scaffolds. Int J Comput Appl Technol 36:1
Bückmann T, Stenger N, Kadic M, Kaschke J, Frölich A, Kennerknecht T, Eberl C, Thiel M, Wegener M (2012) Tailored 3D mechanical metamaterials made by dip-in direct-laser-writing optical lithography. Adv Mater 24(20):2710–2714
Buxboim A, Discher DE (2010) Stem cells feel the difference. Nat Methods 7(9):695
Chan CKF, Chen CC, Luppen CA, Kraft DL, Kim JB, De Boer A, Wei K, Helms JA, Kuo CJ, Weissman IL (2009) Endochondral ossification is required for hematopoietic stem cell niche formation. Nature 457(7228):490
Chen J, Xu J, Wang A, Zheng M (2009) Scaffolds for tendon and ligament repair: review of the efficacy of commercial products. Expert Rev Med Devices 6(1):61–73
Chen WL, Likhitpanichkul M, Ho A, Simmons CA (2010) Integration of statistical modeling and high-content microscopy to systematically investigate cell-substrate interactions. Biomaterials 31:2489
Chen J, Zjang R, Wang W (2012) Fabricating microporous PDMS using water-in-PDMS emulsion. RSC Publishing Blogs Home. Chips and Tips, 16 April
Clayton RA, Court-Brown CM (2008) The epidemiology of musculoskeletal tendinous and ligamentous injuries. Injury 39(12):1338–1344
Cox SC, Thornby JA, Gibbons GJ, Williams MA, Mallick KK (2015) 3D printing of porous hydroxyapatite scaffolds intended for use in bone tissue engineering applications. Mater Sci Eng: Part C 47:237–247
Díaz Lantada A (2013) Handbook of advanced design and manufacturing technologies for biodevices. Springer
Díaz Lantada A, Mosquera A, Endrino JL, Lafont P (2010) Design and rapid prototyping of DLC coated fractal surfaces for tissue engineering applications. J Appl Phys: Conf Ser 252:012003
Díaz Lantada A, Pareja Sánchez B, Gómez Murillo C, Urbieta Sotillo J (2013) Fractals in tissue engineering: Towards biomimetic cell-culture matrices, microsystems and microstructured implants. Expert Rev Med Devices 10(5):629–648
Díaz Lantada A, Alarcón Iniesta H, Pareja Sánchez B, García-Ruíz JP (2014) Free-form rapid-prototyped PDMS scaffolds incorporating growth factors promote chondrogenesis. Adv Mater Sci Eng 612976
Ekaputra AK, Zhou Y, Cool SMK, Hutmacher DM (2009) Composite electrospun scaffolds for engineering tubular bone grafts. Tissue Eng Part A 15(12):3779
Franzesi GT, Yannas I (supervisor) (2006) Design of a novel anterior cruciate ligament prosthesis. MIT Final degree thesis
Halim AS, Khoo TL, Yussof SJM (2010) Biologic and synthetic skin substitutes: an overview. Indian J Plastic Surg 43:23–28
Infür R, Pucher N, Heller C, Lichtenegger H, Liska R, Schmidt V, Kuna L, Haase A, Stampfl J (2007) Functional polymers by two-photon 3D lithography. Appl Surf Sci 254:836
Johnstone MA, Cucchiarini M, Dodge GR, Eglin D, Guilak F, Madry H, Mata A, Mauck RL, Semino CE, Stoddart MJ (2013) Tissue engineering for articular cartilage repair—the state of the art. Eur Cells Mater 25:248–267
Kadic M, Bückmann T, Stenger N, Thiel M, Wegener M (2012) On the practicability of pentamode mechanical metamaterials. Appl Phys Lett 100(19):191901
Kadic M, Bückmann T, Schittny R, Gumbsch P, Wegener M (2014) Pentamode mechanical metamaterials with independently tailored bulk modulus and mass density. Phys Rev Appl 2:054007

Kurihara T, Sasaki R, Isaka T (2012) Mechanical properties of Achilles tendon in relation to various sport activities of collegiate students. In: 30th international conference of biomechanics in sport, Melbourne

Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920

Laschke MW, Rücker M, Jensen G, Carvalho C, Mülhaupt R, Gellrich NC, Menger MD (2008) Incorporation of growth factor containing Matrigel promotes vascularization of porous PLGA scaffolds. J Biomed Mater Res A 85(2):397

Legnani C, Ventura A, Terzaghi C, Borgo E, Albisetti W (2010) Anterior cruciate ligament reconstruction with synthetic grafts. A review of literature. Int Orthop 34(4):465–471

Lennon DP, Haynesworth SE, Bruder SP, Jaiswal N, Caplan AI (2006) Human and animal mesenchymal progenitor cells from bone marrow: identification of serum for optimal selection and proliferation. In vitro Cell Dev Biol 32:602

Lohfeld S, Tyndyk MA, Cahill S, Flaherty N, Barron V, Mc Hugh PE (2010) A method to fabricate small features on scaffolds for tissue engineering via selective laser sintering. J Biomed Sci Eng 3:138

Maher PS, Keatch RP, Donnelly K, Paxton Z (2009) Formed 3D bio-scaffolds via rapid prototyping technology. In: Presented at 4th European conference of the IFMBE, IFMBE proceedings, vol 22(17), p 2200

Mansour JM (2003) Biomechanics of cartilage. In: Oatis CA (ed) Kinesiology: the mechanics and pathomechanics of human movement. Lippincott Williams and Wilkins

Ogueta S, Muñoz J, Obregon E, Delgado-Baeza E, García-Ruíz JP (2002) Prolactin is a component of the human sinovial liquid and modulates the growth and chondrogenic differentiation of bone marrow-derived mesenchymal stem cells. Mol Cell Endocrinol 190(1–2):51

Ovsianikov A, Mironov V, Stampfl J, Liska R (2012) Engineering 3D cell-culture matrices: multiphoton processing technologies for biological & tissue engineering applications. Expert Rev Med Devices 9(6):613–633

Peng S, Hartley PG, Hughes TC, Guo Q (2012) Controlling morphology and porosity of porous siloxane membranes through water content of precursor microemulsion. Soft Maters 8 (40):10493

Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld V, Cohen S (2003) Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J Biomed Mater Res A 65(4):489

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284(5411):143

Richardson TP, Peters MC, Ennett AB, Mooney DJ (1029) Polymeric system for dual growth factor delivery. Nat Biotechnol 2001:19

Ryan GE, Pandit AS, Apatsidis D (2008) Porous titanium scaffolds fabricated using a rapid prototyping and powder metallurgy technique. Biomaterials 29:3625

Schuster M, Turecek C, Kaiser B, Stampfl J, Liska R, Varga F (2007a) Evaluation of biocompatible photopolymers I: photoreactivity and mechanical properties of reactive diluents. J Macromol Sci A 44:547

Schuster M, Turecek C, Kaiser B, Stampfl J, Liska R, Varga F (2007b) Evaluation of biocompatible photopolymers II: further reactive diluents. Monatsh Chem 138:261

Shores JT, Gabriel A, Gupta S (2007) Skin substitutes and alternatives: a review. Adv Skin Wound Care 20(9):493–508

Stampfl J, Fouad H, Seidler S, Liska R, Schwanger F, Woesz A, Fratzl P (2004) Fabrication and moulding of cellular materials by rapid prototyping. Int J Mater Prod Technol 21(4):285

Stampfl J, Baudis S, Heller C, Liska R, Neumeister A, Kling R, Ostendorf A, Spitzbart M (2008) Photopolymers with tunable mechanical properties processed by laser-based high-resolution stereolitoraphy. J Micromech Microeng 18:125014

Tan JY, Chua CK, Leong KF (2010) Indirect fabrication of gelatin scaffolds using rapid prototyping technology. Virtual Phys Prototyping 5(1):45

Tan JY, Chua CK, Leong KF (2013) Fabrication of channeled scaffolds with ordered array of micro-pores through microsphere leaching and indirect rapid prototyping technique. Biomed Microdevices 15:83

Thomas WE, Discher DE, Shastri VP (2010) Mechanical regulation of cells by materials and tissues. MRS Bull 35:578

Tzezana R, Zussman E, Levenberg SA (2008) Ultra-porous scaffold for tissue engineering, created via a hydrospinning method. Tissue Eng Part C 14(4):281

Warnke PH, Douglas T, Wollny P, Sherry E, Steiner M, Galonska S, Becker ST, Springer IN, Wiltfang J, Sivananthan S (2009) Rapid prototyping: porous titanium alloy scaffolds produced by selective laser melting for bone tissue engineering. Tissue Eng Part C: Methods 15(2):115

Yang N, Zhou K (2014) Effective method for multi-scale gradient porous scaffold design and fabrication. Mater Sci Eng, C 43(1):502–505

Yang N, Quan Z, Zhang D, Tian Y (2014) Multi-morphology transition hybridization CAD design of minimal surface porous structures for use in tissue engineering. Comput Aided Des 56:11–21

Yang N, Gao L, Zhou K (2015) Simple method to generate and fabricate stochastic porous scaffolds. Mater Sci Eng, C 56(1):444–450

Yuen PK, Su H, Goral VN, Fink KA (2011) Three-dimensional interconnected microporous poly (dimethylsiloxane) microfluidic devices. Lab Chip 11(8):1541

Chapter 19
Tissue Engineering Scaffolds for Osteochondral Repair

Andrés Díaz Lantada, Graciela Fernández Méjica, Miguel de la Peña, Miguel Téllez, Josefa Predestinación García Ruíz and Hernán Alarcón Iniesta

Abstract Articular repair is a very relevant and challenging area for the emerging fields of tissue engineering and biofabrication, as expertise regarding the repair of both soft and hard tissues is required. The need of significant gradients of properties, for the promotion of osteochondral repair, has led to the development of several families of composite biomaterials and scaffolds, using a wide range of potentially effective approaches, although a perfect solution has not yet been found. Further research is needed to address the advantages of combining different technologies for manufacturing enhanced, even personalized, scaffolds for tissue engineering studies and extra cellular matrices with outer geometries defined as implants for tissue repair, as the niche composition and 3D structure play an important role in stem cells state and fate. In this chapter we present some design and manufacturing strategies for the development of knowledge-based tissue engineering scaffolds with radical variations of mechanical properties, aimed at complex articular tissue engineering applications. These functionally graded scaffolds constitute a key development in the areas of tissue engineering and biofabrication, as their mechanical properties can be tuned to those of the tissues and biological structures being repaired. A couple of complete cases of studies, one linked to a composite scaffold for osteochondral repair, focused on the repair and regeneration of the extremes of large bones, and one linked to a composite scaffold for spine injuries, oriented to the repair and regeneration of both vertebrae and inter-vertebral discs, are also presented to illustrate the proposed strategies.

A. Díaz Lantada (✉) · G. Fernández Méjica · M. de la Peña · M. Téllez
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

J.P. García Ruíz · H. Alarcón Iniesta
Department of Molecular Biology, Universidad Autónoma de Madrid, Madrid, Spain

A. Díaz Lantada (ed.), *Microsystems for Enhanced Control of Cell Behavior*,
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19.1 Introduction: Complex Needs in Articular Repair

A universal methodology for tissue engineering scaffold development is not yet available, first of all due to the complexity of biological materials and systems, but also due to all the possible design resources, manufacturing technologies and related materials available, whose results have not been systematically compared. For instance, additive manufacturing technologies allow precise control of final geometries from the design stage; however such designs are normally obtained by combining Euclidean based (simple) geometries and final result does not mimic adequately the complexity of biomaterials. On the other hand, scaffolds obtained by phase separation and more "traditional" processes typically lead to more biomimetic sponges, even though their final outer form and repeatability are more difficult to control, than using computer-aided strategies linked to rapid prototyping using additive processes.

Therefore, further research is needed to address the advantages of combining different technologies (Tan et al. 2013) for manufacturing enhanced, even personalized, scaffolds for tissue engineering studies and extra cellular matrices with global (outer) geometries defined as implants for tissue repair. In addition, increasing data show that progenitor cell-niche formation is absolutely needed for tissue development and repair (Chan et al. 2009). Indeed, the niche composition and 3D structure play an important role in stem cells state and fate. The niche is created by the specific combination of trophic factors produced by progenitor cells to maintain the capability for tissue repair and regeneration and by a specific extracellular matrix. Recent studies have helped to highlight the extreme relevance of the incorporation of adequate growth factors, within the scaffold, for promoting biological regulation, cell differentiation, angiogenesis and final tissue viability (Richardson et al. 2001; Perets et al. 2003; Laschke et al. 2008). Such inclusion of biochemical effects, derived from the incorporation of growth factors, adds additional uncertainties to the already complex to understand interactions between scaffolds' structure, morphology and mechanical properties. Consequently, studies addressing the synergies between ECMs and growth factors and their impact on tissue viability are needed, in the quest for a general methodology for tissue engineering scaffold development.

The aforementioned tissue engineering challenges are even greater in applications aimed at articular repair, where several types of tissues (bones, cartilages, ligaments, tendons…) must be replaced, if possible using a single multifunctional scaffold able of promoting cell adhesion, growth, migration and differentiation into different types of tissues. The need of relevant gradients of properties for the promotion of osteochondral repair has led to the development of composite scaffolds using different approaches previously reviewed (Moutos and Guilak 2008; Nooeaid et al. 2012). Typical methods for the manufacture of scaffolds with functional gradients of properties include: the use of embedded (nano-)fibres and textiles within polymeric matrixes (Moutos and Guilak 2008; Nooeaid et al. 2012; Kon et al. 2009), the combination of rigid lattice structures with cell-carrying

hydrogels (Moutos and Guilak 2008; Nooeaid et al. 2012; Caterson et al. 2001), the use of multi-layered constructs (Sheerwood et al. 2008; Scheck et al. 2005) (normally requiring adhesives within layers), and computer-aided tissue engineering constructs (Sun et al. 2005).

Among the most promising approaches, towards stable and effective composite scaffolds, it is important to note the combination of: (a) phase separation or leaching processes, normally for obtaining the soft chondral phase, with (b) computer-aided rapid prototyping technologies based on additive manufacturing, usually for manufacturing the rigid bony phase (Da et al. 2013). In spite of the very positive results shown by metallic rapid-prototyped prostheses and scaffolds for bone repair (Alvarez and Nakajima 2009), most successful composite scaffolds for osteochondral repair are based on polymer-ceramic composites (Sheerwood et al. 2008; Da et al. 2013; Zhang et al. 2014), polymer-polymer composites (Kang et al. 2012), ceramic-ceramic composites (Hakamatsuka and Irie 1989), ceramic-metal composites (Zhao et al. 2013) and metal-metal composites (Staiger et al. 2006). Interestingly, metal-polymer composites, which could benefit from the stiffness of metals for the bony phase and from the elasticity of polymers for the chondral phase, are not so common.

In this chapter we present some design and manufacturing strategies for scaffolds with radical variations of mechanical properties aimed at complex articular tissue engineering applications. A couple of complete cases of studies, one linked to a composite scaffold for osteochondral repair and one linked to a composite scaffold for spine injuries, are also presented towards the end of the chapter.

19.2 Design Strategies for Scaffolds with Radical Variations of Mechanical Properties

The design of tissue engineering scaffolds for the repair of complex biological structures conformed by different types of tissues, as happens in articulations, in the spine, in the bone marrow, among other biostructures, can be supported by computer-aided design resources. The possibility of designing knowledge-based functionally graded scaffolds, with gradients of mechanical properties adapted to the biomechanical features of human tissues has been previously detailed, mainly in Chap. 16. Similar strategies, based on the use of concurrent trusses, on the employment of variations of porosities and thicknesses along different directions and on the linkage between medical images and design resources, can be also used for the development of scaffolds with radical variations of mechanical properties, if such changes are amplified.

However, the use of complementary approaches, based on novel metamaterials, such as pentamode mechanical metamaterials (Kadic et al. 2012; Fernández Méjica and Díaz Lantada 2013), which can be also gradually tuned and provide fluid-like behaviors in spite of being solid structures, can promote these desired radial

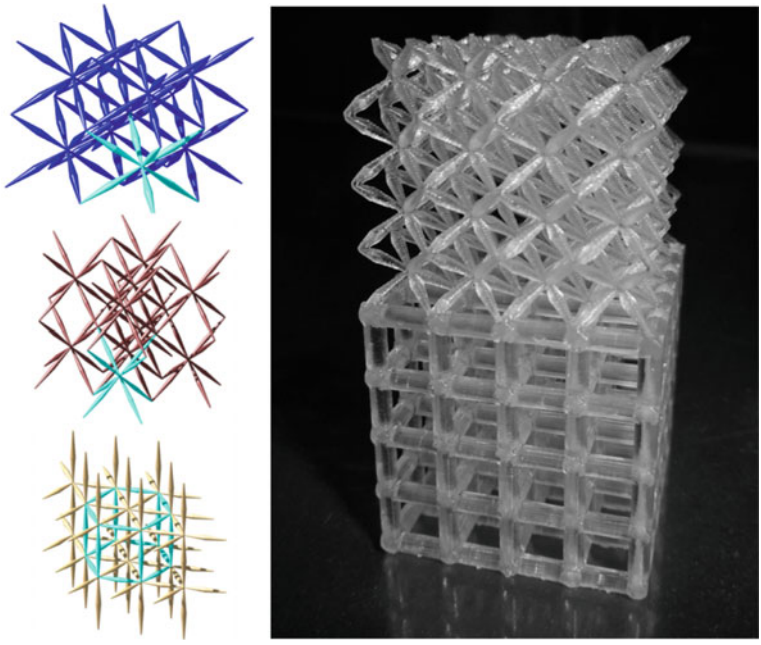


Fig. 19.1 Different types of pentamode mechanical metamaterials for designing solid scaffolding constructs with a very high relationship between bulk and shear moduli, hence promoting fluid-like behaviours. Tuning the dimensions of the nodes of these constructs or connecting pentamodal lattices with conventional scaffolds, as in the epoxy prototype shown, which is obtained by laser stereolithogrpahy, leads to radial variations of mechanical properties

variations of mechanical properties within a scaffolding structure made of just one material. These pentamodes are lattice structures with almost punctual contacts between trusses, which provide them with a very remarkable flexibility.

The incorporation of geometrical gradients to such contacts between trusses can lead to very rigid lattices in some parts of the scaffold and to very flexible lattices in other regions. In addition, the combination of different types of lattice structures and the use of conventional lattices, together with pentamode-based structures, can be also used for controlling the mechanical properties of a single scaffold in a range of several orders of magnitude. The use of FEM models may help to select the adequate parameters for desired ranges of mechanical properties (Fig. 19.1).

Finally, designs based on the use of different lattices or porous structures made of different materials, hence leading to composite scaffolds, provides an additional degree of control regarding the mechanical properties of artificial biomimetic biomaterials. Several manufacturing challenges arise, which are discussed in the following Sect. 19.3 and in the provided cases of study.

19.3 Manufacture of Scaffolds with Radical Variations of Mechanical Properties

The manufacture of the aforementioned design solutions towards scaffolds with radical variations of mechanical properties can be accomplished, based on the 3D geometries from CAD files, using additive manufacturing resources, as common manufacturing option for complex and porous geometries, as detailed in Chaps. 8 and 15–18. Options based on the use of porogens, as those described in Chap. 18 are also possible, although they must be additionally tuned for the promotion of gradients of mechanical properties. One interesting possibility is the employment of particles of different sizes as porogens, so as to obtain controlled variations of pore sizes. Another option is the incorporation of compounding particles to the mixture of pre-polymerized PDMS and porogens, and the final casting of different mixture with different materials and proportions during the polymerization process, for finally obtaining functionally graded constructs as shown in Fig. 19.2.

19.4 Case Study: Composite Ti-PDMS Scaffold for Osteochondral Repair

This section presents the development of a composite scaffold that stands out for having a functional gradient of density and stiffness in the bony phase, which is obtained in titanium by means of computer-aided design combined with additive manufacture using selective laser sintering. The chondral phase is achieved by sugar leaching, using a PDMS matrix and sugar as porogen, and is joined to the bony phase during the polymerization of PDMS, therefore avoiding the use of supporting adhesives or additional intermediate layers.

The mechanical performance of the construct is biomimetic and the stiffness values of the bony and chondral phases can be tuned to the desired applications, by means of controlled modifications of the computer-aided designs, of the materials used, of the rate of porogen employed, among other options that promote the versatility of the proposed approach. Cell culture results, carried out using h-MSCs with the help of growth factors generated by the own progenitor stem cells, provide relevant information regarding the viability of the composite scaffolds used and help us to plan forthcoming research activities.

Additional details can be found in the recent publication by our team: "Composite scaffolds for osteochondral repair obtained by combination of additive manufacturing, leaching processes and h-MSC-CM functionalization", in: *Materials Science and Engineering C* (Díaz Lantada et al. 2016).

In this chapter we are presenting a more complete methodology, linked to the development of multi-scale and multi-material scaffolds, for obtaining the radical variations of properties needed for repairing the damaged biological structures where two or more types of tissue are present, as happens in articular repair when

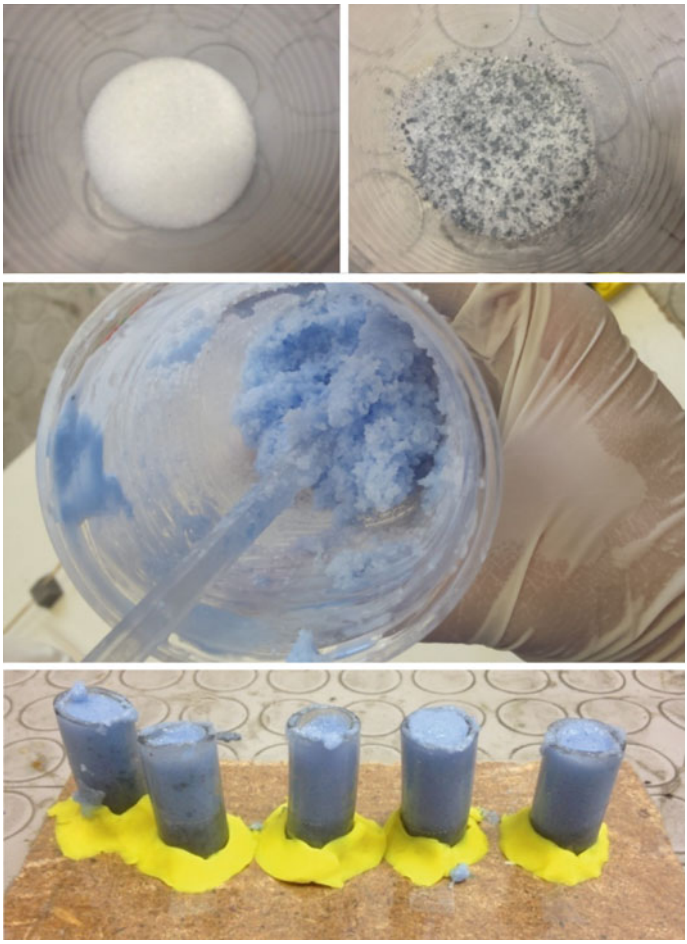


Fig. 19.2 Sugar and sugar mixed with SiC powder for their use as porogens towards the manufacture of functionally graded porous PDMS (*blue*) scaffolds (color online)

trying to recover bone and cartilage. We rearrange the previous document for providing a case of study within the complete design and development methodology for tissue engineering constructs aimed at osteochondral repair.

The proposed composite scaffold is obtained by combining design and manufacturing approaches detailed in the previous subsections and following the schematic process of Fig. 19.2. The functionally graded titanium lattice is obtained by computer-aided design using convergent trusses, as shown in Fig. 16.4, and subsequently manufactured by selective laser sintering from titanium powder for achieving a 3D prototype, included in Fig. 16.5.

The different geometries are simulated using the finite-element method capabilities of NX-8.5 (Siemens PLM Solutions) for studying the mechanical

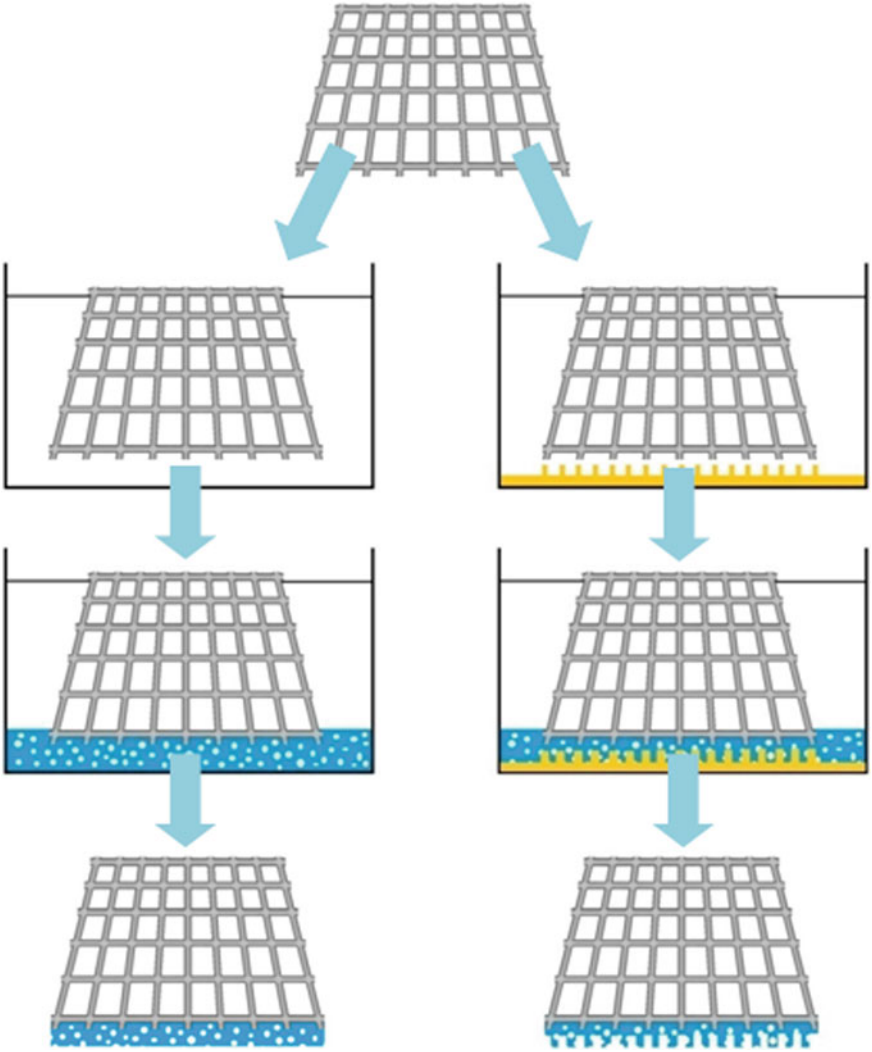


Fig. 19.3 Schematic process for the manufacture of composite Ti (*grey*)—PDMS (*blue*) lattice/porous tissue engineering scaffolds for osteochondral repair (color online)

performance of different scaffold designs, for design optimization tasks, for studying the performance of a porous PDMS layer and for analyzing the potential applications in tissue repair. Simulation results are summarized in Fig. 19.3, which shows the displacement and stress fields. The displacement and stress fields are drawn upon the deformed structure, showing the deformation in absolute magnitude, and the compression of the soft phase can clearly be appreciated.

The applied force of 500 N upon the upper surface of the chondral phase (15×15 mm^2 in the simulated construct) is similar to a pressure applied of 2 MPa and mimics the conditions that the construct should withstand, once implanted. Under such pressure, the chondral phase deforms importantly (around a 90 %, which can be clearly seen in the figures), as should happen in vivo, being the cartilage a shock absorber. The stress values are limited in the chondral phase to around 10 MPa, clearly below the PDMS limit used for the prototypes. The bony titanium phase deforms only a 0.03 % and its stress values reach 135 MPa, also well below the Ti limit.

These results can be used for evaluating the equivalent Young moduli of the bony and chondral phases, for additional comparison with the biomaterials to be substituted. Simulation results lead to a Young modulus of the chondral phase of around 2 MPa, which corresponds to the typical order of magnitude of cartilage moduli. The Young modulus of the titanium lattice varies gradually from 2.7 to 6.6 GPa, which is also in the range of the transitions from trabecular to cortical bone, which the lattice would aim to replace or repair.

In consequence, according to our results, the mechanical performance of the construct is biomimetic and the stiffness values of the bony and chondral phases can be tuned to the desired applications, by means of controlled modifications of the computer-aided designs, of the materials used, of the rate of porogen employed, among other options that promote the versatility of the proposed approach. The design parameters used for these first prototypes provide results in the expected ranges of mechanical properties of the biomaterials to be repaired or replaced.

The chondral (more flexible) phase is obtained by a sugar leaching using a Neukasil PDMS. First of all, the Neukasil RTV-20 pre-polymer and the Neukasil A2 cross-linker are mixed and degasified. Then, the PDMS and sugar mixture is obtained with the help of a Taurus vertical mixer—blender spinning at 1200 r.p.m. during 1 min just before casting. We use a mixture of 50–50 % of PDMS-sugar in weight for obtaining a very flexible support for the chondral phase. The mixture is casted into a rapid mold, where the Ti lattice has been previously introduced, until the lower layers of the lattice are embedded with the PDMS-sugar mixture.

A rapid-prototyped insert can be also placed into the mold for modifying the surface of the chondral phase, for example for obtaining vertical pores aimed at the promotion of vascularization, as proposed elsewhere. The construct is left at room temperature during 24 h for adequate polymerization.

Once the PDMS mixtures are polymerized, de-molding is accomplished for obtaining the desired composite scaffolds, after adequate cutting and leaching. Particle leaching, for the desired phase separation, is achieved by water immersion and systematic squeezing of the chondral phase. Final drying leads to the desired implants, as shown in Fig. 19.4.

Regarding cell culture trials, some results from hMSCs interacting with the composite Ti-PDMS scaffold are shown in the four images of Fig. 19.5. To this end, composites were exposed to conditioned medium produced by hMSC, then seeded with cells and incubated with DMEM low glucose and 10 % FBS during 48 h. The interface between the Ti lattice and the PDMS network is shown.

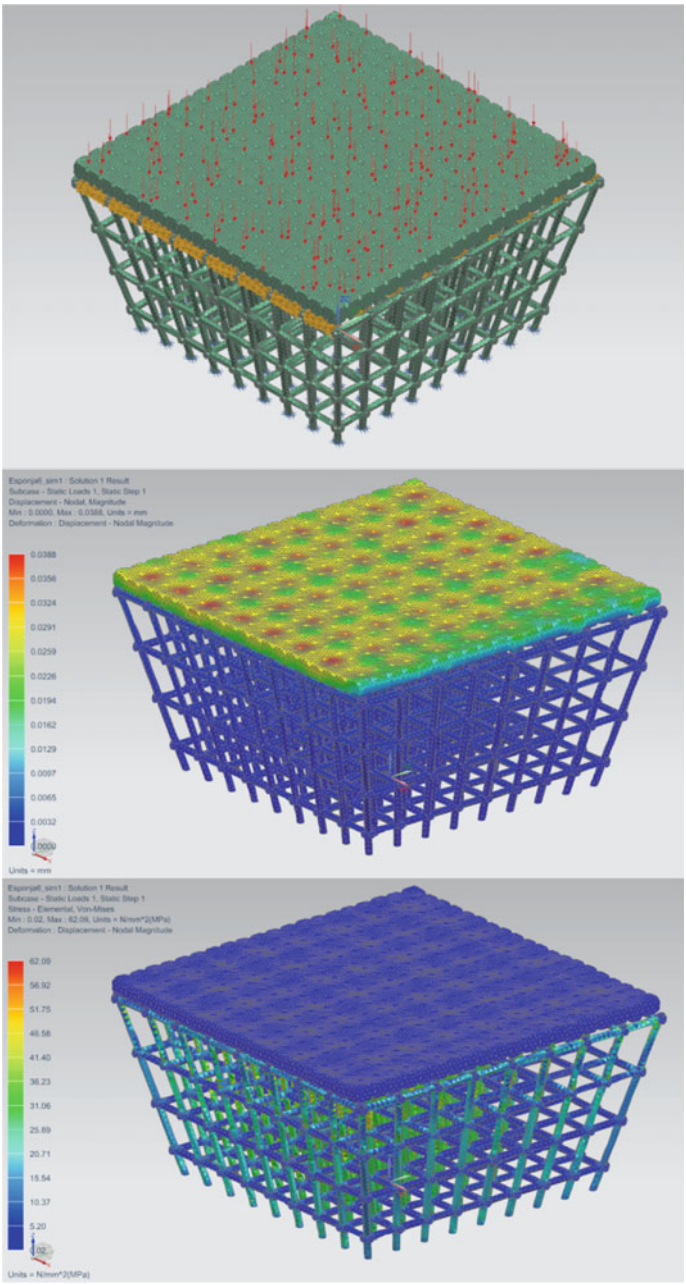


Fig. 19.4 FEM-based assessment of the mechanical performance of composite Ti-PDMS tissue engineering scaffolds for osteochondral repair

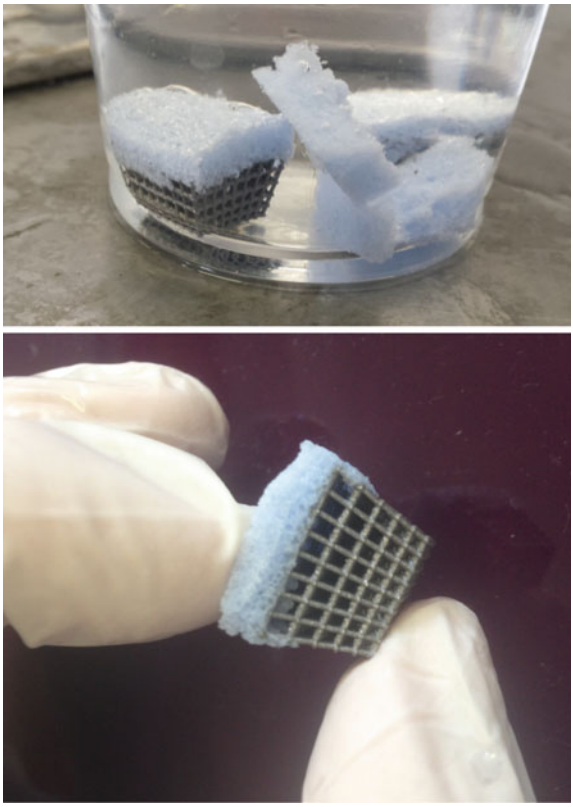


Fig. 19.5 Sugar leaching for obtaining a composite tissue engineering scaffold for osteochondral repair with a porous PDMS layer. The Ti lattice constitutes the rigid bony phase, while the porous PDMS constitutes the flexible chondral phase

The nuclei are stained in blue (DAPI) and their rounded forms without blebs indicating that the cells are in healthy conditions and well adhered to the porous scaffold. It is important to note that more than 50 cells/mm^2 can be appreciated. Several spherical—ellipsoidal cell aggregations can be appreciated, with potential application for promoting differentiation into osteocytes for bone repair, as part of the global osteochondral repair strategy. Thus, CM-hMSC and composite scaffolds are sponges with excellent porosity for hMSC to adhere.

Our results show that the cells and the composites scaffolds are excellent companions for potential tissue repair strategies. In case of tissue damage, the hMCSs-seeded lattice and porous scaffolds may constitute a functionally graded support, in order to allow the permeation of nutrients and debris, to promote oxygenation, to enable adaptation and to provide cellular communication systems, capable of locally inhibiting the immune system and of activating tissue repair, following the fluid dynamic, although additional assessments, both in vitro and in vivo, need to be performed (Fig. 19.6).

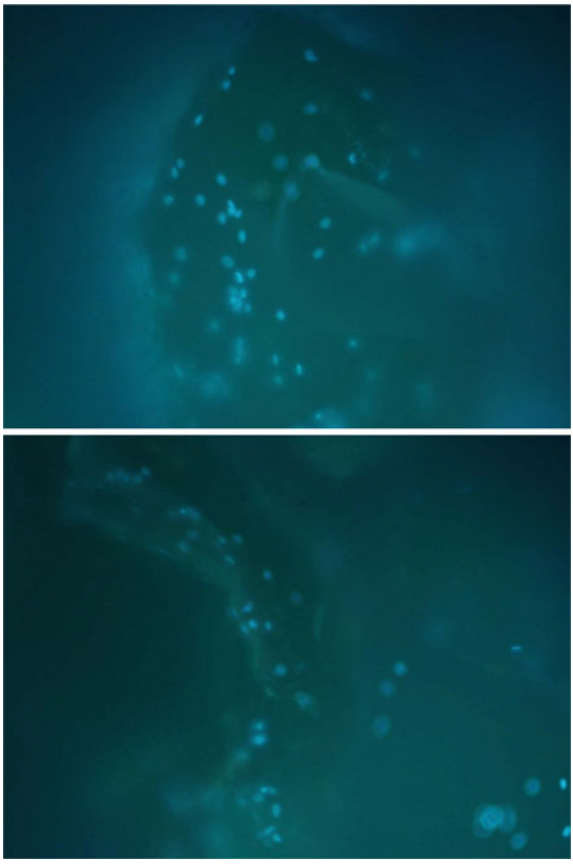


Fig. 19.6 hMSCs cultured upon the Ti-PDMS composite tissue engineering scaffolds for osteochondral repair. Healthy nuclei are shown in blue. The colonization of the rigid Ti lattice (*upper image*) and of the PDMS flexible layer (*lower image*) can be clearly perceived

19.5 Case Study: Scaffolds for Repairing Vertebrae and Spinal Discs

Another biological structure made of different types of tissues including radical variations of mechanical properties is the human vertebral column (backbone or spine). The vertebral column consists of 24 articulating vertebrae and 9 fused vertebrae in the sacrum and coccyx. The vertebrae in the column are separated from each other by intervertebral discs, very flexible cartilage elements that act as shock absorbers. There are also ligaments involved in linking the vertebrae to their companions. The vertebral column houses and protects the spinal cord and acts as a complex multi-body articulation. Spinal injuries affecting the discs are usually surgically treated with the help of spinal fixation devices, which may be improved by means of hybrid implant-scaffold geometries, as those described for the dental implants in Chap. 17, for improving osseointegration and osteoconductivity for

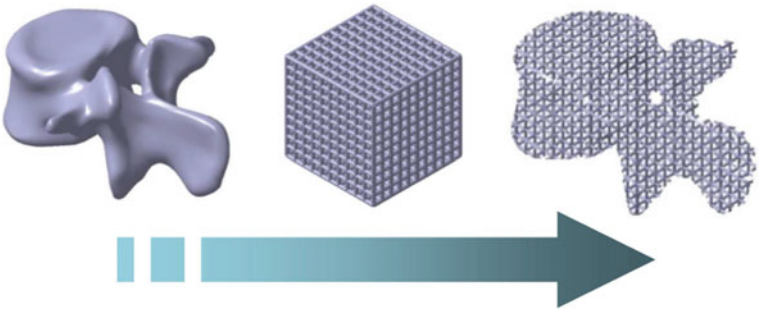


Fig. 19.7 Computer-aided design process showing the application of Boolean operations to obtain a lattice scaffold for vertebral repair

enhanced long-term performance. In other cases, injures affect both vertebrae and discs and more complex artificial vertebrae are needed. In any cases, state-of-the-art solutions are not as biomechanically adequate as desired; patient motility is compromised and the elasticity of the constructs does not usually match that of the original biostructure.

Tissue engineering of vertebrae and intervertebral discs would provide novel solutions to these problems, even with personalized approaches for enhanced in vivo performance, not just mechanically, but also biologically. Recent advances in computer-aided tissue engineering have helped to implement bioinspired design methodologies for these solutions (Wettergreen et al. 2005) and some promising mile-stones have been achieved (Kandel et al. 2008). Further research in the field will lead to additional progresses.

Here we present an alternative design and manufacturing procedure, based on the use of medical imaging and computer-aided design resources, together with additive manufacturing technologies, for the development of lattice biomimetic vertebral scaffolds. Following a similar integration procedure as the one explained in Sect. 19.4, the intervertebral disc can be obtained in porous PDMS by casting of an unpolymerized mixture of PDMS and porogens in a biomimetic mold, to which the vertebral construct is also incorporated. Final polymerization, extraction and particle leaching leads to a multi-material construct, with a rigid lattice for the vertebral scaffold and a porous and flexible PDMS for the intervertebral disc. The following Figs. 19.7, 19.8, 19.9, 19.10, 19.11, 19.12, 19.13, 19.14, and 19.15 help to summarize the procedure. The preliminary prototypes obtained help to validate the conceptual approach. Future prototypes, using titanium for the bony phase and again porous PDMS for the flexible layer, will help us to assess the performance in vitro, focusing on the colonization by cells and on the potential promotion of osseointegration and osteoconductivity.

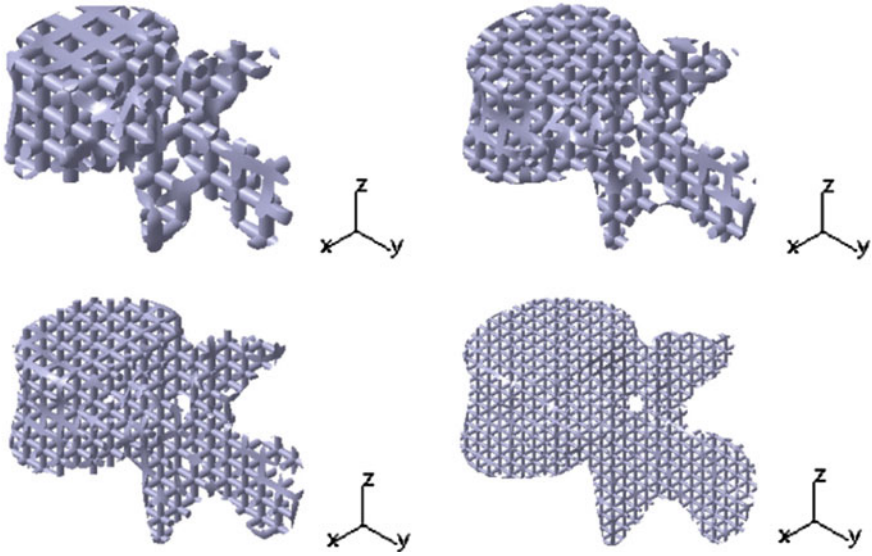
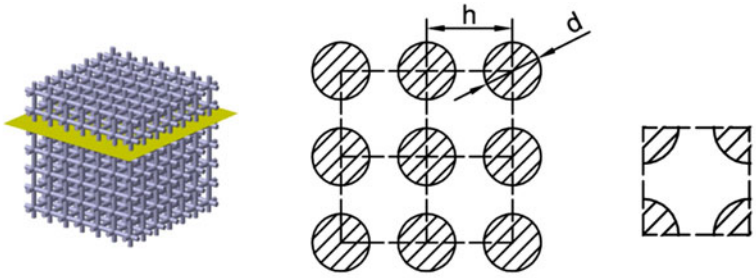


Fig. 19.8 Influence of lattice thickness on final scaffolding geometry



$$E' = \frac{\sigma'}{\varepsilon} \Rightarrow E' = \frac{F/A'}{\sigma/E} = \frac{F/A'}{(F/A)/E} \Rightarrow E' = E\frac{A}{A'} \Rightarrow \frac{E'}{E} = \frac{\pi}{4}\left(\frac{d}{h}\right)^2$$

Fig. 19.9 Simple relationships between scaffold's truss thicknesses (d); distance between trusses (h); porosity, which is connected with the ratio between the cross section of the porous construct (A') and the cross-section of the compact material (A); and Young's modulus of the porous construct (E') compared to the Young's modulus of the compact material (E)

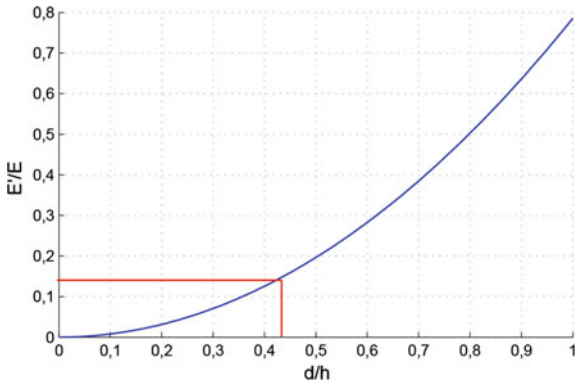


Fig. 19.10 Adequate selection of scaffold's truss thickness and distance between trusses for a biomechanical performance resembling that of bone. The selection is made considering its potential manufacture using a titanium alloy with a Young's modulus E = 11 GPa, and aiming at a final Young's modulus of the biomimetic construct similar to that of bone E′ = 15 GPa

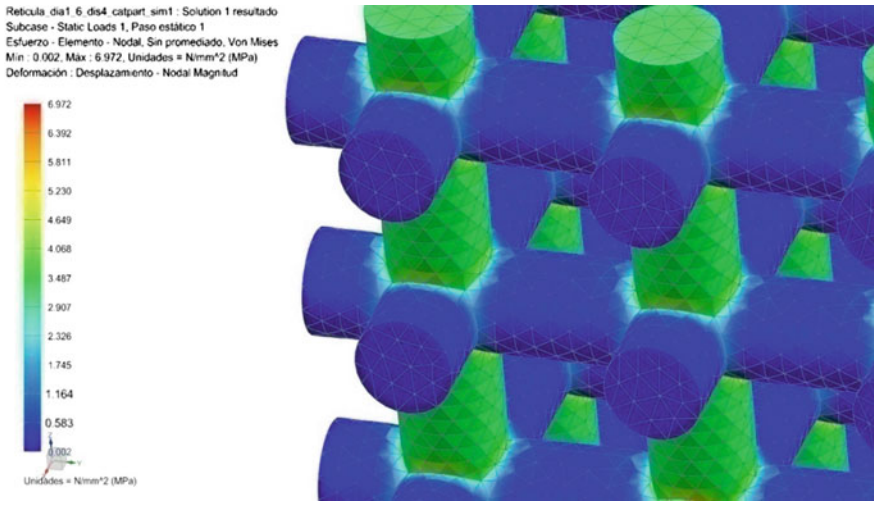


Fig. 19.11 FEM-based assessment of the mechanical performance of a tissue engineering scaffold for bone repair. These detailed simulations help to validate the adequate performance of the lattice selected according to the results from the previous figures and to analyze the impact of design optimization on the reduction of stress concentration factors for improved long-term fatigue behavior

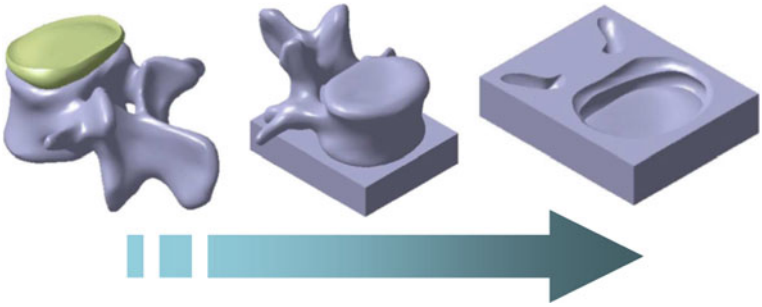


Fig. 19.12 Computer-aided design process for the development of a mold for helping with the incorporation of the intervertebral disc to the vertebral scaffold

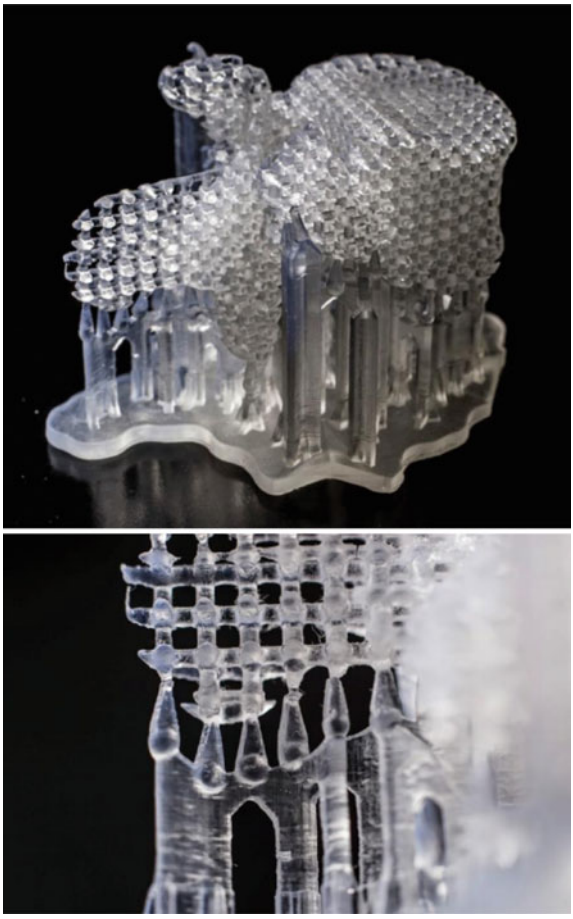


Fig. 19.13 Rapid prototype of a tissue engineering scaffold for vertebral repair. Manufactured by means of example using a Form1+ machine from Formlabs

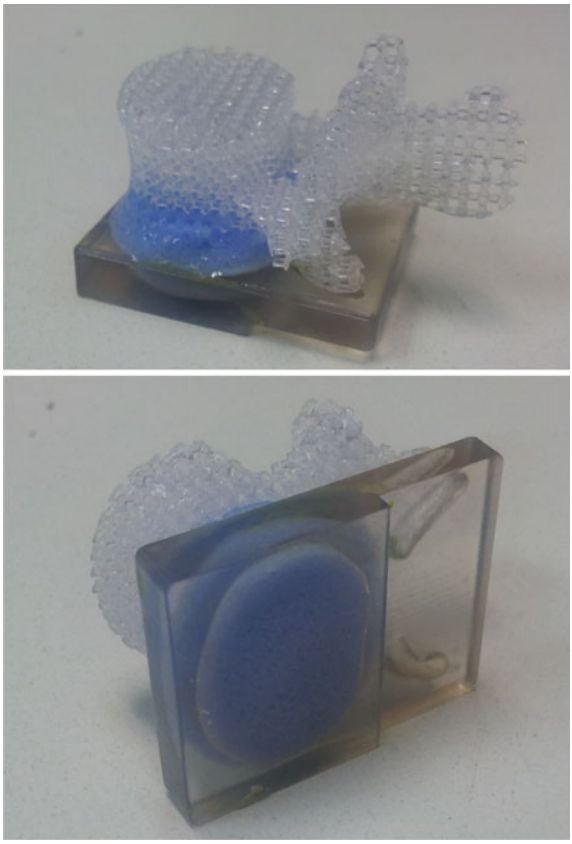


Fig. 19.14 Integration of the flexible intervertebral disc of a composite scaffold for spine injuries by PDMS casting incorporating sugar as porogen. The PDMS-sugar mixture embeds the rigid additively manufactured lattice (bony phase) and finally a porous and flexible PDMS layer (disc) is obtained by sugar leaching

19.6 Main Conclusions and Future Research

Articular repair is a very relevant and challenging area for the emerging fields of tissue engineering and biofabrication, as expertise regarding the repair of both soft and hard tissues is required. The need of significant gradients of properties, for the promotion of osteochondral repair, has led to the development of several families of composite biomaterials and scaffolds, using a wide range of potentially effective approaches, although a perfect solution has not yet been found.

Further research is needed to address the advantages of combining different technologies for manufacturing enhanced, even personalized, scaffolds for tissue engineering studies and extra cellular matrices with outer geometries defined as implants for tissue repair, as the niche composition and 3D structure play an important role in stem cells state and fate.

In this chapter we have presented some design and manufacturing strategies for the development of knowledge-based tissue engineering scaffolds with radical variations of mechanical properties, aimed at complex articular tissue engineering

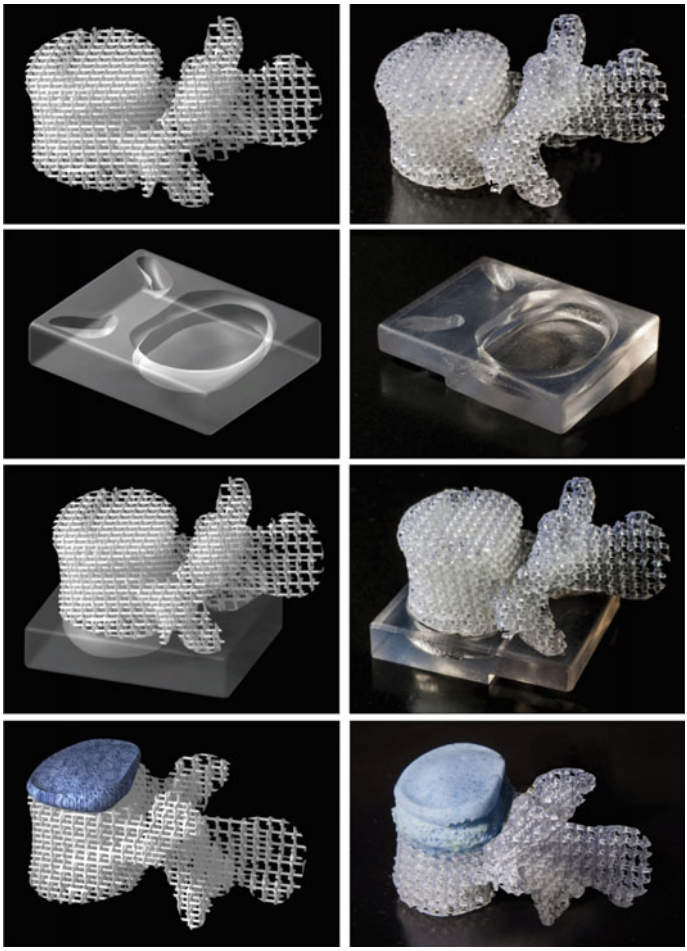


Fig. 19.15 Complete development of composite scaffolds for spine injuries oriented to the repair and regeneration of both vertebrae and inter-vertebral discs. *Left column* Computer aided designs. *Right column* Prototypes

applications. These functionally graded scaffolds constitute a key development in the areas of tissue engineering and biofabrication, as their mechanical properties can be tuned to those of the tissues and biological structures being repaired.

A couple of complete cases of studies, one linked to a composite scaffold for osteochondral repair, focused on the repair and regeneration of the extremes of large bones, and one linked to a composite scaffold for spine injuries, oriented to the repair and regeneration of both vertebrae and inter-vertebral discs, have been also presented to illustrate the proposed strategies.

References

Alvarez K, Nakajima H (2009) Metallic scaffolds for bone regeneration. Materials 2:790–832

Caterson EJ, Nesti LJ, Li WJ, Danielson KG, Albert TJ, Vaccaro AR, Tuan RS (2001) Three-dimensional cartilage formation by bone marrow-derived cells seeded ion polylactide/alginate amalgam. J Biomed Mater Res 57:394–403

Chan CKF, Chen CC, Luppen CA, Kraft DL, Kim JB, De Boer A, Wei K, Helms JA, Kuo CJ, Weissman IL (2009) Endochondral ossification is required for hematopoietic stem cell niche formation. Nature 457(7228):490

Da H, Jia S-J, Meng G-L, Cheng J-H, Zhou W, Xiong Z, Mu Y-J, Liu J (2013) The impact of compact layer on osteochondral tissue engineering. PLoS ONE 8(1):e54838

Díaz Lantada A, Alarcón Iniesta H, García-Ruíz JP (2016) Composite scaffolds for osteochondral repair obtained by combination of additive manufacturing, leaching processes and h-MSC-CM functionalization. Mater Sci Eng C 59(1):218–227

Fernández Méjica G, Díaz Lantada A (2013) Comparative study of pentamode mechanical metamaterials. Smart Mater Struct 22(11):115013

Hakamatsuka Y, Irie H (1989) Prosthetic artificial bone having ceramic layers of different porosity. US Patent 5152791 A

Kadic M, Buckmann T, Stenger N, Thiel M, Wegener M (2012) On the practicability of pentamode mechanical metamaterials. Appl Phys Lett 100(19):191901

Kandel R, Roberts S, Urban JPG (2008) Tissue engineering and the intervertebral disc: the challenges. Eur Spine J 17(4):480–491

Kang H-W, Park JH, Kang T-Y, Seol Y-J, Cho D-W (2012) Unit cell-based computer-aided manufacturing system for tissue engineering. Biofabrication 4:015055

Kon E, Mutini A, Arcangeli E, Delcogliano M, Filardo G, Aldini NN, Pressato D, Quarto R, Zaffagnini S, Marcacci M (2009) Novel nanostructured scaffold for osteochondral regeneration: pilot study in horses. J Tissue Eng Regen Med. doi:10.1002/term.243

Laschke MW, Rücker M, Jensen G, Carvalho C, Mülhaupt R, Gellrich NC, Menger MD (2008) Incorporation of growth factor containing Matrigel promotes vascularization of porous PLGA scaffolds. J Biomed Mater Res A 85(2):397

Moutos FT, Guilak F (2008) Composite scaffolds for cartilage tissue engineering. Biorheology 45 (3–4):501–512

Nooeaid P, Salih V, Beier JP, Boccaccini AR (2012) Osteochondral tissue engineering: scaffolds, stem cells and applications. J Cell Mol Med 16(10):2247–2270

Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld V, Cohen S (2003) Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J Biomed Mater Res A 65(4):489

Richardson TP, Peters MC, Ennett AB, Mooney DJ (2001) Polymeric system for dual growth factor delivery. Nat Biotechnol 19:1029

Schek RM, Taboas JM, Hollister SJ, Krebsbach PH (2005) Tissue engineering osteochondral implants for temporomandibular joint repair. Orthod Craniofac Res 8:313–319

Sheerwood JK, Riley SL, Palazzolo R, Brown SC, Monkhouse DC, Coates M, Griffith LG, Landeen LK, Ratcliffe A (2008) A three-dimensional osteochondral composite scaffold for articular cartilage repair. Biomaterials 23:4739–4751

Staiger MP, Pietak AM, Huadmai J, Diaz G (2006) Magnesium and its alloys as orthopedic biomaterials: a review. Biomaterials 27(9):1728–1734

Sun W, Starly B, Nam J, Darling A (2005) Bio-CAD modeling and its application in computer-aided tissue engineering. Comput Aided Des 37(11):1097–1114

Tan JY, Chua CK, Leong KF (2013) Fabrication of channeled scaffolds with ordered array of micro-pores through microsphere leaching and indirect rapid prototyping technique. Biomed Microdevices 15:83

Wettergreen MA, Bucklen BS, Sun W, Liebschner MAK (2005) Computer-aided tissue engineering of a human vertebral body. Ann Biomed Eng 33(10):1333–1343

Zhang X, Chang W, Lee P, Wang Y, Yang M, Li J, Kumbar SG, Yu X (2014) Polymer-ceramic spiral structured scaffolds for bone tissue engineering: effect of hydroxyapatite, composition on human fetal osteoblasts. PLOS ONE 9(1)

Zhao C, Zhang H, Cai B, Wang G, Fan H, Zhang X (2013) Preparation of porous PLGA/Ti biphasic scaffold and osteochondral defect repair. Biomater Sci 1(7):703–710

Chapter 20
Fluidic Microsystems: From Labs-on-Chips to Microfluidic Cell Culture

Andrés Díaz Lantada, Beatriz del Valle Sesé, Josefa Predestinación García Ruíz and Hernán Alarcón Iniesta

Abstract Lab-on-chip microfluidic devices or "labs-on-chips" are aimed at integrating the complex operations and procedures typical from biochemical and biological laboratories in just a few cm^2, by taking advantage of microfluidic operation, which promotes reaction speed, sustainability due to the use of low fluid and sample volumes, and repeatability, thanks to multiplexing and automation, as already mentioned. Even if further research in the field will promote additional miniaturization and integration of capabilities, lab-on-chip microdevices incorporating cells and tissue samples are already very interesting for disease modeling, for studying in depth the biomechanical and biochemical aspects of disease and for obtaining models of physiological structures of the human body, normally by co-culturing different cell types, for final in vitro assessment of drugs and for a better understanding about the mechanisms of life. This chapter provides an introduction to labs-on-chips aimed at cell culture stimulated by means of microfluidic stimuli. Design, modeling and manufacturing strategies, for the development of labs-on-chips capable of helping researchers with cell co-culture for studying the interactions of different cell types and for the development of in vitro models of physiological structures, are covered. In addition, a complete case of study of a versatile lab-on-a-chip for cell co-culture is detailed. These types of microfluidic systems constitute the basic infrastructures for the development of other more complex devices such as cell-based sensors, cell-based actuators and organs-on-chips, covered in detail in the following chapters.

A. Díaz Lantada (✉) · B. del Valle Sesé
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

J.P. García Ruíz · H. Alarcón Iniesta
Department of Molecular Biology, Universidad Autónoma de Madrid, Madrid, Spain

A. Díaz Lantada (ed.), *Microsystems for Enhanced Control of Cell Behavior*,
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20.1 Introduction: Labs-on-Chips and Enhanced Microfluidic Cell Culture

As the size of a device gradually gets smaller and enters the micrometric range, the surface to volume ratio increases and the surface forces become the ones that dominate the behaviour of these microdevices. On the other hand, in the macroscopic world, the volume forces (gravity, among others) dominate. For example, capillary and electrostatic forces play a fundamental role in microscopic objects and micro-fluid mixing presents a real challenge, since there is limited convection and diffusion becomes the key method of transport to consider, when designing and operating microfluidic devices.

Depending on the predominant propulsion method of the fluids in a microsystem, there are different basic operational modes (Jenkins and Mansfield 2013), as listed below:

- Capillary-force operated microsystems. When the size of the channels in a microsystem is reduced to a few hundreds of microns, the surface forces begin to dominate the system response and the aqueous solutions flow along the capillary walls, without any need to apply external pressure. The advantage of these devices is their simplicity and the fact that no external pumping systems are required to operate them. These are currently the most commercially successful microfluidic systems. The use of additive manufacturing technologies for their monolithic fabrication, with inner channels and compact surfaces, especially when using laser stereolithography, is noteworthy (Waldbaur et al. 2011).
- External pressure-operated microsystems. This kind of device requires external pumps to move the samples and reagents to the mixing and reacting parts of the system. These are versatile systems where the use of an external pumping system lets a larger number of transport, mixing, reaction and separation functions, among others, to be performed. Microsystems usually use laminar flow, which means the mixing processes are slow and sometimes mixers need to be incorporated to boost turbulence, hence reducing mixing distances and minimizing system size. One of the disadvantages of these external pump-operated microsystems is the need for additional components and the difficulties linked to making the connections and avoiding leakage.
- Microsystems operated by active elements or materials. An alternative to using external pumps or micropumps is to include certain transducers inside the microfluidic systems whose job is to make conversions, usually electromechanical or electro-acoustic ones, to promote fluid movement.
  In general, using active or "intelligent" materials that exhibit a controlled response to external stimuli and link different physical-chemical domains helps integrate the functionalities in a microsystem, reduces the number of components and simplifies the design, although it is important to emphasise the need to perform modelling tasks and characterise these materials, due to their innovative nature (Díaz Lantada 2012). Some of the most widely used active materials in

microfluidics are piezoelectric materials, such as ceramics and polymers capable of producing mechanical stimuli when a voltage is applied to their surfaces. These piezoelectrics can be fabricated in the form of a film to produce active membranes. When these have been integrated into a microfluidic system, they help the active parts of the microdevice to behave as diaphragm micropumps. On occasions, if it is sufficiently flexible, the casing of the device can perform as an active component.

- Centrifugal force-powered microsystems. These microfluidic platforms are usually disk-shaped, which is why they are also called lab-on-CDs, and operate through the effect of inertial forces due to the rotation of the disk which make the fluid go from the central container to the reservoirs at the edge of the disk. A motor is required but no external pumps or complicated connections, which means they are also microsystems that are simple to use.
- Electromagnetic field-powered microsystems. These are microsystems that are governed by electroosmotic, electrophoretic, dielectrophoretic and magnetofluidic processes and electrical wetting control. An electroosmotic flow is produced as a result of the fixed charges present on the surface of the microchannels. These charges are capable of causing a separation inside the solution to form a dual electric layer near the channel walls. The subsequent application of a magnetic field brings about a movement of charges that sweeps along the fluid. Electrophoresis is the movement of molecules and particles that are charged through the application of a constant external electrical field to separate the macromolecules as proteins and nucleic acids in microfluidic systems. Dielectrophoresis is a similar process but under the action of a non-constant electrical field. The electrical surface wetting control processes let hydrophilic surfaces change to hydrophobic surfaces and vice versa, by applying a voltage. The production in sequence of these changes in different zones of a microsystem produces a net flow of fluid in a desired direction. The use of ferrofluids that can change their viscosity and move through the action of external fields is also of interest, for both diagnostic and therapeutic devices.

The continuous improvement in the design and fabrication processes of microfluidic devices means that new diagnostic functionalities can be incorporated so that they can be used for ever more complex purposes. One of the most relevant medical problems is the need to have diagnostic elements and platforms that more and more closely resemble human physiology. This is because, in the body's response to certain conditions, not only do pathogens and therapeutic elements play a fundamental role, but also the morphology and behaviour of the different organs and the way they interact.

Tissue engineering strives to reproduce complete organs in vitro, based on cultivating and differentiating embryonic stem cells from patients to form viable tissues, with the support of certain three-dimensional scaffold structures to grow the tissue with the aid of suitable growth factors. Producing artificial organs in laboratories in the future will obviously be vital for therapeutic actions (organs for transplants), but there are also vital implications that are linked to improvements in

diagnostic procedures. Although the growth of small tissue patches is currently common practice, the construction of complete organs with adequate vascular networks that can deliver nutrients to the cells and properly eliminate any waste is still a subject of research. Only small three-dimensional tissue patches have been achieved, as well as some simple tubular structures such as urethras and parts of the trachea.

While research is being carried out into the artificial generation of organs through tissue engineering, the use of microfluidic devices can again be extremely effective. The simple schematic designs of different interconnected channels and the use of semi-permeable membranes, between the multiple layers of a lab-on-chip, and similar strategies are leading to qualitative representations of how different organs work, which can thus be studied quickly and simply.

In fact, one of the major current challenges of labs-on-chips is to go more deeply into these representations of the human body and to obtain comprehensive libraries of physical prototypes, for modeling and studying human physiology and physiological structures. In consequence, new organ-on-chip microfluidic systems are beginning to be defined, each of which can represent an organ or parts of an organ (please see Chap. 22 for additional details about this field). By connecting organ-on-chip devices, apparatuses and complete systems can be modelled for even reaching the "life-on-chip" concept. There are already successful experiences regarding qualitative models of livers, kidneys, lung alveoli, blood-brain barriers and even complete digestive systems (Huh et al. 2011, 2013).

Future research in this field will be based on simple microfluidic systems, which will be as simple as already commercial microsystems and which will be designed and manufactured taking into account similar strategies as those applied for the development of the cases of study presented in this chapter.

20.2 Overview of Existing and Commercial Devices

Microfluidic devices have seen an explosive growth in the last 20 years. This is due to the convergence of clinical diagnostic techniques (blood gas analyses, molecular biology-based trials, the use of immunoanalysis) and the fact that microfabrication technologies have reached maturity, leading to the production of micrometric size channels and reservoirs for fluids in a wide variety of materials (silicon, glass, poly (dimethylsiloxane) or silicone (PDMS), poly(methyl methacrylate) or PMMA, among others).

Miniaturizing the functions of a chemical, biochemical or microbiology laboratory has clear immediate advantages, which include: a dramatic decrease in the reagents and samples used for the analysis; faster results due to working on a micro-scale (because there is an increase in surface to volume ratio, which speeds up the physical-chemical reactions); lower production costs, as the phases can be integrated and automated; and, finally, lower testing costs and increased efficiency, as is usually the case when any kind of microsystem is used.

Remarkable recent progresses are linked to the possibility of using these microfluidic devices at the patient point of care or ("POC" *testing devices*), thanks to their small size, ease of use (providing the design is right) and fast response. Using these microdevices at the patient point-of-care is also highly positive. It can give the patient an almost immediate response or speed up part of the diagnosis; it reduces false positives and negatives, due to the test being given in the doctor's own surgery, instead of having to use external laboratories, with the successive stages of transport and handling. It also means that the treatment can be achieved earlier, particularly in areas that have difficult access to laboratories or large hospitals, such as sparsely populated rural areas, and developing countries. Using these devices at the patient point-of-care also reduces the stress suffered by patients when waiting for the results of a diagnosis.

Moreover, the versatility of these microfluidic systems means they can be used for biochemical, microbiological and other molecular biology-based trials to detect different viruses, bacteria, conditions or a predisposition to conditions on the basis of genetic analysis. This has an impact on the stages of prevention, diagnosis and therapeutic action, thereby improving practically every aspect and phase linked to clinical practice. Even the use of cells from the patients, to understand their interactions with pathogens, to find disease mechanisms and treat them in a personalized way, is enabled thanks to the use of biomedical microfluidic devices capable of interacting at cellular level.

Before going into the detail of the operating principles, designs, fabrication processes and successful cases in industry of microfluidic systems and devices for in vitro diagnosis, in many cases aimed at or based on interacting with cells and tissues, let us concentrate on the some relevant types of microfluidic devices for patient point-of-care diagnosis, which can be also further researched and probably improved by the incorporation of cells:

- Laboratories on a chip or "labs-on-chips". These are platforms for self-sufficient diagnosis, where, in principal, all the diagnostic measures can be performed by mixing the different reagents that flow through the device and react in the appropriate chambers to provide a simple reading regarding the diagnosis, usually by visual examination.
  However, at present, the "lab-on-chip" concept in most present cases means "chip-on-lab" devices. That is, a simple microsystem, but one requiring a set of machines, pumps, readers, support structures…, that are available in the testing laboratory if they are to work properly. Current trends are oriented to obtaining a greater autonomy for these microfluidic devices so they can be used without problem at the patient point-of-care.
- Micro Total Analysis Systems or "μ-TAS". They can be thought of as advanced labs-on-chips or with more highly integrated functions due to the use of thermo-optic-electro-mechanics with which to control a greater number of physical-chemical domains and obtain more exact responses or more information. In principal, we will use the term lab-on-chip as a practical synonym for μ-TAS, in the present state of technology.

- Diagnostic strips. These are microfluidic devices for a more extensive and simpler diagnosis at the patient point-of-care. They are visually oriented to "all or nothing" diagnoses, such as "the patient has an infection", "pregnancy tests" and the like. They are usually made of paper or very economical materials (polymers) and function through the immersion of one of their ends in the sample under study. By diffusion or capillarity the sample reaches the different areas of the test strip and causes a change of colour in the event of a positive diagnosis. Recent research is looking into making the step from the standard qualitative diagnosis using diagnostic strips to diagnoses that have a certain quantification.
- Fluid cartridges and integrated platforms. These are complex systems comprising hardware (desktop machine) with its own software to control the analytical process. Fluid cartridges prepared with all the reagents and the sample to be processed (as with an ink cartridge for a printer) are placed in the machine. They have communication ports to connect to the hospital information systems and are generally used for complex diagnoses, usually in the field of genetics or molecular Biology and the like.

Generally speaking, microfluidic diagnostic devices are being developed to carry out diagnoses such as: glucose level monitoring, blood gas and electrolyte analysis, drug addiction or drug abuse studies, pregnancy and fertility, the detection of urinary infections, detection of gastrointestinal infections, detection of cancer markers, detection of antibodies, evaluation of levels of haemoglobin, cholesterol, urine albumin, detection of acidity or basicity and reaction to polymerase, among others.

Microfluidic systems for patient point-of-care diagnosis have the potential to reduce costs and improve the results in a whole range of processes and areas related to the biosanitary sector. In the field of microfluidics, in spite of the enormous advances achieved since the end of the 80s, the many proposals for use and the success of some technology-based companies, we have still not achieved a universal or definitive application that will let a few companies dominate the situation, as happened in many other sectors where technology plays a fundamental role.

Maybe the application of microfluidic systems to patient point-of-care (POC) diagnosis will turn out to be that definitive application or range of applications for having a greater industrial and social impact. The novel field of organs-on-chips (see Chap. 22) may also contribute in a very significant way to these social and industrial successes.

In spite of the interest in microfluidic systems and the huge advantages of using them for patient point-of-care diagnosis, as previously stated, and the research efforts in the field, a key factor for the future success of these devices is for doctors to become involved (unquestionably the final users) in their development. This involvement may be decisive for minimising the initial reluctance of doctors to change their procedures, which are currently to send samples to the central laboratories in charge of making detailed reports to support the diagnosis.

The use of simple effective designs with which it is easy to interact (promoting the ergonomics, diagnosis through colour change…) will help doctors to move on from the standard systems to patient point-of-care diagnosis since the advantages are clear, not only from a diagnostic point of view but also with a view to preventive actions and monitoring the evolution of disease.

At present, the most successful companies in the microfluidic diagnostic system field contribute solutions, based on interactions at cellular level, mainly intended for:

(a) cell capture and count to detect cancer,
(b) rare cell capture for prenatal diagnosis,
(c) CD4 lymphocyte counts for HIV,
(d) biochemical and microbiology studies at the patient point-of-care and
(e) molecular diagnostics and genetic analysis.

20.3 Design Strategies for Microfluidic Devices Aimed at Interacting with Cells

Another key challenge for the future of microfluidic diagnostic systems is to progressively reduce their mass production costs. Having machines, such as micro-injectors, hot-embossing systems, laser micro-machining, to name but a few, requires high investment of the order of thousands of euros when it comes to setting up a workshop to produce these devices. Of course, once tens of thousands of microsystems have been produced the high cost of the machines will only have an impact of a few euros on the final parts.

However, the access to these technologies is hindering development in many regions where a few euros difference in the final price can be decisive for their use in developing countries. Therefore, a gradual lowering of costs of the means of production and the materials used in these microsystems is today's major challenge.

New trends, particularly those promoted by the Whitesides' team at Harvard (Whitesides et al. 2001; Martínez et al. 2010), pioneers in the field, are actively striving to reduce costs by using very economical materials that can take advantage of low-cost technologies to be found in any home or office (inkjet printer, sewing machine…). For example, paper impregnated with different reagents and chromophore agents is being used in the development of numerous devices (diagnostic strips, in general) to measure anomalous levels of glucose, protein, acidity or basicity in urine as a way of qualitatively diagnosing certain conditions. Outstanding among the successful paper-based solutions that have been commercially available for some time are diagnostic strips to detect pregnancy and other diagnostic strips to detect urinary infections that also have interesting applications in paediatrics apart from adult patients.

Ideally, low-cost devices could even be fabricated at home or in doctors' surgeries directly at the patient point-of-care. Recent proposals have shown the feasibility of using printers similar to inkjet printers, but which use solid wax cartridges which when they melt can apply fine layers and generate letters, lines and grids on paper (Xerox patent), to develop microfluidic systems. In these systems, the zones where the ink is deposited are hydrophilic, which means channels can be constructed to guide the patient's samples. The complexity of the devices for the separating, mixing and control functions can be achieved with various layers of wax-covered paper and by using such simple tools as scissors, guillotines, adhesive tape, punches and hole punches (Phillips and Thom 2013).

Other research has clearly shown that it is possible to obtain microfluidic systems by using cotton threads tied to one another and then coated with the help of a laminator or sewn directly to a frame or paper. In general, different threads immersed in diverse reagents and then suitably dried are knotted to other threads whose job is to transport the sample by diffusion. When the dry threads become wet again, through a colour change brought about by a chemical reaction, they can reveal the presence of some kind of pathology or inappropriate levels of glucose, albumin, acidity, basicity and nitrites, among others (Reches et al. 2010).

To support the gradual cost reduction of diagnostic microfluidic devices, we also need to encourage collaboration among researchers due to the peculiar multidisciplinary nature of these microsystems, which require a knowledge of Biology, Medicine, Pharmacy, Physics, Chemistry and Engineering, to name just a few of the major disciplines.

The "Chips and Tips" web, designed by the Royal Chemical Society, is one of the main forums for scientists in the labs-on-chips field. It provides ideas and solutions, while also offering the possibility to interact in real time regarding practical problems that are frequently encountered in the laboratories dedicated to the design and development of these microdevices. However, there are many relevant problems that are not always dealt with in scientific publications, which usually summarise the process followed and focus on the final results, rather than on the difficulties encountered during the process.

Another key issue, for boosting the industrial and social impact of patient point-of-care diagnostic microfluidic systems, is to promote teaching activities in this field to train the future researchers, designers, producers and even marketers of these kinds of solutions. Currently, mention is only made of microfluidic systems in the programmes of certain specialised subjects such as "Fluid Mechanics", and such mention is usually brief without going into modelling, simulation, fabrication and testing, which are fundamental if satisfactory solutions are to be achieved. Some Master's and Doctoral programmes are beginning to introduce these notions. However, it is important to have infrastructures for practical sessions and modular kits for development that can simply illustrate the basic concepts. In this respect, the work carried out at the MIT is outstanding. They have developed low-cost kits for diagnostic-oriented microfluidic systems that are even compatible with LEGO so as to be able to construct the lab-on-chip more easily. The kits have been so successful that they have even led to spin-off MEDIkits (Gómez Marquez 2011).

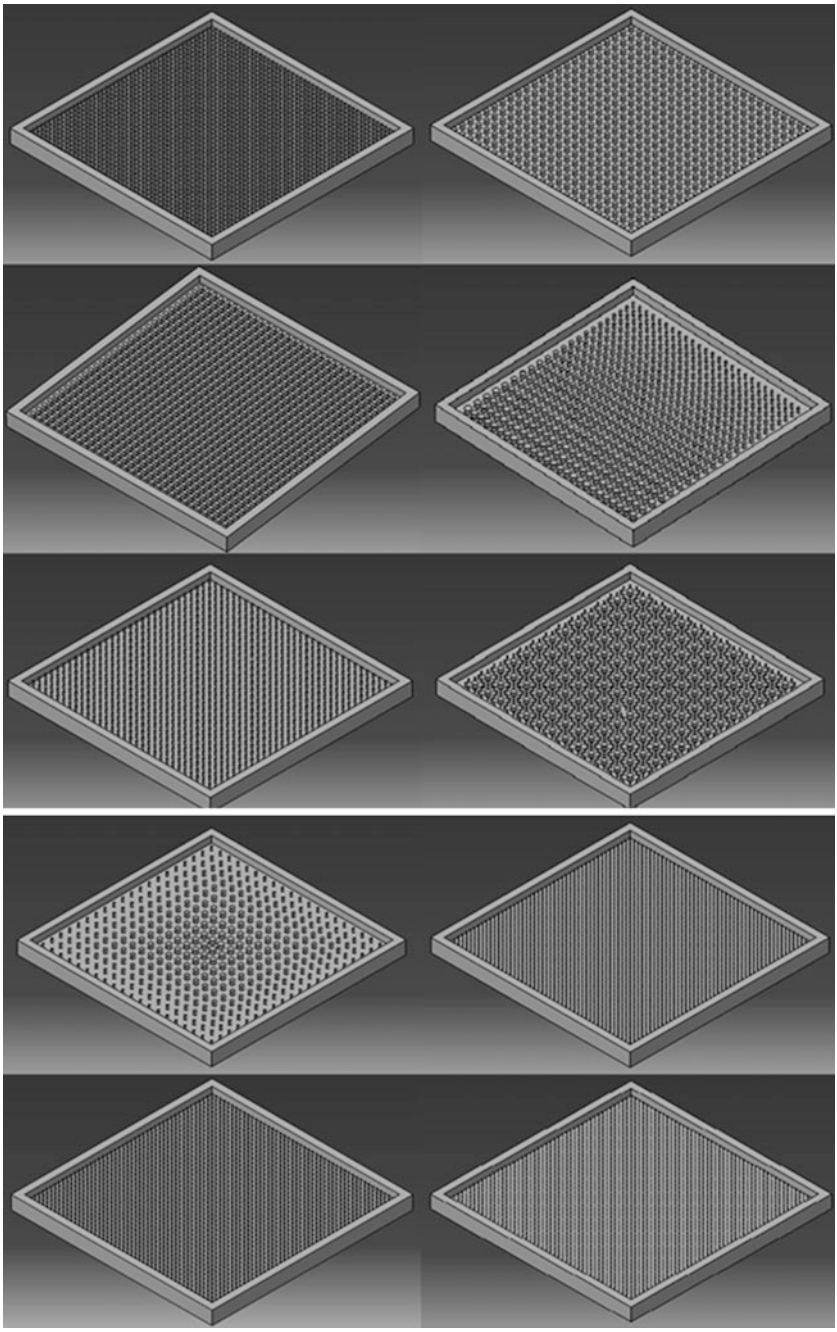

Fig. 20.1 Computer-aided design library of different molds for the manufacture of microporous membranes with functional gradients of porosities

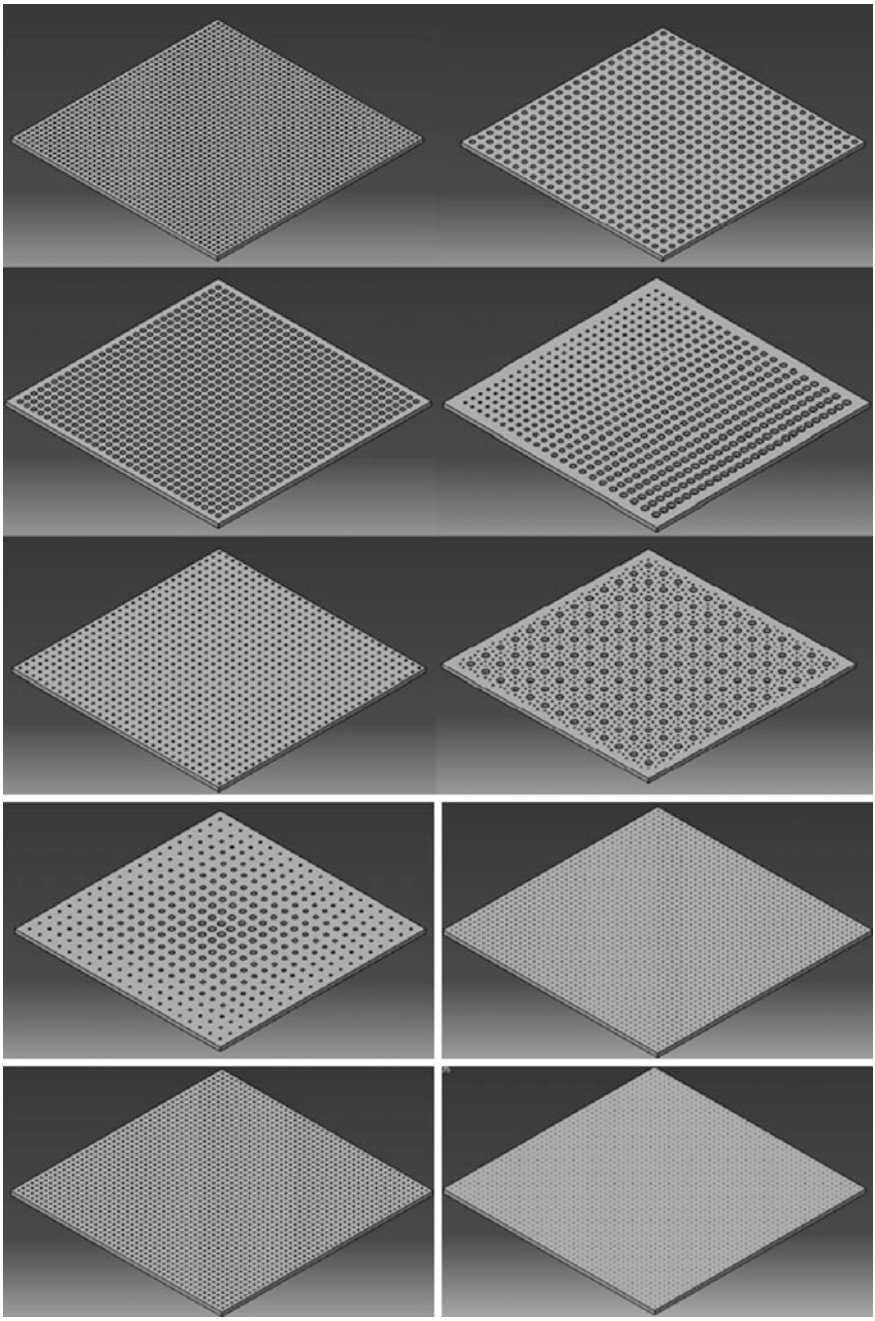

Fig. 20.2 Computer-aided design library of different microporous membranes with functional gradients of porosities

The incorporation of different cell types and living micro-organisms to these micro-fluidic devices is also a challenge, especially regarding the long-term endurance of the micro-organisms within these devices. The use of computer-aided design, engineering and manufacturing resources also helps to speed up the process and leads to lower cost systems. The use of systematic procedures, which we try to illustrate by means of a case of study linked to the development of a multi-layered micro-fluidic system for the co-culture of different cell types, is also relevant. First it is necessary to start with the computer-aided design of potentially adequate geometries and to concentrate on the main components.

Figures 20.1 and 20.2 illustrate this step by showing a library of different molds for the manufacture of microporous membranes with functional gradients of porosities and the related membranes, which are the key components of the microsystem under development, as detailed in the following sections.

20.4 Manufacture Strategies for Microfluidic Devices Aimed at Interacting with Cells

The manufacture of microfluidic devices aimed at interacting with cells can benefit from the wide set of technologies described in Chaps. 8–10. Linking computer-aided designs, improved by means of computer-aided engineering, with computer-aided manufacturing resources, such as automated high precision CNC machining, laser ablation technologies and additive manufacturing tools, is very appropriate for the rapid development of prototypes and master models for the assessment of performance, before focusing on mass production. It is relevant to note that, not just for mass production, but also for prototypes aimed at conceptual validation, the use of adequate biomaterials is required.

It is important to recall that rapid prototyping (RP), based on additive manufacturing processes, is typically very well suited for single prototypes with complex geometries, but normally inadequate for mass production, due to the excessive cost and time involved, in comparison with replication technologies, such as injection molding and compression molding. In addition, the polymers used in the most precise rapid prototyping technologies, which are based on photo-polymerization processes, are typically toxic or inadequate for biomedical applications, what limits enormously the span of final applications. For instance, common thermoplastics used for the mass production of medical devices, including poly(methyl methacrylate) (PMMA) or polycarbonate (PC), cannot be processed using conventional additive manufacturing technologies.

Recent research has achieved groundbreaking improvements in the bio-compatibility of rapid prototyping materials (Baudis et al. 2009, 2010) and dramatically helped to increase the manufacture speed and the attainable precision of these technologies (Stampfl et al. 2008). Nevertheless, for efficient and economic mass production of polymeric microdevices, especially for the biomedical industry,

mass replication technologies still have no rival. Other moldable thermoplastics can be of interest for further specific applications in mechanical engineering, aeronautics, electronics… taking advantage of engineering polymers with enhanced thermal, electrical or mechanical behaviors, which cannot be found among the typical properties of RP polymers.

Exploring cooperative strategies, for taking advantage of the complexity of geometries attainable via rapid prototyping, while also benefiting from the possibility of manufacturing large low-cost series using mass replication techniques, is a relevant industrial need and can be a source of novel procedures for supporting research and innovation in several fields.

Among mass production technologies, micro-injection molding provides a high efficiency concerning the replication of micro- or even nano-sized structures. Description of the so-called micro injection molding process and its advantages can be found in previous references (Piotter et al. 2008, 2010; Piotter and Prokop 2014), which highlight the possibilities of obtaining multi-component and multi-material microsystems.

The use of micro-injection molding is important; not only for mass production, but also as a support to the development process of novel microfluidic devices, when carrying out systematic in vitro and even in vivo trials may constitute the key to validating a novel diagnostic or therapeutic approach. Additive manufacturing technologies are also providing solutions for the testing of novel biodevices and may complement or substitute micro-injection molding in some cases.

The interesting work of Bissacco and colleagues (2014) describes different sequential processes, depending on the number of parts needed, for obtaining microinjection molding and hot-embossing tools. Typically such procedures include combinations of photolithography, etching, laser ablation, high-precision milling or EDM-milling upon soft surfaces, and subsequent electroforming or electrodeposition processes (by chemical or physical vapor deposition or electroplating) for obtaining the mold insert.

Regarding precision, probably the most precise approach towards fabrication of microinjection molding tools is the LIGA process, whose high aspect ratio is also noteworthy (real 3D parts can be obtained, while processes based on surface micromachining by chemical etching typically lead to 2D½ features), but its use is limited due to the expensive hard X-ray radiation needed during the process (Gad-el-Hak 2003).

All these industrial processes can be complemented by means of previously mentioned low-cost microfluidics, especially for the first steps of the development of new microfluidic devices, including prototyping tasks for first trials and first evaluations for conceptual validation. The works from Whitesides' team and from several researchers in the field are continuously providing novel solutions based on very economical materials (paper, cotton, wax…) for obtaining dramatic cost reductions (Whitesides et al. 2001; Martínez et al. 2010). Soft-lithography procedures, based on replicating micromanufactured master models using PDMS, for an enhanced biological interaction and for enabling cell culture, have reshaped the industry of biomedical devices. In some cases, these low-cost devices are even mass

produced and help to change diagnostic paradigms, as has already happened with paper-based diagnostic strips. Ideally, low-cost devices could even be fabricated at home or in doctors' surgeries directly at the patient point-of-care.

Additional details linked to mass-production of biomedical microsystems, also taking into account microfluidic devices, has been covered in deeper detail in Chap. 10.

The following case of study, linked to a microfluidic device with multiple cell culture chambers, helps to put forward the significance of employing different manufacturing technologies and taking advantage of their mutual synergies, for the efficient and effective development of microsystems for interacting at cellular level.

20.5 Case Study: Development of a Microfluidic Device with Several Cell Culture Chambers

In this Sect. 20.5 we present the complete development process of a dynamic cell culture system for studying interactions between the vasculature and the basic tissular cells from different organs. The endothelial cells conforming the vascular networks irrigating our organs usually grow in a healthier biomimetic way if shear stresses from circulating fluids act upon them (Li et al. 2005). On the other hand, the cells of different organs, in spite of benefiting from the exchange of nutrients and debris with the surrounding vasculature, typically growth under more static culture conditions. In consequence, we propose a biomedical device consisting of a lower static cell culture chamber, an intermediate micro-porous membrane and an upper structure of channels for dynamic culture. The cells from different tissues can be cultured in the lower chamber and interact, through the micro-pores, with the endothelial cells cultured dynamically in the upper channelled structure.

Regarding the design of the proposed microsystem, Fig. 20.3 shows the computer-aided design of the microfluidic device, which includes two cell-culture layers, one for the tissue and one for the vasculature, separated by a microporous membrane. The microporous membrane can be selected from the CAD library of microporous membranes shown in Fig. 20.2, which can be obtained by casting PDMS upon the molds from Fig. 20.1. The design is versatile and easy to use, as includes "plug-and-play" connections for the pumping system, can be used for dynamic culture, as well as for static culture, can be loaded with very different types of cells and can support the development of "organs-on-chips", advanced microfluidic devices for mimicking the physiology of human organs, as described in Chap. 22.

The design can be optimized and the supporting external devices can be selected by means of computer-aided engineering resources, normally based on FEM simulations. The performed FEM simulations help to assess the most adequate flow rate for reaching certain fluid velocities, directly linked with the most appropriate shear stresses for the dynamic cell culture. These simulation results help to evaluate

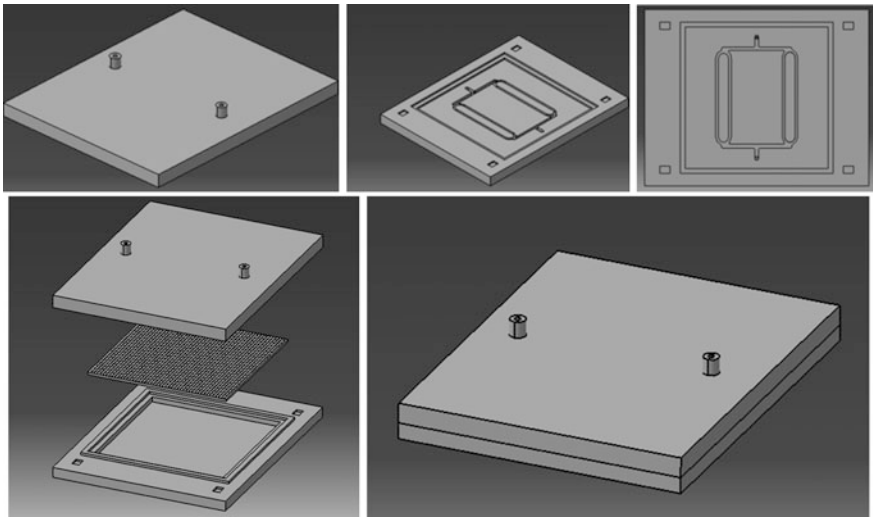

Fig. 20.3 Computer-aided design of a microfluidic device with two cell-culture layers, one for the tissue and one for the vasculature, separated by a microporous membrane

the pressure losses along the microsystem, so as to select the more adequate pumping system. In addition, FEM simulations help to evaluate the impact of membrane pore size on the interactions between the upper and lower chambers and to select the adequate pore size for enabling active culture on one side and passive culture on the other side. Figure 20.4 shows that membranes with large pore sizes

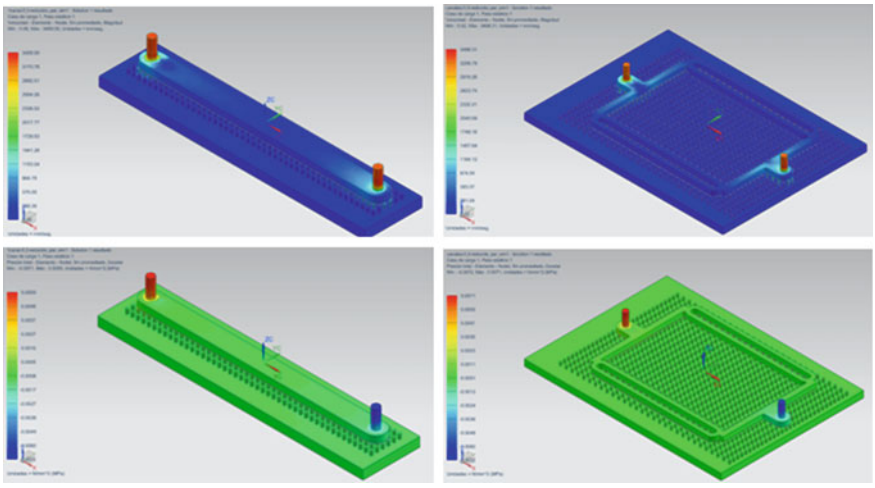

Fig. 20.4 FEM-based assessment of the fluidic performance of a microfluidic device with two cell-culture layers. Membranes with large pore sizes (>75 μm) do not adequately promote the independence between the cell culture layers

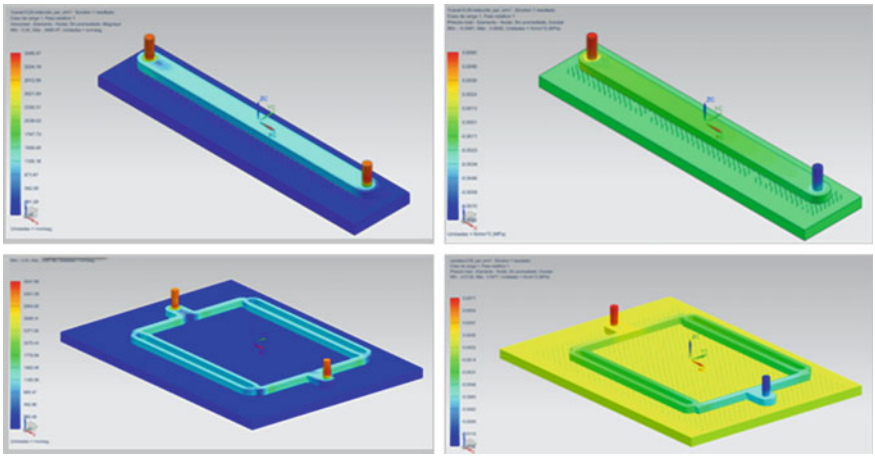

Fig. 20.5 FEM-based assessment of the fluidic performance of a microfluidic device with two cell-culture layers. Membranes with smaller pore sizes (<25 μm) promote the independence between the cell culture layers and help to culture the cells of the lower chamber in static conditions and the cells of the upper layer in dynamic conditions, as required for the promotion of vasculature

(>75 μm) do not adequately promote the independence between the cell culture layers, while Fig. 20.5 shows that membranes with smaller pore sizes (<25 μm) promote the independence between the cell culture layers and help to culture the cells of the lower chamber in static conditions and the cells of the upper layer in dynamic conditions, as required for the promotion of vasculature.

After desing optimization, prototypes for validation trials have been obtained by directly linking computer-aided designs with rapid prototyping tools based on additive manufacturing. Laser stereolithography has been obtained for the first master models, aimed at dimensional verifications. Rapid molds, designed with the help of Boolean operations, have been also obtained for further application of soft-lithography procedures towards biologically adequate PDMS prototypes of the housings and membranes.

Figure 20.6 shows the aforementioned rapid prototypes, including some epoxy masters and PDMS replicas. The microfluidic device includes two cell-culture layers separated by a flexible and microporous PDMS membrane. Figure 20.7 shows a preliminary fluidic testing of the microfluidic device with two cell-culture layers separated by a flexible and microporous PDMS membrane. The flexibility of the membrane is remarkable. The manufacturing process is extremely direct and enables the manufacture of a complete functional prototype in just 2 days, one for the additive manufacture of master models and molds and one for the rapid casting, polymerization, demolding and final implementation. The use of the original epoxy housings for the preliminary functional trials or the manufacture of PDMS replicas, using a transparent PDMS, allows for the inner monitoring of the final cell culture trials.

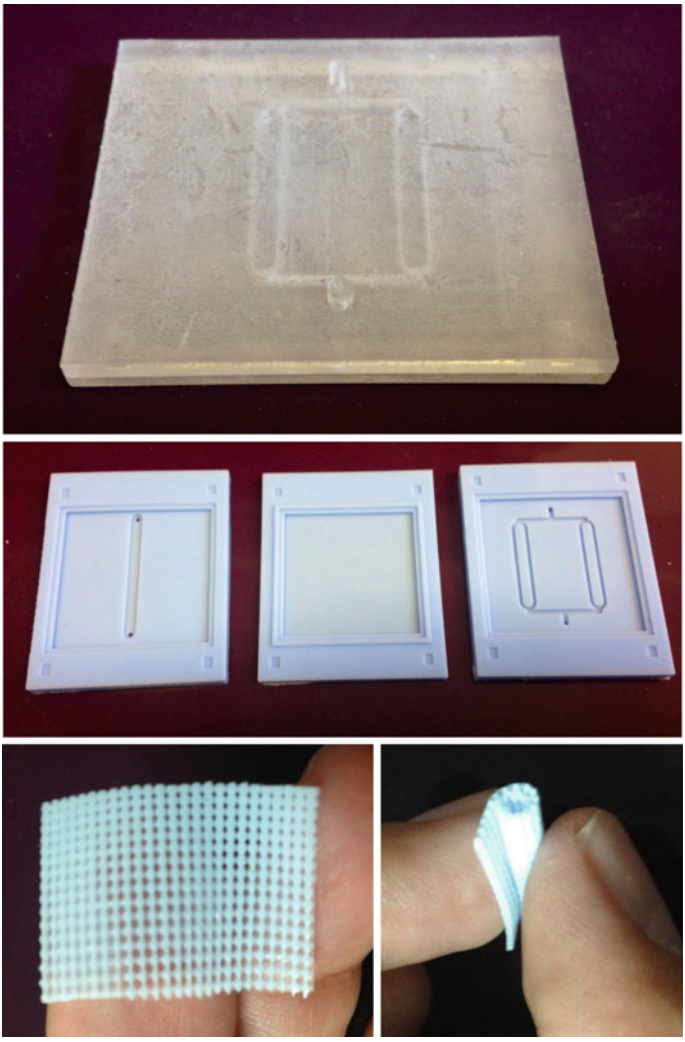

Fig. 20.6 Rapid prototypes, including epoxy masters and PDMS replicas, of a microfluidic device with two cell-culture layers separated by a flexible and microporous PDMS membrane

Regarding in vitro assessment with human mesenchymal stem cells, the images provided in Figs. 20.8, 20.9 and 20.10, help to provide relevant information for future applications of the proposed biodevice. Cell culture has been performed as described in previous experiences by our team with slight modifications (Díaz Lantada et al. 2014, 2016). For example, Fig. 20.8 shows some detailed images of different prototypes of microporous PDMS membranes, as well as of the hMSCs

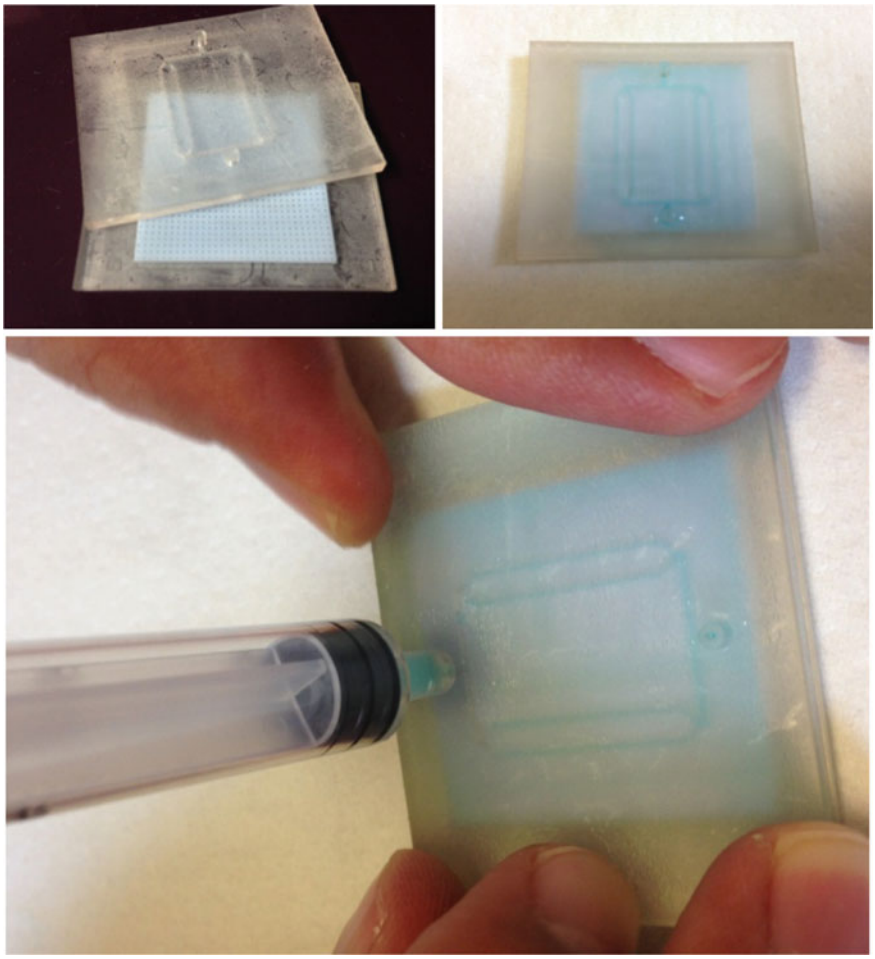

Fig. 20.7 Preliminary fluidic testing of a microfluidic device with two cell-culture layers separated by a flexible and microporous PDMS membrane

cultured upon them showing cells adequately attached to the membranes. The colonization of the pores and the possible interaction between both sides of the membrane, thus mimicking the interactions between cell types in real tissues, can be also appreciated. Nuclei are stained in blue (central images) and complete cells show a good energetic behavior in violet (lower images). Figures 20.9 and 20.10 shows some detailed microscopies of different pores, taken at different depths of focus, to show the colonization of the micropores by the cultured hMSCs and the possible interaction between both sides of the membrane.

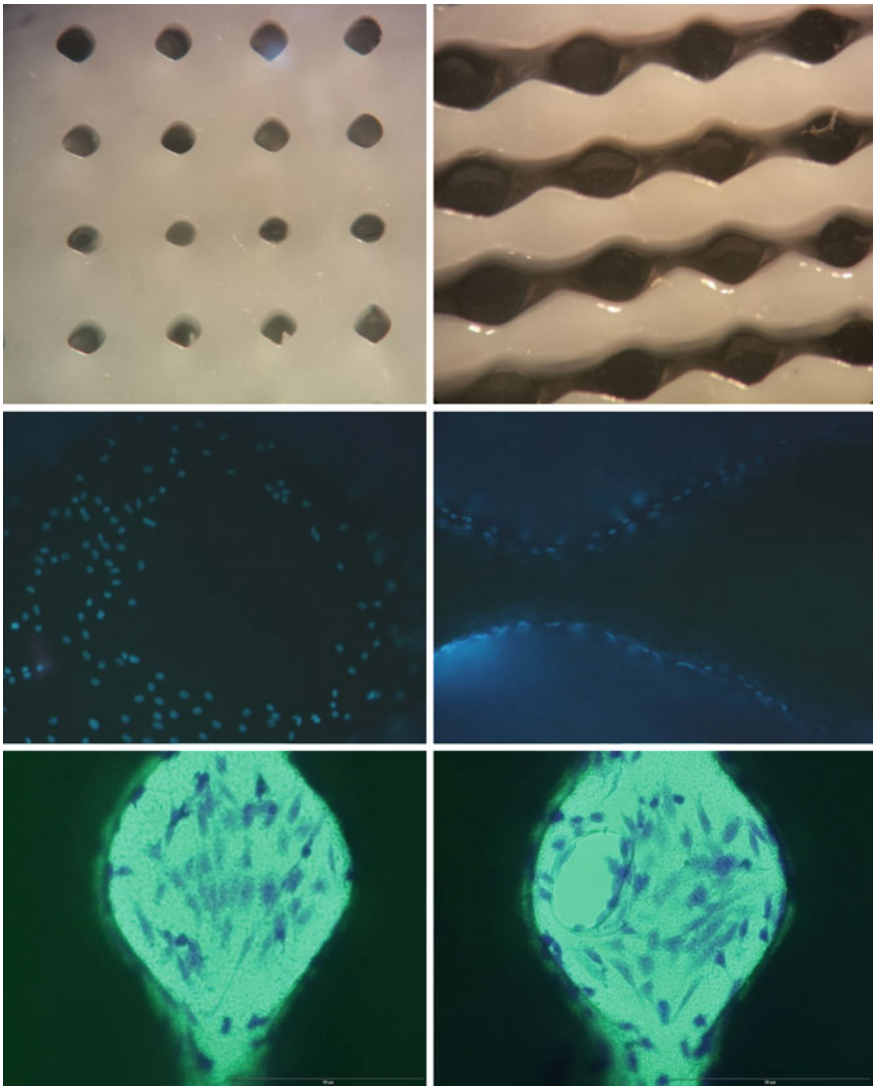

Fig. 20.8 Details of different prototypes of microporous PDMS membranes and hMSCs cultured upon them showing cells adequately attached to the membranes, the colonization of the pores and the possible interaction between both sides of the membrane, thus mimicking the interactions between cell types in real tissues. Nuclei are stained in *blue* (*central images*) and complete cells, which are showing a good energetic behaviour, are stained in *violet* (*lower images*) (color online)

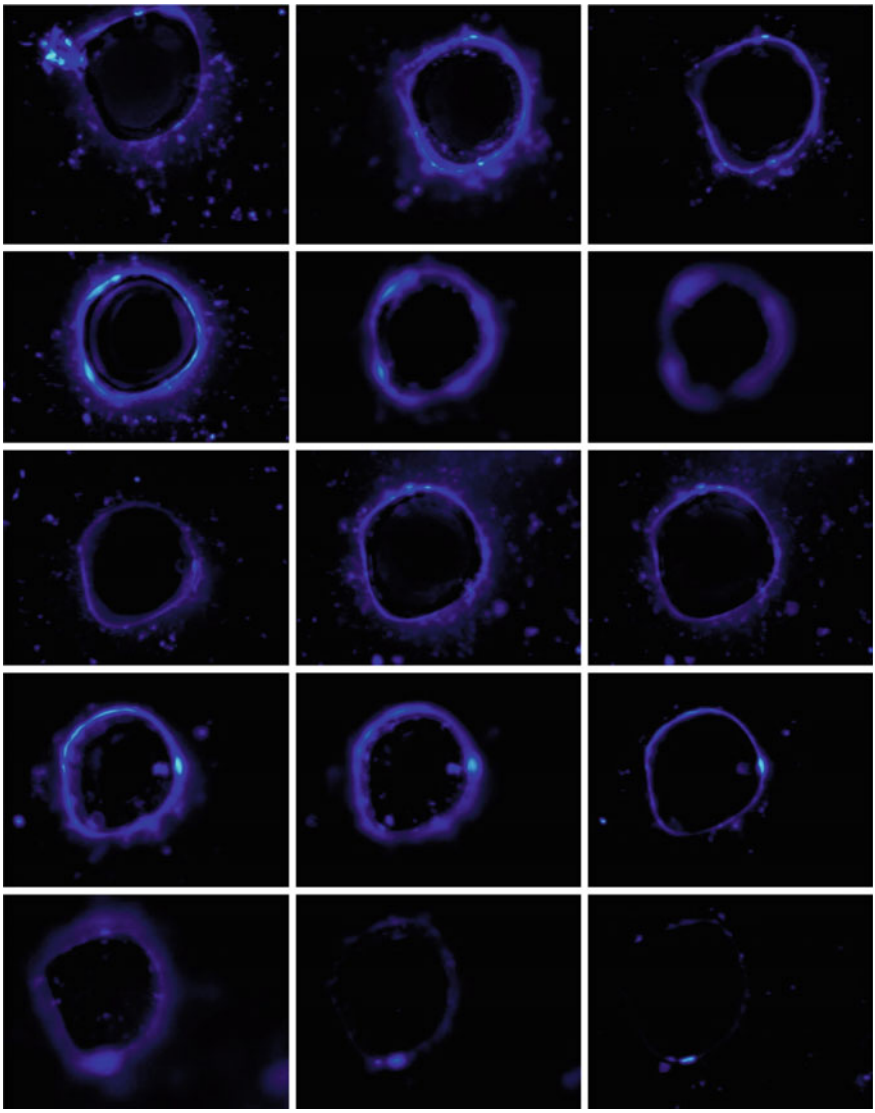

Fig. 20.9 Detailed microscopies of different pores, taken at different depths of focus, to show the colonization of the micropores by the cultured hMSCs and the possible interaction between both sides of the membrane

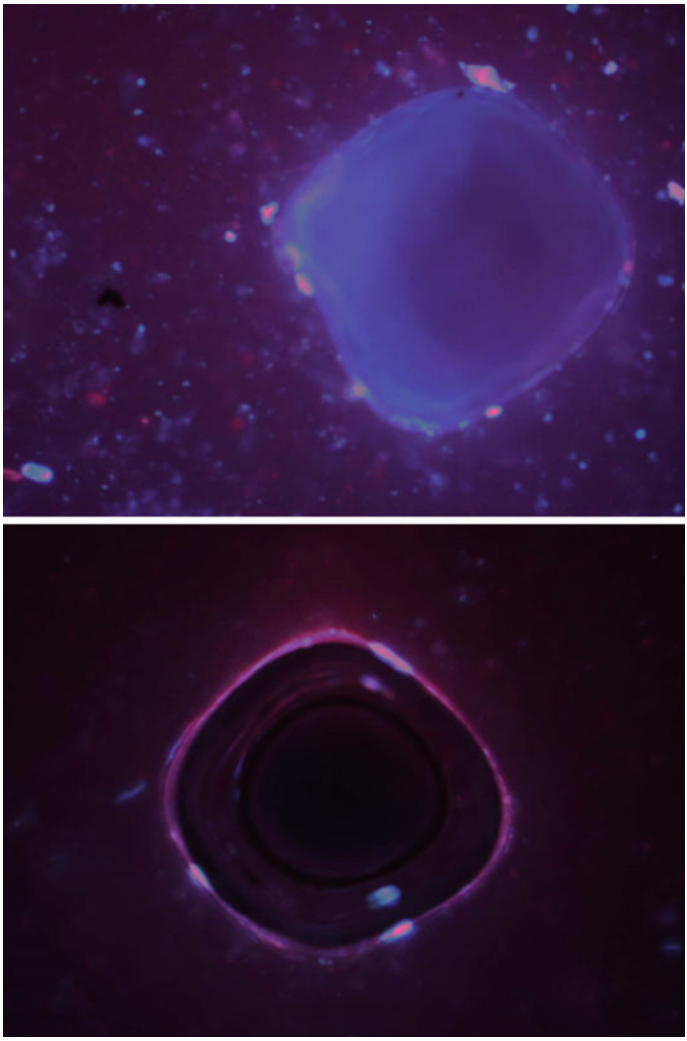

Fig. 20.10 Detailed microscopies of different pores, taken at different depths of focus, to show the colonization of the inner parts of the micropores by the cultured hMSCs. The good energetic and healthy state of the cells can be appreciated, with some cells even reproducing themselves, while the images where taken

20.6 Main Conclusions and Future Research

Lab-on-chip microfluidic devices or "labs-on-chips" integrate the usually very complex operations and procedures typical from biochemical and biological labs in just a few cm^2, by taking advantage of microfluidic operation. Hence, operation speed, sustainability due to the use of low volumes and repeatability, thanks to the

promotion of multiplexing and automation, are boosted. Although further research in the field will promote additional miniaturization and integration of capabilities, lab-on-chip microdevices incorporating cells and tissue samples are already very interesting for all types of tasks linked to understanding cell behavior.

This chapter has provided an introduction to labs-on-chips aimed at cell culture stimulated by means of microfluidic stimuli. Design, modeling and manufacturing strategies, for the development of labs-on-chips capable of helping researchers with cell co-culture for studying the interactions of different cell types and for the development of in vitro models of physiological structures, have been covered. In addition, a complete case of study of a versatile lab-on-a-chip for cell co-culture is detailed.

The use of labs-on-chips with different chambers, one for passive culture of different cell types and tissue samples and one with microchannels for modeling the micro-vasculature, separated by a microporous membrane that allows the mutual interaction of the different cell types, constitutes a versatile approach. The combined use of computer-aided design, engineering and manufacturing resources together with rapid prototyping procedures, allows for the efficient development of these types of solutions.

References

Baudis S, Heller C, Liska R, Stampfl J, Bergmeister H, Weigel G (2009) (Meth)acrylate-based photoelastomers as tailored biomaterials for artificial vascular grafts. J Polym Sci Part A: Polym Chem 47(10):2664–2676

Baudis S, Steyrer B, Pulka T, Wilhelm H, Weigel G, Bergmeister H, Stampfl J, Liska R (2010) Photopolymerizable elastomers for vascular tissue regeneration. Macromol Symp 296(1):121–126

Bissacco G, Hansen HN, Tang PT, Fugl J (2014) Precision manufacturing of tools for injection molding of microfluidic systems. http://www.aspe.net/publications/Spring_2005/05SPAbs/Bissacco-1676.pdf. Accessed 26 Aug 2014

Díaz Lantada A (2012) Handbook of active materials for medical devices. PAN Stanford Publishing

Díaz Lantada A, Alarcón Iniesta H, Pareja Sánchez B, García-Ruíz JP (2014) Free-form rapid-prototyped PDMS scaffolds incorporating growth factors promote chondrogenesis. Adv Mater Sci Eng ID 612976

Díaz Lantada A, Alarcón Iniesta H, García-Ruíz JP (2016) Composite scaffolds for osteochondral repair obtained by combination of additive manufacturing, leaching processes and h-MSC-CM functionalization. Mater Sci Eng C 59(1):218–227

Gad-el-Hak M (2003) The MEMS handbook. CRC Press, New York

Gomez-Marquez J (2011) Design for hack in medicine: MacGyver nurses and Legos are helping us make MEDIKits for better health care. Make Magazine Ultimate Kit Guide

Huh D, Hamilton GA, Ingber DE (2011) From 3D cell culture to organs on chips. Trends Cell Biol 21(12):745–754

Huh D, Kim HJ, Fraser JP, Shea DE, Khan M, Bahinski A, Hamilton GA, Ingber DE (2013) Microfabrication of human organs-on-chips. Nat Protoc 8:2135–2157

Jenkins G, Mansfield CD (2013) Microfluidic diagnostics: methods and protocols. Springer, New York

Li YS, Haga JH, Chien S (2005) Molecular basis of the effects of shear stress on vascular endothelial cells. J Biomech 38(10):1949–1971

Martínez AW, Phillips ST, Whitesides GM (2010) Diagnostics for the developing world: microfluidic paper-based analytical devices. Ann Chem 82:3–10

Phillips ST, Thom NK (2013) Three-dimensional, paper-based microfluidic devices containing internal timers for running time-based diagnostic assays. In: Jenkins G, Mansfield CD (eds) Microfluidic diagnostics, methods and protocols. Methods in Molecular Biology 949, Springer, New York, pp 185–196

Piotter V, Prokop J (2014) Galvanic deposition in partially conductive plastic molds for manufacture of finely detailed microcomponents. J Microsyst Technol 20:169–174

Piotter V, Bauer W, Hanemann T, Heckele M, Müller C (2008) Replication technologies for HARM devices—status and perspectives. J Microsyst Technol 14:9–11, 1599–1605

Piotter V, Prokop J, Ritzhaupt-Kleissl H-J, Ruh A, Haußelt J (2010) Multi-component micro injection moulding—trends and developments. Int J Adv Manuf Technol 47:63–71

Reches M, Mirica KA, Dasgupta R, Dickey MD, Butte MJ, Whitesides GM (2010) Thread as a matrix for biomedical assays. ACS Appl Mater Interfaces 2(6):1722–1728

Stampfl J, Baudis S, Heller C, Liska R, Neumeister A, Kling R, Ostendorf A, Spitzbart M (2008) Photopolymers with tunable mechanical properties processed by laser-based high-resolution stereolithography. J Micromech Microeng 18:125014

Waldbaur A, Rapp H, Länge K, Rapp BE (2011) Let there be chip—towards rapid prototyping of microfluidic devices: one-step manufacturing processes. Anal Methods 3:2681–2718

Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE (2001) Soft-lithography in biology and biochemistry. Annu Rev Biomed Eng 3:335–373

Chapter 21
Cell-Based Sensors and Cell-Based Actuators

Andrés Díaz Lantada

Abstract Cells and tissues can be seen, from the perspective of Materials Science and Engineering, as "smart materials and structures". In fact, cells and tissues are able to perceive and respond to several environmental stimuli and gradients of them, including the presence of biochemical cues and microorganisms, the mechanical and topographical properties of the extra cellular matrix and surfaces upon which they lie, the application of vibrations and the surrounding electromagnetic fields, to cite just a few, as already detailed in several chapters of the Handbook. Advances in technologies for manipulating, culturing and monitoring single cells, together with progress in the fields of modeling, simulation, prototyping and testing, have led to a better understanding of how cells respond to several types of stimuli and accurate predictions about the behavior of cells and tissues are already possible. In consequence, cells and tissues can be employed as living transducers for the development of (micro-)sensors and (micro-)actuators, as it is possible to predict and control their responses. This chapter provides and introduction to the development of cell-based sensors and actuators and to current main challenges in this novel area. Once such challenges are solved, the frontiers between biological systems, machines and synthetic engineering systems in general will start to fade away. An approach for a more rapid solution of the aforementioned challenges, towards a wide-spread use of cell-based sensors and actuators, may rely on the use of systematic libraries with CAD models of conceptual cell-based sensors and actuators, both for modeling and prototyping strategies, as proposed in a final case study included in present chapter.

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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Studies in Mechanobiology, Tissue Engineering and Biomaterials 18,
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21.1 Introduction: Bio-hybrid Systems and the Future of Engineering

Numerous active, multipurpose, multifunctional or "intelligent" materials have appeared in recent decades, all capable of responding in a controllable and in many cases also in a reversible way to different external physical and chemical stimuli by changing some of their properties. Due to their sensitivity or activity, these materials can be used to design sensors, actuators and multipurpose systems with a wide range of applications for biomedical device design (with applications ranging from diagnosis and monitoring, to surgery and therapy). One of the most remarkable advantages of integrating multiple functions into a system, transducer or materials, is the ensuing reduction in size, the increase in production runs and the reduction in costs of materials and processes. Active materials are playing a decisive role in numerous industrial applications and will reshape the future of Engineering, as they embrace electrical, thermal, chemical, optical, magnetic and mechanical properties. These active materials emerge in varied natures and with diverse features: inorganic, metallic and organic, natural and synthetic, inert and "living", and are sensitive to a wide range of physical and chemical phenomena (Díaz Lantada 2012)

Cells and tissues can be seen, from the perspective of Materials Science and Engineering, as "smart materials and structures". In fact, cells and tissues are able to perceive and respond to several environmental stimuli and gradients of them, including the presence of biochemical cues and microorganisms, the mechanical and topographical properties of the extra cellular matrix and surfaces upon which they lie, the application of vibrations and the surrounding electromagnetic fields, to cite just a few, as already detailed in several chapters of the Handbook.

In fact, living cells are being already incorporated to engineering products for the development of biofilms capable of responding to different inputs from the surrounding environment. These living biofilms can reshape themselves, interact with pathogens, improve the response and functionalities of inert materials and perform collaboratively. Novel self-healing approaches for multifunctional materials and engineering systems are hence enabled. Even the manufacturing industry can be completely reconfigured, by the possibility of using living cells and microorganisms for processing and constructing advanced multi-scale controlled materials resorting to bottom-up approaches, instead of following the much more conventional top-down strategies, to cite some examples (Chen et al. 2014).

Advances in technologies for manipulating, culturing and monitoring single cells, together with progress in the fields of modeling, simulation, prototyping and testing, have led to a better understanding of how cells respond to several types of stimuli and accurate predictions about the behavior of cells and tissues are already possible. In consequence, cells and tissues can be employed as living transducers for the development of (micro-)sensors and (micro-)actuators, as it is possible to predict and control their responses.

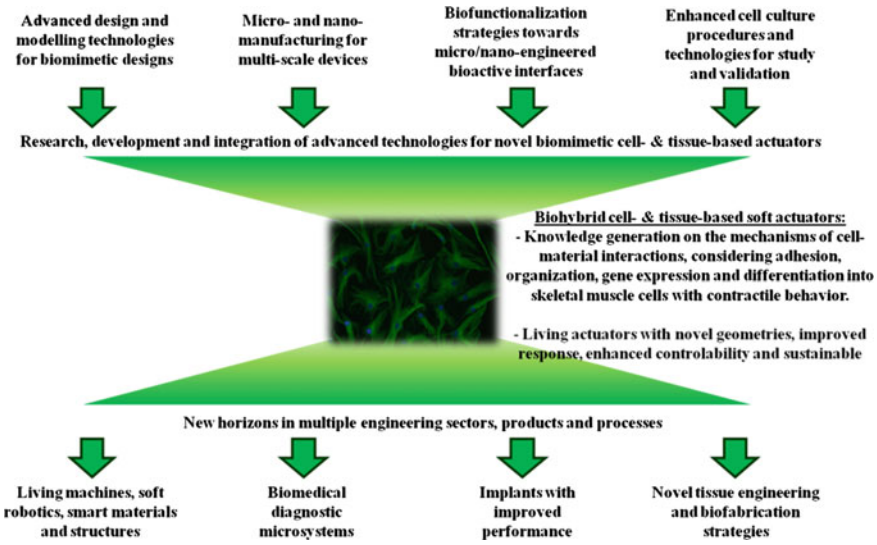

Fig. 21.1 Schematic representation of convergent technologies and expanded horizons in several fields of biology and medicine, thanks to emergent research linked to biohybrid cell-based actuators and sensors

Thanks to the progressive employment of cell-based sensors and actuators, novel diagnostic and therapeutic paths will be opened in areas including biomedical microsystems, biomedical micro-robotics, tissue engineering and biofabrication. Several fields of product and process engineering will benefit from the discoveries in this area, as schematized below in Fig. 21.1.

The following sections provide and introduction to the development of cell-based sensors and cell-based actuators and to current main challenges in this novel area. Once such challenges are solved, the frontiers between biological systems, machines and synthetic engineering systems in general will start to fade away. An approach for a more rapid solution of the aforementioned challenges, towards a wide-spread use of cell-based sensors and actuators, may rely on the use of systematic libraries with CAD models of conceptual cell-based sensors and actuators, both for modeling and for prototyping strategies.

Again the employment of FEM-based simulations constitutes a very valuable companion for studying the most diverse engineering systems, accounting for multi-physical phenomena and even working at different scale levels. Micro- and nano-manufacturing resources (see Chaps. 8–10) provide the technological background for materializing these novel types of bio-microsystems.

21.2 Main Accomplishments and Current Challenges

The field of biohybrid sensors and actuators is steadily advancing thanks to the support of recent technological transformative breakthroughs, which affect almost all areas of biomedical engineering, including:

- The advances in biomedical systems capable of monitoring and analyzing physiological signals, which have enabled patients to be more precisely controlled, both in the short term (i.e. during surgical operations and post-ops) and in the long term (for studying the evolution of pathologies and modeling human behavior) (Deutsch et al. 2007, 2008; Cerutti 2008).
- The development of systems (initially inert and currently also biohybrid) capable of interacting between computers and the nervous systems of living organisms, typically by means of two-way implants for receiving electric signals from the body and for supplying current to the nervous system (Gasson et al. 2005; Warwick 2008).
- New micro- and nano-manufacturing techniques have led to vast reductions in the end-size of implantable devices, even promoting interactions at a single cellular level. The additional possibility of fitting them with micro-instrumentation to endow them with "intelligence" improves versatility (Gad-el-Hak 2003; Schwartz 2006).
- The optimization of engineering design processes thanks to a combination of computer-aided design, engineering and manufacturing resources, which speed up the production of devices by reducing intermediate stages and minimizing costs, while promoting sustainability (Kucklick 2006).
- The development of new bioabsorbable materials, which degrade a certain time after being implanted, while only producing non-toxic matter that can be eliminated or metabolized by the body. Outstanding progress has been made in the synthesis of bioabsorbable and biodegradable polymers for controlled drug-delivery (Lendlein and Langer 2002) and for tissue repair and engineering (Freed et al. 1994).
- The discovery of new active materials that enable multi-functionalities and open up new horizons for the development of active implantable medical devices, thanks to their potential use as sensors and actuators (Davis 2003; Wong and Bronzino 2007; Peterson and Bronzino 2008).

These advances benefit each other and their synergies can multiple solutions to health issues, especially if further improved by means of incorporating living cells to them and by taking benefit from their capabilities as multi-functional units. The adequate application of the aforementioned breakthroughs to the development of cell-based biohybrid actuators is still challenging. The satisfactory control of cell behavior and response within these systems is also a key aspect requiring further research. Some cases of success and strategies towards effective, efficient and sustainable biohybrid systems and actuators are detailed in the following sections, while also highlighting more specific challenges for the future.

21.3 Developing Cell-Based Sensors

Cells are capable of living and maintaining their functions thanks to their outstanding capabilities for responding very rapidly and with a remarkable degree of sensitivity and efficiency to the presence of potential harms and to changes in the external environment in general. As previously mentioned cells and tissues can be seen as active materials, with detection and actuation capabilities, for adequately interacting with external elements, either inert or living. In consequence, the incorporation of living cells to engineering sensing systems will potentially promote the development of faster and more sensitive devices for all imaginable types of industries, sectors and applications.

These cell-based sensors can incorporate, either prokaryotic or eukaryotic cells, together with inert organic or inorganic materials. In principle, eukaryotic cells are more desirable, as their higher degree of complexity provides them with additional detection and actuation mechanisms. In addition, as regards animal and human compatibility, these eukaryotic cell-based sensors are much more adequate, especially if mammalian cells are used, although they are more complex to implement, due to more demanding culture conditions. Other eukaryotic microorganisms, such as yeast, can also be used, due to their easier culturing procedures, but they share potential dangerous impacts with prokaryotic bacterial-based sensors. In any case, these "intelligent" living sensors are clearly opening new horizons in the medical and pharmaceutical industries, highlighted as the two clearest examples of application fields affected by these breakthroughs, as these highly specific sensors can be used for evaluating novel technologies and their cytotoxical, biological and environmental performances.

The relevance and present interest in the topic can be additionally put forward considering the recently devoted special issue on "Live cell-based sensors", published by "Sensors" journal (Ed. Taniguchi 2012).

Interesting advances have demonstrated the possibility of using algae immobilized in silica hydrogels for environmental monitoring (Ferro et al. 2012); of employing microbial sensors for disease management, typically connected with antibiotic selection tasks, based on colorimetric or fluorescent methods (Park et al. 2013); and of using rat cells for monitoring pollution in aquatic environments (Kubisch et al. 2012).

In spite of these promising advances, there are still some essential unsolved questions, which require a deeper knowledge about the basic science involved in cell-material interactions and related cell- and tissue-based sensors.

The most relevant unanswered questions, which represent key scientific and technological groundbreaking challenges, some of them common with the more relevant concerns linked to the development of cell-based actuators, include:

(a) How can we promote long-term performance of these biohybrid devices, linked to the continuous improvement of cell culture procedures, encapsulation systems, lasting nutrients and required hydratation?

(b) Which are the fundamental aspects linked to the automation of electro-optical sensing devices based on luminescent cells and to the promotion of long-term transparency and viability of hydrogels loaded with cells (Choi et al. 2013)?
(c) How can we enlarge the ranges of application of these solutions, enabling sensing at higher temperatures, pressures and in more aggressive environments, without compromising cells and their fates, at least during the adequate time-spans needed for carrying out the detection?
(d) Which types of combinations between cells and supporting extracellular matrices, growth factors and electroding materials provide the more adequate sensing responses regarding effectivity, speed, specificity, repeatability, biological compatibility and long-term stability?
(e) Is it possible to implement (i.e. via multi-layered fabrication and cell culture approaches) truly multi-functional biohybrid sensors and actuators with cell layers or zones aimed at detection or diagnostic purposes and other cell layers or regions focused on actuation or therapeutic tasks?
(f) Will biofabrication (see Chap. 23) and related systems, including bioprinters and cell-printers provide the desired responses towards effective, efficient and sustainable biohybrid devices?

In order to generate the required knowledge and the technological advances to make available successful answers to the previous questions, it is necessary to work among fields, ranging from traditional engineering specialties, to molecular biology and cell science, as well as to integrate advanced design, modeling/simulation, manufacturing and cell-culture technologies and processes, many of which have been covered along the whole Handbook and explained in detail by means of several cases of study.

Some additional strategies focused on the development of biohybrid systems, mainly linked to controlling the geometries and topologies of biomedical microsystems and biomaterials, are discussed at the end of next Sect. 21.4, when dealing with the development process of biohybrid actuators. As several current challenges are common for the future of cell-based biohybrid sensors and of biohybrid actuators and taking into account that these multi-functional systems may become additionally relevant by integration of both sensing and actuation abilities, it is interesting to benefit from and to promote synergies between these types of biohybrid systems, as sensing and actuation are two sides of the same coin.

21.4 Developing Cell-Based Actuators

Actuation is an essential function of any artificial or living machine, allowing its controlled movement and interaction with the surrounding environment. Living muscles have evolved over millions of years within animals as nature's premier living generators of force, showing unique features in comparison with standard artificial actuators. State-of-the-art actuators represent a relevant bottleneck in many

industrial applications, including most fields of robotics, transport, energy and biomedical engineering.

Among the current limitations it is important to note: inertia and back-drivability, stiffness control, power consumption, repeatability and environmental safety. The development of novel actuators able to imitate or even to overcome living muscle performance would open new horizons in several engineering areas (Cvetkovic et al. 2014). Even the use of multi-functional, "smart/intelligent" materials, sometimes referred to as "artificial muscles", is not always providing the adequate responses to the already mentioned actuation limitations and, due to their basic chemistry, biomimetic approaches are prevented (Díaz Lantada 2012).

A very innovative solution to achieve these goals is represented by the merging between artificial and living entities, which will lead to the implementation of biomimetic and biohybrid devices. Combining biological components, such as cells and tissues, with soft robotics can indeed allow the fabrication of biological machines with the ability to sense, process signals and produce force. An intuitive concept of a biological machine is one that can produce motion in response to controllable external signalling.

Even though cardiac cell-driven biological actuators have been demonstrated, their behaviour is quite random and biological machines should respond to external stimuli in a controlled way. Therefore, the use of skeletal muscle cells, which are the primary generators of actuation in animals, is gaining interest in this emerging field as contractile power source (Cvetkovic et al. 2014; Ricotti and Menciassi 2014). Undoubtedly, cell- and tissue-based soft robotic devices will have a transformative impact on our ability to design all types of (bio-)engineering systems that can dynamically sense and respond to a range of complex environmental signals (Ricotti and Menciassi 2014).

In spite of the enormous potential of bio-hybrid cell- and tissue-based soft actuators and taking account of some preliminary results, mainly based on self-beating cells such as cardiomyocytes, there are still some essential unsolved questions, which require a deeper knowledge about the basic science involved in cell-material interactions and related cell- and tissue-based actuators.

The most relevant unanswered questions, some of them common with the challenges linked to the development of cell-based sensors, which represent key scientific and technological groundbreaking challenges, include:

(a) How can we develop engineered substrates and structures, in order to promote and control cell adhesion and organization, using adequate biomaterials and micro-/nano-metric geometries, so as to compliantly favour cell contraction and consequent actuation?
(b) How can we engineer the surfaces of substrates and structures, which will act as the synthetic parts of bio-hybrid actuators, so as to motivate the expression of certain genes and proteins at cellular level, which will allow for cell differentiation and motivate controlled contraction?

(c) Which cell lines are more adequate to promote the desired controlled contractions and overcome the initial more random results obtained using cardiac self-beating cells?
(d) How can we incorporate the generated knowledge, regarding cell-material interactions, as input for the design stage of bio-hybrid actuators, which is a key aspect of all kind of systematic development of complex engineering systems?
(e) How can we promote long-term performance of these biohybrid devices, linked to the continuous improvement of cell culture procedures, encapsulation systems, lasting nutrients and required hydratation?
(f) Which combinations between cell types and extracellular matrices, growth factors and electroding materials provide the best actuations regarding effectivity, speed, precision, repeatability, biocompatibility and long-term stability?
(g) Is it possible to implement (i.e. via multi-layered fabrication and cell culture approaches) truly multi-functional biohybrid sensors and actuators with cell layers or zones aimed at detection or diagnostic purposes and other cell layers or regions focused on actuation or therapeutic tasks?
(h) Will biofabrication (see Chap. 23) and related systems, including bioprinters and cell-printers provide the desired responses towards effective, efficient and sustainable biohybrid devices?

In order to generate the required basic knowledge to provide effective solutions to the previous questions, it is necessary to work among fields, ranging from mechanical and materials engineering, to molecular biology and cell science, and to integrate advanced design, modeling, manufacturing and cell-culture technologies and processes. Working towards the convergence of emerging technologies in the nano-info-bio-cogno fields, will for sure provide answer to these challenging questions and lead to relevant related advances in life quality and environmental sustainability (Bainbridge and Roco 2006).

More specifically, in the micro- and nano-field, but also directly linked to the biomedical field, engineered materials have been recently more and more utilized in order to deeply understand the fine molecular mechanisms responsible for contractile muscle bundle formation and maintenance, getting several insights, mainly concerning the effects of topography on the expression of certain genes and proteins at cellular level which allow for cell contraction (Ricotti and Menciassi 2014; Díaz Lantada et al. 2013). Nevertheless, no substrates match, at present, all the requirements needed to engineer mature skeletal muscle tissues; consequently, no suitable matrices are described in the literature to serve as components of potentially long-term bio-hybrid actuators (Cvetkovic et al. 2014; Ricotti and Menciassi 2014).

Several studies have focused on the importance of surface topography for promoting positive effects in all kinds of biodevices, from implantable prosthesis to scaffolds for cell and tissue growth. These textures have a significant influence in the biological response of prostheses, in cell proliferation and in tissue growth, given that those cells and tissues seem to be more “comfortable”, when faced with

biodevices with similar surface properties (Choi et al. 2009; Thomas et al. 2010; Buxboim and Discher 2010; Longoni and Sartori 2010).

However, the process of introducing desired roughness on the surfaces of biodevices are still mainly linked to carrying out machining operations, laser processing, chemical attacks and coatings, which cannot be easily controlled from the design stage (Naik et al. 2009; Pulsifier and Lakhtakia 2011; Díaz Lantada et al. 2013).

In addition, the potential of (bio-)engineering surfaces for the development of advanced biohybrid cell- and tissue-based soft actuators has not yet been addressed, even though it is clearly perceived as a key approach for solving the previously mentioned challenges.

The development and use of novel design, modeling, simulation and manufacturing strategies for obtaining biodevices capable of interacting at a cellular level,

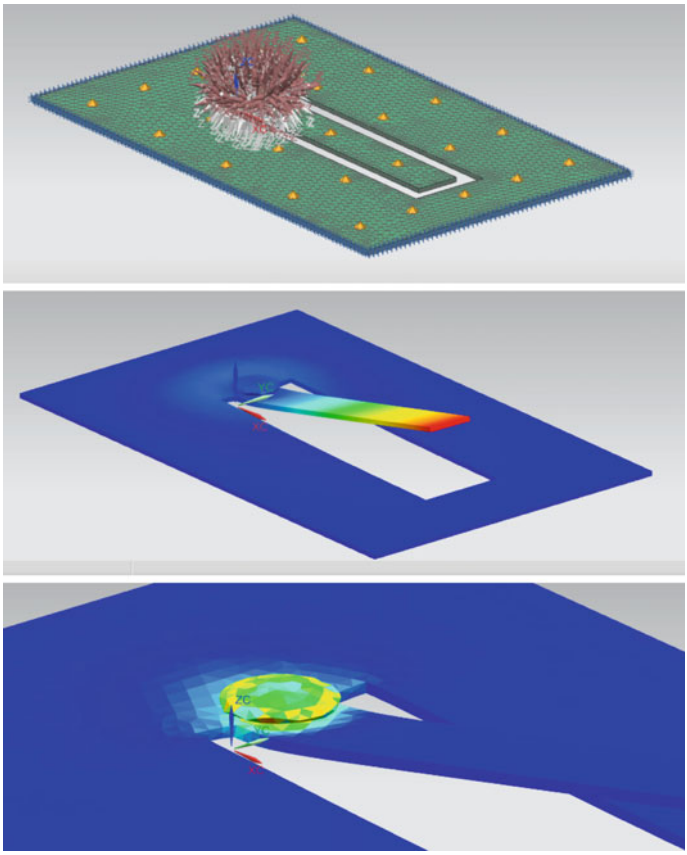

Fig. 21.2 Finite element model, displacement results and stresses upon the cell during its contraction, which leads to the elevation of the microcantilever. The FEM-based simulations help with the conceptual design of the microsystem. Performed with the help of NX-8.5 (siemens PLM solutions)

following some of the methods put forward along the Handbook and thus focusing on "the shape of things to come" (Lipson 2012), will be key for further progress in the field.

Figure 21.2 helps to show the impact of simulations for the development of cell-actuated micromechanical systems and biomedical microdevices. The images show, from top to bottom, the finite element model, the displacement results and the stresses upon the cell during its contraction, which leads to the elevation of the micro-cantilever. In this configuration, the cell is attached to the upper part of the cantilever and may contract due to electrical or biochemical stimuli, hence leading to a traction upon the micro-cantilever and to the desired elevation. The geometry presented may serve as conceptual micro-valve for biomedical microdevices. The use of cell-based actuation is of special relevance for the development of active mechanical microsystems, due to the required degree of precision, even down to micrometric and submicrometric strokes.

21.5 Case Study: Design Library of Conceptual Cell-Based Actuators

Figure 21.3 shows a schematic computer-aided design library and the related actuation mechanisms of several types of potential biohybrid micromechanical systems actuated by cells (in green). The geometries are based on common micro-thermo-electro-magneto-mechanical systems (in short MEMS) (Gad-el-Hak 2003), normally obtained in silicon by means of chemical micro-manufacturing approaches or in polymers by resorting to additive manufacturing or to polymeric micro-manufacturing processes.

The different conceptual actuators from this CAD library include: cantilever and bridged micro-actuators, micro-pincers, micro-mechanical valves and some resonant micro-structures. It has been developed with the valuable help of NX-8.5 (Siemens PLM Solutions) and further processed with common picture editing software, although the different simple geometries can be designed with the help of all state-of-the-art computer-aided design resources. Regarding the use of such geometries for simulation tasks, only advanced design and engineering software is capable of linking the obtained geometries with FEM-based modeling.

Among these interesting engineering software resources, NX-8.5, Catia v.5, Autodesk Inventor and Solid Works, provide interesting combinations between design capabilities and in silico assessment tools, being NX-8.5 probably the more versatile thanks to its broad capabilities for performing mechanical (both statical and dynamical), thermal, fluidical and coupled (thermo-mechanical, fluid-mechanical and thermo-fluid) multi-physical analyses. Its capabilities have been put forward in several cases of study along the whole Handbook.

These simplified CAD libraries are useful for the conceptual design stage, which is especially relevant in extremely novel fields of study, such as the field of

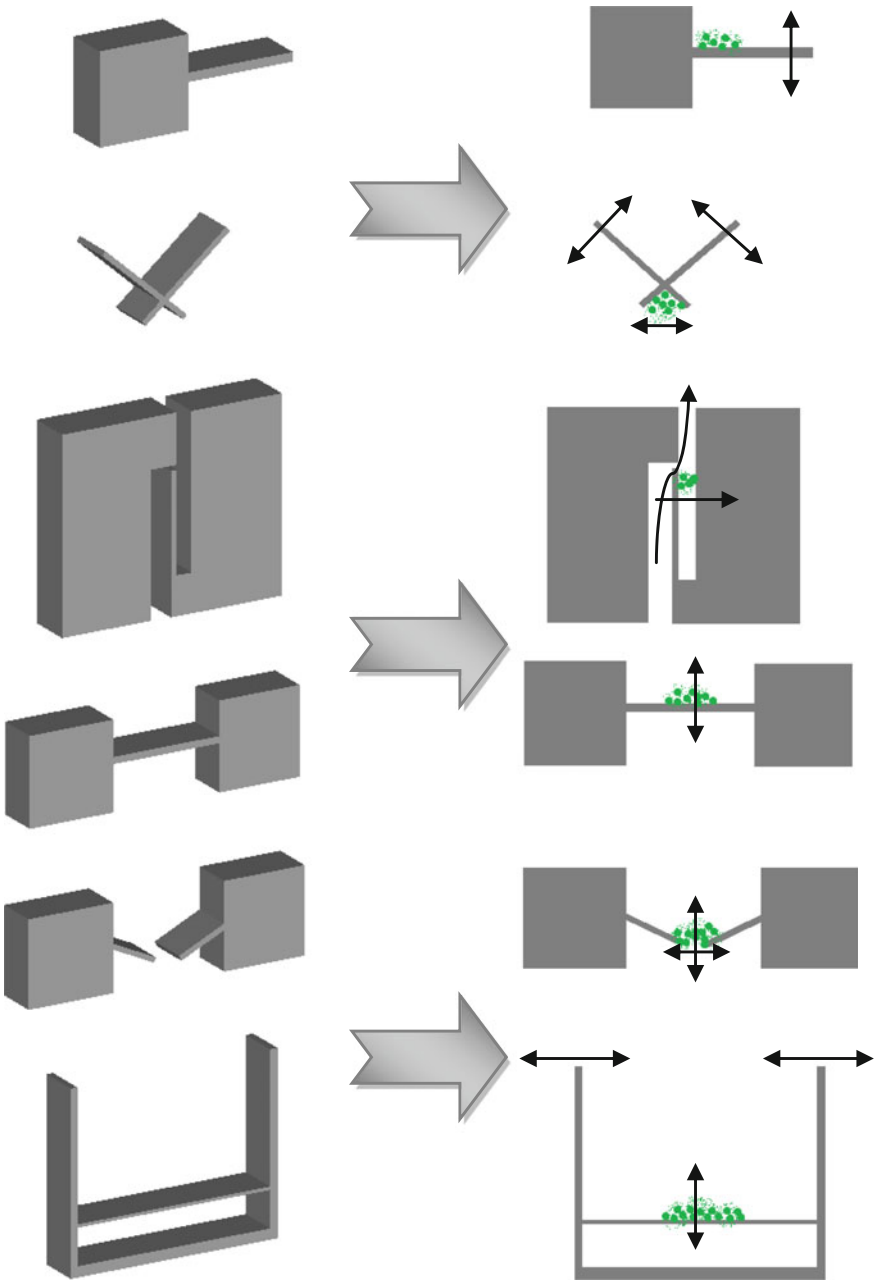

Fig. 21.3 Schematic computer-aided design library and actuation mechanisms of biohybrid micromechanical systems moved by cells (*in green*)

cell-based sensors and actuators or biohybrid intelligent systems. Such libraries of computer-aided designs, for example, help to discuss potential modes of sensing and actuating, support and simplify the communication among project participants from different backgrounds, provide input for engineering design tasks and serve as basic geometries for FEM-based simulations, with which the more fundamental design optimization, material selection and performance assessment activities can be carried out.

Rapid prototyping is also possible, directly from the information of CAD files, for the preliminary validations and comparative purposes, as well as for detecting potential improvements. Rapid-prototyping alternatives, with outstanding degree of precision and even capable of enabling sustainable mass-production of the final designs, have been recently put forward for metallic and polymeric biomaterials (Hengsbach and Díaz Lantada 2014; Muslija and Díaz Lantada 2014; Díaz Lantada et al. 2015).

21.6 Main Conclusions and Future Research

Cells and tissues can be seen, from the perspective of Materials Science and Engineering, as "smart materials and structures". In fact, cells and tissues are able to perceive and respond to several environmental stimuli and gradients of them, including the presence of biochemical cues and microorganisms, the mechanical and topographical properties of the extra cellular matrix and surfaces upon which they lie, the application of vibrations and the surrounding electromagnetic fields, to cite just a few, as already detailed in several chapters of the Handbook.

Advances in technologies for manipulating, culturing and monitoring single cells, together with progress in the fields of modeling, simulation, prototyping and testing, have led to a better understanding of how cells respond to several types of stimuli and accurate predictions about the behavior of cells and tissues are already possible. In consequence, cells and tissues can be employed as living transducers for the development of (micro-)sensors and (micro-)actuators, as it is possible to predict and control their responses.

Due to the micro- and nano-geometries of cells and tissues and to the highly specificity and speed of several of their biochemical and biological responses, the sensors and actuators based on them are extremely precise and can provide high throughput, thanks to the possibilities of multi-plexing. Such biohybrid cell-based devices have the potential to outperform most types of already existing sensors and actuators based on inorganic components.

This chapter has provided and introduction to the development of cell-based sensors and actuators and to current main challenges in this novel area. Once such challenges are solved, the frontiers between biological systems, machines and synthetic engineering systems in general will start to fade away. An approach for a more rapid solution of the aforementioned challenges, towards a wide-spread use of

cell-based sensors and actuators, may rely on the use of systematic libraries with CAD models of conceptual cell-based sensors and actuators, both for modeling and prototyping strategies, as has been proposed in the final case study.

References

Bainbridge WS, Roco MC (2006) Managing nano-bio-info-cogno innovations: converging technologies in society. Nat Sci Found, Springer

Buxboim A, Discher DE (2010) Stem cells feel the difference. Nat Methods 7(9):695

Cerutti S (2008) Multivariate, multiorgan and multiscale integration of information in biomedical signal processing. In: International conference on biomedical electronics and devices biostec 2008—biodevices, keynote lecture. IEEE Engineering in Medicine and Biology Society, INSTICC Press

Chen AY, Deng Z, Billings AN, Seker UOS, Lu MY, Citorik RJ, Zakeri B, Lu TK (2014) Synthesis and patterning of tunable multiscale materials with engineered cells. Nat Mater 13:512–523

Choi M, Choi JW, Kim S, Nizamoglu S, Han SK, Sun SK (2013) Light-guiding hydrogels for cell-based sensing and optogenetic synthesis in vivo. Nat Photonics 7:987–994

Choi JW et al (2009) Fabrication of 3D biocompatible/biodegradable micro-scaffolds using dynamic mask projection microstereolithography. J Mater Process Technol 209:5494–5503

Cvetkovic C et al (2014) Three-dimensionally printed biological machines powered by skeletal muscle. PNAS 111(28):10125–10130

Davis JR (2003) Handbook of materials for medical devices. ASM International, United States

Deutsch A, Brusch L, Byrne H (2007) Mathematical modeling of biological systems. volume I: cellular biophysics, regulatory networks, development, biomedicine and data analysis. Series Model Simul Sci Eng Technol. Birkhäuser

Deutsch A, De la Parra R, De Boer RJ (2008) Mathematical modeling of biological systems. volume II: epidemiology, evolution and ecology, immunology, neural systems and the brain, and innovative mathematical methods. Series Model Simul Sci Eng Technol. Birkhäuser

Díaz Lantada A (2012) Handbook on active materials for medical devices: advances and applications. PAN Stanford Publishing

Díaz Lantada A et al (2013) Fractals in tissue engineering: towards biomimetic cell-culture matrices, microsystems and microstructured implants. Expert Rev Med Devices 10(5):629–648

Díaz Lantada A, Muslija A, García-Ruíz JP (2015) Auxetic tissue engineering scaffolds with nanometric features and resonances in the megahertz range. Smart Mater Struct 24:055013

Ferro Y, Perullini M, Jobbagy M, Bilmes SA, Durrieu C (2012) Development of a biosensor for environmental monitoring base don microalgae immobilized in silica hydrogels. Sensors 12 (12): 16879–16891

Freed L, Vunjak-Novakovic G, Biron RJ, Eagles DB, Lesnoy DC, Barlow SK, Langer R (1994) Biodegradable polymer scaffolds for tissue engineering. Bio/Technology 12:689–693

Gad-el-Hak (2003) The MEMS handbook. CRC Press, New York

Gasson M, Hutt B, Goodhew I, Kyberd P, Warwick K (2005) Invasive neural prosthesis for neural signal detection and nerve stimulation. Proc Int J Adapt Control Signal Process 19(5):365–375

Hengsbach S, Díaz Lantada A (2014) Rapid prototyping of multi-scale biomedical microdevices by combining additive manufacturing technologies. Biomed Microdevices 16(4):617–627

Kubisch S, Bohrn U, Fleischer M, Stütz E (2012) Cell-Based sensor system using L6 cells for broad band continuous pollutant monitoring in aquatic environments. Sensors 12(3): 3370–3393

Kuklick TR (2006) The medical device R&D handbook. CRC Press, Taylor and Francis Group, Florida

Lendlein A, Langer R (2002) Biodegradable, elastic shape-memory polymers for potential biomedical applications. Science 296(5573):1673–1676
Lipson H (2012) Frontiers in additive manufacturing, the shape of things to come. Bridge 42(1):5–12
Longoni S, Sartori M (2010) Fractal geometry of nature (bone) may inspire medical devices shape. Nature Proceedings, (available online)
Muslija A, Díaz Lantada A (2014) Deep-reactive ion etching of auxetic structures. Smart Mater Struct 23:087001
Naik VM et al (2009) Super functional materials: creation and control of wettability, adhesion and optical effects by meso-texturing of surfaces. Curr Trends Sci Platin Jubilee Spec 129–148
Park M, Tsai SL, Chen W (2013) Microbial biosensors: engineered micro-organisms as the sensing machinery. Sensors 13(5): 5777–5795
Peterson D, Bronzino J (2008) Biomechanics. principles and applications. CRC Press, Taylor and Francis Group, Florida
Pulsifier DP, Lakhtakia A (2011) Background and survey of bioreplication techniques. Bioinspiration Biomimetics 6(3):031001
Ricotti L, Menciassi A (2014) Bio-hybrid muscle cell-based actuators. Biomed Microdevices 14:987–998
Schwartz M (2006) New materials, processes and methods technology. CRC Press, Taylor and Francis Group, Florida
Taniguchi A (2012) Live cell-based sensors. Special Issue, Sensors 12(12)
Thomas WE, Discher DE, Shastri VP (2010) Mechanical regulation of cells by materials and tissues. MRS Bull 35:578
Warwick K (2008) Outthinking and enhancing biological brains. In: International conference on biomedical electronics and devices biostec 2008—biodevices, keynote lecture. IEEE Engineering in Medicine and Biology Society, INSTICC Press
Wong J, Bronzino J (2007) Biomaterials. CRC Press, Taylor and Francis Group, Florida

Part IV
Present Challenges and Future Proposals

Chapter 22
Towards Reliable Organs-on-Chips and Humans-on-Chips

Andrés Díaz Lantada, Gillian Begasse, Alisa Morss Clyne, Stefan Hengsbach, Volker Piotter, Peter Smyrek, Klaus Plewa, Markus Guttmann and Wilhelm Pfleging

Abstract The artificial production of complete three-dimensional vascularized functional organs is still a research challenge, although recent advances are opening up new horizons to the treatment of many diseases by combining synthetic and biological materials to produce portions of veins, capillaries, arteries, skin patches and parts of bones and soft organs. Counting with artificially obtained completely functional replicas of human organs will constitute a benchmark for disease management, but there is still a long way to achieve the desired results and produce complete organs in vitro. In the meantime, having at hand simple biomimetic microsystems capable of mimicking the behaviour of complete complex organs, or at least of some of their significant functionalities, constitutes a realistic and very adequate alternative for disease modeling and management, capable of providing even better results than the use of animal models. These simplified replicas of human organ functionalities are being developed in the form of advanced labs-on-chips generically referred to as "organs-on-chips" and are already providing interesting results. This chapter provides an introduction to this emerging area of study and details different examples of organs-on-chips and their development process with the aid of computer-aided design and engineering technologies and with the support of rapid prototyping and rapid tooling resources.

A. Díaz Lantada (✉) · G. Begasse
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

A. Morss Clyne
Vascular Kinetics Lab – Mechanical Engineering and Mechanics,
Drexel University, 3141 Chestnut Street, Philadelphia, PA 19104, USA

S. Hengsbach · V. Piotter · P. Smyrek · K. Plewa · M. Guttmann · W. Pfleging
Karlsruhe Nano Micro Facility, Karlsruhe Institute of Technology,
Hermann von Helmholtz Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

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22.1 Introduction to Organs-on-Chips and Related Design and Manufacturing Technologies

The artificial production (or "biofabrication", see Chap. 23) of complete three-dimensional vascularized functional organs is still a research challenge, although recent advances are opening up new horizons to the treatment of many diseases by combining synthetic and biological materials to produce portions of veins, capillaries, arteries, skin patches and parts of bones and soft organs. Counting with artificially obtained completely functional replicas of human organs will constitute a benchmark for disease management, but there is still a long way to achieve the desired results.

In the meantime, having at hand simple biomimetic microsystems capable of mimicking the behaviour of complex organs, or at least of some of their significant functionalities, constitutes a realistic and very adequate alternative for disease modeling and management, capable of providing even better results than more conventional animal models. These simplified replicas of human organ functionalities are being developed in the form of advanced lab-on-chip devices generically called "organs-on-chips", by combining the aforementioned strategies and technologies, and are already providing interesting results (Huh et al. 2011, 2013).

Most of the already developed organs-on-chips in fact focus on specific interactions among a couple of cell types cultured together, help to assess the effect of chemicals and drugs on cells cultured upon channel networks resembling the organization of more complex organs, or mimic concrete fluid-cell interfaces.

Among the most remarkable experiences published so far, we would like to highlight studies linked to replicating, to some extent, the behaviour of several human organs and physiological structures including: liver (Ho et al. 2006a, b), heart (Domian et al. 2009), lung (Huh et al. 2011) and blood-brain barrier (Wilhelm et al. 2011), among other interesting proposals. Disease development has been also studied and predicted by means of organs-on-chips, as some experiences linked to real-time monitoring of kidney stone formation show (Wei et al. 2012). Some promising results have even led to the establishment of spin-off companies oriented to the commercialization of organ-on-chip platforms (normally the microfluidic structure for further cell culture carried out in the researchers' or clients' labs), such as Nortis Inc. (www.nortisbio.com) and Mimetas (www.mimetas.com).

As detailed in previous chapters, rapid prototyping and manufacturing technologies allow researchers to obtain physical parts in a short period (usually hours or days), directly from the designs created with the help of computers using computer-aided design, engineering and manufacturing resources. Such technologies significantly help to optimise the design iterations, allowing for the early detection of errors and speeding up the whole development process. They are also playing a relevant role in the exponential growth and development speed of organ-on-chip platforms and related industry, as several examples included in present chapter may help to understand.

These technologies are generally based on automatic additive or layer-by-layer manufacturing processes (and they are also referred as layer manufacturing technologies or "LMT"). In some cases, very fast manufacturing processes involving material removal, such as high speed numerical control machining, are also included within the concept of rapid prototyping, or "RP", although the term is normally linked to additive processes. The various technologies available can operate and manufacture prototypes using a wide range of metals, ceramics or polymers, both with synthetic and biological origin, and with remarkable precision and applications in all several areas of biomedical engineering are noteworthy (Kuclick 2006; Wohlers 2010; Díaz Lantada and Lafont Morgado 2012).

Remarkable technologies (and associated materials) with a significant impact on the evolution of the rapid prototyping industry include: laser stereolithography (photosensitive polymers); selective laser sintering (polymer powder, usually nylon, or ceramic powder); 3D printing (powder with binder, liquid resins); or fused deposition modeling (thermoplastics). All of these are based on deposition processes or layered manufacturing approaches, which facilitates the creation of complex geometries, including inner details, carried out directly from the associated CAD files. Biomedical microdevices also benefit from these set of technologies, especially from the combined use of stereolithography with PDMS replication, following soft-lithography processes (Whitesides et al. 2001).

The complex issue of obtaining precise rapid prototypes using biomaterials, for enabling cell culture and promoting adequate cell-material interactions, is also being progressively solved resorting to different strategies. First of all, high precision technologies, mainly two-photon polymerization, are being combined with biophotopolymers for directly obtaining the desired prototypes (Infur et al. 2007; Stampfl et al. 2008). Indirect approaches are also useful, such as using an additive manufactured prototype for obtaining a mold and subsequently casting a biomaterial for replicating the original model, or using an additive manufactured mold for obtaining the desired part by biomaterial casting (Stampfl et al. 2004; Chopra et al. 2012). Adaptations of inkjet and 3D printers into "bioplotters" and "cell-printers", for the three-dimensional deposition of cells and support materials or hydrogels loaded with cells, are also opening new horizons in the field of biofabrication and benefit the microsystems for disease modeling covered in present study (Mironov et al. 2009; Kanani and Gaudette 2010; Jakab et al. 2010; Huang et al. 2012).

A great advantage of using these resources is the possibility of directly obtaining prototypes from CAD models, for rapidly validating results from simulations and for conceptual trials aimed at design improvement, before focusing on the production stage. The additional option of linking rapid prototypes with production tools is also of great interest for the field of microfluidics. The following sections are devoted to providing different examples of organs-on-chips developed with the aid of computer-aided design and engineering tools and with the support of rapid prototyping and rapid tooling resources.

22.2 Case Study: Development of a Blood-Brain Barrier Platform

The blood-brain barrier (BBB) is an active interface between the circulation and the central nervous system (CNS) with a dual function: the barrier function restricts the transport from the blood to the brain of potentially toxic or harmful substances; the carrier function is responsible for the transport of nutrients to the brain and removal of metabolites. The development of microsystems for in vitro studies linked to such BBB is a relevant research challenge not yet resolved, as most microsystems linked to BBB have several culture layers, which are difficult for mass production, or concentrate on very particular effects with just one type of cells and a couple of parallel channels.

Figure 22.1 shows, as example, the rapid prototype of a microsystem for modeling the blood brain barrier, directly obtained from the CAD file. A negative model of the lower functional substrate is obtained using laser stereolithography (SLA-3500 machine from 3D Systems) and further replicated using transparent PDMS for enabling cell culture (as the original stereolithographic epoxy is inadequate due to toxic acrylate components). The upper case is directly obtained using stereolithography, a technology widely used for helping to integrate microsystems with the surrounding accessories, such as tubes, connections, clamps… and

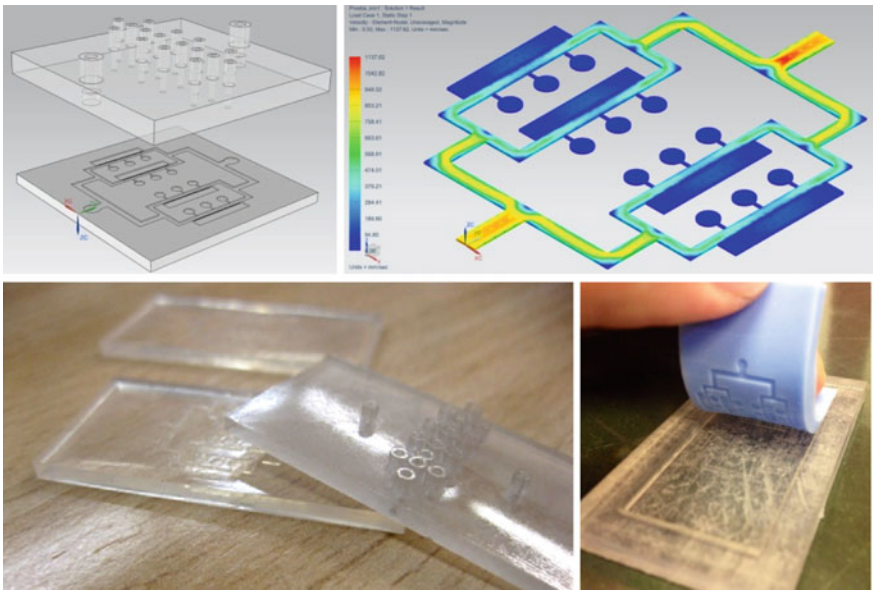


Fig. 22.1 Complete development process of an organ-on-chip aimed at studying the interactions between cell types conforming the blood-brain barrier. Computer-aided design, FEM-based assessment of fluidic performance, rapid prototypes and PDMS (*blue*) replicas for direct cell culture (color online)

supporting equipments, including pumps, optical measuring systems (as the parts are almost transparent) and sensor matrices (Waldbaur et al. 2011).

This approach differs from the more typical organ-on-chip designs inspired on the common Transwell® permeable culture supports (Huh et al. 2011, 2013). Transwell® devices include lower and upper culture chambers, for culturing different cell types, separated by micro-porous membranes, and provide effective solutions for co-culture of cells. However, in the conceptual design presented below, cells are cultured on the same layer and separated by micromanufactured gates, thus simplifying device complexity and providing similar functionalities, using just one culture layer and an upper case, instead of two culture layers and an intermediate membrane. We think that similar designs may promote mass production of organ-on-chip solutions and lead to more economic devices.

The proposed approach is also interesting because different cells, relevant for the physiological behavior of the blood-brain barrier, can be cultured in the same device. In addition the proposed design is dynamic, i.e. endothelial cells can be excited by means of a tunable fluid flow. The number of channels and lateral chambers, for neural cells, growth factors and other drugs, allows testing several conditions in just one experiment, as well as analyzing the effect of different gradients of drugs and growth factors. A final advantage of such a device is its simplicity, while being potentially biomimetic and accurate. Some devices based on co-culture of endothelial cells and astrocytes require the use of a chamber for the endothelial cells, a middle membrane and an upper chamber for neural cells, thus being complex and difficult to manipulate.

The proposed design includes just one chip, with all the required chambers, and a glass cover-slip, what may promote future industrialization as testing device.

Figure 22.1 also includes the computer-aided design and the related FEM-based simulation for performance prediction of the vitro blood-brain barrier model, which we are currently developing, aimed at the co-culture of endothelial and neural cells for improved disease study. Endothelial cell growth benefits from culture under a dynamic flow that produces shear stresses and promotes angiogenesis, while neural cells are usually cultured in more static conditions. The FEM-based simulation helps to validate the interest of our approach, as the vascular channels for endothelial cell culture (clear blue in the simulation) are filled with fluid flowing at a speed of c.a. 400 mm/s, while the rectangular chambers for neural cell culture (dark blue in the simulation) are filled with almost static fluid and not affected by the fluid flowing within the microsystem. In our system, the vascular channels and the rectangular chambers are connected by 20-μm width openings, which let the different cell types connect with each other and help to establish different flow and, therefore, culture conditions.

In addition, as different cell types grow under different optimal shear stresses and shear stresses are linked to fluid velocity and flow rate, simulations help to select the more adequate pumping systems and working conditions for microfluidic biodevices aimed at cell culture for improved diagnosis. However, as several aspects such as cell-cell and cell-material interactions are complex to model and

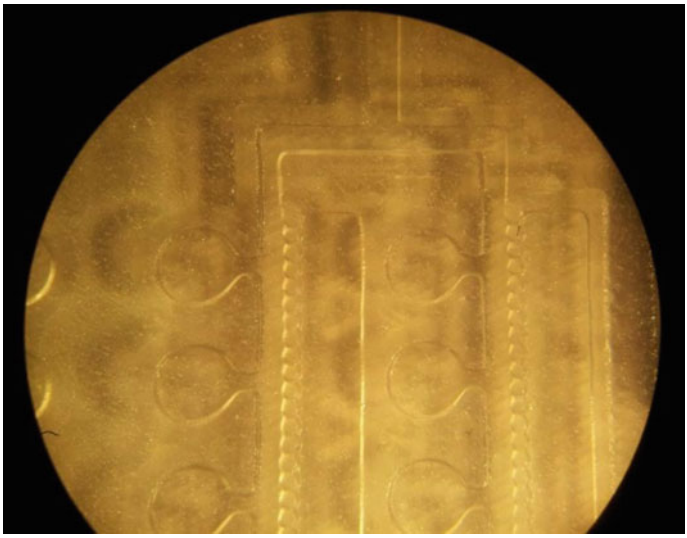


Fig. 22.2 Microscopy of the active layer of an organ-on-chip aimed at studying the interactions between cell types conforming the blood-brain barrier. Micropillars help to separate the passive culture chamber for the neural cells from the active culture channels for the endothelial cells aimed at generating the microvasculature. Some wells provide the opportunity of including drugs and toxins

simulate, the results from simulations must be always handled with care and validated with the help of support prototypes.

After manufacture of the first prototypes in epoxy resin, aimed at validating the functionality of the fluidic microsystem, at verifying mounting aspects and the connections with surrounding systems and at generating rapid molds for further PDMS casting towards functional microsystems apt for the first in vitro trials, we have carried out visual verifications to assess manufacturing precision. Figure 22.2 shows a microscopy of the active layer of an organ-on-chip aimed at studying the interactions between cell types conforming the blood-brain barrier. As can be appreciated, the micropillars help to separate the passive culture chamber for the neural cells from the active culture channels for the endothelial cells aimed at generating the microvasculature. Some wells provide the opportunity of including drugs and toxins.

These rapid prototypes are adequate for the first trials, as well as for obtaining the initial functionally adequate devices by soft-lithography, but mass-production typically requires alternative approaches (as already described in Chap. 10) and an additional degree of precision in this example, for interacting at single cellular level. In this example we resort to a high-precision Heidelberg DW-66 Laser Writer system, based on a femtolaser for enhanced details, available at the Karlsruhe Institute of Technology (via the Karlsruhe Nano- Micro-Facility) for obtaining

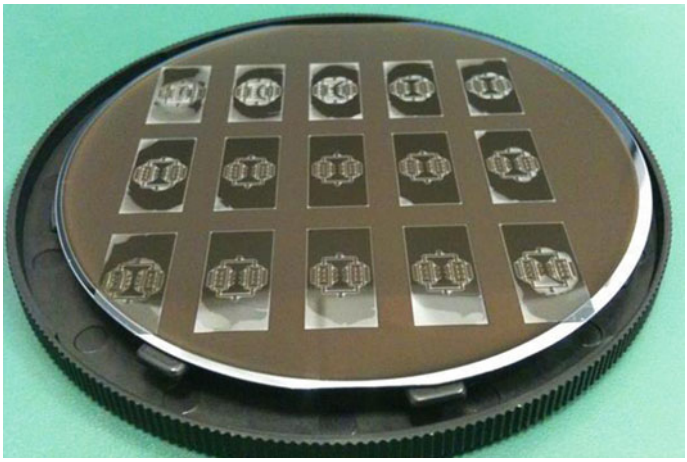


Fig. 22.3 Mass-produced micro-chips for the active layer of an organ-on-chip aimed at studying the interactions between cell types conforming the blood-brain barrier. Obtained with the support of KNMF facilities

several blood-brain barrier chips by photopolymerization of SU-8 upon silicon wafers, as can be seen in Fig. 22.3.

The detailed views from Fig. 22.4 help to highlight the degree of precision obtained. Micropillars with almost perfect rounded geometries and with cross-section diameters of 75 μm can be clearly perceived. Clearances between the pillars of 25 μm can be also precisely obtained and would allow just cell-to-cell interactions between cells from the chambers and cells from the adjacent channels without letting massive migrations from chambers to channels or viceversa. Final validations with several types of cells cultured will be carried out in the near future and help us to find potential improvements towards more effective models. However we believe that the attainable degree of precision and the option of including all functional parts of the physiology being mimicked are important advances in the field. Combining these types of multifunctional layers with other multifunctional layers separated by porous membranes, in the more typical fashion of organs-on-chips, may help us obtain an additional degree of complexity and promote the modeling of even more complex organs and biological structures.

Regarding alternatives for mass-production of organs-on-chips, Sect. 22.4, describing the complete design and development process of the micro-fluidic structure for a liver-on-chip, provides additional insights. Inbetween, Sect. 22.3 focuses on computer-aided design and engineering resources for optimizing the design of a lung-on-chip device with an alternative approach as that used by aforementioned preliminary research linked to such biodevices.

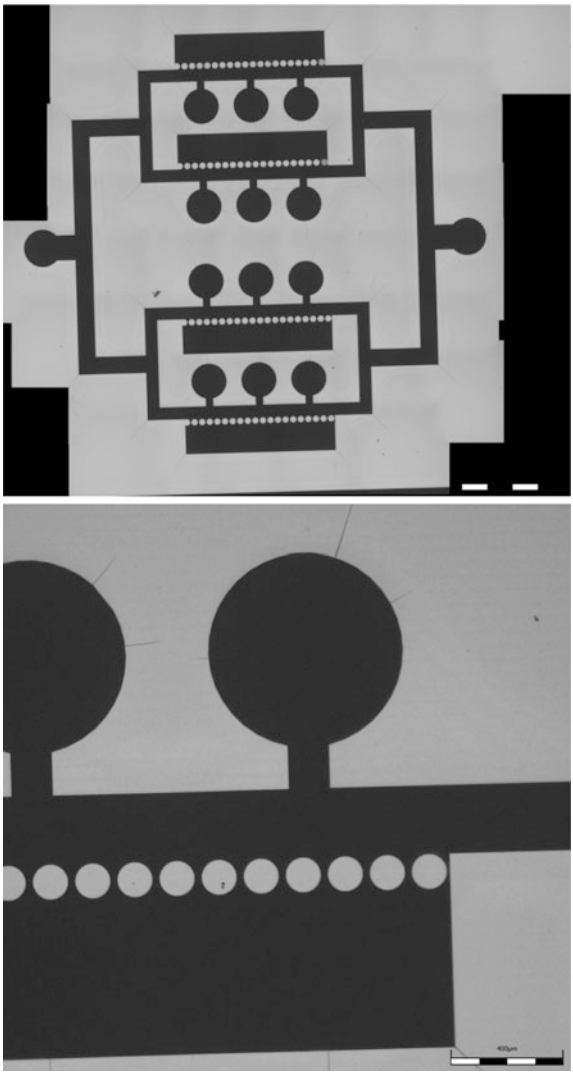


Fig. 22.4 Detailed view of mass-produced micro-chips for the active layer of an organ-on-chip aimed at studying the interactions between cell types conforming the blood-brain barrier. Obtained with the support of KNMF facilities

22.3 Case Study: Development of a Lung-on-Chip Platform

The development of in vitro systems for adequately mimicking the physiology of lungs is necessary for carrying out systematic studies, linked to lung disease and cancer, in a sustainable, low-cost and rapid way. Such systems have already helped to study the dynamics of pathogens within structures similar to those of real lungs, thanks to culturing cells in compliant substrates, and can be used to address the efficacy of potential treatments (Huh et al. 2011).

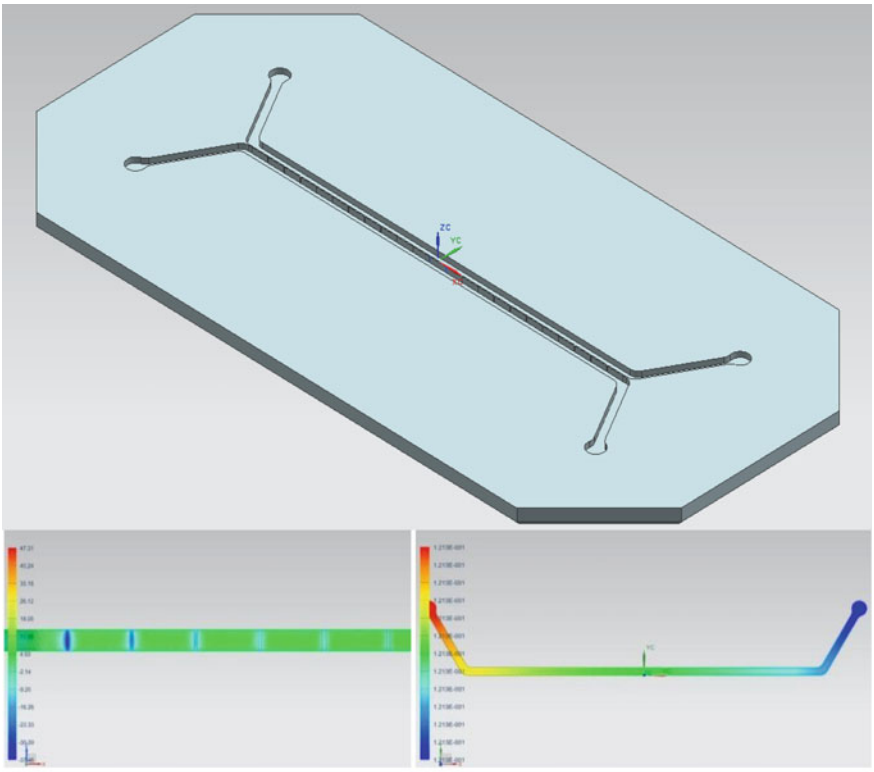
Here we present an alternative geometry, which again differs from the more typical organs-on-chips inspired on the common Transwell® permeable culture supports (Huh et al. 2011, 2013). Transwell® devices include lower and upper culture chambers, for culturing different cell types, separated by micro-porous membranes, and provide effective solutions for co-culture of cells. However, in the conceptual design presented below, cells are cultured on the same layer and separated by micromanufactured gates, thus simplifying device complexity and providing similar functionalities, using just one culture layer and an upper case, instead of two culture layers and an intermediate membrane. We think that similar designs may promote mass production of organ-on-chip solutions and lead to more economic devices.

Figure 22.5 presents the computer-aided design and FEM-based simulation of the micro-fluidic response of a lung-on-a-chip. One channel is filled with PBS buffer for representing the blood and one with pressurized air for imitating the bronchioles. The effects of interconnecting pores and pressures of the channel representing the airway on the leakage from the blood-channel to the air-channel are analyzed.

The manufacturing process has followed previous descriptions (De la Guerra et al. 2012) with some modifications. In short, we have used glass as substrate material due to its transparency and to the fact that we will omit in this case the etching stage (glass should be etched with HF, but here we are just focusing on structuring a microchannel network). For the manufacture of the microchannels we have followed several steps including:

- Initial preparation of the glass coverslip by washing out in ultrasonic cube for around 30 min and subsequent drying.
- Coating of the substrate using Dupont Riston PM-100 photoresin.
- Exposure of the photoresin to UV light by means of the SF-100 equipment from Intelligent Micro Patterning LLC. As previously mentioned, this process is known as mask-less photolithography, as the employment of programmable light filters prevents from using a physical mask.
- Development, using a Na_2CO_3 0.85 % w. solution, for eliminating the uncured photoresin in those pattern zones that are going to be chemically etched.
- Washing out debris and drying.
- Final dimensional verification.

These processes lead us to the results from Fig. 22.6, in which a final glass substrate with a network of polymerized channels can be appreciated. As already mentioned, such obtained microchannelled surfaces can be also used for micro-replication activities, in a family of processed normally referred to as “soft lithography techniques” (see Sect. 8.4 for additional details). Soft stamps can be also obtained by casting PDMS upon these glasses with micro-textures or microchannel networks, following “rapid form copying” procedures. The PDMS replica would include the engraved channel structure and let cells interact.



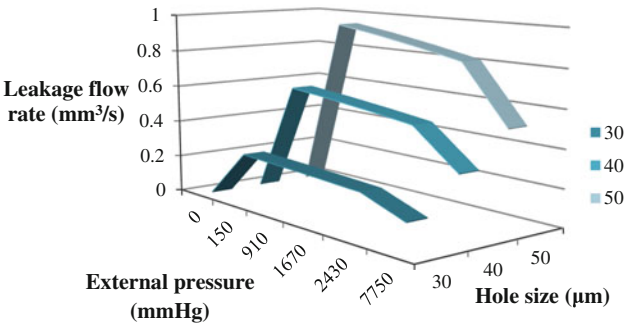


Fig. 22.5 Computer-aided design and FEM-based simulation of the micro-fluidic response of a lung-on-a-chip. One channel is filled with PBS buffer for representing the blood and one with pressurized air for imitating the bronchioles. The effect of interconnecting pores and pressure on leakage are analyzed

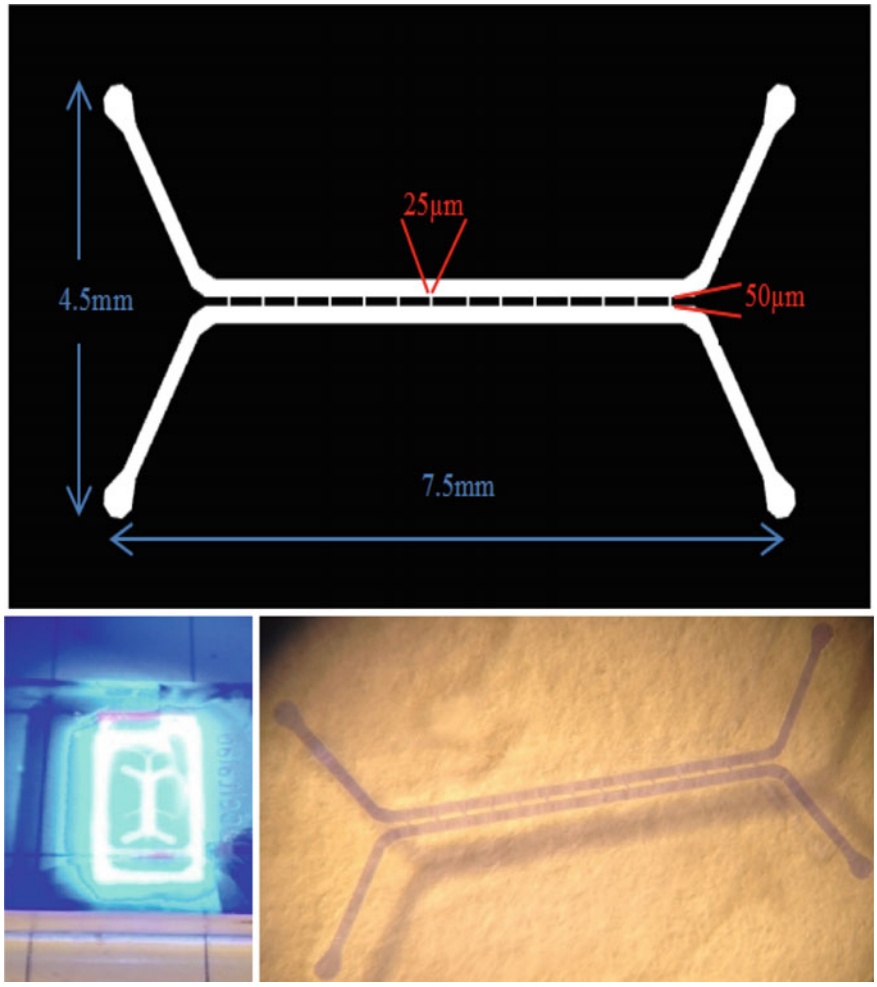


Fig. 22.6 Mask for the manufacture of a lung-on-a-chip by means of UV photolithography, UV light exposure and final prototype obtained

22.4 Case Study: Development of a Liver-on-Chip Platform

As an additional example of a development in progress, Fig. 22.4 shows the computer-aided design of the functional microfluidic layer of a liver-on-chip device, including a central channel for hepatocyte culture and a couple of lateral channels for endothelial cell culture. The central channel includes an entrance for the hepatocytes, small textured (or CVD/PVD functionalized) discs for fixing the hepatocytes in pairs along the channel and, at the other extreme, an outlet for collecting

billiard secretion. The lateral endothelial culture channels include inlets and outlets for culture under maintained flow, which promotes cell growth and angiogenesis. The central and the lateral channels are separated by thin walls with 20-μm gates or openings for allowing interactions between the endothelial cells and the hepatocytes and for letting the hepatocytes obtain nutrients from the lateral channels.

This approach again differs from the more typical organ-on-chip designs inspired on the common Transwell® permeable culture supports (Huh et al. 2011, 2013). Transwell® devices include lower and upper culture chambers, for culturing different cell types, separated by micro-porous membranes, and provide effective solutions for co-culture of cells. However, in the conceptual design presented below, cells are cultured on the same layer and separated by micromanufactured gates, thus simplifying device complexity and providing similar functionalities, using just one culture layer and an upper case, instead of two culture layers and an intermediate membrane. We think that similar designs may promote mass production of organ-on-chip solutions and lead to more economic devices.

The proposed microsystem, shown schematically in the computer-aided design of Fig. 22.7, is aimed at the co-culture of hepatocytes and endothelial cells. The 2D 1/2 design includes no undercuts and is oriented to micro-injection molding, as having several replicas of the proposed device will allow us to culture under different conditions and to carry out several experiments in parallel, so as to obtain the desired results and more relevant information. Previous micro-injection molded microsystems (in PMMA and PC, as shown in Chap. 13) have been adequate for cell-culture, so the final materials attainable with micro-injection molding are perfect.

The microsystem includes some 200 μm width channels separated by thin walls with some openings of around 20–40 μm, depending on the available manufacturing precision. The openings are aimed at allowing endothelial cells and hepatocytes interact with each other. We propose the combination of additive and subtractive processes, such as laser stereolithography for building the main structure, and laser ablation, for obtaining the smallest features upon the master model or upon the mold cavity, prior to micro-injection molding. The central channel includes some parallel dots or circular zones for promoting the attachment of hepatocytes, which work in pairs for the generation of biliar liquid. Such circular zones would ideally be textured circles, similar to some textures attainable by using laser material processing or texturing.

Even though the potentials of combining rapid prototypes obtained by means of additive manufacturing technologies and micro-injection molding for mass-production, after converting the original masters into metallized mold inserts, has been previously put forward in Chap. 10, here we provide an additional turn of the screw: the use of laser ablation after the additive manufacture towards mold inserts with an additional degree of precision.

With this design and the proposed combination of technologies, we expect to obtain a microsystem with a remarkable degree of precision and the potential for mass-production, including several benefits regarding other more complex devices

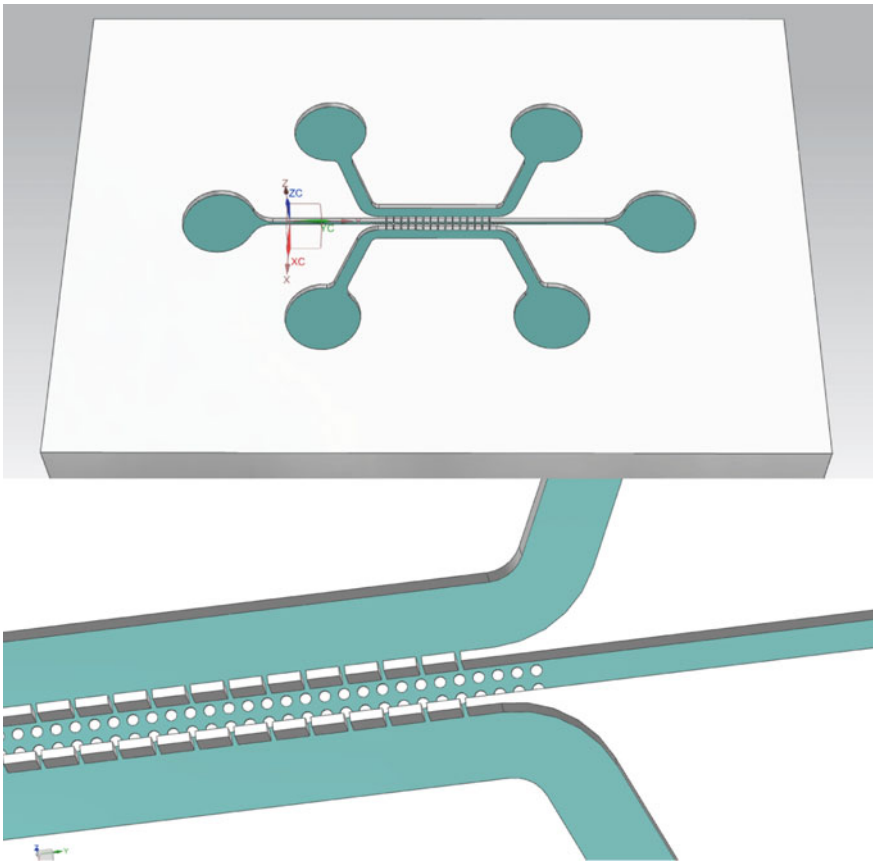


Fig. 22.7 Computer-aided design of a liver-on-chip device. The lateral channels are aimed at the culture of endothelial cells for constructing the vasculature. The inner channel, with pairs of microtextured circles for fixing cells is designed for coupling the hepatocytes and letting them interact in a biomimetic way

with several culture layers. The use of just one central channel for hepatocyte culture will probably allow us to have enhanced cell localization and control, when compared to previous approaches using more intricate spiral designs (Ho et al. 2006a, b).

First models are obtained after designing the geometries with the help of state-of-the-art CAD software and after preparing the additive manufacture of the parts of interest with the help of the Preform software (by Formlabs co.). Figure 22.8 shows the designs distributed in the building platform and with the supporting structures, as well as the prototyping results. Prototypes of the original channelled structure and of the negative mold are obtained in epoxy resin by laser stereolithography, for subsequent post-processing aimed at achieving the smaller features of the microsystem and final replication towards biomaterials more

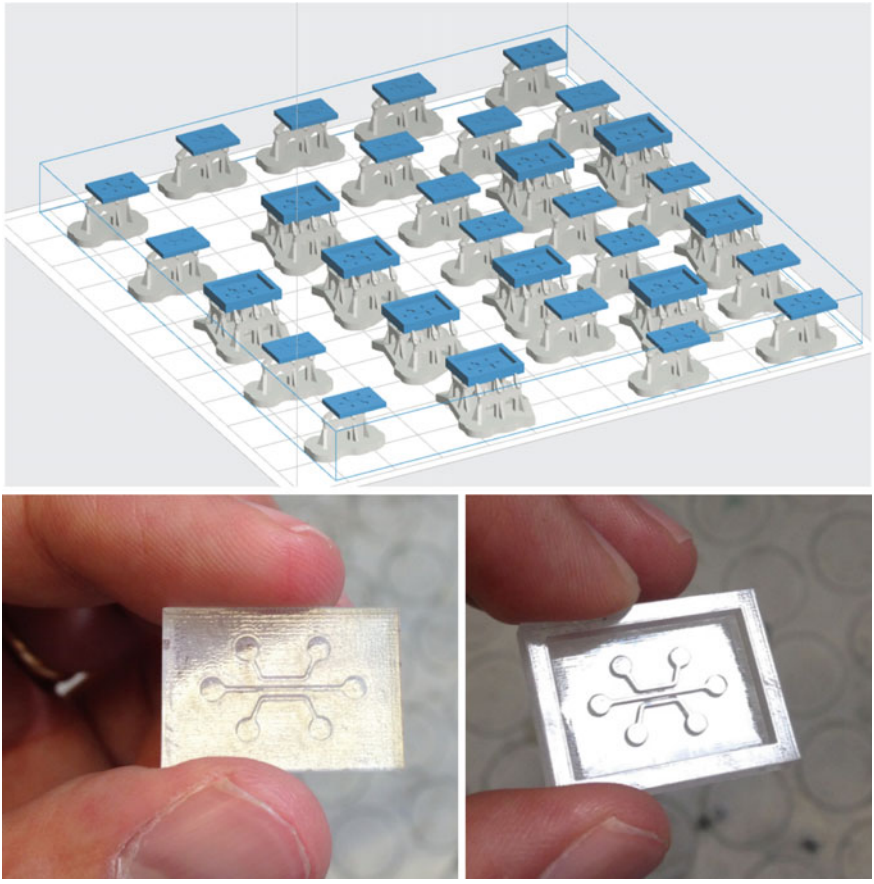


Fig. 22.8 Computer-aided manufacture of a liver-on-chip device. Designs and prototypes of the original channelled structure and of the negative mold obtained in epoxy resin by laser stereolithography for subsequent post-processing aimed at achieving the smaller features of the microsystem and final replication towards biomaterials more adequate for cell co-culture processes

adequate for cell co-culture processes. Figure 22.9 presents a detailed view of an epoxy master prototype, with the overall channel structure for the liver-on-chip device, prepared for subsequent post-processing.

Even though several technologies allow for the micromanufacture of 2D ½ and 3D geometries for biodevices, one of the most relevant among subtractive micromachining technologies is based on laser ablation (or laser machining/drilling), as it can work with a wide range of materials and help to obtain complex geometries. Among the main advantages of the process it is important to highlight the chemical-free process, the simple automation and the minimal heating and damage to surrounding zones of the material (very important for polymers and composites and also relevant for metals) (Pfleging and Roth 2011; Pfleging et al. 2013, Pfleging and Pröll 2014). In

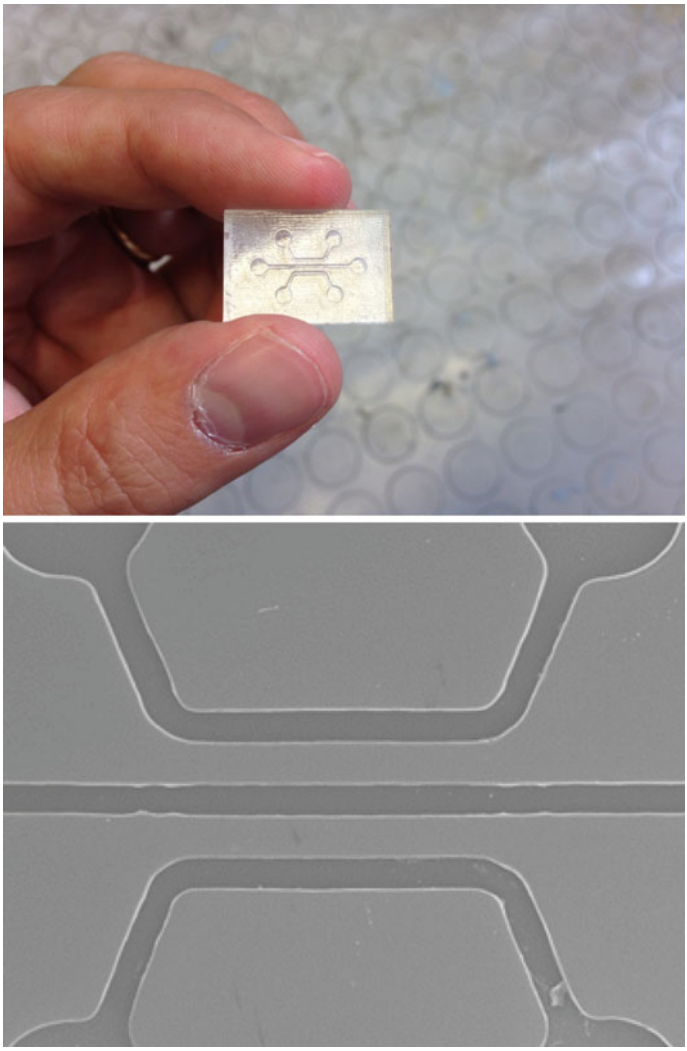


Fig. 22.9 Detailed view of an epoxy master prototype, with the overall channel structure for the liver-on-chip device, prepared for subsequent post-processing. Obtained by laser stereolithography using a SLA-3500 machine from 3D Systems

principle, the lower the heat-affected zone or "HAZ", the higher the quality, as less phase changes are promoted. In addition, thanks to the possibility of robotizing the laser movement, complex geometries such as curved shapes are attainable. In recent research the use of ultrafast laser for micromachining becomes of increasing interest. With decreasing laser pulse length the heat impact into the material can be completely suppressed while the ablation efficiency can be increased (Pfleging and Pröll 2014). Several devices such as auto-expandable stents, normally made of Nitinol, and many

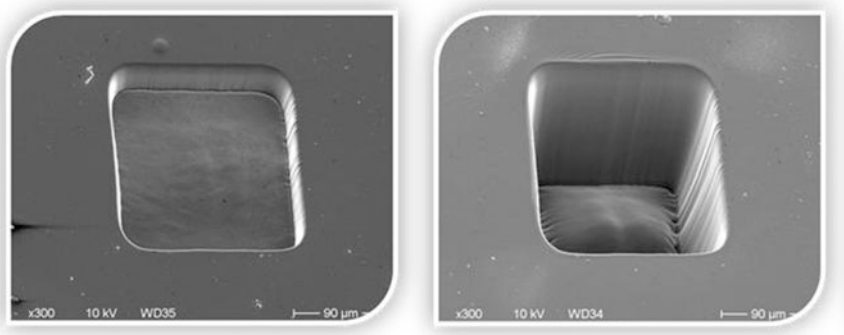


Fig. 22.10 Preliminary trial of laser ablation upon epoxy sample. With support from the KNMF—Karlsruhe Nano-Micro Facility (http://www.knmf.kit.edu/)

"lab-on-a-chip" solutions are manufactured by means of laser ablation or micro-machining (Gad-el-Hak 2003; Kucklick 2006; Queste et al. 2010).

Here we combine laser ablation with previously rapid prototyped masters for a multi-scale approach. The overall structure is additively manufactured by laser stereolithography. Laser ablation is applied to the incorporation of the micro-gates, in the walls separating the different channels, and the micro-patterns, upon the central channel. The process must first of all be adjusted, normally carrying out preliminary trials upon flat samples, as shown in Fig. 22.10. Once understood how the material behaves under the action of a laser, more complex operations can be performed, even in an automated way for repetitive operations and patterns, as further shown in Fig. 22.11. Main results from laser ablation upon an epoxy master can be clearly appreciated. The desired connections between channels and the circular textured discs, for fixing the hepatocytes of the liver-on-chip microsystem, are shown. Details down to 50 µm are present in the prototype mold masters.

After the prototype mold masters are obtained in epoxy resin, by combination of laser stereolithography for constructing the structure and laser ablation for the smaller details, the mold inserts are obtained following a process validated in prior research (Wissmann et al. 2010, 2015; Díaz Lantada et al. 2015).

In short, the procedure starts from additively manufactured and further ablated rapid prototypes, continues with a thin-film (via PVD) deposition technique for improving their surface conductivity, follows with an electroplating process for obtaining mold inserts and ends up with the mold adjustment and with the mass production using micro injection molding. The proposed process stands out for the attainable degree of detail, even capable of working at several scale levels, for the versatility of final materials, for the manufacturing speed and for the possibility of obtaining final low-cost replicas (Díaz Lantada et al. 2015).

Figure 22.12 shows the final liver-on-chip mold insert (already integrated into the mold structure) obtained by metallization of master models for mass-production by micro-injection molding with thermoplastics. Final validations with several types of cells cultured will be carried out in the near future and help us to find

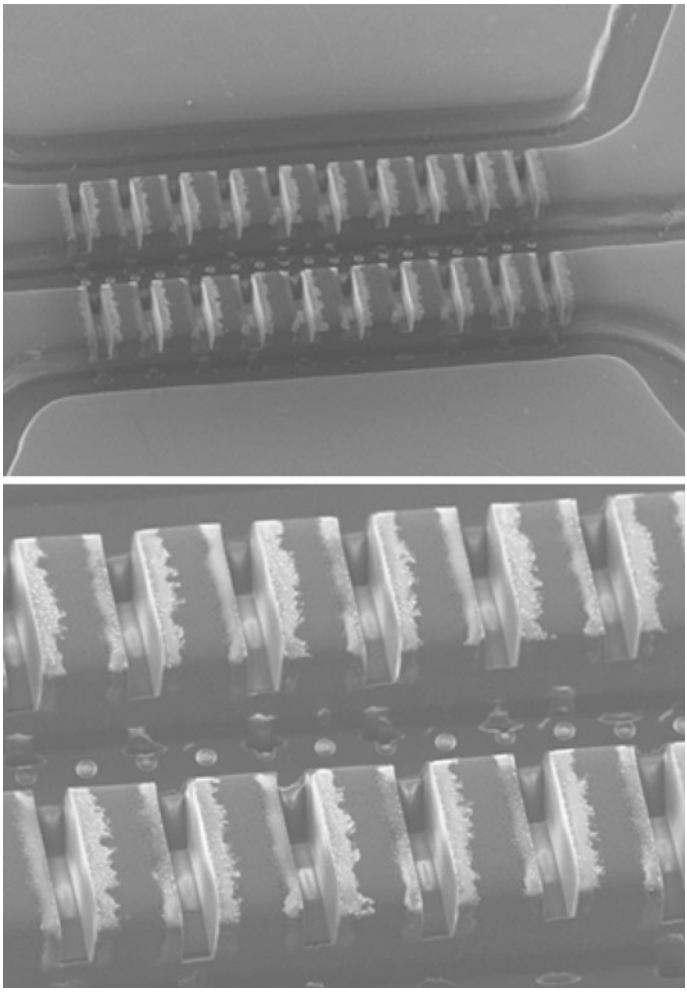


Fig. 22.11 Micromanufacture by laser ablation upon the epoxy master of the connections between channels and of the circular textured discs for fixing the hepatocytes of the liver-on-chip microsystem. Process developed in collaboration between Universidad Politécnica de Madrid and the Karlsruhe Institute of Technology, with support from the KNMF—Karlsruhe Nano-Micro Facility (http://www.knmf.kit.edu/)

potential improvements towards more effective models. However we believe that the attainable degree of precision and the option of including all functional parts of the physiology being mimicked, in just one layer, are important advances in the field towards its sustainability.

To our knowledge, the presented combination of additive, subtractive, deposition and mass-production technologies, applied to the complete development of a single microsystem for biomedical applications, is novel and may bring inspiration to the field, especially as regards rapid connection from the concept to the market.

Fig. 22.12 Final liver-on-chip mold insert obtained by metallization of master models for mass-production by micro-injection molding with thermoplastics. Process developed in collaboration between Universidad Politécnica de Madrid and the Karlsruhe Institute of Technology, with support from the KNMF—Karlsruhe Nano-Micro Facility (http://www.knmf.kit.edu/)

22.5 From Organs-on-Chips to Reliable Humans-on-Chips

A generalization of the previously covered organ-on-chip devices are the "life-on-chip" and "body-on-chip" concepts, aimed at the development of microsystems integrating several organs-on-chips, towards in vitro models of complete human systems (Lindstrom and Meldrum 2003; Huh et al. 2011; Shuler 2012).

Recently the Wyss Institute for Biologically Inspired Engineering at Harvard University has announced a relevant DARPA grant (to our knowledge the most relevant and inspiring in the field of biomimetic microsystems) to develop an automated instrument that integrates 10 human organs-on-chips to study complex human physiology outside the body.

The human-on-chip device may include models of the heart, lung, gut, brain, blood-brain barrier, liver, kidney, bone, bone marrow and skin and, once developed, should be a an accurate alternative to traditional animal testing models that often fail to predict human responses and a very promising approach for rapidly assessing responses to new drug candidates and further increasing knowledge regarding multiple diseases.

Apart from the typical problems present in any kind of integration and to the conventional demands present in microfluidic systems and cell culture devices, correctly designing individual organs and scaling them relative to each other, so as to make a functional microscale human analog is indeed challenging, and a generalized approach has yet to be identified. However the use of metabolically supported functional scaling is starting to provide interesting results, as has been recently put forward (Moraes et al. 2013).

22.6 Main Conclusions and Future Research

Although the artificial production of complete three-dimensional vascularized functional organs is still a research challenge, researchers already count with a wide set of interesting biomimetic microsystems capable of mimicking the behaviour of complete complex organs, or at least of some of their significant functionalities.

These simplified replicas of human organ functionalities are being developed in the form of advanced labs-on-chips generically referred to as "organs-on-chips" and constitute a realistic and very adequate alternative for disease modeling and management, capable of providing even better results than the use of expensive and ethically concerning animal models.

This chapter has provided an introduction to the emerging area of organs-on-chips and detailed different complete development examples. The advantages of combining computer-aided design and engineering technologies with rapid prototyping and rapid tooling resources, for a more straightforward development, have been also analyzed.

Acknowledgements We gratefully acknowledge the support for the manufacture of high-precision prototypes and mass-produced replicas, which was carried out by the Karlsruhe Nano Micro Facility (KNMF, http://www.knmf.kit.edu/) a Helmholtz research infrastructure at the Karlsruhe Institute of Technology (KIT). Proposal KNMF-2013-010001532 (*In vitro model of the blood brain barrier (BBB on chip): Microsystem for dynamic co-culture of endothelial and neural cells under different conditions*), linked to the manufacture of precise blood-brain barrier chips, and proposal KNMF-2014-011002991 (*Liver on chip: Microsystem for co-culture of hepatocytes and endothelial cells*), linked to replicating the liver chips by micro-injection molding, and the co-authors and their teams that made them possible are acknowledged.

References

Chopra K, Mummery PM, Derby B, Gough JE (2012) Gel-cast glass-ceramic tissue scaffolds of controlled architecture produced via stereolithography of moulds. Biofabrication 4(4):045002

De la Guerra Ochoa E, Del Sordo Carrancio D, Echávarri Otero J, Chacón Tanarro E, Díaz Lantada A, Lafont Morgado P (2012) The influence of textured surfaces on the lubrication of artificial joint prostheses. In: Biodevices 2012—international conference on biomedical electronics and devices. IEEE Engineering in Medicine and Biology Society

Díaz Lantada A, Lafont Morgado P (2012) Rapid prototyping for biomedical engineering: current capabilities and challenges. Annu Rev Biomed Eng 14:73–96

Díaz Lantada A, Piotter V, Plewa K, Barie N, Guttmann M, Wissmann M (2015) Towards mass production of biomedical microdevices: linking rapid prototyping with micro-injection molding. Int J Adv Manuf Technol 76:1011–1020

Domian IJ, Chiravuri M, Van der Meer P, Feinberg AW, Shi X, Shao Y, Wu SM, Parker KK (2009) Generation of functional ventricular heart muscle from mouse ventricular progenitor cells. Science 326(5951):426–429

Gad-el-Hak (2003) The MEMS handbook. CRC Press, New York

Ho CT, Lin RZ, Chang WY, Chang HY, Liu CH (2006a) Rapid heterogeneous liver-cell on-chip patterning via the enhanced field-induced dielectrophoresis trap. Lab Chip 6:724–734

Ho CT, Lin RZ, Chang WY, Chang HY, Liu CH (2006b) Rapid heterogeneous liver-cell on-chip patterning via the enhanced field-induced dielectrophoresis trap. Lab Chip 6:724–734

Huang G, Wang L, Wang SQ, Yan H, Wu J, Zhang Q, Xu F, Liu TJ (2012) Engineering three-dimensional cell mechanical microenvironment with hydrogels. Biofabrication 4 (4):042001

Huh D, Hamilton GA, Ingber DE (2011) From 3D cell culture to organs on chips. Trends Cell Biol 21(12):745–754

Huh D, Kim HJ, Fraser JP, Shea DE, Khan M, Bahinski A, Hamilton GA, Ingber DE (2013) Microfabrication of human organs-on-chips. Nat Protoc 8:2135–2157

Infür R, Pucher N, Heller C, Lichtenegger H, Liska R, Schmidt V, Kuna L, Haase A, Stampfl J (2007) Functional polymers by two-photon 3D lithography. Appl Surf Sci 254:836–840

Jakab K, Norotte C, Marga F, Murphy K, Vunjak-Novakovic G, Forgacs G (2010) Tissue engineering by self-assembly and bio-printing of living cells. Biofabrication 2:022001

Kanani C, Gaudette GR (2010) Cell printing: a novel method to seed cells onto biological scaffolds. PhD thesis, Worcester Polytechnic Institute

Kucklick TR (2006) The medical device R&D handbook. CRC Press

Kuclick TR (2006) The medical device R&D handbook. CRC Press, Taylor and Francis Group

Lidstrom ME, Meldrum DR (2003) Life-on-a-chip. Nat Rev Microbiol 1(2):158–164

Mironov V, Trusk T, Kasyanov V, Little S, Swaja R, Markwald R (2009) Biofabrication: a 21st century manufacturing paradigm. Biofabrication 1(2):022001

Moraes C, Labuz JM, Leung BM, Inoue M, Chun TH, Takayama S (2013) On being the right size: scaling effects in designing a human-on-a-chip. Integr Biol 5(9):1149–1161

Pfleging W, Roth S (2011) Generating micro- and nanopatterns on polymeric materials, Weinheim, Wiley-VCH, pp 141–162. ISBN 978-3-527-32508-5

Pfleging W, Kohler R, Südmeyer I, Rohde M (2013) Laser micro and nano processing of metals, ceramics, and polymers. In: Majumdar JD, Manna I (eds) Laser-assisted fabrication of materials, Springer-Verlag. ISBN 3642283594

Pfleging W, Pröll J (2014) A new approach for rapid electrolyte wetting in tape cast electrodes for lithium-ion batteries. J Mater ChemA 14918–14926

Queste S, Salut R, Clatot S, Rauch JY, Khan Malek CG (2010) Manufacture of microfluidic glass chips by deep plasma etching, femtosecond laser ablation, and anodic bonding. Microsyst Technol 16(8–9):1485–1493

Shuler ML (2012) Modeling life. Ann Biomed Eng 40(7):1399–1407

Stampfl J, Fouad H, Seidler S, Liska R, Schwanger F, Woesz A, Fratzl P (2004) Fabrication and moulding of cellular materials by rapid prototyping. Int J Mater Prod Technol 21(4):285–296

Stampfl J, Baudis S, Heller C, Liska R, Neumeister A, Kling R, Ostendorf A, Spitzbart M (2008) Photopolymers with tunable mechanical properties processed by laser-based high-resolution stereolitoraphy. J Micromech Microeng 18:125014

Waldbaur A, Rapp H, Länge K, Rapp BE (2011) Let there be chip—towards rapid prototyping of microfluidic devices: one-step manufacturing processes. Anal Methods 3:2681–2718

Wei ZJ, Amponsah PK, Al-Shattic M, Nie ZH, Bandyopadhyay BC (2012) Engineering of polarized tubular structures in a microfluidic device to study calcium phosphate stone formation. Lab Chip. doi:10.1039/C2LC40801E

Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE (2001) Soft-lithography in biology and biochemistry. Annu Rev Biomed Eng 3:335–373

Wilhelm I, Fazakas C, Krizbai IA (2011) In vitro models of the blood-brain barrier. Acta Neurobiol Exp 71:113–128

Wissmann M, Guttmann M, Hartmann M, Beiser M (2010) Alternative technology for fabrication of nano- or microstructured mould inserts used for optical components. Proceedings of the SPIE, vol 7590

Wissmann M, Barié N, Guttmann M, Schneider M, Kolew A, Besser H, Pfleging W, Hofmann A, Van Erps J, Beri S, Watté J (2015) Mould insert fabrication of a single-mode fiber connector alignment structure optimized by justified partial metallization. J Micromech Microeng 25:035008

Wohlers T (2010) The Wohlers' report. Wohlers' Associates

Chapter 23
Towards Effective and Efficient Biofabrication Technologies

Andrés Díaz Lantada

Abstract The artificial production, in laboratories, of biological structures and even complete organs, by adequately placing and combining ex vivo cells, synthetically produced tissue patches and supporting biomaterials, including but not limited to tissue engineering scaffolds, is no more a matter of science fiction but a present relevant research challenge already providing promising results, included under an innovative area called "biofabrication". If larger biological structures and complete organs could be synthetically obtained, patients would benefit from more rapid surgical interventions, compatibility would be highly promoted, as they would be produced ex vivo from the own patient's cells, and aspects such as organ piracy would be limited. It is important to highlight that nowadays around 10 % of organs used for transplantation worldwide comes from illegal activities. The socio-economical impact of synthetic organ production is comparable to that of the whole pharmaceutical industry, what explains the interest it has arisen in the last decades, with several new companies and research centres worldwide aiming at improving state-of-the-art tissue engineering procedures for starting 3D tissue construction and organ biofabrication. In addition novel scientific journals and book series are being devoted to these advances and related concepts and techniques are starting to be included in the syllabuses of teaching programs at universities, what will for sure be very positive for the evolution of this area. This chapter provides a brief introduction to this field of research, discussing most relevant advances on materials science, design tools and manufacturing technologies that being combined for making biofabrication a viable alternative to conventional therapeutic procedures. Main present difficulties and remarkable research challenges are also discussed. It constitutes an updated version of "Chap. 14: Biofabrication: Main advances and Challenges" from Springer's "Handbook on Advanced Design and Manufacturing Technologies for Medical Devices" also by Andrés Díaz Lantada (Díaz Lantada 2013).

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory,
Universidad Politécnica de Madrid, c/José Gutiérrez Abascal, 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

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23.1 Introduction: The Manufacture of Biological Systems

We have seen that in the context of medical device development and the biomedical industry, one of the major areas of application for these rapid prototyping technologies is tissue engineering. Since the 1980s, outstanding researchers like Eugene Bell and Robert S. Langer, both professors at the MIT, began looking at how to produce scaffolds with materials and geometries that were suitable for cell culture and tissue growth and could be used in surgical operations (Langer and Vacanti 1993).

The gradual progress in the field of biodegradable polymers together with the advances in more flexible rapid manufacturing technologies, means that at present, complex geometry scaffolds can be obtained to which living cells with growth factors adhere, and which multiply until they cover the scaffold. Having reached this stage, the set (scaffold + coating) is implanted into the damaged parts of the body. After being implanted, the cells adapt to their environment and reproduce the functions of the surrounding tissue, while the scaffold is gradually reabsorbed (Hollister 2005; Gómez Ribelles et al. 2010). All this has led to important changes in the approach to solving many surgical problems.

Further research efforts, with a view to obtaining three-dimensional biological structures, will one day lead to the additive fabrication of complex human organs (Atala and Yoo 2015). The firm EnvisionTec GmbH has already developed the Bioplotter® with which small three-dimensional structures are being obtained by the "layer by layer" deposit of cells together with biocompatible material, and the initial use of the concept of a "bioprinter" looks promising. The company Lithoz stands out for high precision ceramic manufacture in an additive way, with which bone-like structures can be manufactured with micrometric precision. A larger number of printing technologies based on appropriately modified conventional RP technologies are being sought, which is progressively leading to more affordable machines (DigiLab Inc., Formlabs…).

This progress may open up new horizons to the treatment of many diseases by combining synthetic and biological materials to produce veins, capillaries, arteries, bones and soft organs, or at least part of them. By using machines with several heads that can deposit different materials biological tissue could be directly obtained with synthetic implants pre-integrated into them. This would endow the newly generated tissue with mechanical consistency.

However, there is still a long way to go, not only regarding the precision of these "bioprinters" and the biological and biomedical materials they are capable of depositing, but also regarding the manufacture of structures larger than 1 cm^3. It would appear that the development of a capillary network to provide the newly generated three-dimensional cell structures with nutrients is currently one of the major limitations (Mironov et al. 2009; Bartolo and Bidanda 2008; Bartolo et al. 2009). There is also an important need for further progress in the design field, so as to obtain more adequate biomimetic CAD files, for subsequent manufacture of biostructres.

Relevant progresses in the field of high-precision medical imaging, together with software for handling such medical images as design inputs, are proving to be a key for further developments in the field.

Organising specific work sessions to facilitate information exchange among researchers is a particularly useful idea, usually within a framework of Bioengineering congresses, where rapid prototyping applications in the medical sector, especially those oriented to biofabrication, can be discussed, and people can join forces to go forward together. Worth mentioning are the "World Bioprinting Congresses", the "International Workshop on Bioprinting and Biopatterning" and the "International Conferences on Biomedical Electronics and Devices—Biodevices 2008–2015. Relevant journals in this novel field include "Bioinspiration and Biomimetics" and "Biofabrication".

This chapter provides a brief introduction to these topics, after discussing main applications of biofabrication for the biomedical field in next section.

23.2 The Potential of Biofabrication and Its Application Fields

The final objective of research in the biofabrication area is the artificial production of organs and biological structures in laboratories, by adequately placing and combining ex vivo cells, synthetically produced tissue patches and supporting biomaterials. If organs could be artificially produced patients would benefit from more rapid surgical interventions, compatibility would be highly promoted, as they would be produced ex vivo from the own patient's cells, and aspects such as organ piracy would be limited. The applications in Medicine, if this final objective is achieved, are endless, however partial results, in the way to final achievement, are already providing interesting applications briefly discussed here.

Advances in the field of biofabrication are actually improving tasks and procedures linked to Tissue Engineering, as novel machines allow for the combined manufacture of biosubstrates with incorporated living cells and nutrients, hence enhancing cell growth and tissue formation for transplantation (Jakab et al. 2010).

Some biodevices for surgical interventions, such as sutures, are being seeded with cells (normally hMSCs) with the help of 3D printers designed ad hoc, thus accelerating tissue repair and recovery from surgical procedures (Kanani 2012).

New materials and biomaterials are continuously being discovered, in the search for more adequate substrates and supports for cell growth, and special attention is being paid to unconventional biomaterials as candidates for Tissue Engineering, as well as for other fields of technology, such as secretions from animals and plants (spider silk, plant resins…) (Lenaghan et al. 2011).

In addition, progresses on imaging technologies, aimed initially at improving diagnosis, if adequately combined with design and modeling tools, are also being of

help for promoting biomimetic designs, but also for replicating the structures of novel bio- and meta-materials and in silico assessing their behaviour, as detailed in next section.

23.3 Advances and Challenges Linked to Biomaterials

Materials Science has devoted great efforts in the last decades of 20th Century to the development (mainly synthesis/extraction and processing) of new materials and material families (such as polymers, polymer-matrix composites, metallic foams, super alloys…) and main advances during the first decade of the 21st Century focus also on that direction (artificial muscles, biopolymers, materials from natural origin…). These advances have completely changed the engineering world and reshaped the whole product development process, with outstanding impact in several fields including automation, aeronautics, architecture, design, electronics, information and communication technologies, energy and biosciences.

Parallel advances in design and simulation tools are providing very adequate resources for modeling such novel and often complex materials, whose behaviour is in many cases not yet fully characterized or understood. Therefore, besides the continued search for new materials capable of producing biocompatible devices, additional challenges linked to characterization and precise simulation are also needed for promoting the global biodevice development process.

Some relevant characterization tools (both oriented to biomaterials and to more specific biodevices) are normally oriented to an assessment of overall long-term mechanical performance and stability. More linked to modeling tasks are advances in medical imaging technologies, especially micro-CT, whose current precision, reaching around 25–50 μm, is high enough for the detailed reconstruction of most corporal structures (Shi et al. 2008; Guo et al. 2010).

The use of micro-CT technology to the 3D reconstruction of complex materials (and biomaterials), for subsequent modeling and simulation linked to studies in the field of Materials Science, is already common place. Once reconstructed, these materials can also be used, thanks to Boolean operations, for designing the inner structure of several biomimetic biodevices and prostheses. The linkage between medical imaging, CAD programs and FEM-based simulation modules can be a great help for assessing the adequate performance of a biomimetic structure, once adapted to the geometry of novel prostheses and biodevices, before the investing in the manufacture of prototypes for pre-production validation trials.

Reconstructions of the glass fibers of composite materials, of nickel foams and of porous woods can be found as cases of study in the website of SkyScan micro-CT company. Similar results can be obtained from micro-CT of polymers, ceramics, bioplolymers and other biomaterials such as bone, as well as biostructures, what can help to design biomimetic scaffolds for tissue engineering and supports for biofabrication strategies.

23.4 Advances and Challenges Linked to Biodesign Tools

Advances for promoting biofabrication approaches are not only linked to finding more adequate materials and processing technologies compatible with cell deposition, but also to additionally exploring design processes capable of providing alternative approaches or complementary solutions, to those based on medical imaging-based reconstructions.

The term "Biomimesis" (from Greek "bios"—life and "mimesis"—imitation) is linked to the study of Nature's models, principles, designs and processes to imitate them or find new inspiration for solving human problems (Benyus 2002). Main applications of biomimesis are aimed at finding new ways of producing food or energy, novel methods of manufacture, innovative therapeutic solutions and overall management of mankind and its relations.

As already detailed in cases of study along the Handbook, three important possibilities for promoting the development of biomimetic devices are: (1) the use of multi-scale mathematical modeling of biostructures, (2) the use of computer-aided design resources and recursive procedures towards biomimetic fractal geometries and (3) the use of medical imaging-based reconstructions. Figure 23.1 shows a biomimetic vascular model manufactured by means of additive technologies and designed by means of recursive procedures towards a fractal-like biostructure. The model is just aimed at providing an idea of the complexity of geometries capable of being additively manufactured and of the potential of these resources towards solving the problem of vascularization, which constitutes an unsolved challenge in the field of tissue engineering, repair and regeneration (Boccaccini et al. 2012).

The use of multi-scale manufacturing approaches is also enabling the production of biomedical microsystems with biomimetic features for enhanced interaction at a cellular level (see Chap. 8). Chapter 13 also already introduced the possibility of using fractal models for the design of biodevices with controlled surface properties or geometries imitating those from the body for an optimized biomimetic response.

In any case it seems clear that the incorporation of novel design tools into the conventional computer-aided design resources is a relevant need for supporting industrial designers facing the challenge of designing biomimetic devices. Even resources coming from the cinematographic industry may be of help, as several software used for the incorporation of artificial textures in digitally designed 3D geometries may be adapted to industrial design and rapid prototyping tasks.

It is necessary to note that, thanks to advances in the linkage between medical imaging technologies and CAD-CAE-CAM resources, once more multipurpose and effective 3D bioprinters are developed (capable of manufacturing even whole implantable organs), the reconstructions will probably be carried out on the basis of original information taken from the patient's body, so as to provide personalized solutions.

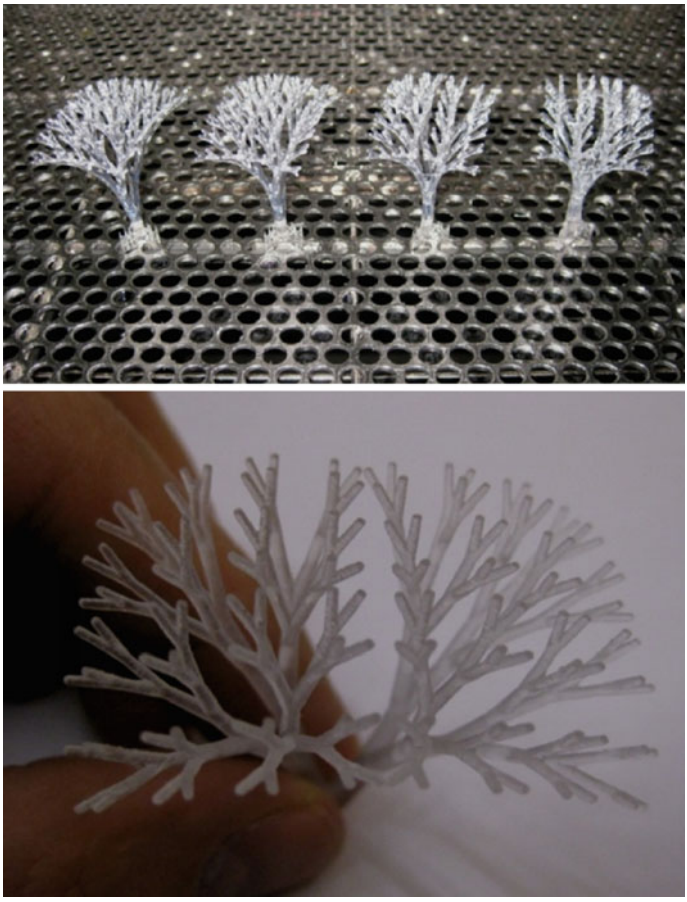

Fig. 23.1 Example of self-supported branched networks based on fractal biomimetic designs carried out by multi-scale computer-aided design

23.5 Advances and Challenges Linked to Biomanufacturing Resources

Conventional desktop-printers deposite micro-bubbles of ink, with remarkable precision, for writing documents and state-of-the-art very simple 3D printers (see information provided by the wiki of the "RepRap" project) are also capable of extruding fused polymers, gels and even molten chocolate, for obtaining three-dimensional prototypes with complex geometries in different materials.

Therefore the technology for depositing cells, coming within a liquid or gel-like matrix, and further constructing sheets and three-dimensional tissues, already exists. A simple combination of already available resources and additional research,

focused on supporting such cell growth, through an adequate vascularization and nutrient supply, are making biofabrication a reality.

Relevant recent results have already been obtained by using alternative methods, such as laser printing of cells into 3D scaffolds, which uses the propulsive force from laser-induces shock wave to propel cells gently into a substrate (Ovsianikov et al. 2010) or layer-by-layer extrusion of gelatin/alginate with seeded stem cells, for bioprinting small 3D biostructures (Norotte et al. 2009; Li et al. 2009; Marga et al. 2012).

The use of concurrent additive manufacture of scaffolding structures based on biodegradable thermoplastics and cells suspended in gels (different extruders would print different materials, as support, and provide also cells and nutrients), has also been proposed (Melchels et al. 2012).

In fact some commercially available resources already exist, which are already providing excellent support to research tasks linked to further advances in these directions, as detailed further on.

In Europe, EnvisionTec GmbH provides its "3D-Bioplotter™" (already in its 4th generation). The "3D-Bioplotter™" stands out for its versatility, as it can build parts by combining up to 5 materials with automated tool change, for its fast plotting speed, while maintaining appropriate accuracy, and for the possibility of printing up to 5 types of cells per object. Actually the "3D-Bioplotter™" has the capacity of fabricating scaffolds using the widest range of materials of any singular rapid prototyping machine, from soft hydrogels and biomaterials (agar, alginate, fibrin, chitosan, collagen, gelatin), over polymer melts (PLLA, PCL, PLGA), up to hard ceramics (hydroxyapatite, tricalcium phosphate) and metals (titanium), although these last harder materials require a sintering post-process.

In the United States, Digilab Inc. offers its "Cell Jet Cell Printer", which stands out for its special focus on gentle cell deposition and for handling and delivering cell suspensions. Some tailoring to final application is also affordable. The current and potential uses of the cell printer include, but are not limited to:

- Delivering cell suspensions into micro-fluidic chips/high throughput cell based assay platforms.
- Dispensing cell suspensions in customized patterns to form microarrays on standard or custom microscope slides (or other substances), most commonly for developing/performing cell based assays.
- Delivering cell suspensions (in cell culture media or hydrogels) at defined locations in 2 and 3 dimensions onto pre-formed scaffolds (such as biological sutures/tissue construct scaffold), in order to populate the scaffold.
- Dispensing cell suspensions in custom patterns on a surface, for migrational studies or to study interaction of cells amongst each other or with growth factors.
- Delivering cell suspensions to micro-wells in a various diagnostic/research devices made of silicon, PDMS, or other substances, where manual delivery of sample is difficult, time consuming or simply impossible.

- Dispensing other reagents or growth factors or biologically relevant substances in a suspension, in addition to cells, to targeted locations/patterns in a similar manner.
- De novo biofabrication of relatively simple tissue constructs.

Researchers wishing to obtain additional information on related advances may wish to visit Digilab's website (www.digilabglobal.com) and have a more detailed look at the conference papers and publications linked to the use of the "Cell Jet Cell Printer" and related "synQUAD Technology" (capable of dispensing drop-by-drop down to 20 nL and up to several microliters of fluids).

Main challenges of bioplotters and cell printers are still linked to constructing more complex and bigger tissue constructs mimicking the complex structures of complete organs. Combined advances in medical imaging, design technologies and materials science will surely find solutions to such challenges, as the "hardware" (automated bio-manufacturing machines) for biofabrication is already working properly and providing effective solutions.

23.6 Main Conclusions and Future Research

The artificial production, in laboratories, of organs and biological structures, by adequately placing and combining ex vivo cells, synthetically produced tissue patches and supporting biomaterials, is no more a matter of science fiction but a present relevant research challenge already providing promising results, included under an innovative area called "biofabrication".

If organs could be artificially produced patients would benefit from more rapid surgical interventions, compatibility would be highly promoted, as they would be produced ex vivo from the own patient's cells, and aspects such as organ piracy would be limited. The actual socio-economical impact of synthetic organ production is even comparable to that of the whole pharmaceutical industry, what clearly explains the interest it has arisen in the last decade, with several new companies aiming at improving state-of-the-art tissue engineering procedures for starting 3D tissue construction.

This chapter has aimed to provide a brief introduction to this field of research, discussing some relevant advances on materials science, design tools (either based on analytical modeling or on digital reconstruction) and manufacturing technologies that are currently working for making biofabrication a viable alternative to conventional therapeutic procedures.

Even though main challenges of bioplotters and cell printers are still linked to constructing more complex and bigger tissue constructs, mimicking the complex structures of complete organs, combined advances in medical imaging, design technologies and materials science are already providing interesting solutions and the "biomanufacturing machines" are already commercial and effective. Final whole organ printing is just a matter of time.

The promotion of collaboration between researchers may prove essential for reaching final objectives of biofabrication in perhaps a couple of decades, for instance following the example of the "RepRap" project collaborative wiki, which is encouraging many researchers to introduce additive manufacture as an additional support for their research.

These kinds of "do-it-yourself" rapid prototyping machines can also be adapted to 3D printing of biomaterials and cells, as an easy and affordable way of obtaining resources for conceptual validations linked to tissue engineering and biofabrication.

References

Atala A, Yoo JJ (2015) Essentials of 3D biofabrication and translation. ISBN 9780128010150, Academic Press, Elsevier

Bartolo PJS, Bidanda B (2008) Biomaterials and prototyping applications in medicine. Springer

Bartolo PJS, Almeida H, Laoui T (2009) Rapid prototyping and manufacturing for tissue engineering scaffolds. Tissue Eng 36(1):1–9

Benyus JM (2002) Biomimicry. innovation inspired by nature. Harper Collins Publishers

Boccaccini A, Kneser U, Arkudas A (2012) Scaffolds for vascularized bone regeneration: advances and challenges. Expert Rev Med Devices 9(5):457–460

Díaz Lantada A (2013) Handbook of advanced design and manufacturing technologies for biomedical devices. Springer

Gómez Ribelles JL, Monleón Pradas M, García Gómez R, Forriol F, Sancho-Tello M, Carda C (2010) The role of three-dimensional scaffolds in the regeneration of joint cartilage. In: Biodevices 2010—international conference on biomedical electronics and devices: special session on rapid prototyping for improving the development of biodevices. IEEE Eng Med Biol Soc, 20–23 Jan 2010 (in Valencia)

Guo X, Liu X, Zhang B, Hu G, Bai J (2010) A combined fluorescence and microcomputer tomography system for small animal testing. IEEE Trans Biomed Eng 58:2876–2883

Hollister SJ (2005) Porous scaffold design for tissue engineering. Nat Mater 4:518–524

Jakab K, Norotte C, Marga F, Murphy K, Vunjak-Novakovic G, Forgacs G (2010) Tissue engineering by self-assembly and bio-printing of living cells. Biofabrication 2:022001

Kanani C (2012) Cell printing: a novel method to seed cells onto biological scaffolds. Ph.D. Thesis, Worcester Polytechnic Institute

Langer RS, Vacanti JP (1993) Tissue engineering. Science 260:920–926

Lenaghan SC, Serpersu K, Xia L, He W, Zhang M (2011) A naturally occurring nanomaterial from the Sundew (Drosera) for tissue engineering. Bioinspir Biomim 6:0460009

Li SJ, Xiong Z, Wang XH, Yan YN, Liu XH, Zhang RJ (2009) Direct fabrication of a hybrid cell/hydrogel construct by a double-nozzle assembling technology. J Bioact Biocompat Polym 24:249–265

Marga F, Jakab F, Khatiwala C, Shepherd B, Dorfman S, Hubbard B, Colbert S, Gabor F (2012) Toward engineering functional organ modules by additive manufacturing. Biofabrication 4:022001

Melchels FPW, Domingos MAN, Klein TJ, Malda J, Bartolo PJ, Hutmacher DW (2012) Additive manufacturing of tissues and organs. Prog Polym Sci (in press). doi:10.1016/j.progpolymsci.2011.11.007

Mironov V, Trusk T, Kasyanov V, Little S, Swaja R, Markwald R (2009) Biofabrication: a 21st century manufacturing paradigm. Biofabrication 1(2):022001

Norotte C, Marga FS, Niklason LE, Forgacs G (2009) Scaffold free vascular tissue engineering using bioprinting. Biomaterials 30:5910–5917

Ovsianikov A, Gruene M, Pflaum M, Koch L, Maiorana F, Wilhelmi M, Haverich A, Chichkov B (2010) Laser printing of cells into 3D scaffolds. Biofabrication 2:014104
Shi H, Farag AA, Fahmi R, Chen D (2008) Validation of finite element models of liver tissue using micro-CT. IEEE Trans Biomed Eng 55:978–985

Some Interesting Related Websites

http://reprap.org/wiki/RepRap
http://www.digilabglobal.com
http://www.digilabglobal.com/celljet
http://www.envisiontec.de
http://www.skyscan.be

Chapter 24
Project-Based Learning in the Field of Biomedical Microdevices: The CDIO Approach

Andrés Díaz Lantada, Milagros Ramos Gómez, José Javier Serrano Olmedo, Miguel Ángel Cámara Vázquez and Borja Domínguez Nakamura

Abstract In this chapter we present the complete development of a novel course on "Biomedical Devices", in the framework of the "Biomedical Engineering" Degree at Universidad Politécnica de Madrid (TU Madrid). The course is based on the "CDIO: Conceive, Design, Implement, Operate" approach, as we consider it a very remarkable way of promoting student active learning and of integrating, with impact, novel concepts into ongoing curricula. During the course, groups of students live through the complete development process of different biomedical devices aimed at providing answers to relevant social needs. Computer-aided engineering and rapid prototyping technologies are used as support tools for their designs and prototypes, so as to rapidly reach the implementation and operation phases. Main benefits, lessons learned and challenges, linked to this CDIO-based course, are analyzed, considering the results from 2014–2015 academic year. Some of the most remarkable biodevices developed by students are linked to the field of biomedical microdevices for interacting at a cellular level, the central topic of present Handbook. The complete development of two bioreactors, which have led to Master's Degree Theses, after additional tasks carried out in parallel to the course, is also schematized and presented as one of the most remarkable results of the teaching-learning strategy.

A. Díaz Lantada (✉)
Mechanical Engineering Department – Product Development Laboratory, Universidad Politécnica de Madrid (UPM), c/José Gutiérrez Abascal 2, 28006 Madrid, Spain
e-mail: adiaz@etsii.upm.es

M. Ramos Gómez · J.J. Serrano Olmedo · M.Á. Cámara Vázquez · B. Domínguez Nakamura
Centre for Biomedical Technology, Universidad Politécnica de Madrid, UPM Scientific and Technological Park, Montegancedo Campus, Crta. M40 - Km 38, 28223 Pozuelo de Alarcón, Spain

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24.1 Introduction

Biomedical engineering a quite recent engineering field, as the first Biomedical Engineering programmes appeared at US universities in the late 1950s, with Drexel University, the Johns Hopkins University, the University of Pennsylvania and the University of Rochester as outstanding pioneers. In the late 1960s and 1970s other relevant universities followed them, including: Boston University, Carnegie Mellon, Harvard and MIT, Ohio State University, the University of Illinois, among other interesting examples (Fagette 1999). Biomedical Engineering is aimed at the application of engineering principles, methods and design concepts to medicine and biology for healthcare purposes, mainly as a support for preventive, diagnostic or therapeutic tasks. Biomedical Engineering professionals are expected to achieve, during their studies and professional practice, considerable knowledge of both health sciences and engineering. Studying Biomedical Engineering programmes or combining pre-graduate studies in life sciences with graduate studies in engineering, or vice versa, are typical options for becoming qualified biomedical engineering professionals, although there are additional interesting alternatives. According to our experience, graduates and post-graduates from more traditional and multidisciplinary engineering programmes, especially industrial and telecommunications engineers can play varied and very relevant roles in the biomedical industry and in extremely complex biomedical device development projects. In any case, the impact of future professionals of the biomedical engineering field can be importantly increased, by means of an adequate integration of biomedical engineering design concepts, methodologies and good practices into traditional and modern engineering curricula.

In fact, according to the Biomedical Engineering Society, a biomedical engineer uses traditional Engineering expertise to analyze and solve problems in Biology and Medicine, providing an overall enhancement of healthcare. Students choose the Biomedical Engineering field to be of service to people, to partake of the excitement of working with living systems and to apply advanced technology to the complex problems of medical care. The biomedical engineer works with other healthcare professionals including physicians, nurses, therapists and technician. Biomedical Engineers may be called upon in a wide range of capacities: to design instruments, devices and software, to bring together knowledge from many technical sources, to develop new procedures, or to conduct research needed to solve clinical problems (BMES). The aforementioned duties are directly connected to the traditional corpus of Engineering (in its broadest sense) and, being applied tasks in direct relation with real and complex problems (pathologies) and systems (human body), can potentially be taught and promoted by means of project-based learning approaches. The "CDIO" (Conceive—Design—Implement—Operate) cycle is probably the most complete project-based learning approach for the systematic promotion of complex problem solving (Crawley et al. 2007) and may impact the future of Biomedical Engineering, as it is already doing in several Engineering fields.

In this chapter we present the complete development of a novel course on "Biomedical Devices", in the framework of the "Biomedical Engineering" Degree at Universidad Politécnica de Madrid (TU Madrid). The course is based on the CDIO approach, as we consider it a very remarkable way of promoting student active learning and of integrating, with impact, novel concepts into ongoing curricula. During the course, groups of students live through the complete development process of different biomedical devices aimed at providing answers to relevant social needs. Computer-aided engineering and rapid prototyping technologies are used as support tools for their designs and prototypes, so as to reach the implementation and operation phases more rapidly. Main benefits, lessons learned and challenges, linked to this CDIO-based course, are analyzed, considering the results from 2014–2015 academic year.

Some of the most remarkable biodevices developed by students are linked to the field of biomedical microdevices for interacting at a cellular level, the central topic of present Handbook. The complete development of two bioreactors, which have led to Master's Degree Theses, after additional tasks carried out in parallel to the course, is also schematized and presented as one of the most remarkable results of the teaching-learning strategy. These complete developments help to highlight the benefits of the proposal regarding student motivation. The acquired skills and professional outcomes are also noteworthy. The possibility of focusing on the development of microdevices for interacting at a cellular level, as main development topic for future editions of the course, will be further explored by the team of teachers.

24.2 The "Biomedical Devices" Course of the "Biomedical Engineering" Degree at TU Madrid

The "Biomedical Engineering" Degree at TU Madrid is a 240-ECTS-credit programme (1 credit corresponds to 25 h of student workload according to the European Credit Transfer System) aimed at training the "biomedical engineers of the future", not just as a support for the development of the biomedical industry in Spain, but as key players in an international context. The programme started in academic year 2011–2012, just after the implementation of the European Area of Higher Education, and the first graduates have just finished, after academic year 2014–2015. It is important to note that TU Madrid has been training programmes and courses related to the field of biomedical engineering almost since its foundation in 1971 and carries out relevant research linked to biomaterials, biodevices, biomechanics, biomedical signals and biomedical equipments, all of which has played a relevant role in the implementation of the Degree.

In short, the design and contents of the Degree are structured so as to provide students with the necessary concepts, knowledge, skills and professional outcomes required to improve or develop new products, processes and services with impact in the biomedical industry and in the socio-medical services. Being able to face and

solve real medical problems, normally working in multidisciplinary teams, is central to the Degree. The structure includes three semesters of basic courses (mainly Maths, Physics, Chemistry and Biochemistry, Biology and ICTs), three semesters of compulsory courses (mainly Physiology, Signals and Algorithms, Biomechanics, Biomaterials, Cellular and Tisular Biology, Clinical Engineering and Management) and two final semesters for the specialization. Four specializations are available, namely: "Biodevices, biomaterials and biomechanics", "Biomedical informatics", "Biomedical signals" and "Telemedicine".

The "Biomedical Devices" course is placed in the last semester and is compulsory for those students taking the "Biodevices, biomaterials and biomechanics" specialization, although it can be also selected as optional course by students from other itineraries. The course is aimed at providing students with a systematic methodology for the successful and straightforward development of biomedical devices and systems, including prostheses, ortheses, technical helps and laboratory equipment, among other options. The course is also planned as a possibility for students to tackle, for the first time, the complete development of biomedical devices, linked to real diagnostic and therapeutic challenges. Hence, the experience acquired within the course may help them to improve the planning and development of their final degree projects, which are normally linked to new biomedical engineering products and systems, and have a positive impact on their future professional practice. The course counts with the following moduli:

- Introduction to biomedical devices and to the biomedical industry.
- The systematic development process of engineering product and systems: Biomedical engineering design.
- Conceptual design, promotion of creativity and intellectual property issues.
- Sustainable development of biomedical devices.
- Design and simulation of biomedical devices.
- Prototyping and testing of biomedical devices.
- Management of signals and energy.
- Integration, maintenance and recyclability issues.
- Standardization and security issues.
- Recent advances and future perspectives.

The different lessons are planned with an introductory theoretical part followed by different hands-on activities and by the illustration of several cases of study linked to real development of medical devices carried out by the group of teachers and their research teams. Among the different developments used as cases of study it is important to mention the use of designs, simulations and prototypes of expandable stents, active annuloplasty rings, personalized heart valve prostheses, personalized hip prostheses, instrumented splints for monitoring bruxisms, labs-on-chips and organs-on-chips and several types of scaffolds for tissue engineering and cell culture platforms, many of which have already been detailed in different chapters of present Handbook. The idea is to incorporate the products developed by students themselves as additional cases of study for forthcoming editions.

24.3 Learning Objectives, Desired Outcomes and Teaching Methodology: The "CDIO" Approach

According to the central purpose of the course detailed in previous section, some learning objectives include making students: (a) aware of main types of medical devices and their applications; (b) capable of understanding and applying systematic methodologies for the development of medical devices; (c) able to take advantage of advanced design and manufacturing technologies for the development of biomedical devices; (d) conscious of the social relevance of biomedical devices and of the relevance of taking into account sustainability along the whole life cycle.

The general and specific outcomes of the course are also well integrated within the strategy of the whole degree and are listed below, including the codes used in the "Biomedical Engineering" Degree Report used for verification by the Spanish Agency of Accreditation (ANECA).

General outcomes of the course:

- CG1. To develop autonomous learning abilities.
- CG2. To understand and master the basics of Biomedical Engineering.
- CG6. To solve engineering problems using multi-objectvie approaches.
- CG7. To apply the scientific method to solving biomedical problems.
- CG13. To be able to work in multidisciplinary teams.
- CG18. To acquire a social and ethical compromise.

Specific outcomes of the course:

- CE38. To understand and apply principles and techniques for measuring the most relevant magnitudes in the field of Biomedical Engineering.
- CE39. To develop and use sensors and actuators for solving diagnostic and therapeutic challenges in the field of Biomedical Engineering.
- CE40. To understand the relevance of biomedical devices and to acquire an overview of the devices used for integral health management.
- CE41. To develop biomedical devices taking advantage of computer-aided design, engineering and manufacturing resources.

In our opinion, as the learning objectives and expected outcomes of the subject involve several domains and levels, according to Bloom's taxonomy (Bloom et al. 1956) the best option is to promote a project-based learning approach. As the course is linked to the field of medical devices, it seems very appropriate to divide the students into groups and let each group develop a different medical device, while addressing a relevant diagnostic or therapeutic challenge. As we want the student to live the whole product life-cycle or, at least, the most relevant parts of the life-cycle from an engineering perspective, we decide to follow the "CDIO: Conceive-design-implement-operate approach", following CDIO standards, with the hope of reaching some prototypes for initial validations of novel concepts.

Being a one-semester subject, we were conscious of the difficulty of living the whole cycle only in the framework of the course, so we let students work on tasks

related to their final degree projects, hence focusing on the design, implementation and operation stages, after an already existing concept. Another option was to use the course for finding a relevant concept and obtaining a very detailed design, for eventual further development after the subject, which could lead to the manufacture and exhaustive testing of prototypes required for completing a final degree thesis. We let students freely decide the biodevices to develop, as the "Biomedical Engineering" Degree is extremely multidisciplinary and we wanted them to exploit and promote their more relevant personal skills. At the beginning, students were advised that those selecting more complex devices would normally focus on the "conceive" and "design" stages, while those proposing more simple products would be expected to reach and concentrate on the "implementation" and "operation" steps.

The teaching-learning methodology is also based on previous very successful experiences by our team (Díaz Lantada et al. 2013) and follows systematic strategies for the promotion and assessment of professional outcomes (Shuman et al. 2005; Hernández Bayo et al. 2014). Main results from the first implementation are detailed in the following section.

24.4 Results Obtained: Designs, Prototypes, Trials

During academic year 2014–2015 a total of 13 students took part in the course and a total of 7 biomedical engineering products or systems were developed, six of them by students working in couples and one of them by a single student, who preferred to work on his own for entrepreneurial reasons. During the first 2 weeks of the semester, students were presented with an overview of biodevices focusing on very different medical problems and faced with many of the most relevant medical challenges, as listed by the World Health Organization, in order to make them aware of possible needs to focus on. After the first couple of weeks, they were asked to think and propose the biomedical devices they would like to develop during the subject. The products selected and developed are listed below:

- Medical device for rapid assessment of gluten-free food.
- Device for assisted drinking and control of fluid intake.
- Self-sensing napkins for dependent patients.
- Electrically assisted wheelchair.
- Portable infant warmer for newborns.
- Bioreactor for electrical stimulation of cell culture processes.
- Mobile phone application for assisted dermatology.

The first six devices were developed in couples. The last one is a software-based system, which was developed by just one student. The "mobile phone app", the "bioreactor for cell culture" and the "device for assisted drinking" were further developed and led to a total of four final degree theses.

Table 24.1 Summary of the degree of development reached and of the final marks obtained by the different groups of students

Biodevice	Development stage reached by students				Mark obtained (out or 10)
	Concept	Design	Prototype	Operation	
Rapid gluten detector	x	x			9.25
Aided drinking device	x	x	x		9.5
Self-sensing napkins	x	x			9.75
Assisted wheelchair	x				9
Portable infant warmer	x	x			8.5
Mobile app. for dermatology	x	x	x	x	9.9
Bioreactor for cell culture	x	x	x	x	10

Table 24.1 includes a summary of the degree of development reached and of the final marks obtained by the different groups. It is important to note that the mark is not directly related to the development step reached, as some devices are extremely complex to develop. We mainly focus on student workload, on their degree of innovation and on the level of detail demonstrated in every step of the product development process. The marks are finally individualized by considering participation during the lessons, individual homework and public presentation of results. The projects developed and assessed, with a single mark for each group (that of Table 24.1), account for a 70 % or the final mark, while the individual components account for a 30 %.

Interestingly, the best development was linked to a biomedical microdevice for cell culture, based on a concept by Prof. Milagros Ramos, in which a couple of students (Miguel Ángel Ramos and Borja Domínguez) were already working at the beginning of the subject as topic for their respective final degree theses. They found that the subject might provide some interesting lessons and resources for design and prototyping tasks and proposed to carry out two different designs. To sum up, they developed a couple of multi-chamber cell culture devices with the possibility of electrical stimulation for controlling cellular response. Figures 24.1 and 24.2 schematically show the design and prototyping process of two alternative bioreactor designs. A first set of prototypes and molds was obtained by means of laser stereolithography (SLA-3500 by 3D Systems) and the final chambers for cell culture were obtained by vacuum casting of PDMS (blue) in the epoxy molds.

After prototyping, both devices were tested at the facilities of the Centre for Biomedical Technology at TU Madrid with positive results regarding cell culture viability, although further improvements were carried out and incorporated to the Master Theses of Miguel Ángel and Borja. It is very important to highlight that this teaching-learning experience has led to novel research collaborations between different departments and faculties at TU Madrid. Typically, research activities are

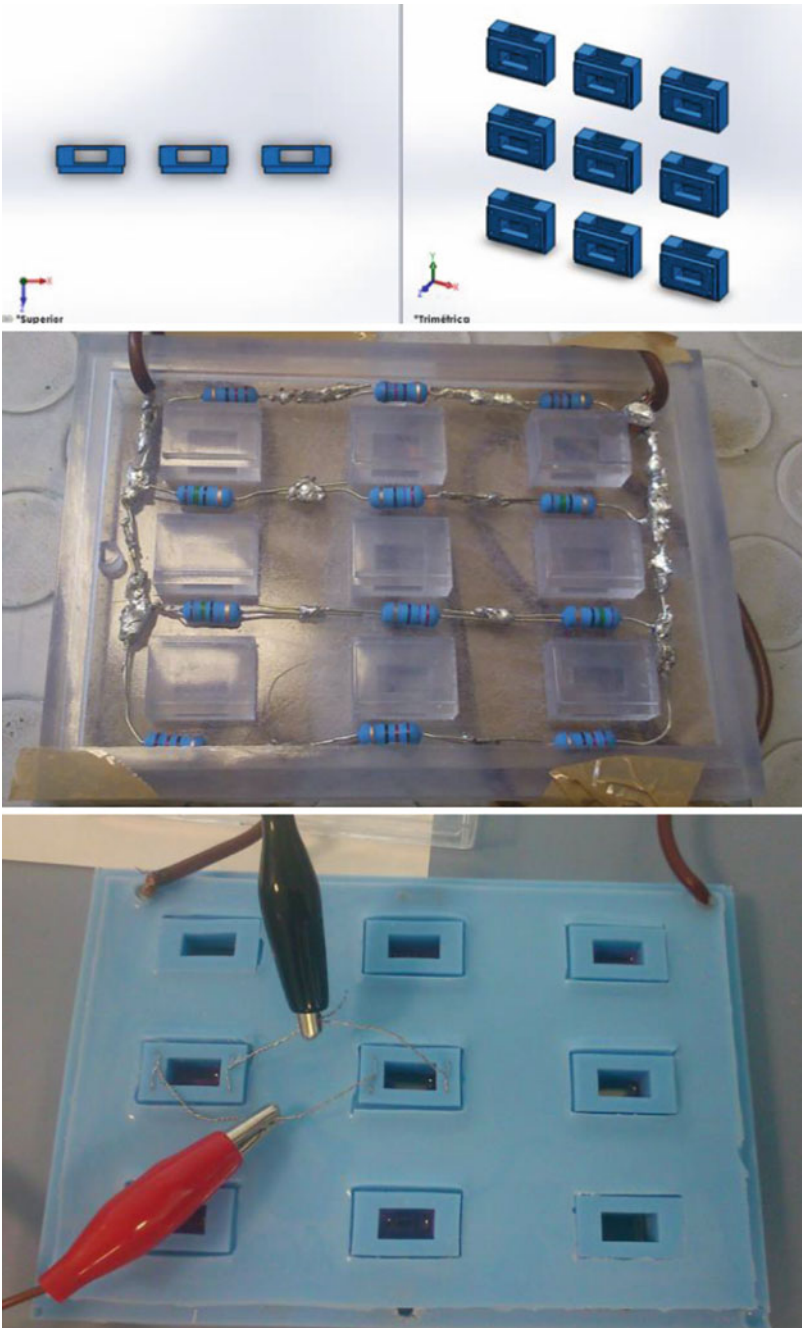

Fig. 24.1 Development process of a bioreactor for the electrical stimulation of cell culture processes. Computer-aided designs and rapid prototypes. Designs: Miguel Ángel Cámara, under the advice of Prof. Milagros Ramos. Prototyping: Product Development Lab UPM

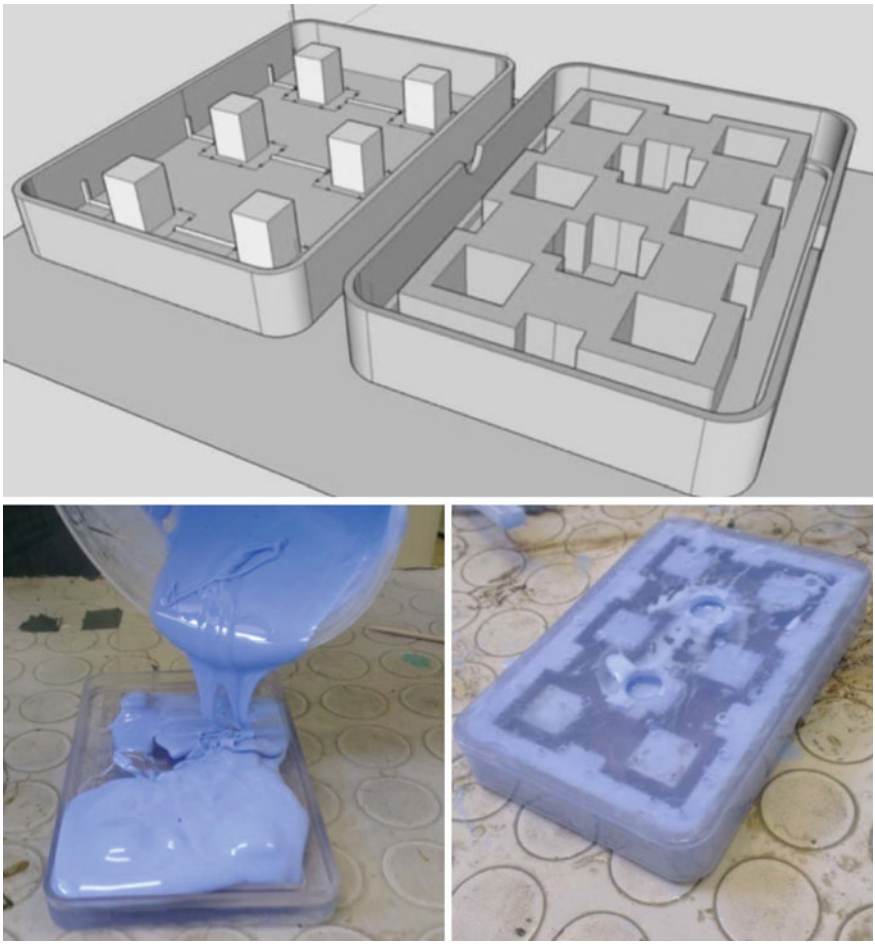

Fig. 24.2 Development process of a bioreactor for the electrical stimulation of cell culture processes. Computer-aided designs and rapid prototypes. Designs: Borja Domínguez, under the advice of Prof. Milagros Ramos. Prototyping: Product Development Lab UPM

clearly considered to promote the quality of teaching, due to the fact that teachers carrying out research in their fields of knowledge are able to explain avant-garde topics in their courses. However it is also true that well-arranged teaching-learning activities, concentrating on students' active learning, are a remarkable strategy for the promotion of new contacts and research collaborations among the professors of different departments and centres. The impact of students as drivers of change should always be taken into consideration.

24.5 Assessment of the Experience: Personal Views

The positive effect of employing CDIO related methodologies can be clearly appreciated taking into account: the interesting concepts selected as topics for their projects, the degree of development reached by the teams of students and the student success ratio. Values such as a 100 % of students having passed the course and an overall attendance to lectures above 85 % are clear indicators of students' engagement. It is necessary to indicate that the benefits affect not only learning and acquisition of outcomes, but also student and teacher motivation and mutual relation in a very special way, which is starting to influence the overall ambience of learning, collaboration and respect present in our "Biomedical Engineering" Degree at TU Madrid. In addition, the establishment of new synergies between teaching and research activities has already been put forward as one of the most relevant effects of the course.

Thanks to implementing the CDIO approach, students taking part in our subject lived, for the first time, through the complete development process of an engineering system and are now better prepared for their final degree theses, as students themselves have highlighted in several occasions during the subject. In addition, they received, again for the first time, training in relevant engineering resources and improved their comprehension and application of several professional skills, all of which adds to the learning outcomes of the subject. Some seminars organized for the promotion of learning outcomes and professional skills include:

- Introduction to computer-aided design and engineering (4 h).
- Introduction to the relevance of IP management and protection (4 h).
- Introduction to sustainable engineering design (4 h).
- Professional skills: communication, teamwork, creativity (6 h).

Regarding student assessment, we are facing and managing the typical problems that arise when assessing teamwork activities. First of all, the proposed biodevices are complex enough to promote positive interdependence between members of the team, so that each of the members is needed for the overall success and that there is enough workload to let all students work hard and enjoy the experience, thanks to learning a lot. In addition, we are encouraging individual assessment, complementing the teamwork activities with individual deliveries and during the public presentations of their final results (which account for a 30 % of the global qualification). The evaluation of professional skills counts with the help of ad hoc designed rubrics, as part of an integral framework for the promotion of engineering education beyond technical skills, consequence of recent educational innovation projects (Hernández Bayo et al. 2014).

Considering slight modifications, we would like to incorporate the developed products of 2014–2015 academic year as case studies, in a continuous improvement cycle, which hopefully will help us complete a large library of biomedical devices. Counting with a library of designs and prototypes of several biodevices will be positive, not only for teaching purposes, but also for research activities.

As for the future, we would like to explore the possibility of linking several subjects by means of capstone projects or project-based learning strategies, using a similar methodology but probably focusing on more complex biodevices requiring more dedication and benefiting from the collaborative input of several engineering disciplines. Such "bus projects" have been successfully used for connecting very basic subjects with more applied ones, hence promoting student motivation (Rayegani and Ghalati 2015). The possibility of incorporating students from other countries and carrying out international development teams, as recently described by colleagues counting with the support of EU-funded projects (Malheiro et al. 2015), is also attractive. The good performance of the team working with the cell culture device also encourages us to devote some forthcoming courses to a more concrete special topic, such us "development of labs-on-chips", "development of organs-on-chips" or "development of tissue engineering scaffolds", hence helping to promote a more homogeneous dedication among the different teams of study.

Regarding the performance of the proposed devices, additional cell cultures are needed, so as to assess its effectivity. In any case, electrical stimulation has been shown to modulate embryonic stem cell differentiation towards a neuronal fate (Yamada et al. 2007). The use of the bioreactor for electrical stimulation of cell culture, developed in this experience, will help to understand the molecular mechanisms underlying neural differentiation processes after electrical stimulation.

24.6 Main Conclusions

Present study has detailed the complete development of a novel subject on "Biomedical Devices", in the framework of the "Biomedical Engineering" Degree at TU Madrid. The subject has been implemented with the CDIO approach in mind, as we consider it a very remarkable way of promoting student active learning and of integrating, with impact, novel concepts into new curricula. During the course, groups of students have lived through the complete development process of different complex biomedical devices aimed at providing answers to relevant social needs. Computer-aided engineering and rapid prototyping technologies have been used as support tools for their designs and prototypes, so as to reach the implementation and operation phases more rapidly, in some cases even with enough time for a re-design cycle. Main benefits, lessons learned and challenges, linked to this CDIO-based subject, have been analyzed and discussed, considering the results from 2014–2015 academic year, in which the course was carried out for the first time with the successful performance of 13 excellent students.

The use of rapid prototyping technologies as support resources is already a well-established practice (Díaz Lantada et al. 2007), but its application to Biomedical Engineering is very rewarding and appropriate for the promotion of biomimetic approaches and in some cases even of personalized designs (Díaz Lantada et al. 2010). In addition, it constitutes an excellent opportunity for students

to practice with design and modeling technologies widely used in several industrial branches, from transport and building, to energy and health (Lorenzo Yustos et al. 2010).

To our knowledge, the course constitutes one of the first complete (from the concept, to the operation) project-based learning experiences linked to the field of biomedical devices in Spain, together with some very recent proposals also based on the CDIO approach in Master Degrees linked to Industrial Engineering (Díaz Lantada et al. 2015) and Telecommunications Engineering (Domingo et al. 2015).

References

Biomedical Engineering Society. Planning a career in biomedical engineering. BMES, USA

Bloom BS, Engelhart MD, Furst EJ, Hill WH, Krathwohl DR (1956) Taxonomy of educational objectives: the classification of educational goals. Handbook I: Cognitive domain. David McKay Company, New York

CDIO Standards 2.0 (last access in August 2015) http://www.cdio.org/implementing-cdio/standards/12-cdio-standards

Crawley EF, Malmqvist J, Östlund S, Brodeur DR (2007) Rethinking engineering education: the CDIO approach. Springer, pp 1–286

Díaz Lantada A, Lorenzo Yustos H, Lafont P, Munoz-Guijosa JM, Echavarri Otero J, Muñoz Sanz JL (2007) Teaching applications for rapid prototyping technologies. Int J Eng Educ 23 (2):411–418

Díaz Lantada A, Del Valle Fernández R, Lafont Morgado P, Muñoz García J, Muñoz Sanz JL, Munoz-Guijosa JM, Echávarri Otero J (2010) Development of personalized annuloplasty rings: combination of CT images and CAD-CAM tools. Ann Biomed Eng 38(2):280–290

Díaz Lantada A, Lafont Morgado P, Muñoz-Guijosa JM, Muñoz Sanz JL, Echávarri Otero J, Muñoz García J, Chacón Tanarro E, De la Guerra Ochoa E (2013) Towards successful project-based learning experiences in Engineering Education. Int J Eng Educ 29(2):476–490

Díaz Lantada A, Ros Felip A, Jiménez Fernández J, Muñoz García J, Claramunt Alonso R, Carpio J (2015) Integrating biomedical engineering design into engineering curricula: benefits and challenges of the CDIO approach. In: 11th international CDIO conference, Cheng-du, China, 2015

Domingo T, Ballarín M, Rojano JM, Oliveras A, García-Hernández M, Bragós R (2015) Cooperation framework with a hospital as a design-build projects source. 11th international CDIO conference, Cheng-du, China, 2015

Fagette PH (ed) (1999) The biomedical engineering society: an historical perspective. BMES, USA

Hernandez Bayo A, Ortiz Marcos I, Carretero Díaz A, De la Fuente MM, Lumbreras Martín J, Martínez Muneta ML, Riveira Rico V, Rodríguez Hernández M (2014) Integral framework to drive engineering education beyond technical skills. Int J Eng Educ 30(6B):1697–1707

Lorenzo Yustos H, Lafont Morgado P, Díaz Lantada A, Fernández-Flórez Navidad A, Muñoz Sanz JL, Munoz-Guijosa JM, Muñoz García J, Echávarri Otero J (2010) Towards complete product development teaching employing combined CAD-CAE-CAM technologies. Comput Appl Eng Educ 18(4):661–668

Malheiro B, Silva M, Ferreira P, Guedes P (2015) CDIO and the European project semester: a match for capstone projects?. In: 11th international CDIO conference, Cheng-du, China, 2015

Rayegani F, Ghalati R (2015) Integration of math and science with sheridan engineering program using CDIO tools. In: 11th international CDIO conference, Cheng-du, China, 2015

Shuman LJ, Besterfield-Sacre M, Mc Gourty J (2005) The ABET professional skills, can they be taught? can they be assessed? J Eng Educ 94:41–55

World Health Organization (2010) Innovative technologies that address global health concerns. Outcome of the call. Global initiative on health technologies 2010

Yamada M et al (2007) Electrical stimulation modulates fate determination of differentiating embryonic stem cells. Stem Cells 25:562–570

Appendix

A.I Summary of Especially Relevant References Linked to the Contents

1. Ashby MF (2005) Materials selection in mechanical design, 3rd edn. Butterworth Heinemann
2. Ashby MF, Jones DRH (1996) Engineering materials 1, 2nd edn. Butterworth Heineman
3. Ashby MF, Jones DRH (1998) Engineering materials 2, 2nd edn. Butterworth Heineman
4. Atala A, Yoo JJ (2015) Essentials of 3D biofabrication and translation. ISBN 9780128010150, Academic Press, Elsevier
5. Benyus JM (2002) Biomimicry innovation inspired by nature. Harper Collins Publishers
6. Brinker CJ, Scherer GW (1990) Sol-gel science: the physics and chemistry of sol-gel processing, 1st edn. Academis Press
7. Bunshah RF (1994) Handbook of deposition technologies for films and coatings: science, technology and applications, 2nd edn. Noyes Publications, Materials science and process technology series, Park Ridge
8. Caplan AI (1991) Mesenchymal stem cells. J Orthop Res 9(5):641–50
9. Caplan AI (2008) All MSCs are pericytes?. Cell Stem Cell 3(3):229–30
10. Capolino F (2009) Metamaterials handbook theory and phenomena of metamaterials, vol 1. Taylor and Francis
11. Capolino F (2009) Metamaterials handbook applications of metamaterials, vol 2. Taylor and Francis
12. Davis JR (2003) Handbook of materials for medical devices. ASM International
13. Díaz Lantada A (2012) Handbook on active materials for medical devices: advances and applications. PAN Stanford Publishing
14. Díaz Lantada A (2013) Handbook of advanced design and manufacturing technologies for biodevices. Springer
15. Díaz Lantada A, Lafont Morgado P (2012) Rapid prototyping for biomedical engineering: current capabilities and challenges. Ann. Rev Biomed Eng 14

A. Díaz Lantada (ed.), *Microsystems for Enhanced Control of Cell Behavior*,
Studies in Mechanobiology, Tissue Engineering and Biomaterials 18,
DOI 10.1007/978-3-319-29328-8

16. Drexler E (1990) Enginers of creation: the coming era of nanotechnology (Anchor Books 1986). Oxford University Press
17. Drexler E (1991) Molecular machinery and manufacture with applications to computation. PhD thesis, MIT
18. Feynman RP (1992) There's plenty of room at the bottom (data storage). J Microelectromech Syst 1(1):60–66. doi:10.1109/84.128057
19. Feynman RP (1993) Infinitesimal machinery. J Microelectromech Syst, 2(1):4–14. doi:10.1109/84.232589
20. Gad-el-Hak (2003) The MEMS handbook. CRC Press, New York
21. Jacobs CR, Huang HK,Won RY (2012) Introduction to cell mechanics and mechanobiology. Garland Sci
22. Javed A, Guo B, Hiebert S, Choi JY, Green J, Zhao SC, Osborne MA, Stifani S, Stein JL, Lian JB, van Wijnen AJ, Stein GS (2000) Groucho/TLE/R-esp proteins associate with the nuclear matrix and repress RUNX (CBF(alpha)/AML/PEBP2 (alpha)) dependent activation of tissue-specific gene transcription. J Cell Sci 113 (12):2221
23. Kucklick TR (2006) The medical device R and D handbook. CRC Press
24. Lanza R, Langer R, Vacanti J (2014) Principles of tissue engineering, 4th edn. Elsevier
25. Lorenzo Yustos H. (2008) Aplicación de nuevas tecnologías en la realización de herramientas para moldes de inyección de termoplásticos. PhD Thesis (Advisor: Lafont Morgado P.), Universidad Politécnica de Madrid
26. Madou MJ (2002) Fundamentals of microfabrication: the Science of miniaturization, 2nd edn. CRC Press
27. Maluf N (2000) An introduction to micro-electromechanical systems engineering. Artech House
28. Mandelbrot B (1982) The fractal geometry of nature. W.H. Freeman, San Francisco
29. Mironov V, Trusk T, Kasyanov V, Little S, Swaja R, Markwald R (2009) Biofabrication: a 21st century manufacturing paradigm. Biofabrication 1(2):022001
30. Pahl G, Beitz W (1996) Engineering design: a systematic approach, 2nd edn. Springer, London
31. Romero-Prado M, Blázquez C, Rodríguez-Navas C, Muñoz J, Guerrero I, Delgado-Baeza E, García-Ruiz JP (2006) Functional characterization of human mesenchymal stem cells that maintain osteochondral fates. J Cell Biochem 98:1457
32. Roozenburg N, Eeckels J (1995) Product design: fundamentals and methods. Wiley, New York
33. Saaksvuori A, Immonen A (2008) Product lifecycle management, 3rd edn. Springer, Heidelberg
34. Schwarz M (2005) New materials processes, and methods technology, 1st edn. CRC Press, Taylor and Francis Group
35. Stark J (2004) Product lifecycle management: 21st century paradigm for product realisation. Springer, Heidelberg
36. Ulrich K, Eppinger S (2007) Product design and development, 4th edn. Mc-Graw Hill/Irwin, New York
37. Varadan VK, Jiang X Varadan, VV (2001) Microstereolithography and other fabrication techniques for 3D MEMS. Wiley

A.II Summary of Especially Relevant Websites

On computer-aided design and engineering resources:

More linked to design tasks:

http://usa.autodesk.com
http://usa.autodesk.com/3ds-max
http://usa.autodesk.com/autodesk-inventor
http://usa.autodesk.com/product-design-suite
http://www.3ds.com/es/products/catia
http://www.plm.automation.siemens.com/en_us/products/nx/nx8
http://www.plm.automation.siemens.com/en_us/products/velocity/solidedge
http://www.rhino3d.com
http://www.solidworks.com

More linked to calculation tasks:

http://www.3ds.com/products/simulia/portfolio/abaqus/overview
http://www.ansys.com
http://www.comsol.com
http://www.mscsoftware.com/products/cae-tools/msc-nastran.aspx
http://www.mscsoftware.com/products/cae-tools/patran.aspx
http://www.mathworks.com

On medical imaging and design based on medical images:

http://biomedical.materialise.com/mimics
http://biomedical.materialise.com/mis
http://www.amira.com
http://www.mathworks.com (Users community for open access software)
http://www.skyscan.be

On additive manufacturing (rapid prototyping) resources:

http://reprap.org/wiki/RepRap
http://www.3dsystems.com
http://www.additive3d.com
http://www.bitsfrombytes.com

http://www.digilabglobal.com
http://www.digilabglobal.com/celljet
http://www.dimensionprinting.com
http://www.envisiontec.de
http://www.fabathome.org
http://www.makerbot.com
http://www.nanoscribe.de
http://www.stratasys.com
http://www.zcorp.com/en/home.aspx

On micro- and nano-manufacturing:

http://e.drexler.com
http://www.ceramed.pt
http://www.efds.org
http://knmf.kit.edu
http://www.intelligentmp.com
http://www.nanoscribe.de
http://www.oxfordlasers.com
http://www.svc.org

On systems for characterization trials and biological models:

http://biomedical.materialise.com/anatomical-models
http://biomedical.materialise.com/heartprint
http://biomedical.materialise.com/other-anatomical-models
http://simbionix.com
http://worldwide.bose.com
http://worldwide.bose.com/electroforce/en/web/home/page.html
http://www.3bscientific.es
http://www.cae.com/en/healthcare/home.asp
http://www.simulab.com

A.III Summary of Relevant Standards and Associations

Main organizations

International Organization for Standarization "ISO" (www.iso.org).
The World Medical Association (www.wma.net).

"New approach" Directives related to the Medical Industry

Directive 93/42/EEC related to "Medical devices".
Directive 90/385/EEC related to "Active implantable medical devices".
Directive 98/79/EC related to "Medical devices for "in vitro" diagnosis".

Standards related to the development of medical devices

ISO 10993 standard on "Biological evaluation of medical devices".
ISO 13485 standard on "Sanitary products. Quality management and regulatory affairs".
ISO 13488 standard on "Quality systems. Medical devices, sanitary products and especial requirements for applying ISO 9002 standard".
ISO 14971 standard on "Application of risk management to medical devices and sanitary products".
ISO 15223 standard on "Symbols used for labelling and information provided together with medical devices".

Standards and associations related to medical imaging

DICOM standard—Digital Imaging and Communications in Medicine: Strategic Document (http://medical.nema.org).
Medical Imaging and Technology Alliance (www.medicalimaging.org).
NEMA—The Association of Electrical and Medical Imaging Equipment Manufacturers (www.nema.org).

Additional documents of interest

Council of Europe "Convention for the protection of Human Rights and dignity of the human being with regard to the application of biology and medicine: Convention on Human Rights and Biomedicine" (1994).
UNESCO "Universal Declaration on the Human Genome and Human Rights" (1997) and "Guidelines for Implementation" (1999).
World Medical Association "Declaration of Helsinki. Ethical principles for medical reserach involving human subjects" (current revised edition 2008).

A.IV Relevant Scientific Journals Linked to Medical Microdevices and Related Topics of Interest

Listed below there are several high-quality scientific journals, linked to the different topics covered within the Handbook, where researchers can find additional information on biodevices and medical devices, as well as on recent advances on Materials Science and Technology for further promoting such advances.

"Acta Biomaterialia", Elsevier.
"Annals of Biomedical Engineering", Springer.
"Annual Review of Biomedical Engineering", Annual Reviews.
"Annual Review of Materials Research", Annual Reviews.
"Biochip Journal", Springer.
"Biofabrication", IOP Publishing.
"Bioinspiration and Biomimetics", IOP Publishing.
"Biomaterials", Elsevier.
"Biomechanics and Modeling in Mechanobiology", Springer.
"Biomedical Engineering", Springer.
"Biomedical Engineering Letters", Springer.
"Biomedical Engineering Systems and Technologies", Springer.
"Biomedical Microdevices", Springer.
"Cardiovascular Engineering and Technology", Springer.
"Cellular and Molecular Bioengineering", Springer.
"Computer Methods in Biomechanics and Biomedical Engineering", Taylor and Francis.
"IEEE Transactions on Biomedical Engineering", IEEE.
"International Journal of Advanced Manufacturing Technologies", Springer.
"International Journal of Biomedical Engineering and Technology", Inderscience.
"International Journal of Mechanics and Materials in Design", Springer.
"Journal of 3D Printingi in Medicine", Springer.
"Journal of Applied Physics", American Institute of Physics.
"Journal of Biomedical Engineering", Elsevier.
"Journal of Biomimetics, Biomaterials and Tissue Engineering", Scientific.net.
"Journal of Materials Science: Materials in Medicine", Springer.
"Journal of Microelectromechanical Systems", IEEE ASME.
"Journal of Nano Research", Scientific.net.
"Journal of Physics D: Applied Physics", IOP Science.
"Journal of Tissue Engineering and Regenerative Medicine", Wiley.

"Materials and Design", Elsevier.
"Materials Science and Engineering C: Materials for Biological Applications", Elsevier.
"Nature Materials", Nature Publishing Group.
"Nature Nanotechnology", Nature Publishing Group.
"Plasma Processes and Polymers", Wiley.
"Polymers", MDPI Publishing.
"Rapid Prototyping Journal", Emerald.
"Science Translational Medicine", Science AAAS.
"Science Signalling", Science AAAS.
"Sensors", MDPI Publishing.
"Sensors and Actuators A: Physical", Elsevier.
"Sensors and Actuators B: Chemical", Elsevier.
"Smart Materials and Structures", IOP Publishing.
"The Open Biomedical Engineering Journal", Bentham Open.
"Tissue Engineering: Parts A, B and C", Mary Ann Liebert Inc..

A.V Relevant Enterprises Linked to Medical Microdevices

Listed below there are several websites of multinationals and highly relevant companies linked to the development of medical devices and microdevices, as a help for researchers needing information on conventional commercially available biodevices for their own development projects.

http://3dbiotek.com
http://biomet3i.es
http://global.smith-nephew.com/master/6600.htm
http://integralife.com
http://www.3dbiotek.com/web
http://www.admedes.com
http://www.bbraun.de
http://www.biotronik.de
http://www.bostonscientific.com/home.bsci
http://www.ceramed.pt
http://www.clevemed.com
http://www.edwards.com
http://www.gehealthcare.com
http://www.gpc-medical.com
http://www.healthcare.philips.com
http://www.hitec-implants.com
http://www.insphero.com
http://www.jnj.com/connect
http://www.medical.siemens.com
http://www.medtronic.com
http://www.microtissues.com
http://www.sfm.de
http://www.sjm.com
http://www.smiths-medical.com
http://www.sorin.com
http://www.spacelabshealthcare.com
http://www.stryker.com
http://www.valtronic.ch
http://www.zimmer.com
http://www.zyvex.com

A.VI Relevant Enterprises Linked to Labs-on-Chips

Listed below there are several websites of multinationals and highly relevant companies linked to the development of microfluidic devices for the biomedical field, as a help for researchers needing information on conventional commercially available biodevices for their own development projects.

- Abaxis: abaxis.com
- Abbott Point of Care: abbottpointofcare.com
- Achira: achiralabs.com
- Akonni Biosystems: akonni.com
- Axis-Shield: axis-shield.com/Afinion
- Biosite: biosite.com
- Biosurfit: biosurfit.com
- Boehringer Ingelheim: boehringer-ingelheim.de
- Boston Micro fluidics: bostonmicrofluidics.com
- CapitalBio Corporation: capitalbio.com
- Celula: celula-inc.com
- Cepheid: cepheid.com
- Claros Diagnostics: clarosdx.com
- Cleveland Biosensors: clevelandbiosensors.com
- Clondiag: clondiag.com
- Crospon: crospon.com
- Daktari Diagnostics: daktaridx.com
- DEOS Labs: deoslabs.com
- Diagnostics for All: dfa.org
- Diagnoswiss: diagnoswiss.com
- DNA Electronics: dnae.co.uk
- Epocal: epocal.com
- Fluidigm: fluigent.com
- Fluidmedix: fluimedix.com
- FocusDx: focusdx.com
- Helicos Bioscences: helicosbio.com
- Ingeneron: ingeneron.com
- IonTorrent (Life Technologies): iontorrent.com
- LeukoDx: leukodx.com
- Maxwell Sensors: maxwellsensors.com
- Micro2Gen: micro2gen.com

- MicroCHIPS: mchips.com
- Micronics: micronics.net
- Micropointbio: micropointbio.com
- Mode Diagnostics: modedx.com
- Molecular Vision: molecularvision.co.uk
- Mycrolab: mycrolab.com
- Nanobiosym: nanobiosym.com
- Nanomix: nano.com
- Nanosphere: nanosphere.us
- On-Q-ity: on-q-ity.com
- Pathogenetix: pathogenetix.com
- PerkinElmer: perkinelmer.com
- Philips Applied Technologies: apptech.philips.com
- Rheonix: rheonix.com
- Samsung: samsung.com
- Sensivida: sensividamedical.com
- SensLab: senslab.de
- SFC Fluidics: sfc- fl uidics.com
- Siemens: siemens.com
- Siloam Biosciences: siloambio.com
- Smart holograms: smartholograms.com
- T2 Biosystems: t2biosystems.com
- TearLab: tearlab.com
- TECAN: tecan.com
- Veridex: veridex.com

A.VII Some Matlab Programs for Helping Designers

A.VII.1) Program for the design of a fluidic device with microtextured channels:

```
% Microtextured channels based on Brownian surfaces
% Example from Chapter 8 of the Handbook

clc
clear all
close all

% Working in microns

i=0;
j=0;

for ibis = 1:5:151
     i=i+1;
     j=0;
   for jbis = 1:5:3001;
       j=j+1;
       X(i)=ibis;
       Y(j)=jbis;
       suma = 0;
       lambda = 1.5;
       alfa = 0.9;
      for n = 1:1:3
           suma = suma +
(random('norm',0,1))*50*(lambda^(-
alfa*n))*sin((lambda^n)*(i*cos(2*pi*rand(1))+j*sin(2*pi
*rand(1))+2*pi*rand(1)));
       end
       Z(i,j)=suma+800;
   end
end

for ibis = 152:5:302
     i=i+1;
     j=0;
   for jbis = 1:5:3001;
       j=j+1;
       X(i)=ibis;
       Y(j)=jbis;
```

```
        Z(i,j)=-200;
    end
end


for ibis = 303:5:453
     i=i+1;
     j=0;
    for jbis = 1:5:3001;
        j=j+1;
        X(i)=ibis;
        Y(j)=jbis;
        suma = 0;
        lambda = 1.5;
        alfa = 0.7;
        for n = 1:1:3
            suma = suma +
(random('norm',0,1))*50*(lambda^(-
alfa*n))*sin((lambda^n)*(i*cos(2*pi*rand(1))+j*sin(2*pi
*rand(1))+2*pi*rand(1)));
        end
        Z(i,j)=suma+800;
    end
end

for ibis = 454:5:604
     i=i+1;
     j=0;
    for jbis = 1:5:3001;
        j=j+1;
        X(i)=ibis;
        Y(j)=jbis;
        Z(i,j)=-200;
    end
end

for ibis = 605:5:755
     i=i+1;
     j=0;
    for jbis = 1:5:3001;
        j=j+1;
        X(i)=ibis;
        Y(j)=jbis;
        suma = 0;
```

```
        lambda = 1.5;
        alfa = 0.5;
        for n = 1:1:3
            suma = suma +
(random('norm',0,1))*50*(lambda^(-
alfa*n))*sin((lambda^n)*(i*cos(2*pi*rand(1))+j*sin(2*pi
*rand(1))+2*pi*rand(1)));
        end
        Z(i,j)=suma+800;
    end
end

for ibis = 756:5:906
     i=i+1;
     j=0;
    for jbis = 1:5:3001;
        j=j+1;
        X(i)=ibis;
        Y(j)=jbis;
        Z(i,j)=-200;
    end
end

for ibis = 907:5:1058
     i=i+1;
     j=0;
    for jbis = 1:5:3001;
        j=j+1;
        X(i)=ibis;
        Y(j)=jbis;
        suma = 0;
        lambda = 1.5;
        alfa = 0.3;
        for n = 1:1:3
            suma = suma +
(random('norm',0,1))*50*(lambda^(-
alfa*n))*sin((lambda^n)*(i*cos(2*pi*rand(1))+j*sin(2*pi
*rand(1))+2*pi*rand(1)));
        end
        Z(i,j)=suma+800;
    end
end
```

```
for ibis = 1059:5:1210
     i=i+1;
     j=0;
   for jbis = 1:5:3001;

       j=j+1;

       X(i)=ibis;
       Y(j)=jbis;
       Z(i,j)=-200;
   end
end

for ibis = 1211:5:1362
     i=i+1;
     j=0;
   for jbis = 1:5:3001;
       j=j+1;
       X(i)=ibis;
       Y(j)=jbis;
       suma = 0;
       lambda = 1.5;
       alfa = 0.1;
       for n = 1:1:3
           suma = suma + (ran-
dom('norm',0,1))*50*(lambda^(-
al-
fa*n))*sin((lambda^n)*(i*cos(2*pi*rand(1))+j*sin(2*pi*r
and(1))+2*pi*rand(1)));
       end
       Z(i,j)=suma+800;
   end
end

for ibis = 1363:5:1524
     i=i+1;
     j=0;
   for jbis = 1:5:3001;
       j=j+1;
       X(i)=ibis;
       Y(j)=jbis;
       Z(i,j)=-200;
   end
end
```

```
for ibis = 1525:5:1676
     i=i+1;
     j=0;
   for jbis = 1:5:3001;
       j=j+1;
       X(i)=ibis;
       Y(j)=jbis;
       Z(i,j)=800;
   end
end

colormap(gray);
superficie = surf(Y,X,Z)
```

A.VII.2) Program for the design of a microdevice with hydrophobic surface:

```
% Microtextured device with hydrophobic surface.
% Based on multi-scale modeling for mimicking the
% surfaces of the lotus flower, famous for self-
% cleaning properties.
% Coupling between trigonometric and fractal surfaces
% Example from Chapter 8 of the Handbook.

clc
clear all
close all

% Working in microns

i=0;
j=0;

for ibis = 1:0.1:150
     i=i+1;
     j=0;
   for jbis = 1:0.1:100
       j=j+1;
```

```
        X(i)=ibis;
        Y(j)=jbis;
        suma = 0;
        lambda = 1.5;
        alfa = 0.9;
      for n = 1:1:3
            suma = suma +
(random('norm',0,1))*(lambda^(-
alfa*n))*sin((lambda^n)*(i*cos(2*pi*rand(1))+j*sin(2*pi
*rand(1))+2*pi*rand(1)))/7;
        end

Z(i,j)=suma+10*(sin(pi*ibis/10)*sin(pi*jbis/10)*1)+sin(
pi*ibis)*sin(pi*jbis);
    end
end

colormap(gray);
superficie = surf(Y,X,Z)
```

A.VII.3) Program for designing fractional Brownian fractal surfaces:

```
%Fractal surface (based on fractional Brownian fractal
model)

clear all
close all

for ibis = 1:0.1:31

for jbis = 1:0.1:31

i=ibis*10;
j=jbis*10;

X(i)=ibis;
Y(j)=jbis;

sum = 0;
lambda = 1.5;
alfa = 0.8;
```

```
% Fractal dimension is given by 3-alfa

for n = 1:1:100

sum = sum + (random('norm',0,1))*(lambda^(-
alfa*n))*sin((lambda^n)*(i*cos(2*pi*rand(1))+
+ j*sin(2*pi*rand(1))+2*pi*rand(1)))/100;

end

Z(i,j)=sum;

end

end

surface = surf(X,Y,Z)
```

A.VII.4) Program for fractal surfaces based on the Mandelbrot-Weierstrass model:

```
% Mandelbrot-Weierstrass fractal surface

clear all,
close all,
clc;

x=[0:0.1:10];
y=[0:0.1:10];

A=5;
B=5;
D=[0:0.5:2];
gamma=[1:1:4];

z=zeros(length(x),length(y),length(gamma),length(D));
```

```
for jj=1:length(D)
    for ii=1:length(gamma)
        for i=1:length(x)
            for j=1:length(y)
                for n=1:50

z(i,j,ii,jj)=z(i,j,ii,jj)+A^(D(jj)-
1)*cos(2*pi*gamma(ii)^n*x(i))/(gamma(ii)^((2-
D(jj))*n))+B^(D(jj)-
1)*sin(2*pi*gamma(ii)^n*y(j))/(gamma(ii)^((2-
D(jj))*n));

                end
            end
        end
    end
end

figure;

a=1;


for i=1:length(D)
    for j=1:length(gamma)



subplot(length(D),length(gamma),a)

mesh(x,y,z(:,:,j,i));title(['D =',
num2str(D(i),'%10.1f'),' Gamma =',
num2str(gamma(j),'%10.1f')]); xlabel('x'); ylabel('y');
zlabel('surface');

        a=a+1;
    end
end
```

A.VII.5) Program for designing fractal spheres:

```
%Fractal spheres

clear all
close all

i = 0;
j = 0;

[X,Y,Z]=sphere(50);

Xbis = X;
Ybis = Y;
Zbis = Z;

l1=0;
l2=0;

for numespx=0:1:2
for numespy=0:1:2

for i = 1:1:51
for j=1:1:51

radius = 1;
lambda = 1.5;
alfa = 0.1;
sum = 0;

for n = 1:1:5

sum = sum + (random('norm',0,1))*(lambda^(-
alfa*n))*sin((lambda^n)+2*pi*rand(1))/5;

end

r=radius+sum;

Xbis(i,j) = Xbis(i,j)*sqrt((r^2)/(Xbis(i,j)^2+
Ybis(i,j)^2+ Zbis(i,j)^2));

Ybis(i,j) = Ybis(i,j)*sqrt((r^2)/(Xbis(i,j)^2+
Ybis(i,j)^2+ Zbis(i,j)^2));
```

```
Zbis(i,j) = Zbis(i,j)*sqrt((r^2)/(Xbis(i,j)^2+
Ybis(i,j)^2+ Zbis(i,j)^2));

Xbis2(i,j) = Xbis(i,j)*40+l1*110;

Ybis2(i,j) = Ybis(i,j)*40+l2*110;

Zbis2(i,j) = Zbis(i,j)*40;

end
end

surf(Xbis2, Ybis2, Zbis2)
hold on

l2=l2+1;

end

l2=0;

l1=l1+1;

end

% surf(Xbis,Ybis,Zbis)

surf(Xbis2, Ybis2, Zbis2)
hold on
```

A.VII.6) Program for designing fractal circumferences (and cylinders and cones):

```
%Fractal circumference

clear all
close all

i = 0;
j = 0;
k = 1;

FI = 0;
RO = 10;
Z = 0;

k = k+1;
```

```
for fi = 0:3.1415/320:2*3.1415
j=j+1;

radius = 10;
lambda = 1.5;
alfa = 0.2;
sum = 0;

for n = 1:1:20

sum = sum + (random('norm',0,1))*(lambda^(-
alfa*n))*sin((lambda^n)+2*pi*rand(1))/10;

end

r = radius + sum;

FI = [FI; fi];
RO = [RO;r];

[Xint,Yint] = pol2cart(FI,RO);

end

plot(Xint,Yint)
```

A.VII.7) Program for cellular random walks in the 3D space

```
clear all
close all

r=[0 0 0];
for t= 0:0.1:200

    a=rand;
```

```
    if (a<0.16)
        rnew=r+[0 1 0];

    elseif (a<0.33)
        rnew=r+[0 -1 0];

    elseif (a<0.5)
         rnew=r+[1 0 0];

    elseif (a<0.66)
         rnew=r+[-1 0 0];

    elseif (a<0.85)
         rnew=r+[0 0 1];

    elseif (a<0.99)
         rnew=r+[0 0 -1];

    else
        rnew=r+[0 1 0]

    end

    hold on;
    plot3([r(1) rnew(1)],[r(2) rnew(2)], [r(3)
rnew(3)]);

hold on

    r=rnew;
end
```