Chapter 20
Fluidic Microsystems: From Labs-on-Chips to Microfluidic Cell Culture

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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.

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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).

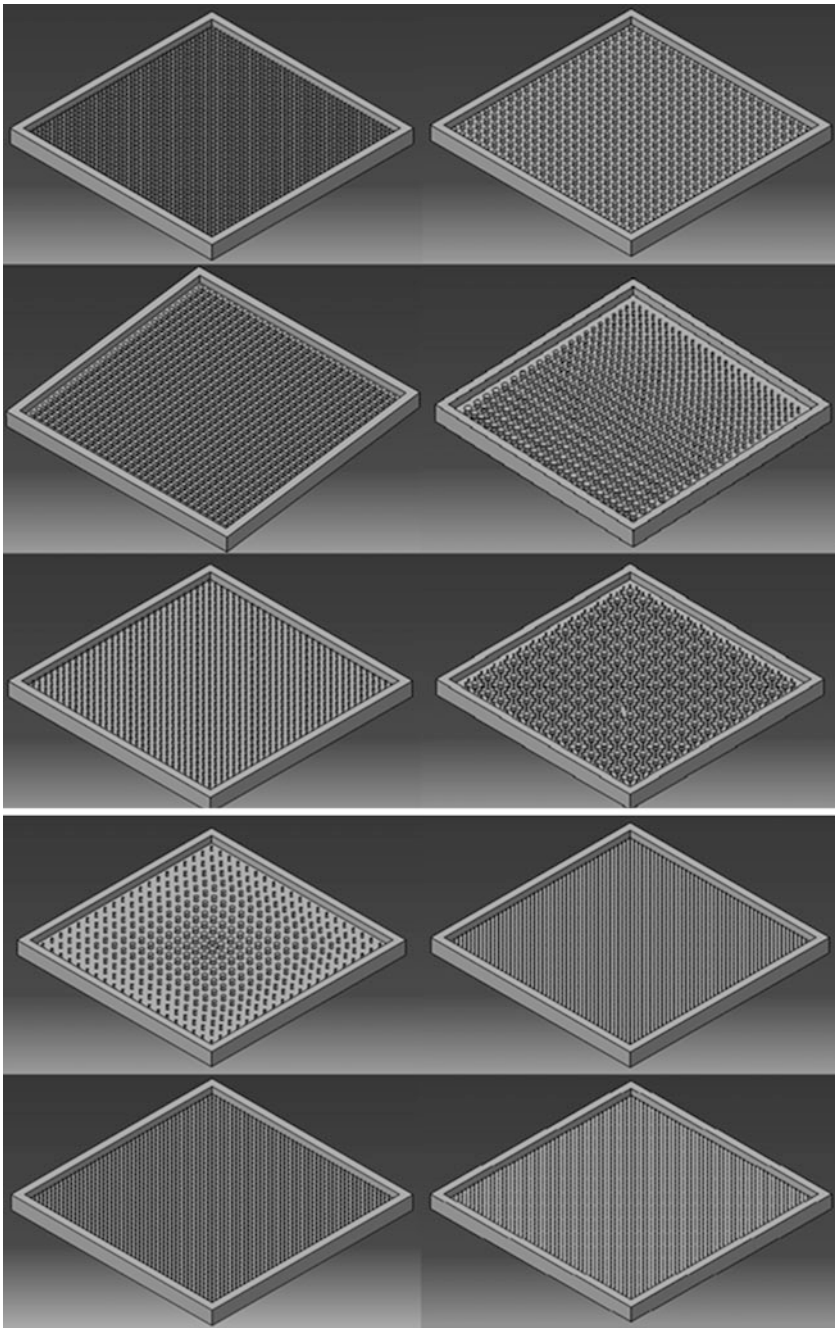

Fig. 20.1 Computer-aided design library of different molds for the manufacture of microporous membranes with functional gradients of porosities

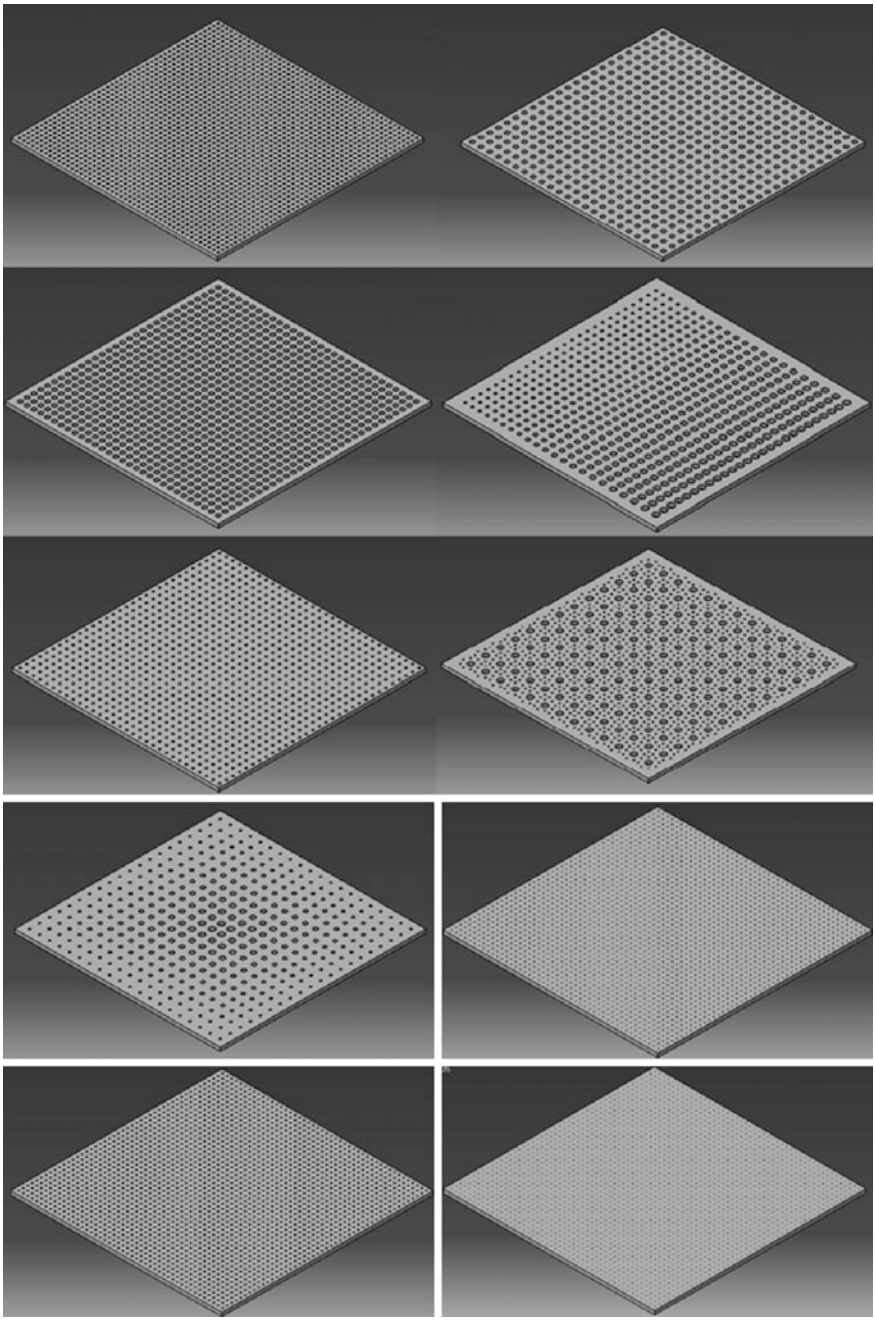

Fig. 20.2 Computer-aided design library of different microporous membranes with functional gradients of porosities

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

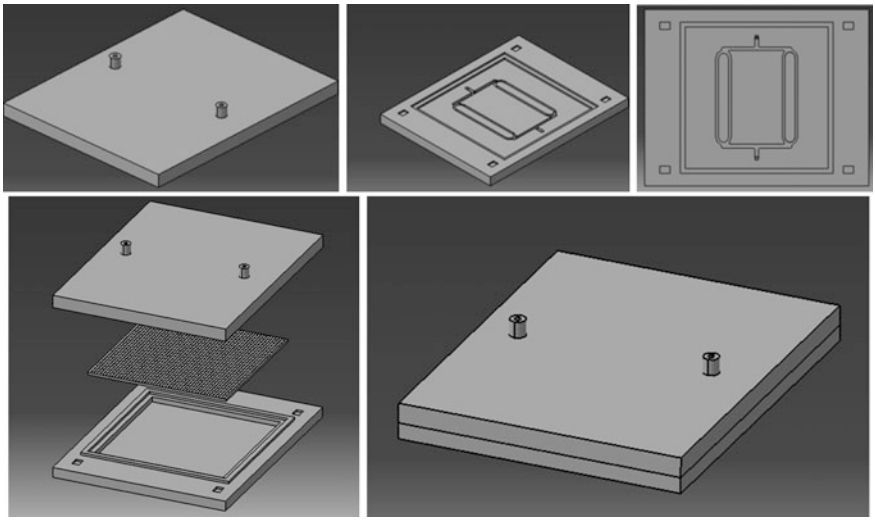

Fig. 20.3 Computer-aided design of a microfluidic device with two cell-culture layers, one for the tissue and one for the vasculature, separated by a microporous membrane

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

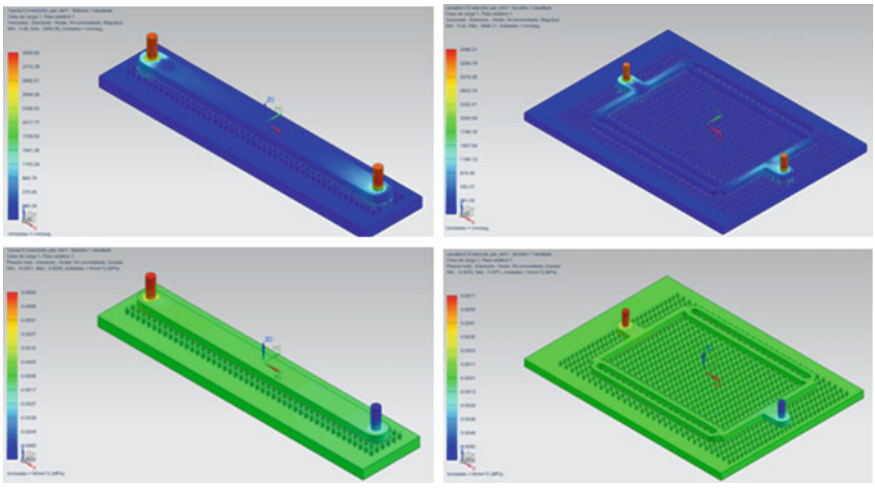

Fig. 20.4 FEM-based assessment of the fluidic performance of a microfluidic device with two cell-culture layers. Membranes with large pore sizes (>75 μm) do not adequately promote the independence between the cell culture layers

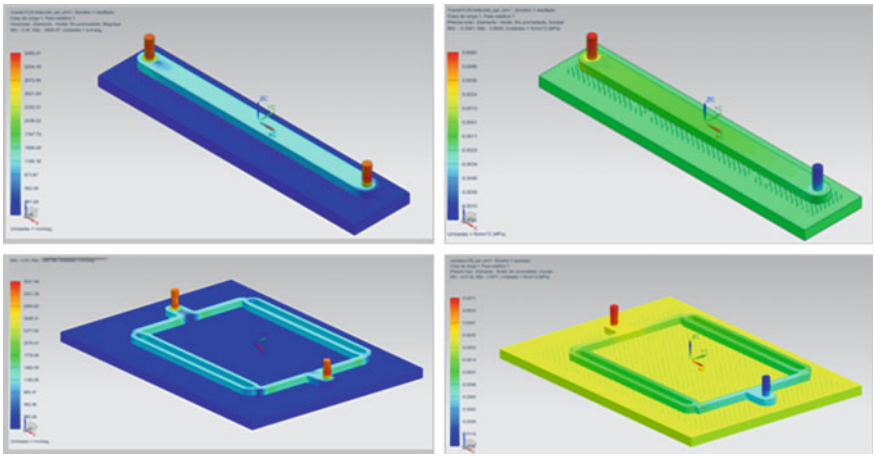

Fig. 20.5 FEM-based assessment of the fluidic performance of a microfluidic device with two cell-culture layers. Membranes with smaller pore sizes (<25 μm) promote the independence between the cell culture layers and help to culture the cells of the lower chamber in static conditions and the cells of 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.

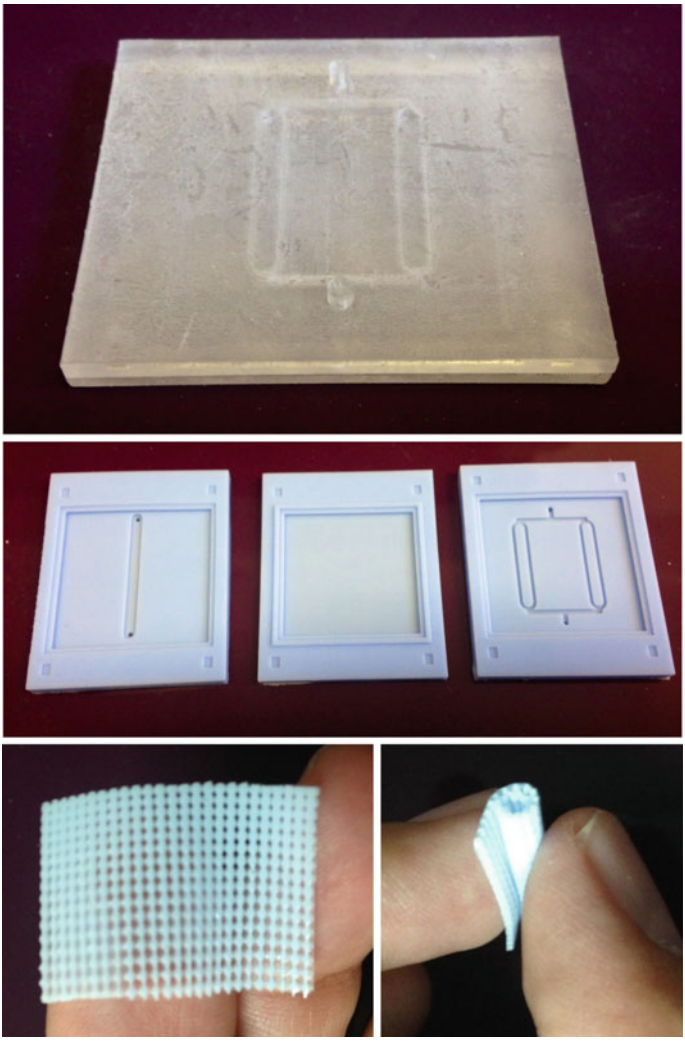

Fig. 20.6 Rapid prototypes, including epoxy masters and PDMS replicas, of a microfluidic device with two cell-culture layers separated by a flexible and microporous PDMS membrane

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

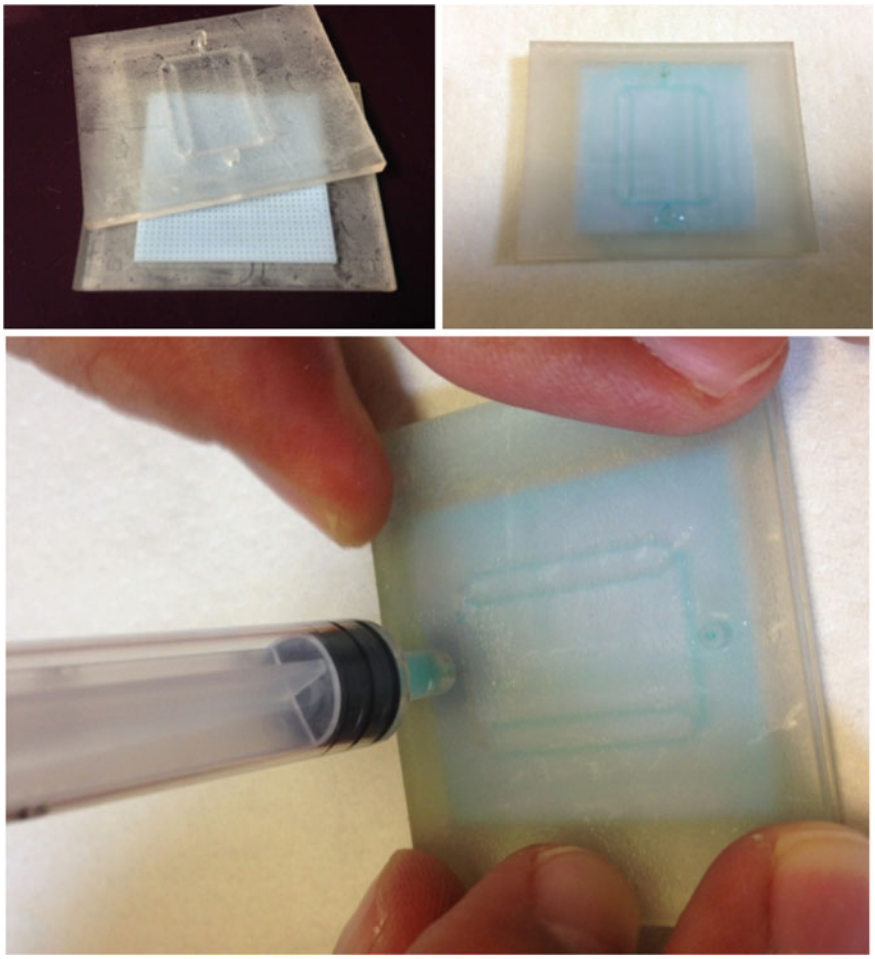

Fig. 20.7 Preliminary fluidic testing of a microfluidic device with two cell-culture layers separated by a flexible and microporous PDMS membrane

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.

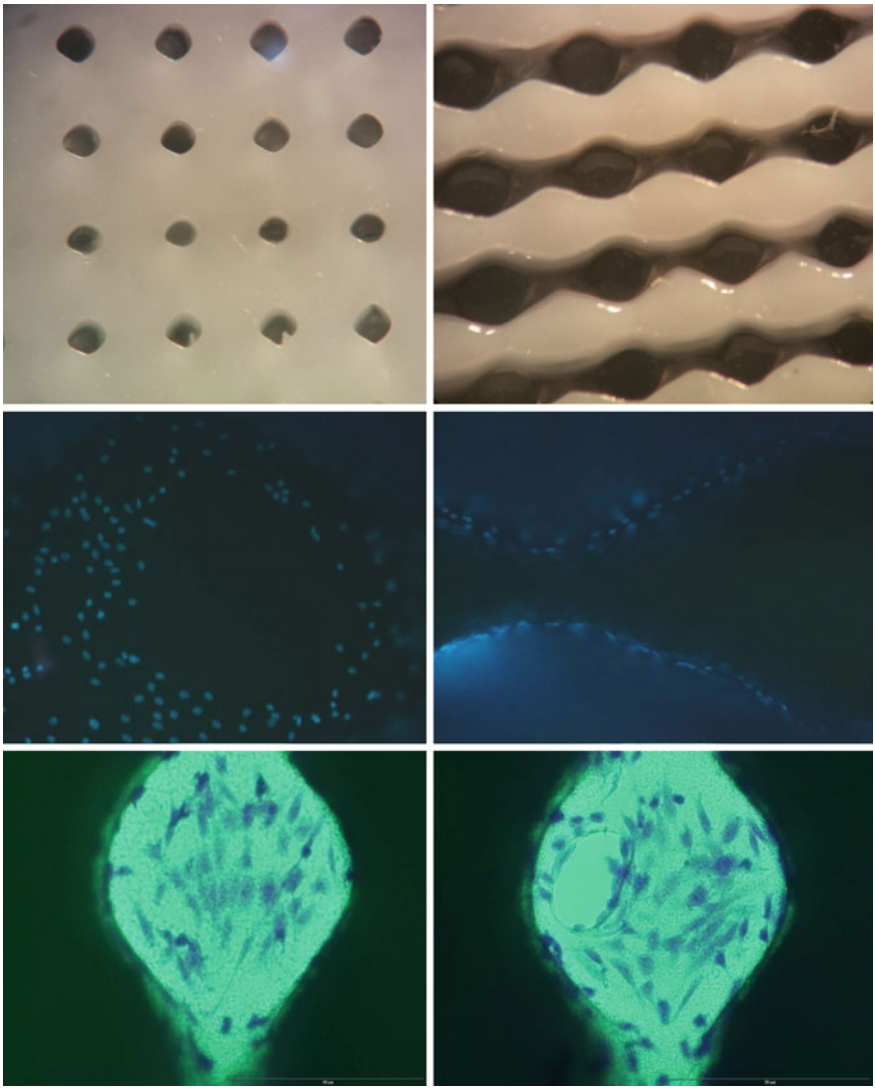

Fig. 20.8 Details of different prototypes of microporous PDMS membranes and hMSCs cultured upon them showing cells adequately attached to the membranes, the colonization of the pores and the possible interaction between both sides of the membrane, thus mimicking the interactions between cell types in 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)

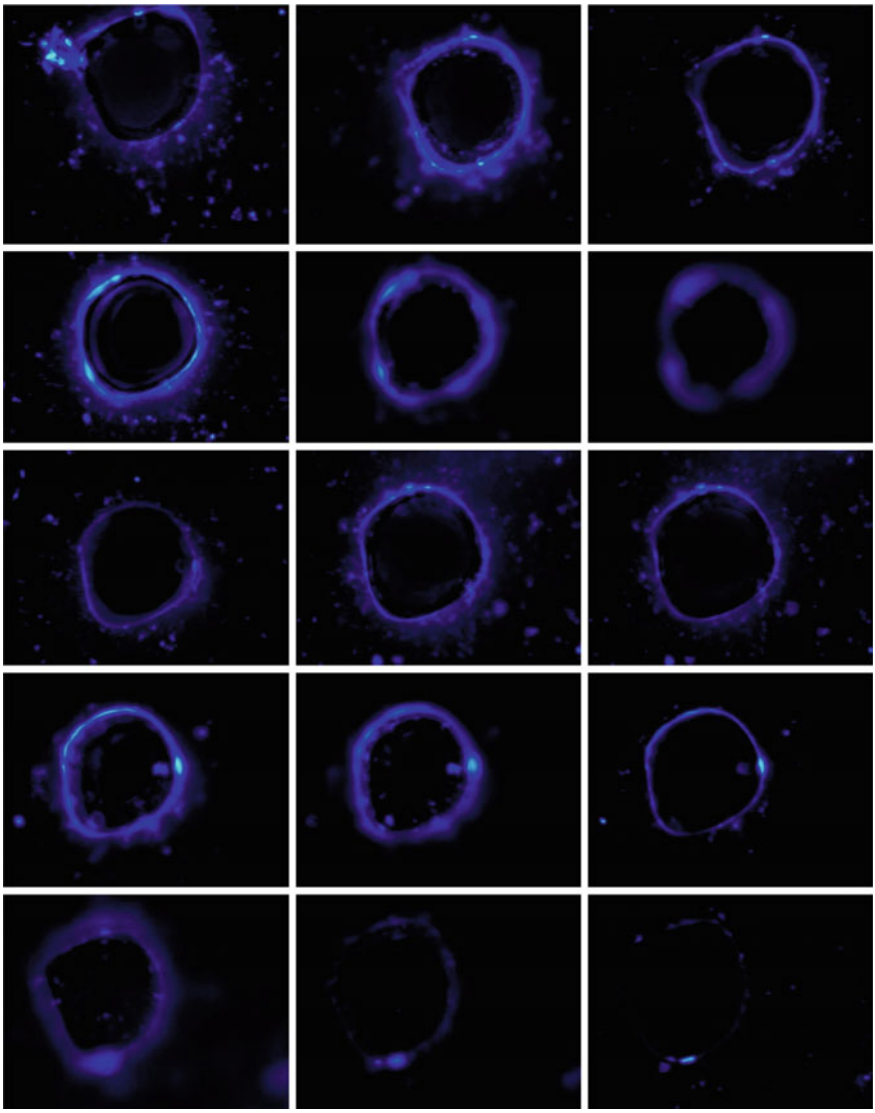

Fig. 20.9 Detailed microscopies of different pores, taken at different depths of focus, to show the colonization of the micropores by the cultured hMSCs and the possible interaction between both sides of the membrane

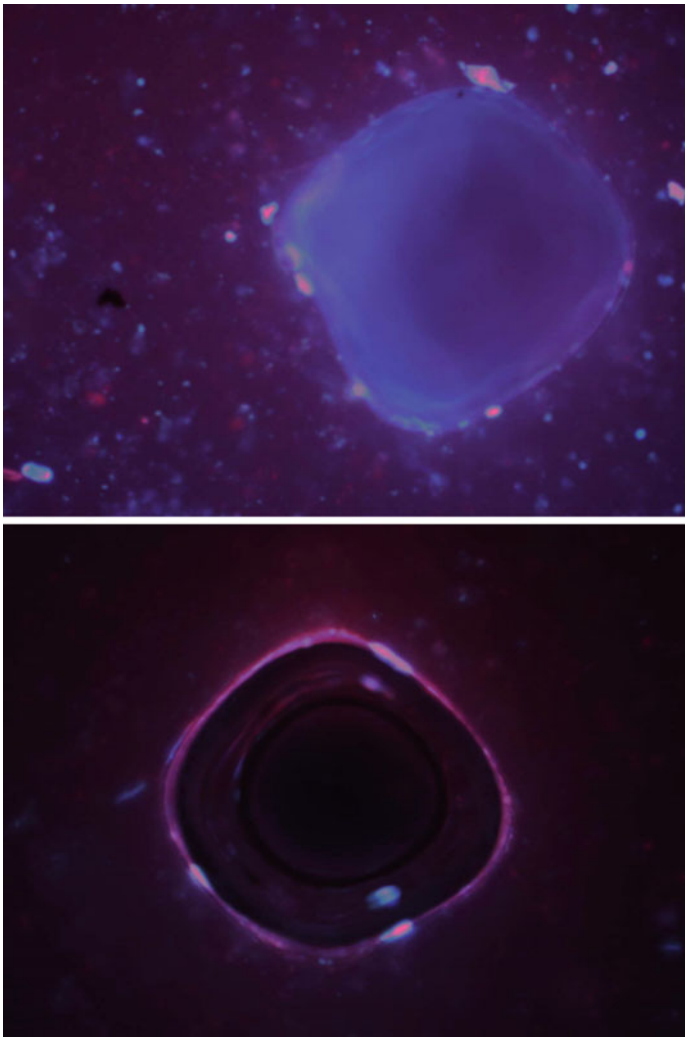

Fig. 20.10 Detailed microscopies of different pores, taken at different depths of focus, to show the colonization of the inner parts of the micropores by the cultured hMSCs. The good energetic and healthy state of the cells can be appreciated, with some cells even reproducing themselves, while the images 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.

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