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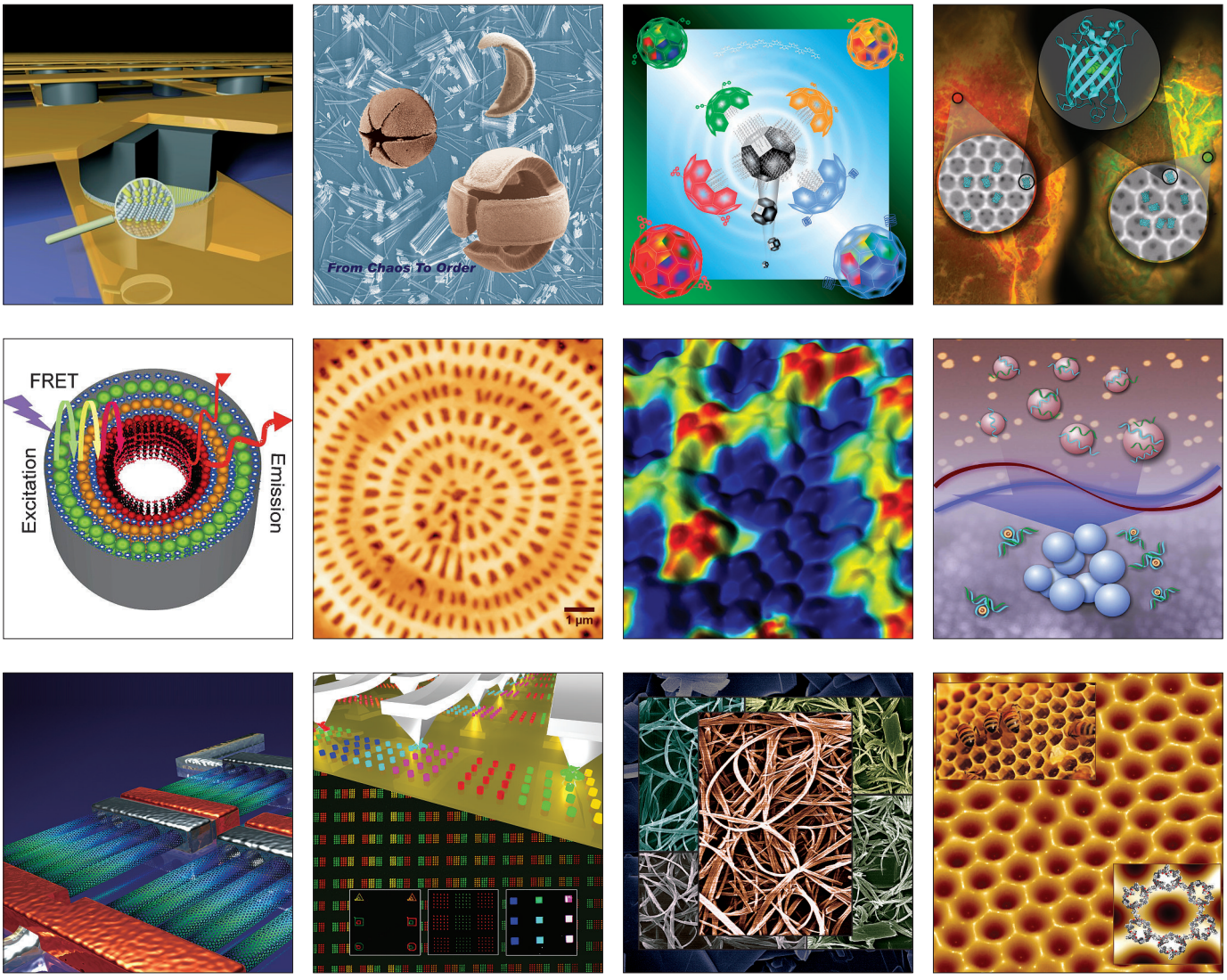

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Improving Biocompatibility of Implantable Metals by Nanoscale Modification of Surfaces: An Overview of Strategies, Fabrication Methods, and Challenges

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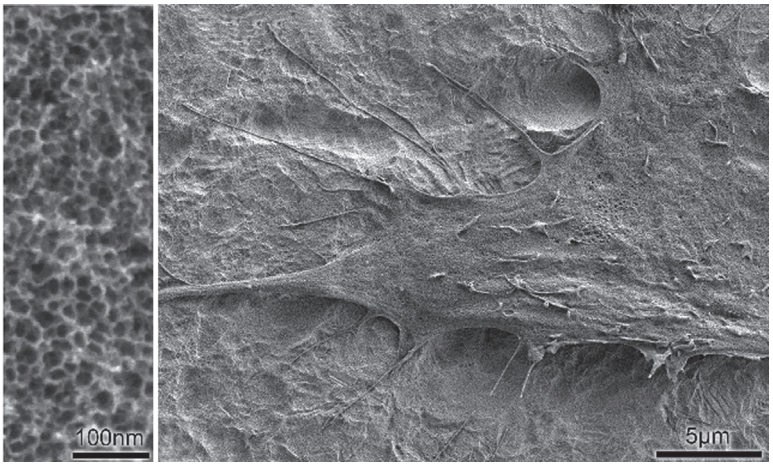

From the Contents

The human body is an intricate biochemical–mechanical system, with an exceedingly precise hierarchical organization in which all components work together in harmony across a wide range of dimensions. Many fundamental biological processes take place at surfaces and interfaces (e.g., cell–matrix interactions), and these occur on the nanoscale. For this reason, current health-related research is actively following a biomimetic approach in learning how to create new biocompatible materials with nanostructured features. The ultimate aim is to reproduce and enhance the natural nanoscale elements present in the human body and to thereby develop new materials with improved biological activities. Progress in this area requires a multidisciplinary effort at the interface of biology, physics, and chemistry. In this Review, the major techniques that have been adopted to yield novel nanostructured versions of familiar biomaterials, focusing particularly on metals, are presented and the way in which nanometric surface cues can beneficially guide biological processes, exerting influence on cellular behavior, is illustrated.

Frontispiece adapted from Reference [94].

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1. Introduction

The development of new biomedical devices with optimal performance and functionality requires careful consideration of many parameters. For example, materials used for making load-bearing implants must be mechanically strong[1] and must possess high resistance to corrosion and wear to prevent weakening of the mechanical strength and the release of potentially dangerous metallic ions[2,3] or debris[4] in the human body. Materials for implants must also provide the ability to create diverse shapes, from simple rods to complex geometries. Importantly, implantable materials must exhibit natural biocompatibility[5] to minimize allergic immune reactions, which could eventually compromise fixation of the implant and reduce its load-bearing capacity.[6] Some devices must permit substantial deformations, as in the case of cardiovascular stents.[7] Among the three principal classes of materials adopted in medicine (metals, ceramics, and polymers),[8] biologically compatible metals best satisfy the requirements for implants. For this reason, they are commonly used in orthopedics, dentistry, cardiology, and other areas of medicine.[9] The most widely used ones are stainless steels (such as 316L), as well as titanium and its alloys (e.g., TiAl and shape-memory TiNi alloys used in orthopedics[10] and cardiology,[11] respectively). In addition, CrCo alloys (such as CrCoMo and CoNiCrMo) are also encountered, especially in applications where low friction and high wear resistance are needed, such as in the components of knee and hip joints.[12,13] Tantalum is also advantageously exploited to create an interconnected three-dimensional (3D) support that favors the ingrowth of bone and effective integration of the implant.[14]

Resistance to corrosion and biocompatibility are two fundamental properties of implantable metals that are intimately correlated. Together, they determine the interaction between the metal and the human body from an electrochemical and biological point of view. The response is typically mediated by the presence of a superficial oxide layer such as TiO_2 (for titanium and its alloys), Cr_2O_3 (for stainless steels and CrCo alloys), Nb_2O_5 (for niobium), and Ta_2O_5 (for tantalum). The outer layer insulates the reactive underlying metal from the external environment.[15] And vice versa, the role of the superficial oxide is not only to protect the metal but also to add further benefits needed for biomedical applications. In the case of Ti-based materials, for example, TiO_2 is believed to enhance bioactivity by providing a surface with a moderate negative charge at physiological pH. These attributes ensure that proteins are not denatured when their hydrophilic outer shell interacts with the TiO_2 surface.[16] In addition, a negative charge is also expected to attract Ca^{2+} ions to the surface when it is exposed to bodily fluids. While such a phenomenon is advantageous for orthopedic and dental implants by favoring their osseointegration,[17,18] it is not desirable for cardiovascular stents, where a charged surface could seed precipitation of insoluble mineral aggregates, ultimately contributing to recurrence of narrowing of vessels (*restenosis*).

As a result of their intrinsic bioactivity, mechanical properties, and high resistance to wear and corrosion,[8] the above implantable metals have become widely used in medicine. In particular, Ti-based metals have received much attention and represent an attractive model system for exploring how nanotechnology can be exploited to create even more effective implantable devices. For these reasons, this Review focuses on recent research that has begun to offer a new generation of metals characterized by surfaces with physico/chemical features that have been modified on the nanoscale (i.e., in the 1–100-nm range). In the following section, we describe the role of surfaces and interfaces in biology, specifically in the context of implantable biomaterials. Section 3 describes approaches currently used for surface modification of biocompatible metals and is divided in two parts, grouping together physical and chemical methods. Finally, Section 4 summarizes the main points of the Review and provides perspectives for future work in this field.

2. Surface Science and Biology

Recent developments in nanoscience have created exciting opportunities for collaborative ventures in which materials scientists and biologists work closely together and pool their knowledge to develop a deep understanding of how cells respond to foreign surfaces.[19–25] Variations in biological activity at the interface between materials and host tissue can be correlated with specific surface properties. Chemical

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composition, energy, roughness, and topography[26–29] are all believed to help determine the activity of different cell lines, acting either separately or synergistically. The connections between the physico/chemical properties of surfaces and cellular responses are still not fully understood. Once they are elucidated, however, they will improve our understanding of fundamental biological processes. At the same time, the ability to tailor surfaces to control cellular events and guide the fate of cells along predetermined pathways will pave the way for the rational design of a new generation of biomaterials that can be integrated in the human body more effectively and beneficially. The classical conception of conventional biologically inert or biocompatible materials will gradually be replaced by a more ambitious vision of a new generation of ''smart'' materials with intelligent surfaces able to interact decisively with the biological environment, and that may even initiate selective reactions in response to differential cues.

The properties of surfaces of materials can be modified on a range of scales by various techniques,[30,31] with the common aim of collecting information to unravel the link between surface cues and cellular response.[32,33] Straightforward surface treatments that have frequently been used to modify the behavior of biocompatible metals include polishing, grinding, blasting, and machining.[34–36] Such purely mechanical methods, used either individually or in combination with other treatments, mainly cause the formation of different topographies with inhomogeneous micrometric features. Nevertheless, these features have been demonstrated to have an impact on cellular activities and on osseointegration.[37–41] Although simple and partially effective, modifications introduced by these methods are unlikely to be optimal and can be questioned, largely because they are too coarse to directly influence events on the spatial scale at which cells function. In fact, it is increasingly recognized that interactions between biomaterials and host tissues are controlled by nanoscale features.[42,43] Cells grow on nanostructured extracellular matrices,[44–46] and biological events such as signaling and cell–substrate interactions occur at the nanometric level.[47–49] In addition, adsorbed proteins and their aggregates are a few nanometers in dimension. These arguments have been strongly reinforced by recent reports that have highlighted the creation of nanoscale surface cues[32,50] and have begun to assess their effects on biological activity.[51–56] In certain cases, however, cells respond to features greater than 100 nm.[57] For example, it was reported that smooth muscle cells respond more efficiently to submicrometric than to nanometric features.[21,58]

The most widely used cell types for studies of bioactivity are osteoblasts (bone cells), fibroblasts (connective tissue cells), endothelial cells (blood vessel cells), smooth muscle cells, bone marrow cells, and stem cells (pluripotent undifferentiated cells).[59] The choice of the cell model depends on the application intended for the material (e.g., osteoblastic cells are used to evaluate the osseointegration of materials for future orthopedic and dental implants, and endothelial cells are used to test whether a material for cardiovascular applications has anti-thrombosis properties) and/or on the biological activity under investigation (such as matrix mineralization or cellular differentiation). In many cases, the cells used are transformed cell lines (e.g., MC3T3, UMR106, MG63, NIH3T3, BHK21, and A7r5). In large part reflect the activity of the cell type from which they derive but that may not necessarily yield similar bioactivity outcomes. In fact, these cells are usually referred to by terms such as ''osteoblastic'' and ''fibroblastic,'' for instance. Transformed cells are often selected because they can be maintained for a long time without losing their potential. Primary cultures with cells isolated from tissues prior to use are more difficult to grow, and they generally lose phenotypic specificity. Also, they exhibit greater phenotypic variability than transformed cell lines, a feature which actually reflects more closely the in vivo biological situation. For these reasons, although in vitro studies provide useful indications, ultimately in vivo analyses must be carried out to unequivocally assess the bioactivity and performance of a material under the multifactorial conditions found in the body.

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Antonio Nanci received his Ph.D. (1982) in anatomy and cell biology from McGill University and was a postdoctoral fellow in craniofacial molecular biology at the University of Southern California. He is now Professor of Cell Biology and Director of the Laboratory for the Study of Calcified Tissues and Biomaterials at the Faculty of Dentistry of the Université de Montréal. In 2007, he was elected Director of the Department of Stomatology at the Université de Montréal. His research focuses on the study of basic cell-biological processes of normal and pathological calcified tissues and on the exploitation of nanotechnology-based strategies to functionalize the surfaces of biomaterials to improve their integration in the body.

3. Approaches for Surface Modifications

This Review does not report an exhaustive analysis of the literature pertaining to nanostructured biomaterials but rather focuses on the main methods adopted until now to give implantable metals new biological functionalities on the nanoscale by using surface-modification approaches. These methodologies have been selectively chosen to reflect commonly used strategies and have been divided into

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chemical approaches (Section 3.1) and physical approaches (Section 3.2).

3.1. Chemical Modifications

Recent work has established that key biological processes, including protein adsorption, cell proliferation, and gene expression, can be controlled to some extent by using chemical methods to modify the surface properties of biocompatible materials.[60] The most popular and efficient ways to modify surfaces on the nanoscale involve direct chemical modifications with acids and oxidants. Chemical treatments are attractive for large-scale manufacturing because they are simple and provide efficient and uniform access to all surfaces, even on multifaceted devices with complex 3D shapes such as dental screws and cardiovascular stents. In principle, chemical modifications leading to controlled surface functionalization can be also applied to other families of materials such as polymers[61,62] thus extending the scope of the technique. For example, it was reported that poly(lactic-*co*-glycolic acid) can be nanostructured by chemical etching with NaOH, resulting in a material with novel surface features able to enhance the activity of various cell types.[63–65] Together, these characteristics make chemical treatments an advantageous and flexible way to modify biomaterials for commercial applications.

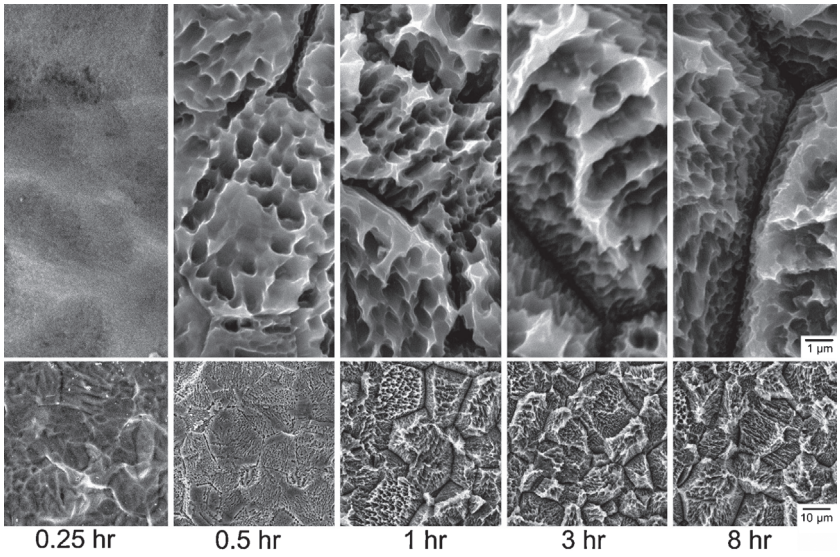

Figure 1. Scanning electron microscopy (SEM) images illustrating various microtextures achieved on Ti after etching in 48% H_2SO_4 at 60 °C for 0.25, 0.5, 1, 3, and 8 h. The upper row displays micrographs with higher magnification. Reproduced with permission from Reference [70]. Copyright 2006, Elsevier.

3.1.1. Oxidative Nanopatterning

Topography is known to help determine how cells respond to surfaces.[33,55,66–69] Different chemical treatments with acids,[70–72] bases,[73] and oxidants[74,75] have been used to create micrometer-scale and submicrometer-scale textures on surfaces (Figure 1). Such studies have revealed that chemically treated surfaces can enhance the adhesion and proliferation of osteogenic cells,[35,76] precipitation of apatite,[77] and the expression of bone-related genes and proteins.[78,79] From these observations, surfaces that are hydrophilic, microrough, and porous appear to have beneficial effects on various biological phenomena.

Although chemical treatments have yielded a variety of microtextured implants with improved clinical outcomes,[80,81] the demonstration in several laboratories that cells respond to nanofeatures has intensified the application of chemical treatments for nanostructuring biometals. A particularly effective method for nanostructuring titanium-based metals is electrochemical oxidation.[53] By adjusting parameters such as the nature of the electrolyte, voltage, and current density, smooth Ti surfaces[82–84] were transformed into nanotubular structures (Figure 2), with diameters less than 100 nm.[85–87] Biological studies carried out on these anodized Ti surfaces revealed a general increase of in vitro activity, measured by enhanced osteoblastic activity[83,85,87] and mineral precipitation.[88] Anodization creates nanoscale topographies, yet it also allows control over other physical properties such as pore size and the thickness of the oxide layer,[89] thus providing a way to conduct targeted experiments (e.g., varying only the dimension of surface features) to reveal how each of these parameters affects cellular behavior.

A different chemical approach for modifying the surface of metals is based on the observation that etching with combinations of strong acids and oxidants can generate a nanotopography. Mixtures of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) have been shown to reproducibly yield networks of nanometer-sized pits around 20 nm in diameter (Figure 3) on Ti[90,91] and Ti6Al4V alloy.[21,92] Surface morphology, wettability, nanoroughness, and the thickness of the TiO_2 overlayer can be controlled by adjusting the length of exposure to the etching solution.[21,93] It is also possible to vary the density of OH groups on the surface,[91] which is believed to influence cell activity.[93] Unlike other methods described so far for structuring surfaces, this chemical treatment of Ti and its alloys creates surfaces with distinctive discriminatory effects on different cell types. In particular, the

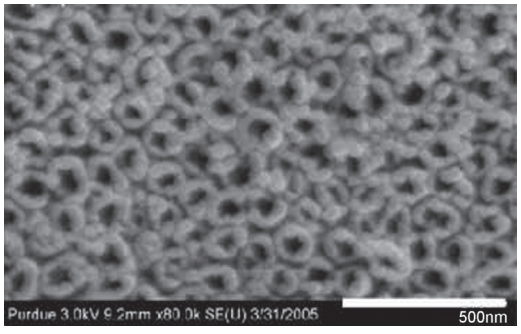

Figure 2. SEM image of nanotubular structures created by anodization of Ti. Reproduced with permission from Reference [87].

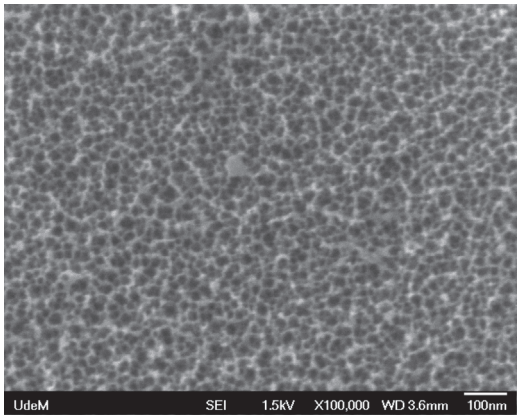

Figure 3. SEM image of the characteristic nanometric sponge-like structure that is achieved by treatment of Ti with H_2SO_4/H_2O_2. Reproduced with permission from References [90, 91]. Reference [91] copyright 2006, Elsevier.

surfaces can promote the expression of bone-related genes and the activity of osteoblastic cells,[94–96] while inhibiting that of fibroblastic cells (Figure 4).[21,92] Such differential effects are important for understanding the basic factors that underlie specific cellular behavior, as well as for creating dental and orthopedic implants with surfaces that optimize the formation of bone yet also limit detrimental fibrous encapsulation.[97,98] On-going studies from our group have demonstrated that oxidative nanopatterning can also be successfully applied to other relevant implantable metals, such as CrCoMo and Ta. In addition, as in the case of anodization,[89] varying the conditions of chemical oxidation (such as the nature of the etching solution) makes it possible to incorporate selected elements in nanotopographic surfaces created by oxidative treatment. One such interesting feature is the introduction of fluorine, which has known antibacterial effects[99] and is believed to favor bone formation.[100]

3.1.2. Other Chemical Approaches

The properties of surfaces can also be modified at the nanometric level by using other processes such as sol–gel[101] and chemical vapor deposition (CVD).[102] In vitro biological tests on materials coated in these ways have demonstrated that

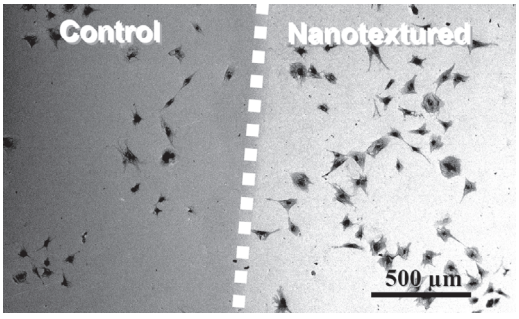

Figure 4. Comparative SEM images of primary osteoblasts, grown for 3 days of culture on side-by-side smooth (control) and nanotextured Ti6Al4V surfaces. This nanotexture obtained by oxidative patterning enhances osteogenic cell growth. Reproduced with permission from Reference [21].

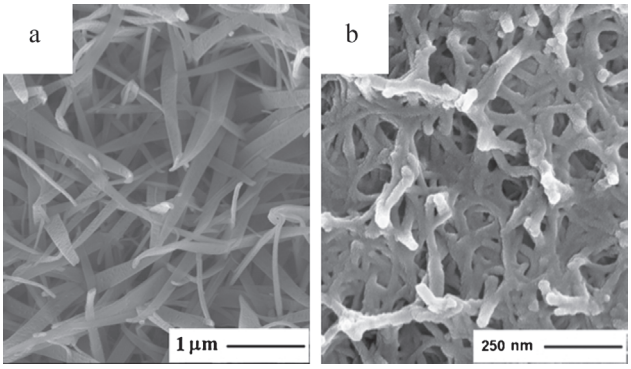

Figure 5. SEM images of rod-like structures resulting from the treatment of Nb with NaOH at a) 60 °C (diameter of the rods in the 100–300-nm range) and b) 80 °C (diameter of the rods in the 50–100-nm range). Reproduced with permission from Reference [108]. Copyright 2008, Elsevier.

the novel surface features have beneficial effects on bone cell activities, including adhesion, spreading, and matrix mineralization.[103,104] Chemical strategies have been efficiently exploited to create nanostructured coatings of materials that are not directly used in implantology. Coatings of nanostructured niobium oxide[103] and diamond-like carbon,[105–107] when deposited by sol–gel or CVD on titanium and other substrates, have demonstrated significant bioactivity, thus providing additional avenues for improved biomaterials. In addition, alkali treatment of bulk niobium has resulted in the formation of nanometric fibers (Figure 5) that favor precipitation of apatite from simulated bodily fluids.[108] Electrochemical deposition makes it possible to create coatings comprised of wirelike nanometric crystals of hydroxyapatite (Figure 6),[109–111] which enhance bone remodeling and maturation.[112] Similarly, composite coatings of hydroxyapatite and multi-walled carbon nanotubes[113] deposited on titanium have been achieved by electrophoretic deposition.

3.2. Physical Modifications

Several physical methods have been used to alter biomaterials and endow them with useful new properties, and such alterations have also shown to favor diverse biological processes.[114,115] Electrostatic and plasma spray,[116,117] as well as physical vapor deposition (PVD) techniques such as electron-beam evaporation and deposition,[118,119] can yield superficially deposited bioactive layers. The deposition of TiO_2 and hydroxyapatite in this way has been shown to enhance the activity of osteoblastic cells and to favor osseointegration in vivo.[114,120] Although these methods generally result in modifications on the micrometer scale, such physical approaches can also be used to create nanostructures.

Nanonodular structures form during PVD of titanium on microroughened biocompatible metals such as Ti and CrCo alloys.[121] The resulting nanostructured surfaces, when implanted in rat femur, showed increased in vivo osseointegration compared to the same microtextured metallic substrates. Nanometric nodules were also created on ceramics and semiconductors, thus extending the applicability of this technique. PVD was also exploited to create substrates for the

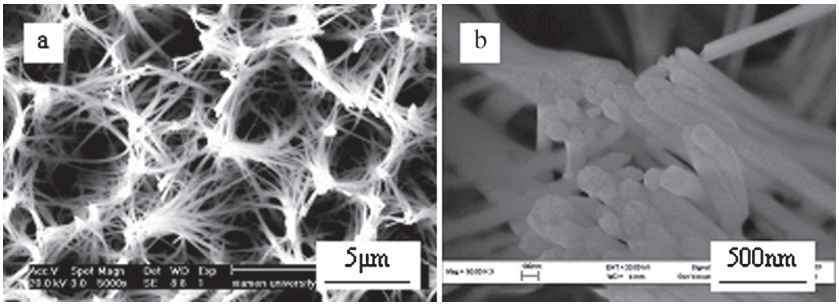

Figure 6. SEM images of an electrochemically deposited coating of calcium phosphate on smooth Ti. a) Microporous structure. b) Crystal grains on the nanometer scale. Reproduced with permission from Reference [110].

study of basic cellular functions. For example, PVD was adopted to modify titanium with different nanotopographical coatings for in vitro studies of the effects of surface roughness on focal adhesion contact formation by fibroblasts.[122] Similarly, titanium layers were deposited by PVD on textured titanium and hydroxyapatite to evaluate the effects of surface chemistry and topography on the cellular and/or tissue response.[123] Although not nanometric in scale, PVD-deposited titanium/silver coatings proved to increase the antibacterial properties of titanium.[124] A nanostructured cluster-assembled TiO_2 film was produced by supersonic beam deposition as a substrate for different applications in cell-based assays, biosensors, and microfabricated medical devices.[125] Other biologically relevant metals have been deposited on different biocompatible materials by physical deposition. For example, electron-gun evaporation has been used to create coatings of other implantable metals, such as films of nanostructured tantalum with well-controlled roughness.[126]

Laser-based approaches have been exploited to produce a coating of calcium phosphate on titanium and its alloys. Such coatings have been reported to have multiphase compositions ranging from the nano- to mesoscale[127] and to enhance in vitro osteogenic cell attachment, growth, and differentiation.[128]

A different approach based on compaction of metallic nanoparticles (Ti, Ti6Al4V, CrCoMo) has been successfully applied to produce nanostructured surfaces[129] (Figure 7). The inherently higher number of particle boundaries in materials prepared from nanoparticles was suggested as an explanation for the observed enhanced adhesion of osteoblastic cells.[129] Moreover, there was more deposition of calcium and phosphorus from simulated bodily fluid, which suggests that mineralization can also be enhanced by appropriate nanostructuring.[130]

4. Summary and Outlook

In this Review, we described mechanical, chemical, and physical methods that have been used to alter the physico/chemical features of the surfaces of metallic biomaterials, thus modifying their impact on different cellular activities and functions. Improved biological activities have been reported on both microstructured and nanostructured surfaces. This Review focuses specifically on chemical and physical methods that have been exploited successfully to create nanometric features on implantable metals (Table 1). Significant progress has been made in this area, and nanoscale surface modifications are expected to usher a new generation of improved implants with more efficient and enhanced biological responses.

Promising evidence is emerging that nanotopographical features on surfaces have unique and important effects that can lead to cell-specific functions. Understanding how physico/chemical features influence biological events, by identifying how cells sense surface cues and the various signaling cascades

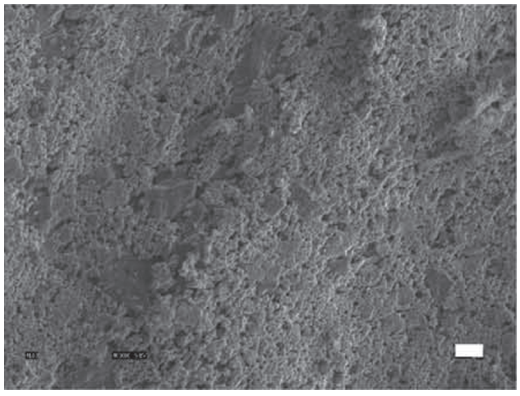

Figure 7. SEM image of nanophase CrCoMo produced by compaction of nanoparticles. Scale bar = 1 μm. Reproduced with permission from Reference [129]. Copyright 2004, Elsevier.

Table 1. Methods for creating nanometric topographical features of biological relevance

Methods	Resulting topographical features
Chemical	
Anodization[85–87]	Nanotubolar structures on Ti
Oxidative nanopatterning[21,90–92]	Nanoporous texture on Ti, Ti6Al4V, Ta, and CrCoMo
Sol–gel and CVD[101–108]	Nanostructured coatings on implantable metals
Molecular self-assembly[31,90,148–153]	Self-assembled monolayers of bioactive molecules
Physical	
PVD[121–124]	Nanostructured coatings on implantable metals
Nanoparticle compaction[128,129]	Nanophase biomaterials (Ti, Ti6Al4V, CrCoMo)
Lithography[20,138–146]	Custom-designed nanometer-sized patterns for the study of substrate–cell interactions

that are involved, is an important step toward improved biomaterials and the creation of surfaces tuned for achieving specific biological outcomes and for attaining overall improved performance. So far, most of the studies have been carried out in cell culture. In vitro conditions cannot accurately represent the complex biochemistry of living systems, yet they can still provide very useful indications about the validity of surface treatments, and can offer improved understanding of basic cellular functions. Such studies will undoubtedly lead to a deeper knowledge of fundamental phenomena that control biological processes such as tissue integration. Even though primary cells may be subjected to more experimental variability, their use, as opposed to transformed cell lines, is advisable to probe signaling mechanisms.

Williams[131] has recently analyzed over 50 years of experience with implantable devices and has concluded that, in the vast majority of circumstances, attempts to introduce biological activity into a biomaterial have not been clinically successful, and that the long-term outcome essentially depends only on chemical and biological inertness. However, a recent review paper by Mendonça et al.[132] as well as some clinical reports[133–137] have indicated that surface modification is a valuable strategy to give metallic implants new biological functionalities for in vivo clinical applications. Cellular response at a site of implantation results from a combination of biochemical signals and physico/chemical cues. Learning how to best integrate these two contributions is thus essential to optimize tissue repair and even achieve regeneration. It is also expected that this will ultimately allow the rational design of implantable devices with precise tailor-made surface properties to assist the natural response of the body. Clearly, more extensive investigations of the behavior of nanostructured surfaces in vivo and clinical evaluations are needed to assess the relevance of such surfaces in the complex biological environment of the body, where implants are exposed to multiple cell types that may not all respond in the same way to a given surface cue. Therefore, it is likely that optimizing implant surfaces will require different levels of topography. Because of their simplicity and applicability to different biocompatible metals, chemical treatments such as oxidative nanopatterning offer an attractive way to obtain surfaces with topographies of various dimensions.

This Review focuses on implantable metals. However both polymers[20,138,139] and ceramics[140–142] with custom-designed nanometer-sized patterns and features (Figure 8) have also been created to improve our understanding of cell–substrate interactions (Figure 9).[140,143–146] These other materials have also been shown to influence cell activity, yet a direct comparison with metallic nanostructured materials is difficult because of the different surface chemistries involved.

An effective way to achieve improved biomaterials is to synergistically combine

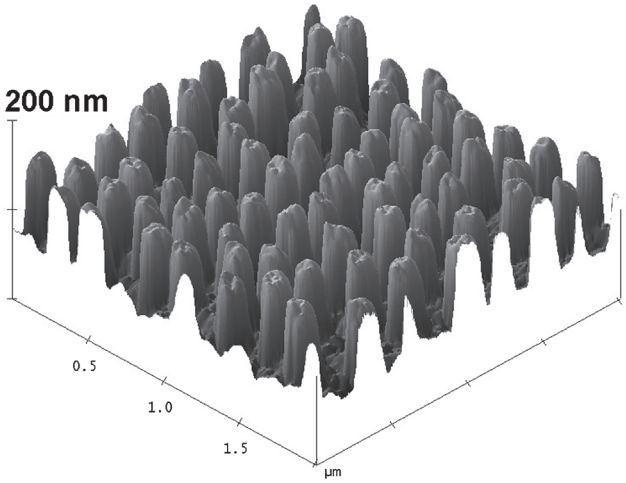

Figure 8. Atomic force microscopy (AFM) image (3D rendering) of 160-nm-high nanocolumns created on a PMMA substrate by colloidal lithography. Reproduced with permission from Reference [146]. Copyright 2004 Elsevier.

different functionalization approaches.[147] For instance, nanopatterning materials can be combined productively with the technique of grafting bioactive molecules to the surface.[31,148,149] Oxyilanes[90,149,150] and phosphonates[151–153] can be used to link bioactive proteins and peptides to the surface oxide layer of metals. These serve primarily as linkers, yet they can stimulate cellular functions on their own.[154] Oxyilane linkers have also been used to graft antibacterial agents[155,156] and antibiotics,[157,158] thus providing a valuable strategy for creating infection-free implants for clinical applications. The physico/chemical properties of the surface affect the degree of coverage, as well as the nature and stability of the linkage to the grafted molecules.[155] Chemical

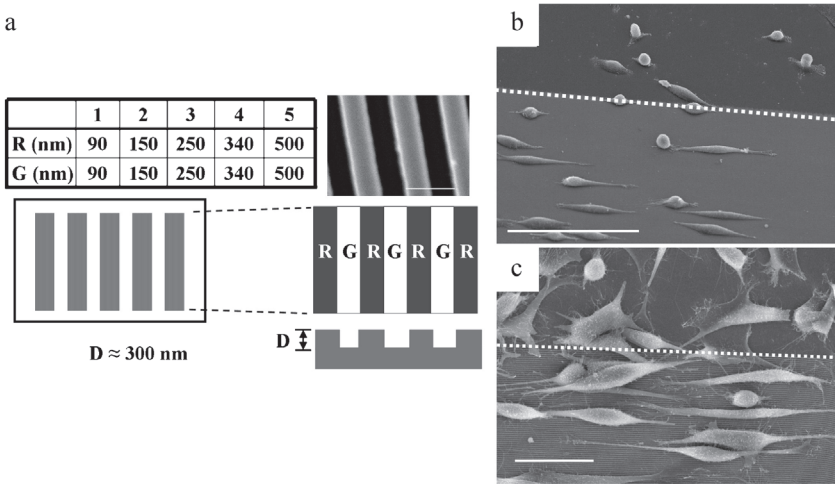

Figure 9. a) Nanopatterned silicon with ridges (R) and grooves (G) of different widths but equal depths. An SEM image of the nanogrooved surfaces is shown in the top-right corner (scale bar = 1 μm). b) MG-63 cells on the nanopatterned surface with 90-nm-wide grooves (scale bar = 50 μm). c) MG-63 cells on the nanopatterned surface with 340-nm-wide grooves (scale bar = 30 μm). The areas above the dashed lines in (b) and (c) are flat regions, whereas those below the dashed lines are the nanopatterned regions. Adapted from Reference [140].

treatments used to nanotexture metals are expected to exert effects by increasing the surface area and/or by providing more binding sites. Such effects presumably result from various factors, such as the removal of contaminants, variations in the oxidation state, and alterations in the spatial distribution of surface functional groups such as OH. This may allow suitably prepared surfaces to simultaneously present cells with nanotopographical features combined with dense ordered arrays of bioactive molecules, linked directly to surfaces or through spacers. For example, the oxidative nanopatterning of titanium can increase the molecular density of adsorbed alkanephosphonic acids.[148] Although studies of simple adsorbed layers of siloxane and phosphonate provide important guidance, the best agents for surface modification are likely to be more complex molecules that form strong links at multiple sites, such as *multidentate* linkers.

Adsorbed proteins are believed to play an important role in controlling cell colonization on implants.[26] In fact, protein adsorption onto surfaces is the initial event during the integration of an implant with surrounding tissue.[47] One question that remains to be addressed is how these adsorbed proteins influence how cells sense and perceive nanostructures on the surface. Non-fouling agents such as poly(ethylene glycol) can be used to render surfaces non-adhesive and thus to control the adhesion of a particular cell type or protein.[159] Integrated with physical cueing, this non-fouling capacity could enhance the bioactivity of a nanostructured surface by controlling protein adsorption from biological fluids on implanted metals and by suppressing non-specific interactions with unwanted cells.[160]

To enhance the biological responsiveness of surfaces, physical modifications can also be applied to further modify nanopatterns created by the various approaches discussed above. For instance, one such synergistic modification is ion implantation with calcium ions to favor mineral deposition on the surface[115] and to activate bone formation in vivo.[161] However, the potential modification of nanostructures by highly energetic processes must be considered when using synergistic approaches such as ion implantation (Figure 10).

Recent evidence has shown that nanometric surface cues can guide stem cells along the differentiation pathway without exposure to molecular signals.[20] A potentially valuable source of stem cells for use in regenerative medicine of the skeletal system could be adipose-derived adult stromal cells. In vitro, these cells demonstrate a capacity for bone formation equal to that of bone marrow.[162–164] They have the distinct advantage of circumventing the complex isolation of bone marrow stem cells and the need to expand and engineer them in vitro before implantation. An exciting new development in biology is the finding that stem cells are present at more sites than expected and can even be created by dedifferentiation of committed cells, thereby paving the way for more routine use of these cells.[165,166] The availability of surfaces that are capable of recruiting and expanding stem cell populations as well as guiding their differentiation along selected pathways has great potential to induce local tissue regeneration at sites of implantation.

Although much work has been done with osteogenic cells, surface modifications are not limited to orthopedic applications. For example, they may also be relevant for cardiovascular applications. In fact, we anticipate that it will be possible to produce biomaterials that have anticoagulant and antiplatelet adhesion capacities by exploiting simple yet effective chemical treatments. Nanoporous networks generated by oxidative patterning can provide physico/chemical cuing to cells, but in principle these networks can also be tailored to regulate the release of drugs as a function of time or distance from the implantation site to control indiscriminate cell growth (*hyperplasia*). In this way, by rationally designing surface properties, it may be possible to create devices that avoid the side effects of current generations of cardiovascular self-eluting stents. Another problem that afflicts implants is bacteria-related infections, which still represent one of the most serious and devastating complications for prosthetic devices.[167] Nanostructured biomaterial surfaces may help solve this problem by offering inherent antimicrobial and antiadhesion properties. For example, nanophase TiO_2 and ZnO[168,169] promise to serve as effective coatings for implants to reduce the risk of bacterial infections.

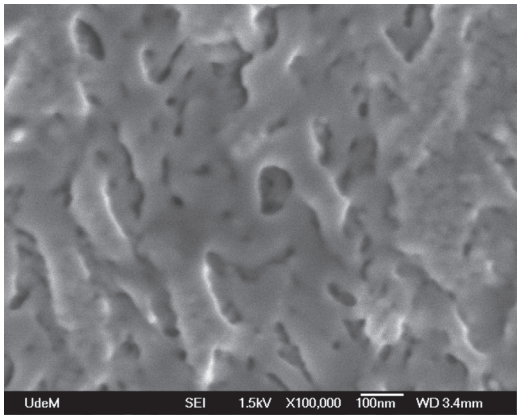

Figure 10. SEM image of a Ti sample nanotextured by oxidative patterning and implanted with Ca^{2+} ions (150 keV, 1×10^{16} Ca^{2+}/cm^2). Implantation under the conditions used alters the characteristic nanoporous surface structure obtained with this chemical treatment (see Figure 3) (unpublished results).

In conclusion, the most effective approaches to create a novel generation of biometals are those that i) confer enhanced biocompatibility directly onto material surfaces, ii) create synergistic effects, iii) selectively influence cells and guide stem cell differentiation, iv) result in surfaces that have more than one medical application, such as orthopedic and cardiovascular, v) simultaneously reach all surfaces in devices with complex geometries, and vi) can be manufactured by large-scale processes.

Efficient functionalization approaches, resulting from concomitant progress in nanotechnology and biological sciences, will undoubtedly help accomplish one of the most challenging missions in science: replacing lost tissues and restoring bodily functions to improve human health. The content of the present review shows that achieving this goal will benefit enormously from the active collaboration of specialists in materials science, chemistry, and biology.

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