Chapter 12
Materials Surface Effects on Biological Interactions

Josep A. Planell, Melba Navarro, George Altankov, Conrado Aparicio, Elisabeth Engel, Javier Gil, Maria Pau Ginebra, and Damien Lacroix

Abstract At present it is well accepted that different surface properties play a strong role in the interaction between synthetic materials and biological entities. Surface properties such as surface energy, topography, surface chemistry and crystallinity affect the protein adsorption mechanisms as well as cell behaviour in terms of attachment, proliferation and differentiation. The aim of this chapter is to show the most relevant processes and interactions that take place during the first stages of contact between the material and the physiological environment. Some examples show that the modification of different biomaterials surfaces affects both protein adsorption and cell behaviour.

Keywords Surface properties • Topography • Surface chemistry • Cell–material interactions

12.1 Introduction

A multiplicity of parameters such as implant location, size, shape, micromotion, surface chemistry and topography, and porosity among others play a very relevant role in the behaviour in service of biomaterials. The host characteristics, namely, age and health condition, are also factors that need to be taken into account. Immediately after a prosthetic device is implanted into the body, a cascade of events is triggered. As soon as a biomaterial becomes implanted in vivo, it gets in direct contact with the physiological environment consisting in a highly corrosive aqueous medium containing different types of ions, different molecules such as proteins,

J.A. Planell (✉), C. Aparicio, E. Engel, J. Gil, M.P. Ginebra,
Technical Univerity of Catalonia (UPC), Av. Diagonal 647,
Barcelona 08028, Spain
email: Joesp.A.Planell@upc.edu

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polysaccharides and enzymes, as well as different types of cells non-adherent an even adherent. The initial events taking place on the biomaterial surface upon implantation will affect very strongly the future life in service of the implant.

The ability of the material to be wetted by the physiological fluids is the first factor to be taken into account immediately after implantation. The initial events occurring at the biomaterial surface are highly ruled by its surface properties and the complex interplay that exists between them. Indeed, the hydrophilicity or the hydrophobicity of the material surface is a consequence of its surface energy which turns out to be related with the electrical charges distribution. At the same time, the distribution of electrical charges is a consequence of the surface chemistry and crystallinity and all these properties are affected by the surface topography.

Cell adhesion requires the presence of an appropriate proteinaceous substrate where cell adhesion receptors such as integrins can be attached and form the cells anchoring points. The formation of the right adhesive layer of proteins or the opsonization of the surface at very early stages depends on the surface properties of the material. Opsonization is the process of coating microorganisms or material surface with plasma proteins such as C3b (activated constituent of the group of proteins circulating in the serum of blood known as the complement system) and IgG (Immunoglobulin G) to label them as a foreign body and target it for attack by phagocytic cells (Tang et al. 1998). This is what happens in the case of most biomaterials used in medical devices.

The foreign body response is basically an inflammatory response that persists as long as there is a foreign body present to respond to. An inflammatory reaction involves the migration of neutrophils and monocytes/macrophages to the injury site by chemotaxis of different cytokines in order to phagocytose all the material labelled as foreign and cellular debris. Neutrophils disappear after finishing their task leaving place to macrophages. A sustained macrophage response is typical of a chronic inflammatory reaction and is common in most implants.

According to some authors, foreign body reaction starts during this persistent response of macrophages (Hunt 2004). The sustained and numerous presences of macrophages lead the formation of multinucleated giant cells or foreign body giant cells (FBGC) as a response to the effort to overcome the frustrated phagocytosis process experienced by single cells (Anderson et al. 1996; Dee et al. 2002). At this stage, macrophages and also fibroblasts release chemotactic factors for the recruitment of more fibroblasts. Macrophages inactivate their attack mechanisms and fibroblasts become the main cell line. At this time, fibroblasts start secreting a collagen I and III-based extracellular matrix that will encapsulate the material. The thickness of this extracellular matrix will vary depending on the movement of the implanted device and will isolate the material from the host tissue.

The foreign body reaction is a serious limitation especially in those cases where materials have to be in direct contact and integrate with the surrounding tissues; it can also lead to chronic pain and eventual device rejection and failure. The result of all the previous considerations is that the implant or medical device surface plays the leading role in its interaction with the biological environment. Consequently, the study and

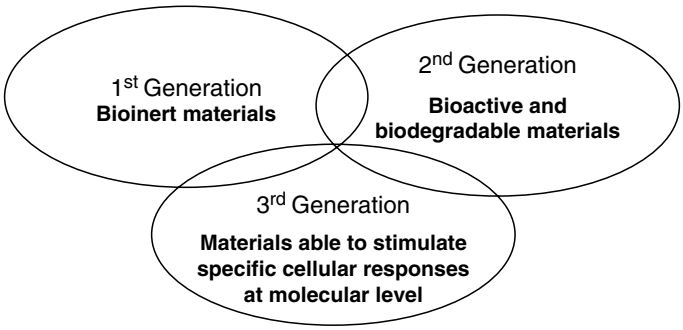

Fig. 12.1 Biomaterials generations

characterization, modification and functionalization of the biomaterials surfaces are probably the main strategies for success of implants and the tissue regeneration.

The development of materials with appropriate bulk and surface properties aiming at different goals such as support, fixation or substitution roles represents a very challenging scientific and technological issue. Most of the materials used for biomedical applications along most of the twentieth century were the same as those successfully used for other industrial sectors such as in the chemistry, energy, automotive, machine tool and aerospace industries.

The history of biomaterials' progress during the last 50 years can be understood in terms of three different generations (Fig. 12.1): a first generation (bioinert materials), a second generation (bioactive and biodegradable materials), and a third generation (materials designed to stimulate specific cellular responses at the molecular level) (Hench and Polak 2002). It is worth noting that this classification of the materials used for biomedical purposes does not imply that the appearance of a new generation of materials would exclude the use of preceding ones. In fact, materials used within the first generation of biomaterials are still successfully used in a wide spectrum of applications. Third generation materials are expected to overcome many limitations that still require adequate solutions, but by no means it is expected that third generation biomaterials should totally replace the materials from preceding ones.

12.1.1 First Generation

The progress of biomaterials has been taking place by continuously adding new demands to the list of required properties. New biomaterials have been developed as a response to these demands meant to cover new needs in the field. Concepts such as foreign body reaction, stress shielding, biocompatibility, biodegradability, bioactivity or osteoinduction are some of the demands that have steered the research for new biomaterials.

At the beginning, the main concern was to develop or select materials that combined the necessary physical properties for the devices in which they are used to match the functionality of the substituted tissue with a minimal toxic response of the host (Hench 1980). Thus, the first generation biomaterials were "inert materials" focused on achieving the minimum immune response and foreign body reaction. These first generation biomaterials consist in materials used and developed for different industrial applications, such as chemistry, food, transport and energy among others, that combine physical and chemical properties meant to endure the body aggressive environment. Among metallic materials only a few families of alloys can be selected, being the most widely used stainless steels, Co-Cr alloys and Ti and Ti alloys. Among ceramic materials, oxidic ceramics that cannot oxidize are the main candidates. Finally, among polymers and polymeric matrices in composites, fully polymerized thermoplastics and thermostables are the most widely used.

12.1.2 Second Generation

Inertness reduces the toxic response of the host. However, it does not eliminate the foreign body reactions and the formation of a fibrous layer that envelops the implant or device avoiding the direct contact between the material surface and the surrounding tissue.

Second generation biomaterials are considered to appear between 1980 and 2000. This second generation was characterized by the development of materials aimed to overcome the formation of a fibrous layer that hindered the surface/tissue interaction. This goal was achieved along two different paths: (a) by promoting a specific biological response, and (b) by using biodegradable materials able to degrade progressively as the new tissue is regenerated. This was the generation of "bioactive materials" and "biodegradable materials". For some authors the term "bioactivity" refers to the capacity of a material to elicit a specific biological response at its interface which results in the formation of a bond between the tissues and the material (Hench and Andersson 1993). Other authors proposed a definition that not only includes the ability of a material to bind to tissues but also their capacity to modulate other biological events (Black 2006).

In the case of materials used for bone applications, the most common expression of bioactivity is related to the formation of a mineral CaP layer that promotes direct binding between the implant and the tissue. Bioactivity was initially easy to associate to calcium phosphate ceramics. These materials do promote the in vivo deposition and formation of a biological hydroxyapatite layer at the material surface, improving in this way the interaction between the material surface and the bone tissue.

These materials have been used in a wide range of dental and orthopaedic applications aiming for bone tissue repair or regeneration. Bioactive materials accomplished clinical use by the mid-1980s in the form of bioactive glasses, ceramics, glass-ceramics, and composite materials.

In the case of metallic materials two strategies have been developed to obtain calcium phosphate bioactive surfaces for bone applications. One consists in coating the metallic implant surface with a calcium phosphate by different means, including plasma spray or other chemical methods, and the other consists in modifying the surface chemistry in order to induce in vivo or in vitro the nucleation of a CaP. A more general approach will consist in the material functionalization that will be described in the case of a polymer substrate.

Polymers bioactivity depends on the functional groups and binding sites available at the material surface. Thus, in the case of polymers, bioactivity can be improved by coupling certain biomolecules to their surface. This same strategy can be also used in the case of metals and ceramic materials.

Biodegradable materials are mainly represented by both natural and synthetic polymers that showed a controlled chemical breakdown and resorption of the polymer chains. The concept of bioabsorbable material was introduced in the late 1960s (Kulkarni et al. 1966; Kulkarni et al. 1971). In the last decades, these materials have been used in several orthopaedic applications such as bone substitution, repair of fractures (including ligament fixation), as sutures, rods, screws, pins and plates (Ciccone et al. 2001), and also in multiple non-orthopaedic applications such as cardiovascular, and nervous regeneration applications (Huang and Huang 2006; Teixeira et al. 2007).

12.1.3 Third Generation

Biomaterials developed during this generation are designed to be able to trigger specific cellular responses at the molecular level (Hench and Polak 2002). During this generation the biodegradability and bioactivity concepts are combined to generate biomaterials that are both degradable and bioactive. In addition to these two properties, it is also sought that materials have the ability to stimulate specific cellular events and behaviour depending on their final application.

The beginnings of this third generation of biomaterials coincide with the development of new 3D scaffolds for tissue engineering. Tissue engineering emerged as an alternative to overcome limitations such as donor site scarcity, rejection, diseases transfer, harvesting costs, and postoperative morbidity due to tissue transplantation (Banwart et al. 1995; Fernyhough et al. 1992; Goulet et al. 1997). The ultimate aim of tissue engineering is to regenerate and return the functionality to damaged tissues or organs. Thus, temporary 3D porous scaffolds are developed to be used as support and to stimulate cellular ingrowth, attachment, proliferation, and differentiation.

There are some tasks that cannot be achieved by the material itself. Therefore, growth factors and peptide sequences among others are used in combination with 3D scaffolds to repair and regenerate tissues and organs mimicking the natural signalling pathway (Hardouin et al. 2000).

Thus, 3D porous scaffolds and functionalized surfaces with biomolecules such as peptide motifs and proteins that simulate the extracellular matrix components as

to trigger specific cell responses are one of the most important achievements during the third generation (Agrawal and Ray 2001; Hutmacher et al. 2000; Temenoff and Mikos 2000).

12.1.4 Biomaterials for Substitution, Repair and Regeneration

Biomaterials development through these three generations has made possible the availability of materials exhibiting physical, chemical and biological properties as to satisfy numerous applications. These applications range from those that require materials for repair or substitution of tissues or organs to those requiring more sophisticated materials for more complex applications such as regeneration tasks. Thus, there are two main approaches that can be distinguished within the final applications of biomaterials. The first one is related to repair and substitution purposes, and the second one is related to regeneration of tissues and organs.

The first approach deals with those materials used for the elaboration of implants and prosthesis that are required to return the functionality of the tissue or organ in a short period of time, and where regeneration will not be possible. These materials are intended to fix, support or substitute the damaged tissue or organ, such as in traumatological treatments and pathologies that require urgent treatment as in the case of accidents. Most of these materials are included in the first and second generation categories of biomaterials.

The repair and substitution approach requires both non-degradable and degradable materials able to integrate and form a direct bond with the tissue as in the case of the osseointegration phenomenon. In this case, the aim is to develop materials whose surfaces stimulate and allow a direct union between the material and the osseous tissue to be formed while hindering the formation of a fibrous protein layer that envelops the implant and avoids proper material/tissue integration. Thus, there is a clear need to create surfaces able to overcome this problem. The strategies used to enhance material-protein and material–cell interactions will be discussed later in this chapter.

The second approach is related to the use of materials for tissue and organ regeneration purposes. Devices used for these applications must provide temporary support until the new tissue is regenerated and the damaged tissue/organ recovers its functionality. Thus, in this case, materials must be biodegradable and its degradation rate should match the healing process of the new tissue. This approach includes mainly materials from the second and third generation.

Another important issue within the regeneration approach is the use of cells, in particular stem cells which have raised great interest in the last years. Given the numerous limitations related to the use of autologous tissue and other alternative tissue sources such as allografts and xenografts, the use of new techniques involving the use of growth factors and stem cells has been boosted. The term “stem cell” implies that: (1) cells are capable of self-renewal, (2) cells have the ability to give rise to different cell lineages, and (3) cells are capable of in vivo functional regeneration of the tissues to which they give rise (Verfaillie 2002). Stem cells possess

the capacity to differentiate into a variety of cell phenotypes. This phenomenon is also known as "potency", and varies depending on the cells source; cells able to differentiate in only one cell phenotype are unipotent cells, while cells able to differentiate into a wide variety of cells are pluripotent cells. Totipotent cells are those able to differentiate in any cell phenotype. The potency ability of stem cells is considered as a promising tool for tissue engineering applications and transplantation.

12.1.5 Stem Cells Sources

Stem cells can be obtained from both embryonic and adult tissues. Embryonic stem cells are collected at very early stages of embryogenesis. In spite of their totipotency, their collection and usage deals with important ethical issues. The potency of adult progenitor cells is more reduced than in the case of the embryonic ones, however, their collection does not involve ethical issues. These cells can be retrieved from bone marrow, brain, and adipose tissue (Stoltz et al. 2006) (Fig. 12.2).

Mesenchymal stem cells (MSCs) are together with muscle-derived stem cells, the most used for tissue engineering applications. The term MSCs is usually used to refer to connective tissue cells in adults tissues namely (myo)fibroblasts, bone, cartilage, fat, tendon, muscles, and nerve tissue. MSCs are a subgroup of stem cells that also have the ability to give rise to different cell lineages (Pittenger et al. 1999). These cells can be isolated from a variety of sources including bone marrow, fat, umbilical cord blood, and also peripheral blood (Chim and Schantz 2006, Fuchs et al. 2005; Kern et al. 2006), and may differentiate into osteoblasts, chondroblasts, myoblasts, and adipocytes.

Among the different MSC sources, bone marrow is the most currently used. Bone marrow is a natural reservoir of skeletal MSCs. These MSCs are found in the stromal compartment of bone marrow and represent a minimal fraction (0.001–0.01%) of the total population of nucleated cells in marrow (Chim and Schantz 2006).

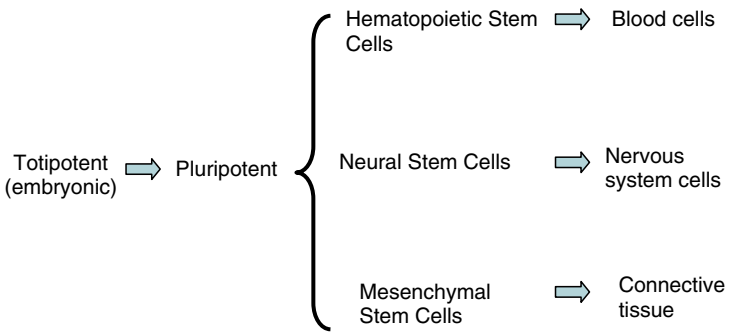

Fig. 12.2 Stem cells hierarchy

Therapies involving the use of progenitor cells require the presence of specific factors or cues that trigger the activation of particular cell behaviour and induce cell differentiation towards a determined cell lineage. Therefore, tissue regeneration supposes that not only materials' bulk properties are important but also their surface properties play a main role. Both topographical and chemical surface characteristics are of paramount importance in the interactions between materials and cells. Thus, it is expected that biomaterials aimed to regeneration purposes combine biodegradability and bioactivity together with surface properties that provide them the ability to stimulate specific cellular responses.

12.2 Surface Modification to Improve Cell–Material Interactions

In general, the success of a biomaterial strongly depends on its interaction with the biological environment. There are applications where a direct and tight contact between the tissue and the materials is required while there are other applications where a rather antifouling behaviour is needed. Within this context, material surface properties are of paramount importance.

Once a material is in contact with physiological fluids, the first interactions that take place are between the material surface and water molecules. The formation of a water coating layer involving the material occurs within a period of nanoseconds. This first stage is highly dependent on the surface properties of the material and will condition and determine which biomolecules and proteins will interact with the surface. After hydration, a second stage occurring from some seconds up to hours after implantation takes place. This stage consists in the interaction of the material surface with sugars, lipids and other macromolecules found in the physiological medium such as proteins. During this stage the "Vroman effect" is held and the material surface is covered by an adsorbed layer of proteins (McFarland et al. 1999). Finally, a third stage takes place after time periods ranging from minutes up to days after implantation. During this third stage, cells make contact with the surface and interact with it. This third stage is characterized by multiple complex interactions between the extracellular matrix proteins, cell membrane proteins and cytoskeleton proteins, surface chemistry and topography, the micro and macrostructure of the material (porosity, pore size and geometry, interconnectivity) and the released degradation by-products of the material if any. The complete process is illustrated in Fig. 12.4.

Thus, in general, biocompatibility and material biological responses are dependent on the protein adsorption process which is highly influenced by the materials' surface properties. Indeed, surface features such as its chemical composition, and surface energy determine the nature of the proteins adsorbed to the surface and their orientation and conformation.

Proteins' orientation and conformation are very important aspects affecting subsequent cell attachment and adhesion. Depending on these two parameters, the

protein peptide sequence availability will vary. Only those peptide sequences exposed to the cell–material interface are accessible to cell membrane receptors while those located in the interior of the protein are not.

Initial protein–material interactions are crucial given that they mediate cell attachment and adhesion processes. The proteins adsorbed on the material surface interact with specific cell adhesion proteins known as integrins. These are cell transmembrane proteins that possess two glycoproteic units (α and β) and three domains (cytoplasmic, transmembrane, and the extracellular one) as shown in (Fig. 12.3). The extracellular domains of the α and β units possess receptors for the specific recognition of cell adhesive peptide motifs that are contained in some adhesive proteins present in the extracellular matrix (ECM) (Siebers et al. 2005). The cytoplasmic domain interacts with the cytoskeleton fibres and other intracellular signalling molecules. Thus, integrins are able to mediate cell attachment and adhesion to the different surfaces. The cell adhesion process triggers some mechanical and chemical signals that affect further cell events such as proliferation and differentiation that indeed determine cell functionality (Anselme et al. 2000).

Cells interaction with the external medium and specifically, with the material surface is carried out through their cytoplasm, in particular, through cell structures known as lamellipodia or pseudopodia depending on the cell type, which are cell extensions formed by actin filaments (Anselme 2000). These lamellipodia possess smaller extensions which are also formed by actin filaments. These are very thin and long structures that sense the extracellular matrix and material surface. Fillopodia are the actuators of the adhesion, spreading and motility processes. Integrins located within these long and thin cytoplasm extensions interact with the substrate surface creating focal contacts that are points where several integrin receptors meet to form stronger adhesion points. Depending on the surface conditions, the fillopodia will receive more or less signals allowing the cell to attach or separate from the surface, to move in one direction or other, to get a very spread or rounded morphology, etc. (Beningo et al. 2001; Magel et al. 1993).

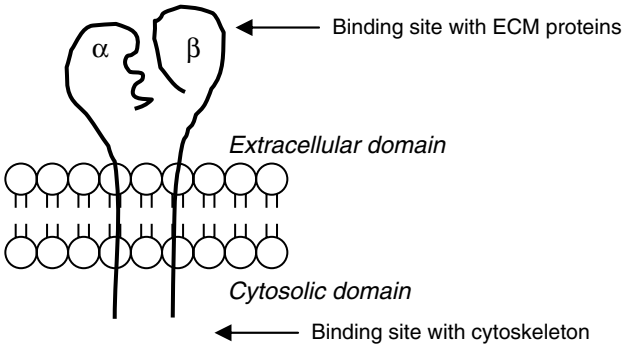

Fig. 12.3 Scheme of the structure of integrins

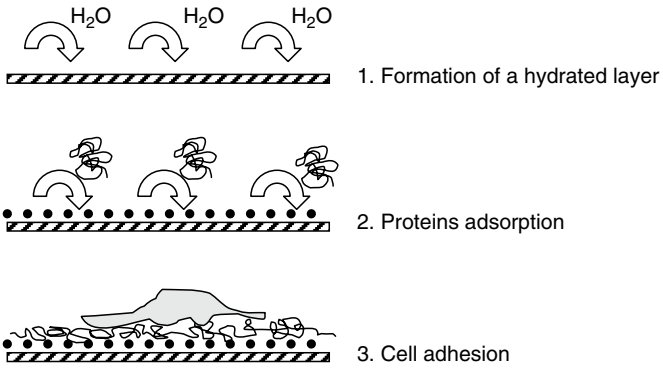

Fig. 12.4 Illustration of the sequence of events taking place at the material surface during the first stages of contact with the physiological medium

In addition to the adhesion process, cell–material interactions may be also controlled by adding some specific cues that induce their proliferation, differentiation or other cellular functions. These cues can be coupled to the materials surface by using different functionalization techniques.

In the cases where proteins adsorption, cell adhesion and formation of direct binding between the tissue and the material surfaces are not desired, materials surfaces must be modified in order to act as antifouling surfaces where proteins do not adsorb and cells do not attach and grow.

Thus, it is clear that materials surfaces are a key issue in order to enhance and control the biological response of biomaterials. Both surface chemistry and surface topography are the most important features affecting the biological response of a biomaterial.

12.2.1 Surface Topography

As shown in numerous works, surface roughness and topography are two important aspects that play a main role in cell response (Healy et al. 1996; Chesnel et al. 1995). It has been accepted that cell attachment, adhesion and proliferation on a surface can be guided by microtopography. In fact, numerous studies have been carried out and corroborate that topography influence cell adhesion (Anselme et al. 2000; Huang et al. 2004), morphology (Chen et al. 1997), migration (Tan and Saltzman 2002), orientation, focal adhesion (Diener et al. 2005), and differentiation (Zinger et al. 2005).

In some applications where cell orientation is critical to achieve a functional tissue, namely tendons, nerves, corneal stroma, and intervertebral disc regeneration, contact guidance of cells by micro–and nano-topographical features is a promising strategy. In the case of nervous tissue, it has been reported that aligned channels in

the micrometer scale are considered as the surface topographical patterns with the best results under in vitro conditions. In the case of bone tissue both micro and nanotopography have shown to induce bone cells response. Nanotopography, specifically random nanostructures have shown to elicit the best cell response to induce osteoblast differentiation for tissue regeneration (Dalby et al. 2007). In the case of implants to substitute and repair bone there seem to be an optimal roughness in the microscale that induces faster and appropriate bone growing in vitro and in vivo on rough titanium surfaces (Aparicio et al. 2003).

The use of micro or nanotopographic features depends mainly on the size of the cell structures to be influenced by the topographic changes and must be in accordance to the biological to be activated. As already mentioned, proteins are the first entities to become adsorbed to the implanted substrates. Microscale topographic structures are too large and can not be felt by proteins, in this case, nanotopographic features are closer to the dimension scale of proteins and adjust better to their size. Thus, it is possible to influence to some extent proteins adsorption behaviour by controlling the nano-topography of the substrates. However, there are many questions that remain unclear still today such as the possibility to control protein configuration upon adsorption, packing density, and arrangement among others.

Protein conformation is a very important issue since it determines the amount and type of functional peptide sequences exposed to the surface/cell interface. It has been shown that topographical changes of nanometric order of magnitude can modify the conformation and activity of the adsorbed proteins (Roach et al. 2007). A wide variety of studies conducted and reported by different research groups, where different substrates, conditions, and types of proteins and cells have been used, have been carried out. These studies have been done in order to substantiate the effects of topography, and more specifically, the relationship between the proteins adsorption mechanism and surface topographic differences. However, they have not been able to provide a clear and unambiguous understanding of the role of topography in the protein adsorption processes.

Cells are entities larger that proteins and therefore, they can be stimulated by using larger features. In general, it is well known that features ranging between 10 and 100 μm do influence cells (Mrksich and Whitesides 1995). Nevertheless, it must be highlighted that not all cell phenotypes react in the same manner to topographical changes; it seems that topographical stimulation is highly cell dependent (Meyer et al. 2005).

12.2.2 Surface Chemistry

Modification of surface chemistry is the most direct way to influence protein adsorption and cell behaviour. By tailoring the functional groups available at the material surface, it is possible to modify the surface properties, and consequently wettability, surface electrical charges, and free energy will change and as a result,

the affinity of some proteins for a particular substrate will be altered. Even though it is well accepted that certain functional groups enhance protein/surface interactions, at present there is no methodology that allows a full control of the protein conformation and orientation after adsorption (Roach et al. 2007).

Surface modification methods to improve the interactions between the material surface and cells have evolved during the last decades. One of the main goals of the second generation of biomaterials was the development of "bioactive materials". Surface bioactivation can be achieved functionalizing surfaces with different biomolecules by means of a variety of methods where both chemical bonding and physical adsorption take place. Metallic and polymeric surfaces were studied and modified using methods such as dip-coating techniques, the formation of self-assembled monolayers (SAMs) and binding polymer chains to the surface to enhance the adhesion of cells, to influence proliferation and differentiation rates, and to achieve faster and more stable integration between the material and the tissue as in the case of dental implants and some orthopaedic prostheses (Blawas and Reichert 1998; Scotchford et al. 1998). Covalent chemical coupling of polymers and biomolecules to the substrates has been achieved through silanized titania surfaces, using amino- and carboxyl-directed immobilization mainly through glutaraldehyde chemistry, and photochemistry by "grafting to" biomolecules with a photoactive group (Xiao et al. 1998, 2001; Colloioud et al. 1993).

During the third generation more sophisticated "bottom-up" and "top-down" techniques have been developed to engineer surfaces with high specificity levels as well as the synthesis and tailoring of new biomolecules for specific applications. The development of more complex biopolymers and biomolecules such as elastin-like biopolymers including peptide sequences that induce mineralization and cell adhesion, or self-assembled amphiphilic peptides that include cell signalling cues are new approaches to mimic the natural process by which collagen induces the assembling of calcium phosphate, and hydroxyapatite crystallites within bone to generate its mineral rigid phase (Rodríguez-Cabello et al. 2007; Sergeant et al. 2008)

To provide evidences about the way in which surface properties affect the biological behaviour, some specific cases showing the importance and effect of surface chemistry and topography in biological response are described below.

12.3 Oxidation Treatment of NiTi Shape Memory Alloys to Obtain Ni-Free Surfaces and to Enhance Biocompatibility

NiTi shape memory alloys have raised great interest for different biomedical applications such as orthodontic wires, vascular, urological and gastroenterological stents, staples for orthopaedics, etc. This is due to their unique properties such as superelasticity, shape memory and their excellent damping characteristics. However, in spite of their attractive properties, these are quite controversial materials since they might cause negative effects such as allergies and potential carcinogenesis,

Table 12.1 Surface properties of the NiTi alloy before and after the oxidation treatment. Mean values ± SD

NiTi alloy	Sa (nm)	Sz (nm)	Ssk	Sku	CA (°)	γ^d (mJ/m^2)	γ_t (mJ/m^2)
Untreated	21.8 ± 4.8	373.4 ± 58.1	−0.5 ± 0.3	10 ± 4.2	63.2 ± 2.6	11.3 ± 2.3	49.4 ± 2.3
Oxidized	103.5 ± 9.7	863.2 ± 93.3	0.1 ± 0.1	3.1 ± 0.2	59.0 ± 2.2	13.3 ± 1.8	52.1 ± 1.9

Sa = spacing between local peaks; Sz =....; Ssk = skewness surface plane; Sku = kurtosis of the surface; CA = Contact angle; γ^d = polar component of the total surface free energy; γ_t = total surface free energy

which are attributed to the Ni release to the surrounding medium (Wataha et al. 2001; Peltonen 1979; Dunlap et al. 1989).

To overcome this limitation and reduce the amount of Ni exposed at the material surface, an oxidation treatment was developed. It is based on a thermal treatment at a pressure of 3×10^{-2} mbar and 400°C during 2 h 30 min that leads to the formation of a stoichiometric TiO_2 almost Ni-free protective layer (Michiardi et al. 2004).

The formation of this TiO_2 layer introduces some important modifications at the material surface. It increases its roughness from a mean Sa = 22 nm to Sa = 103 nm, and enhances the hydrophilic character of the surface, mainly by increasing the polar component of its surface free energy (Michiardi et al. 2006, 2007) (Table 12.1).

It has been shown in previous studies that the surface changes caused by the formation of the TiO_2 layer on the NiTi material surface induce significant differences in protein adsorption and cell behaviour.

In a protein adsorption study performed with fibronectin, which is one of the most important adhesive proteins and with albumin, the results obtained showed that the adsorption of both proteins on the material surface was highly affected by the presence of the TiO_2 layer (Howlet et al. 1994; Altankov and Groth 1994; Grinnel and Feld 1982). In fact, a significantly higher amount of both proteins was adsorbed in the materials with the oxidation treatment than in the material without any treatment. In the case of albumin, a direct correlation between surface energy and the amount of protein adsorbed was observed, showing the highest values of protein when the polar component reached its highest value. However, fibronectin did not show any correlation between the amount of protein adsorbed and the variations of surface energy. These results suggest that in the case of fibronectin there must be other factors besides the polar component that also influence its behaviour (Michiardi et al. 2007).

In vitro biocompatibility studies carried out with MG63 osteoblastic-like cells seeded on treated and untreated NiTi surfaces have also corroborated the influence of surface characteristics in cell behaviour. According to this study, the expression of osteoblastic differentiation markers such as alkaline phosphatase and osteocalcin was higher in the cells seeded in the material with the oxidation treatment than in the NiTi surface without treatment (Michiardi et al. 2008). These differences could be attributed to the physicochemical differences between the treated and non-treated

surfaces such as chemical composition, polarity, and even crystalline oxide structure that affect the adsorbed proteins in the surface and, in turn, affects the cell response, and also to their topographical differences. The Sa value of treated NiTi surfaces was five times greater than the one of the untreated surfaces. Even if both of them are in the nanometer length scale there seems to be an effect of nanotopography on cells behaviour which is in agreement with other studies where a faster differentiation process is enhanced by nanotopographic roughness (Larsson et al. 1996).

Thus, in this case the modification of the material surface leads to a lower release of Ni ions to the medium, to a higher nanoroughness and to changes in the polar component of the free surface energy, and the combination of all these factors enhanced the biocompatibility of the material.

12.4 Surface Characterisation of Fully Biodegradable Composite Scaffolds for Bone Regeneration

Biodegradable poly (α-hydroxyacids), in particular, polylactic acid (PLA) are currently used in diverse biomedical application namely, sutures, pins, screws, and drug delivery systems (Middleton and Tipton 2000; Rokkanen 2000). In addition, PLA is a very interesting candidate for the development of tissue engineering scaffolds due to its degradability that can be tuned according to the percentages of PLA stereoisomers present in the copolymer, and also because its degradation by-products can be metabolized and eliminated from the body following natural pathways in the form of H_2O and CO_2 (Grizzi et al. 1995). However, the use of PLA has been limited to some extent because it can not fully meet the mechanical requirements of some applications such the orthopaedic ones.

To overcome this limitation, PLA matrices have been reinforced with fibres and particles of polymeric and ceramic materials (Adriano et al. 1993; Kasuga et al. 2003). This is the case of the PLA/G5 glass biodegradable composite material, which has been reinforced with particles of a soluble CaP glass coded G5 (Navarro et al. 2003). It has been observed in previous works that the incorporation of bioabsorbable glass particles into the polymer matrix not only improve the mechanical properties of the material but also its bioactivity and biological behaviour (Navarro et al. 2005).

The addition of G5 glass particles (<40 μm, 50% w/w) into the polymer leads to significant changes at the material surface. On one hand, relevant topographical changes took place as shown in Table 12.2. There is a clear increase of Sa values when G5 particles are present. Besides, other roughness values such as the Ssk (surface skewness), Sku (surface kurtosis) concerning the height and the distance between peaks and valleys revealed important differences between both surfaces.

Moreover, remarkable changes in the material's wettability and surface energy were also observed. Water contact angles varied from 73.6 for PLA to 67.6 for PLA/G5, whereas their surface energy varied from 31.1 to 41.7 mN/m.

Table 12.2 Topographical parameters for the PLA and PLA/glass composite material

Material	Sa (nm)	Sku	Ssk	SAI
PLA	74.41 ± 32.64	189.16 ± 365.74	−3.36 ± 8.24	1.01 ± 0.01
PLA/glass	3806.7 ± 587.28	5.01 ± 1.10	−0.407 ± 0.45	1.53 ± 0.16

Sa = spacing between local peaks; Sku = kurtosis of the surface; Ssk = skewness surface plane; SAI = surface area index

As in the case of the NiTi surfaces, variations in topography, and surface chemistry led to interesting differences in protein adsorption, and as a consequence, in cell behaviour. Indeed, it was reported that the amount of proteins adsorbed into the materials surfaces was higher in the case of the composite material than in the case of PLA. Moreover, the total amount of adsorbed protein increased with glass wt% significantly (Charles-Harris et al. 2005). Thus, in this particular case, protein adsorption seems to be sensitive to the chemical effect of the exposed glass particles.

In vitro cell cultures with MG63 osteoblast-like cells on PLA and PLA/G5 surfaces have shown interesting differences between the results obtained in both substrates. According to the reported results, there is a higher adhesion of cells in the case of the polymer reinforced with the glass particles than in plain PLA substrates. Moreover, there is a very clear difference in the morphology of the cells adhered to PLA or to the PLA/G5 composite material. In the case of PLA, cells showed a very well spread and flat morphology, whereas in the case of the composite material, cells adopted a more rounded and more voluminous configuration (Navarro et al. 2008). Topography has an important effect on cell behaviour as already mentioned (Boyan et al. 2001). In fact, surface roughness affect the interactions between the extracellular matrix and cells which in turn affects the formation and total amount of focal contacts as well as their type of adhesion and leads to changes in the cell cytoskeleton and in gene expression (Gronowicz et al. 1996). Thus, according to this statement, surface topography highly affects cell proliferation and differentiation processes.

The presence of glass particles and the interfaces and nonunions between the polymer matrix and the CaP glass particles promoted morphological changes in cells, so they could adapt better to the material topography.

12.5 Micro and Nanopatterned Surfaces for Biomedical Applications

As already discussed and described in the previous sections, there is a clear influence of surface topography and chemistry in cell response (Curtis and Wilkinson 1997). However, there is not a comprehensive understanding of the mechanisms of this effect.

Numerous studies have been done to elucidate the process by which cells are affected by both topography and chemistry. In the case of chemistry, there is a more

direct effect caused by the presence of well known specific functional groups, peptide motifs or proteins that are known to react with certain cell structures and activate some signalling cascades that trigger specific cell behaviours.

In the case of topography, the effect is not so obvious. Nevertheless, it has been observed that in general, the studies reported at the moment suggest that using a determined micro and nanotopography may cause cells to spread and elongate, to align following the direction of the surface pattern (Wilkinson et al. 2002; Charest et al. 2007) to rearrange the extracellular matrix in contact with the surface (Dalby et al. 2004; Johansson et al. 2006), and to internally re-organize cellular components (Gadegaard 2006), leading to variations in cellular responses. Nevertheless, there are well defined differences between surfaces presenting features in the micro or nano length scale.

Cell studies on PMMA non-structured surfaces and on PMMA surfaces structured with posts and holes using the hot embossing technique (Mills et al. 2007) have shown that in general, cells prefer to grow on non-structured surfaces that on the structured ones. In the case of the structured surfaces, there seems to be and slight difference in the cell response whenever posts or holes are used. It seems that cells use the posts as anchorage points to hold themselves to the surface. This work also showed that cells are also affected by the features size and separation between them; this in turn affects cell alignment. As the dimensions of the structures become smaller and the dimension differences between cells and features increase, the cell morphology seems to be less affected by the surface structures. Smaller features with subcellular dimensions affect through smaller cell receptors such as integrins.

Micro and nanopatterning of surfaces has also been combined with chemical patterns in order to study cell behaviour under different conditions. Micro and nanofabrication techniques such as nanoembossing together with surface functionalization techniques like microcontact printing, nanoplotting and dip-pen nanolitography have been used to covalently attach selectively adhesion proteins such as fibronectin to 2D substrates (Martínez et al. 2007).

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