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Nanostructured Implant Materials And Their Surface Interactions

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Abstract
The interactions between solid surfaces and cells are crucial to many biological phenomena for all biomaterials. A material is said to be biocompatible, only when no or minimal adverse reactions ensue at the blood/tissue - material interface, and high resistance to biodegeneration.Nanoscale modification of the implant surface can alter the chemistry and/or topography. Different methods have been described to modify or to embellish titanium substrates with nanoscale features. Such changes alter the implant surface interaction with ions, biomolecules and cells. These interactions can favorably influence molecular and cellular activities and alter the process of osseointegration. Here we present a review of these surface interactions and its applicability in practice.

Key Words
Nanotechnology, Implant Materials, Nanostructured Implant surfaces

Introduction

The biocompatibility of an artificial material in the body is complicated. The artificial implants, once implanted in vivo, induce a cascade of reactions in the biological micro-environment through interaction of the biomaterial with body fluid, proteins, and various cells [1], [2], [3], [4]. The sequence of local events often leads to the classic foreign body response and the formation of a fibrous tissue capsule around an implant. It is clear that a major factor influencing this unfavorable reaction of the body is the biomaterial surface. Both the chemical composition of the surface and the surface topography are believed to be important in bone contacting implants[5].

Primary stability is the first step of the osseointegration of implants. This is related to the implant design, mechanical anchorage and bone structure. The primary stability gives way to secondary anchorage with time, which is characterized by a biological bonding at the bone-implant interface. Thus, the nature of the initial bone-implant interface determines the ultimate success or failure of implant. Tissue compatibility is also an issue of prime importance issue while determining the implant success.

The interactions between solid surfaces and cells are crucial to many biological phenomena for all biomaterials. A material is said to be biocompatible, only when no or minimal adverse reactions ensue at the blood/tissue - material interface, and high resistance to biodegeneration. To be deemed biocompatible, implant materials don't destroy or sensitize the cellular elements of blood, cause adverse immune responses or cause any teratological effects or produce toxic and allergic responses or be affected by sterilization. Till date, no material has been able to satisfy these criteria, so inevitable reaction occurs.

Surface characteristics of dental implants
Biocompatibility is multifactorial as simultaneous stimuli from implant materials properties i.e. morphological, chemical, or electrical surface qualities can elicit reactionary responses from the surrounding biological environment that can affect the host response.

The quality of titanium surfaces has been described in terms of surface chemistry, which refers to the critical surface tension (CST) or surface energy[6]. CST is related to the contact angle of a liquid drop on the surface and, thus, provides an indicator of the potential of cell adhesion or surface wettability[7]. It has been observed that chemically activated and hydrophilic sandblasted and acid-etched (SLA) surfaces resulted in a greater percentage of bone-implant contact in the first weeks of osseointegration[8].

The surface properties of the implants can be changed by different methods of cleaning, sterilization, and storage [2], [9], [10]. For example, it has been observed that discs with an active SLA surface sterilized by gamma irradiation and continuously submersed in isotonic NaCl presented less contamination with hydrocarbons and carbonates from the atmosphere, producing a chemically clean and reactive surface [11].

The chemical composition and surface microstructure can regulate the adsorption of components present in extracellular fluid as a result of alterations in the surface energy. In vitro studies showed that rough and chemically activated surface provides the ideal conditions for direct protein adsorption and alter the adsorption of fibronectin and albumin due to modifications in their ionic state [12].

Titanium is found to be well tolerated and nearly an inert material in the human body environment. Under optimal situations, titanium is capable of osseointegration with bone[1]. Moreover,

titanium forms a highly stable passive layer of TiO2 on its outer surface and provides superior biocompatibility. Even if this passive layer gets damaged, TiO2 is immediately rebuilt. The oxide film protects the metal substrate from corrosion and is of particular importance due to its physicochemical properties such as crystallinity, impurity segregation etc, have been found to be quite relevant.

Advances in surface modification

Various advances have been introduced in the field of surface modification of implants. Few of these are:

1. **Physical approaches**
   a. Compacting nanosized particles of Titanium dioxide onto the metal core.
   b. Ion beam deposition
2. **Chemical approaches**
   a. Acid etching
   b. Sol – gel deposition (colloidal particle adsorption) of calcium phosphate, aluminium, zirconia, titanium and other materials
3. **Lithography and other optical methods.**
   a. Peroxidation
   b. Discrete crystalline depositions which superimposes a nano-topography
4. **Biomimetics**
   a. Alkali treatment
   b. Anodization- Acid etching and exposure to hydrogen peroxide increases the adsorption of RGD and mineralization. Leads to the formation of a titanium gel layer. Sodium titanate is formed allowing the deposition of hydroxyapatite.
   c. Plasmanitriding: Titanium implants are exposed to a gas atmosphere containing a mixture of nitrogen and hydrogen in the ratio of 20:80 at low pressure and ionized by a continuous current, leading to deposition of nitride onto the metallic surface. It has the advantage of reduced treatment time, lower treatment temperature, reduced cost and increased environmental cleanliness.This produces surfaces with increased wettability and hydrophilic characteristics and cell adhesion apart from modifying chemical characteristics and surface topography.

Laser lock technology has introduced implant with a 2 mm wide collar with the uppermost 0.5 mm is smooth and lower 0.7 mm of the implant surface has grooves of 5-8 µ to prevent epithelial downgrowth. The lowermost 0.8 mm of the collar having grooves of 10- 12µ helps in developing a strong bone-implant interface and retains crestal bone.

Studies have demonstrated that calcium phosphate coatings provide osteoconductive surface to the titanium implants[13].The dissolution of calcium phosphate coatings in the peri-implant region increases ionic strength and saturation of blood leading to the precipitation of biological apatite nanocrystals onto the implant surface. This biological apatite layer incorporated proteins and promoted the adhesion of osteoprogenitor cells that producedosteoid. Also, it was shown that osteoclasts were able to degrade the calcium phosphate coatings through enzymatic degradation and created resorption pits on the implant surface[14].

Hybrid implants i.e. titanium implants with zirconia collars demonstrates lower level of crestal bone loss as compared to implants with titanium collars as it enhances the fibroblast and osteoblast adhesion and proliferation.

Flouride modified titanium implants are those that undergo additional cleaning procedure in hydrofluoric acid after the process of blasting. This leads to the formation of fluoridated hydroxyapatite and fluoroapatitein the calcified tissues further leading to increase in bone implant contact.

Nanostructured Biomaterials

Using nanotechnology for regenerative therapy becomes obvious when examining nature[15]. Bone is a nano scaled composite that consists of collagen, non-collagenous proteins (laminin, fibronectin, vitronectin), and water) and hard inorganic components (hydroxyapatite), HA, $Ca_{10}(PO_4)_6(OH)_2$ [16],[17]. 70% of the bone matrix is composed of nanocrystalline HA[18].

Nanostructured biomaterials possess unique surface and mechanical properties similar to the bone and hence are considered to be the future generation biomaterials [22],[23],[24]. Owing to very high number of atoms on the surface, nanograined materials possess large surface energy. Thus, they exhibit entirely different behavior compared to the micron-sized grains. The bone-forming cells generally attach themselves to the surface whose roughness is of nanometer range.

Nano-materials exhibit unique surface properties such as surface chemistry, wettability, and energy, due to their increased surface area and roughness as compared to thetraditional or microstructuredimplant materials. Material surface properties mediate specific proteins (such as fibronectin, vitronectin, and laminin) adsorption and bioactivity, thus regulating the cell behavior and dictating tissue regeneration[16]. Increased alkaline phosphatase levels, increased collagen matrix,increased primary retention of the implant, and greater shear strength is some of the factors that have popularized these materials recently.

On metal surfaces, enhanced cell metabolic activity has been observed, such as the upregulation of bone sialoprotein and osteopontin[19], as well as a threefold increase in osteoblastic cell adhesion as compared with the surfaces without nanostructure. Furthermore, enhancement of calcium and phosphorus deposition has been observed on nanostructured titanium alloys and on CoCrMo surfaces but it was not observed on pure titanium [20],[21].

The nano roughness arises because of the fact that human bones consist of inorganic minerals of grain size varying from 20 to 80 nm long and 2 to 3 nm in diameter[25]. The variation in the surface energy due to the nanosurface roughness leads to desirable cellular responses on nanostructured titanium and other materials resulting in high osseointegration [26],[27],[28],[29],[30],[31]. The cell adhesion behavior on submicron, nanometer structured titanium surface was investigated and the obtained results were compared with a flat smooth titanium surface[26]. The study demonstrated that both nanometer and submicron surfaces have very high surface energy and adhesion of bone cells was very high. Additionally, nanograined alloys made of Cp Ti, Ti–6Al, 4V, and

CoCr as well as nanoceramic biomaterials such as alumina, titania, and hydroxyapatite also exhibit increased cell adhesion [32],[33]. When the grain size was decreased from 167 to 24 nm, 51% increased osteoblast adhesion and fibroblast adhesion responsible for encapsulation was reduced by 235%.

Though different types of cells were utilized for cell culture studies on the alloys and ceramics, the cell density was observed to be relatively higher for the nanomaterials when compared to conventional counterparts.

Apart from the roughness, the pore size on the surface also has an influence on the protein adhesion. The protein, victronectin, is generally adsorbed on pores of smaller sizes on the other hand, the protein that decreases cell adhesion such as laminin, generally adsorbs to bigger pore size[34]. Increased osteoblast adhesion was also observed on nano HA coated Titanium alloy and further bone ingrowth toward implant was noted indicating ceramic surface coatings leading to high osseointegration[35].

Nanostructured Biointerfaces

In vivo, the cell interactions with its surroundings are mediated at the both molecular and macromolecular level. Specific interactions with, for example extracellular matrix components and soluble factors, or macromolecules in the outer membranes of adjacent cells provide necessary signaling and communication routes. Such interfaces have both topographic nanostructure and chemical/ biospecific interaction sites distributed at the nanoscale.

Studies have focused on the effect of surface nanotopography on cell functions such as adhesion, motility, morphology, cytokine release, gene expression, and differentiation[36]. The ability to define interfaces on a length scale that match those of the mediating macromolecules in cell membranes and extracellular matrixes, has the potential to create artificial biointerfaces that are capable of signaling/communicating with the adherent cells. Such artificial biointerfaces are of immediate interest for application in areas such as biomaterials, tissue engineering, scaffolds for cell therapies, and cell-based sensors/electronics.

The role of surface parameters (both bulk chemistry and topography) requires consideration of molecular (ionic and biomolecular) interactions with the surface, cell adhesion phenomenon, and local biomechanical features of the established interface. It is clear that nanoscalemodification affects the chemical reactivity of an endosseous implant surface and alter the ionic and biomolecular interactions with the surface. Proposed changes include enhanced wettability, altered protein adsorption, and potential mineralization phenomenon[37]. Changes in wettability and altered protein adsorption lead to altered cell adhesion, likely involving both integrin and non-integrin receptors. The potential for mineralization and epitaxic crystal growth in support of early bone bonding could dramatically alter the biomechanical environment of the healing implant in favor of stability.

Recently, a set of unique structures ranging from mesoporousnanoscaffolds, nanoflowers, nanoneedles, nanorods, and octahedral pyramids were fabricated by tuning the hydrothermal conditions such as reaction medium composition, concentration, temperature, and time duration systematically[38]. The cytotoxicity of surface modified Ti was assessed using human primary osteoblastic cells, and more than 90% of the cells were found to be viable after 24 h of incubation. Various studies on protein adsorption have revealed that the nano-modified surface structures on titanium adsorbed more proteins, suggesting that these promote cell adhesion/attachment.

Interaction of surfaces and blood

Blood interactions with implant material leads to protein adsorption this being dependent on the surface properties of the implantable material. This occurs through a complex series of steps of adsorption and displacement, more commonly known as the Vroman effect [39] A hydrophilic surface is better than a hydrophobic surfacefor blood coagulation. Consequently, dental implants manufacturers have developed high hydrophilic and rough implant surfaces that in turn exhibited better osseointegration than conventional ones[40]. Adsorption of proteins such as fibronectin, vitronectin on the surface of dental implants has been shown to promote cell adhesion by cell-binding RGD domain[41]. After proteins absorption, the osseointegration is characterized by platelet adhesion and fibrin clots formation at the injured blood vessels site. Previous studies have shown that implants in contact with platelet-rich plasma (PRP) having a platelet concentration of approximately 106 protein/μL have a positive effect on peri implant bone regeneration and osseointegration. At lower concentrations of platelet rich plasma, the effect was not optimal, while higher concentrations resulted in a paradoxically inhibitory effect on peri implant bone regeneration. Few studies that were not in agreement with effect of PRP on the osseointegration of dental implants, have also been documented[42].

Interactions between surfaces and Mesenchymal stem cells

Following clotting around the implants, several cells interact with implant surfaces for healing. Mesenchymal stem cells (MSCs) are attracted to the injured site by chemotactic action of implant with neighboring bone and gingival tissue factors have a determinant role in peri implant tissue healing.

The integration of implant with neighboring bone and gingival tissue depends on successful crosstalk between old tissue and implant surface. The challenge in dental implant research is the capability of the surface to guide cells colonization and differentiation. Cell migration, adhesion, and proliferation on implant surfaces are a prerequisite to initiate the tissue regeneration. Authors have shown that some factors present in tissues and secreted during the inflammatory phase are able to attract MSCs to the injured site [43],[44].

In the microenvironment, MSCs are stimulated by some specific factors to differentiate into the adequate cell line. Under the influence of these factors, MSCs switch to osteoblastic cells in contact to bone tissue while they differentiate into fibroblastic lineage in the gingival tissue region. These two differentiation pathways are in concurrence around dental implants. In some cases, implants are encapsulated by fibrous tissue due to the proliferation and differentiation of MSCs into fibroblastic cells. In response to cytokine, fibroblasts migrate and generate a capsule of collagen, the first step in generation of gingival tissue or rejection on contact to

bone. This fibrous capsule prevents bonding between implant surface and juxtaposed bone and causes a failure of the implant[45]. On the other hand, both the differentiation of MSCs into fibroblastic lineage and the fibroblastic adhesion are desired in the gingival upper part of dental implants. Fibroblasts adhesion has been shown to be lower on nanoscale surface compared to conventional surfaces[46]. Moreover, nanometer size features have been shown to decrease fibroblast adhesion and proliferation [47],[48]. The micro- and nanoscale surface properties of the implant i.e. surface chemistry, surface roughness, and wettability, could affect bone formation [49]. Research has specifically demonstrated that nanorough Ti [50] and nanostructured Ti can enhance osteoblast adhesion and differentiation compared to their nanosmooth control[51]. Furthermore, surfaces with micro- and nanopores have shown to enhance greatly osseointegration[52],[53]. Surface properties may control the steps of adhesion, proliferation, and differentiation of MSCs and, thus, condition tissue integration.

Tissue Integration

Branemark et al. described the osseointegration as a direct structural and functional bone to implant contact under load. The challenge in developing new implant surface consists in increasing the clinical success rate as well as decreasing the tissue healing time for immediate loading of implants, particularly in aesthetic situations. Implant surface with various roughnesses have been used to increase the total area available for osteoapposition. Kubo et al.[54] observed a substantial increase by 3.1 times in bone-titanium interfacial strength by Ti nanotube (300 nm) at 2 weeks of implantation in femur rats. These results suggest the establishment of nanostructured surfaces for improved osteoconductivity. Moreover, Ogawa et al.[55] have prepared titanium nanostructure by physical vapor deposition and tested their osseointegration in the femur of rats. They found an increased surface area by up to 40% and a greater strength of osseointegration for the nanostructured compared to an acid-etched surface [55].

In particular, Le Guehennec et al.[56] studied the osseointegration of 4 implant surfaces in the femoral epiphyses of rabbits after 2 & 8 weeks of healing. In this study, the bone-implant contact and bone growth inside the chambers were compared for four different implant surfaces and shown that biomimetic coating method may enhance the bone apposition onto titanium. In order to prevent coating delamination and implant loosening, the Calcium phosphate coating should dissolve or degrade under osteoclastic activity at a similar rate than bone apposition. The preferred result should be a direct bone-implant coating without the presence of fibrous tissue. Another advantage of these calcium phosphate coatings is related to their preparation by biomimetic methods at physiological temperature and pH from simulated body fluids. Calcium phosphate crystals have characteristics that resemble bone mineral in terms of size and composition. Furthermore, it is possible to incorporate biologically active drugs such as antibiotics or growth factors during the precipitation of calcium phosphate coatings on titanium implants[57]. These molecules could be locally and gradually released in the peri-implant bone region for either preventing bacterial infections or stimulating bone growth.

Effects of nanotopography on osseointegration

Depiction of broad range of nanoscale topography effects observed in cellular protein adsorption is altered by nanoscale modification of bulk material. It is believed that, the changes in initial protein–surface interaction control osteoblast adhesion [58]. When implants come into contact with a biological environment, protein adsorption (e.g. plasma fibronectin) that occurs immediately will mediate subsequent cell attachment and proliferation. Altering the surface energy or wettability of a material is a classical approach to changing cell interactions with the surface. Nanotopography specific effects on cellular behavior have been demonstrated using a wide range of different cell types including epithelial cells, fibroblasts, myocytes, and osteoblasts.

Interestingly, osteoblasts were observed to adhere specifically at particle boundaries. Since nanophase metals have higher percentages of particle boundaries at the surface, this may explain the greater numbers of osteoblasts on nanophase compared to conventional metals.

Both cell specificity and extent of cell adhesion are altered, too. Depending on the nano-architecture of the cell, spreading may be affected. Lim[56] more directly related protein adsorption, cell adhesion and the active process of attachment by measurement of increased focal adhesion kinase (FAK) activity. Surface roughness at the nanoscale is an important determinant of protein interactions that ultimately direct cell activity in control of tissue formation at implant surfaces [59].

Nanotopographicalfeatures of a surface affect both cell adhesion and motility. On comparison of cell morphology and cytokine production on deep grooves and hemispherical nanopillars, the cells appeared partially aligned to the grooves and had a cytokine release similar to that found from cells on flat surfaces. Osteoprogenitor cell adhesion was enhanced on poly-L-lactide (PLLA) and polystyrene (PS) surface with nanoscale and micron-scale roughness compared to smooth surfaces.

Cell proliferation and osteoblast differentiation appears to be enhanced by nanoscale topography, too. Webster [29],[32] observed increased osteoblast proliferation on the nanoscale materials. Several investigators have demonstrated the relative diminution of fibroblast adhesion compared to osteoblast adhesion when nano- and micron-structured surfaces were evaluated [61],[60]. For example, on nano-sized materials, the affinity ratio between osteoblasts and fibroblasts was 3 to 1 compared to conventional materials, the ratio was 1:1 [62]. Bacterial adhesion and proliferation is also diminished on nanophase materials [63]. Decreased bacterial colonization on nanostructured titanium oxide and zinc oxidehas been observed even though these surfaces promote osteoblast differentiation and adhesion.

The topographical and chemical properties of the implant surface strongly influence the properties of the layer. Since cells and proteins range in size from nano- to micrometer, these are relevant length scales for the problem. Equally importantis theability of cells to communicate through the extracellular

matrix by signal molecules. These bioactive signal molecules control the regeneration during tissue healing and some proteins stimulate healing near the implant.

Biocompatibility of Ti-BioceramicNanocomposites

The application of Ti-bioceramicnanocomposites has focused attention on the biocompatibility of synthesized bulk materials. For Dental Implants, hybrid Ti-x vol% 45S5 Bioglass, Ti-x vol% SiO2, and Ti-x vol% HA bionanocomposites (0 = x = 20) were produced by the combination of mechanical alloying (MA) and powder metallurgical process [64],[65],[66],[67],[68],[69].

It has been demonstrated that metal (Ti, Ti6Al4V, and CoCrMo) surfaces utilizing submicron to nanometer particles, due to higher amounts of particle boundaries at their surfaces, promoted the adhesion of osteoblasts as compared to metals composed of respective micron particles [31].

Cytotoxicity tests of the extracts of studied Ti-45S5 Bioglass materials under wear conditions are shown. The relative viability of the cells (RVC) decreases when fraction increases. It is important to note that the RVC of nanoscale Ti-45S5 Bioglass is higher in comparison with microcrystalline titanium. The wear and fretting accelerates the corrosion of the studied samples in a biological environment such as cell culture medium. Two factors may influence cell growth on the disks: adsorbing protein onto the disks and released metal ions from the disks.

A quantification study provided evidence of significant differences in the amount of calcium and phosphorus deposition by osteoblasts as well as their precipitation from culture media between common orthopedic (Ti6Al4V, CoCrMo) alloys due to nanometer particle sizes [31]. Also chromium was detected at the concentration of 4.4 ± 0.7 and 4.1 ± 0.6 mg/L, respectively. Chromium is one of the essential elements for human, so slight amount of this element may contribute to cell proliferation, and resulting in higher cell growth.

Conclusion

Nanoscale surface modification have shown to alter the chemistry and/or topography of the implantable material surface. Various methods have been described to modify titanium substrates with nanoscale features. Such changes have been shown to alter the implant surface interaction with host bio-environment. These interactions have been shown to favorably influence molecular and cellular activities and alter the process of osseointegration.

As the disciplines of immunology continue to understand the process of wound healing, development of biomaterials plays a complementary role as an interdisciplinary approach to developing implant surfaces, which mimic and promote accelerated wound healing processes. At this moment, both a hydrofluoric acid modified titanium endosseous implant with nanoscale features and calcium phosphate nanofeature-modified titanium implants are available for clinical use. The potential risks and benefits of manipulating biomaterial interfaces at the nanoscale will be defined by long-term clinical evaluation of such endosseous devices.

References

1. Freitas Jr., R.A. (1999). Nanomedicine, Volume I: Basic Capabilities, Landes Bioscience, Georgetown, TX.
2. Frayssinet, P., Trouillet, J.L., Rouquet, N., Azimus, E., and Autefage, A. (1993). Osseointegration of macroporous calcium phosphate ceramics having a different chemical composition, Biomaterials, 14, pp. 423–429.
3. Shi, Z., Huang, X., Cai, Y., Tang, R., Yang, D. (2009). Size effect of hydroxyapatite nanoparticles on proliferation and apoptosis of osteoblast-like cells, ActaBiomater.,5, pp. 338–345.
4. Webster, T.J. (2003). Nanophase ceramics as improved bone tissue engineering materials, Am. Ceram. Soc. Bull.,82, pp. 23? 28.
5. Chen, S.C., Mrksich, M., Huang, S., Whitesides, M.G., and Ingber, E.D. (1997). Geometric control of cell life and death, Science, 276, pp. 1425–1428.
6. Keselowsky, B.G., Collard, D.M., and Garcia, A.J. (2003). Surface chemistry modulates fibronectin conformation and directs integrin binding and specificity to control cell adhesion, J. Biomed. Mater. Res., A, 66, pp. 247–259.
7. Degasne, I., Basle, M.F., Demais, V., Hure, G., Lesourd, M., Grolleau, B., Mercier, L., and Chappard, D. (1999). Effects of roughness, fibronectin and vitronectin on attachment, spreading and proliferation of human osteoblast-like cells (Saos-2) on titanium surfaces, Calcio3090;ied Tissue Int., 64, pp. 499? 507.
8. Best, S.M., Porter, A.E., Thian, E.S., and Huang, J. (2008). Bioceramics: Past, present and for the future, J. Eur. Cer. Soc., 28, pp. 1319–1327.
9. Kaplan, F.S., Hayes, W.C., Keaveny, T.M., Boskey, A., Einhorn, T.A., and Iannotti, J.P. (1994). Form and function of bone, in Orthopedic Basic Science (ed. Simon, S.R.), American Academy of Orthopaedic Surgeons, Rosemont, pp. 127? 185.
10. Leigh, S.H., Lin, C.K., and Berndt, C.C. (1997) Elastic response of thermal spray deposits under indentation tests, J. Am. Ceram. Soc., 80, pp. 2093–2099.
11. Yoshinari, M., Oda, Y., Kato, T., and Okuda, K. (2001). Influence of surface modifications to titanium on antibacterial activity in vitro, Biomaterials, 22, pp. 2043–2048.
12. Shi, Z., Huang, X., Cai, Y., Tang, R., Yang, D. (2009). Size effect of hydroxyapatite nanoparticles on proliferation and apoptosis of osteoblast-like cells, ActaBiomater.,5, pp. 338–345.
13. R. G. T. Geesink, "Osteoconductive coatings for total joint arthroplasty," Clinical Orthopaedics and Related Research, no. 395, pp. 53–65, 2002.; S. Leeuwenburgh, P. Layrolle, F. Barrre et al., "Osteoclasticresorption of biomimetic calcium phosphate coatings in vitro," Journal of Biomedical Materials Research, vol. 56, no. 2, pp. 208–215, 2001.
14. S. Leeuwenburgh, P. Layrolle, F. Barrre et al., "Osteoclasticresorption of biomimetic calcium phosphate coatings in vitro," Journal of Biomedical Materials Research, vol. 56, no. 2, pp. 208–215, 2001.
15. Yamamoto, A., Kohyama, Y., Kuroda, D., and Hanawa, T. (2004). Cytocompatibility evaluation of Ni-free stainless steel manufactured by nitrogen adsorption treatment, Mater. Sci. Eng. C, 24, pp. 737–743
16. Webster, T.J., Ergun, C., Doremus, R.H., Siegel, R.W., and Bizios, R.

(2000). Enhanced functions of osteoblasts on nanophase ceramics, Biomaterials, 21, pp. 1803–1810.
17. Yang, B., Uchida, M., Kim, H.M., Zhang, X., and Kokubo, T. (2004). Preparation of bioactive titanium metal via anodic oxidation treatment, Biomaterials, 25, pp. 1003–1010.
18. Jurczyk, K., Niespodziana, K., Stopa, J., and Jurczyk M. (2007). Composite Ti-hydroxyapatite bionanomaterials for application in modern dentistry, Polish J. Environ. Studies, 16, no 2CII, pp. 323? 327.
19. Cooper, L.F., Masuda, T., Yliheikkila, P.K., and Felton, D.A. (1998). Generalizations regarding the process and phenomenon of osseointegration. Part II. In vitro studies, Int. J. Oral Maxillofac. Implants, 13, pp. 163? 174.
20. Lim, J.Y., Hansen, J.C., Siedlecki, C.A., Runt, J., and Donahue, H.J. (2005 Human foetalosteoblastic cell response to polymer-demixednanotopographic interfaces, J. R. Soc. Interface, 2, pp. 97–108.
21. Wan, Y., Wang, Y., Liu, Z., Qu, X., Han, B., Bei, J., and Wang, S. (2005). Adhesion and proliferation of OCT-1 osteoblast-like cells on micro- and nano-scale topography structured poly(L-lactide), Biomaterials, 26, pp. 4453–4459.
22. El-Ali, J., Sorger, P.K., and Jensen, K.F. (2006). Cells on chips, Nature, 442, pp. 403? 411.
23. Ellingsen, J.E. (1998). Surface configurations of dental implants, Periodontology 2000, 17, pp. 36–46.
24. Wang, G.C., Liu, X.Y., and Ding, C.X. (2008). Phase composition and in vitro bioactivity of plasma sprayed calcia stabilized zirconia coatings, Surf. Coat. Technol., 202, pp. 5824–5831.
25. Del Curto, B., Brunella, M.F., Giordano, C., Pedeferri, M.P., Valtulina, V., Visai, L., and Cigada, A. (2005). Decreased bacterial adhesion to surface-treated titanium, Int. J. Artif. Organs, 28, pp. 718–730.
26. Keller, J.C., Draughn, R.A., Wightman, J.P., Dougherty, W.J., and Meletiou, S.D. (1990). Characterization of sterilized cp titanium implants surface, Int. J. Maxillofac. Implants, 5, pp. 360? 367.
27. Kilpadi, D., and Lemons, J. (1994). Surface energy characteriza- tion of unalloyed titanium implants, J. Biomed. Mater. Res., 28, pp. 1419? 1425.
28. Kilpadi, K.L., Chang, P.L., and Bellis, S.L. (2001). Hydroxylapatite binds more serum proteins, purified integrins, and osteoblast precursor cells than titanium or steel, J. Biomed. Mater. Res., 57, pp. 258–267
29. Wang, G.C., Liu, X.Y., Gao, J.H., and Ding, C.X. (2009). In vitro bioactivity and phase stability of plasma-sprayed nanostructured 3Y-TZP coatings, ActaBiomater.,5, pp. 2270–2278.
30. Webster, T.J., Siegel, R.W., and Bizios, R. (1999). Osteoblastadhesionon R32 ; nanophase ceramics, Biomaterials, 20, pp. 1221? 1227.
31. Webster, T.J., Ergun, C., Doremus, R.H., Siegel, R.W., and Bizios, R. (2001). Enhanced osteoclast like cell functions on nanophase ceramics, Biomaterials, 22, pp. 1327–1333.
32. Ward, B.C., and Webster, T.J. (2006). The effect of nanotopography on calcium and phosphorus deposition on metallic materials in vitro, Biomaterials, 27, pp. 3064? 3074.
33. Webster, T.J., Siegel, R.W., and Bizios, R. (2001). Nanoceramic surface roughness enhances osteoblast and osteoclast functions for improved orthopaedic/dental implant efficacy, Scripta Mater.,44, pp. 1639–1642.
34. Albrektsson, T., and Wennerberg, A. (2004). Oral implant sulfaces: part 2 - review focusing on clinical knowledge of different surfaces, Int. J. Prosthodont.,17, pp. 544? 564.
35. Baraton, M.I., Chen, X., and Gonsalves, K.E. (1997). FTIR study of nanostructured alumina nitride powder surface: determination of the acidic/basic sites by CO, CO2, and acetic acid adsorptions, Nanostruct. Mater.,8, pp. 435? 445.
36. Ejiofor, J., and Webster, T.J. (2004). Biomedical implants from nanostructured materials, in Dekker Encyclopedia of Nanoscience and Nanotechnology, (ed. Schwarz, J.A., Contescu, C., and Putyera, K.), Marcel Dekker, Inc., New York, pp. 263–275.
37. Martinez, E., Lagunas, A., Mills, C.A., Rodriguez-Segui, S., Estevez, M., Oberhansl, S., Comelles, J., and Samitier, J. (2009). Stem cell differentiation by functionalized micro- and nanostructured surfaces, Nanomedicine (Lond).,4, pp. 65–82.
38. Damen, J.J.M., Ten Cate, J.M., and Ellingsen, J.E. (1991). Induction of calcium phosphate precipitation by titanium dioxide, J. Dent. Res., 70, pp. 1346? 1349.
39. R. Miller, Z. Guo, E. A. Vogler, and C. A. Siedlecki, "Plasma coagulation response to surfaces with nanoscale chemical heterogeneity," Biomaterials, vol. 27, no. 2, pp. 208–215, 2006
40. T. Sawase, R. Jimbo, K. Baba, Y. Shibata, T. Ikeda, and M. Atsuta, "Photo-induced hydrophilicity enhances initial cell behavior and early bone apposition," Clinical Oral Implants Research, vol. 19, no. 5, pp. 491–496, 2008.
41. G. Balasundaram, M. Sato, and T. J. Webster, "Using hydroxyapatite nanoparticles and decreased crystallinity to promote osteoblast adhesion similar to functionalizing with RGD," Biomaterials, vol. 27, no. 14, pp. 2798–2805, 2006
42. G. Weibrich, T. Hansen, W. Kleis, R. Buch, and W. E. Hitzler, "Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration," Bone, vol. 34, no. 4, pp. 665– 671, 2004.
43. H. Agis, B. Kandler, M. B. Fischer, G. Watzek, and R. Gruber, "Activated platelets increase fibrinolysis of mesenchymal pro- genitor cells," Journal of Orthopaedic Research, vol. 27, no. 7, pp. 972–980, 2009.
44. J. P. Vogel, K. Szalay, F. Geiger, M. Kramer, W. Richter, and P. Kasten, "Platelet-rich plasma improves expansion of human mesenchymal stem cells and retains differentiation capacity and in vivo bone formation in calcium phosphate ceramics," Platelets, vol. 17, no. 7, pp. 462–469, 2006
45. J. A. Hobkirk, "Progress in implant research," International Dental Journal, vol. 33, no. 4, pp. 341–349, 1983
46. E. Eisenbarth, J. Meyle, W. Nachtigall, and J. Breme, "Influence of the surface structure of titanium materials on the adhesion of fibroblasts," Biomaterials, vol. 17, no. 14, pp. 1399–1403, 1996
47. A. Cohen, P. Liu-Synder, D. Storey, and T. J. Webster, "Decreased fibroblast and increased osteoblast functions on ionic plasma deposited nanostructured Ti coatings,"

Nanoscale Research Letters, vol. 2, no. 8, pp. 385–390, 2007
48. D. Miller, R. Vance, A. Thapa, T. Webster, and K. Haberstroch, "Comparaison of fibroblast and vascular cell adhesion to nano structured poly(lactic co glycolic acid) films," Applied Bionics and Biochemics, vol. 2, no. 1, pp. 1–7, 2005.
49. R. M. Streicher, M. Schmidt, and S. Fiorito, "Nanosur- faces and nanostructures for artificial orthopedic implants," Nanomedicine, vol. 2, no. 6, pp. 861–874, 2007
50. S. Puckett, R. Pareta, and T. J. Webster, "Nano rough micron patterned titanium for directing osteoblast morphology and adhesion," International Journal of Nanomedicine, vol. 3, no. 2, pp. 229–241, 2008
51. S. Puckett, R. Pareta, and T. J. Webster, "Nano rough micron patterned titanium for directing osteoblast morphology and adhesion," International Journal of Nanomedicine, vol. 3, no. 2, pp. 229–241, 2008
52. K. H. Frosch, F. Barvencik, V. Viereck et al., "Growth behavior, matrix production, and gene expression of human osteoblasts in defined cylindrical titanium channels," Journal of Biomedical Materials Research A, vol. 68, no. 2, pp. 325–334, 2004
53. S. H. Oh, R. R. Fino M1;nes, C. Daraio, L. H. Chen, and S. Jin, "Growth of nano-scale hydroxyapatite using chemically treated titanium oxide nanotubes," Biomaterials, vol. 26, no. 24, pp. 4938–4943, 2005.
54. K. Kubo, N. Tsukimura, F. Iwasa et al., "Cellular behavior on TiO2 nanonodular structures in a micro-to-nanoscale hierarchy model," Biomaterials, vol. 30, no. 29, pp. 5319–5329, 2009
55. T. Ogawa, L. Saruwatari, K. Takeuchi, H. Aita, and N. Ohno, "Ti nano-nodular structuring for bone integration and regeneration," Journal of Dental Research, vol. 87, no. 8, pp. 751–756, 2008]
56. Le Guehennec, L., Martin, F., Lopez-Heredia, M.A., Louarn, G., Amouriq, Y., Cousty, J., and Layrolle, P. (2008) Osteoblastic cell behavior on nanostructured metal implants, Nanomedicine(Lond), 3, pp. 61?71
57. Y. Liu, K. De Groot, and E. B. Hunziker, "BMP-2 liberated from biomimetic implant coatings induces and sustains direct ossification in an ectopic rat model," Bone, vol. 36, no. 5, pp. 745–757, 2005
58. Anagnostou, F., Debet, A., Pavon-Djavid, G., Goudaby, Z., Helary, G., and Migonney, V. (2006). Osteoblast functions on functionalized PMMA-based polymers exhibiting Staphylococcus aureus adhesion inhibition,Biomaterials, 27, pp. 3912–3919.
59. Niespodziana, K., Jurczyk, K., Miklaszewski, A., and Jurczyk, M. (2010). Hybrid Ti-ceramic bionanomaterials for medical engineering, Phys. Status Solidi C, 7, pp. 1363?1366.
60. Oliveira, P.T., Zalzal, S.F., Beloti, M.M., Rosa, A.L., and Nanci, A. (2007). Enhancement of in vitro osteogenesis on titanium by chemically produced nanotopography, J. Biomed. Mater. Res. A, 80, pp. 554?564
61. Lim, Y.M., Kim, B.H., Jeon, Y.S., Jeon, K.O., and Hwang, K.S. (2005) Calcium phosphate films deposited by electrostatic spray deposition and an evaluation of their bioactivity, J. Ceram. Proc. Res., 6, pp. 255–258
62. Ward,B.C. , and Webster, T.J. (2007). Increased functions of osteoblasts R32; on nanophase metals, Mater. Sci. Eng. C, 27, pp. 575?578
63. Boyan, B.D., Schwartz, Z., and Hambleton, J.C. (1993). Response of bone and cartilage cells to biomaterials in vivo and in vitro, J. Oral Implantol.,19, pp. 116–122.
64. Hyde, F.W., Alberg, M., and Smith, K. (1997). Comparison of fluorinated polymers against stainless steel, glass and polypropylene in microbial biofilm adherence and removal, J. Ind. Microbiol. Biotechnol.,19, pp. 142–149
65. Jakubowicz, J., Jurczyk, K., Niespodziana, K., and Jurczyk, M. (2009). Mechanoelectrochemical synthesis of porous Ti-based nanocomposite biomaterial, Electrochem. Commun.,11, pp. 461?465.
66. Mendonca, G., Mendonca, D.B.S., Araga M1;o, F.J.L., and Cooper, L.F. (2008). Advancing dental implant surface technology — from micron to nanotopography, Review, Biomaterials 29, pp. 3822–3835
67. Miller, D.C., Haberstroh, K.M., and Webster, T.J. (2005). Mechanism(s) of increased vascular cell adhesion on nanostructured poly(lactic-co-glycolic acid) films, J. Biomed. Mater. Res. A, 73, pp. 476–484.
68. Nagano, M., Nakamura, T., Kokubo, T., Tanahashi, M., and Ogawa, M. (1996). Differences of bone bonding ability and degradation behaviourin vivo between amorphous calcium phosphate and highly crystalline hydroxyapatite coating, Biomaterials, 17, pp. 1771–1777.
69. Nanci, A., Wuest, J.D., Peru, L., Brunet, P., Sharma, V., Zalzal, S., and McKee, M.D. (1998). Chemical modification of titanium surfaces for covalent attachment of biological molecules, J. Biomed. Mater. Res., 40, pp. 324–335.

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