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Biomaterials and Biotechnology Schemes Utilizing TiO_2 Nanotube Arrays

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1. Introduction

Ti and Ti alloys are corrosion resistant, light, yet sufficiently strong for utilization as load-bearing and machinable orthopaedic implant materials. They are one of the few biocompatible metals which osseo-integrate, provides direct chemical or physical bonding with the adjacent bone surface without forming a fibrous tissue interface layer. For these reasons, they have been used successfully as orthopaedic and dental implants (Ratner 2004). To impart even greater bioactivity to the Ti surface and enhance integration properties, surface treatments such as surface roughening by sand blasting, formation of anatase phase TiO_2 (Uchida et al. 2003), hydroxyapatite (HAp) coating, or chemical treatments (Ducheyne et al. 1986; Cooley et al. 1992) have been employed. However, these treatments are generally on the micron scale. Webster et al. (Webster et al. 2001; Webster, Siegel, and Bizios 1999) reported that it is even more advantageous to create nanostructured, in particular in the less than 100nm regime, surface designs for significantly improved bioactivity at the Ti implant interface and for enhanced cell adhesion. Since then, advances in biomaterial surface structure and design, specifically on the nanoscale, have improved tissue engineering in general. This chapter is a report on titanium dioxide (TiO_2, or Titania) nanotube surface structuring for optimization of titanium (Ti) implants utilizing nanotechnology.

The main focus will be on the unique 3-D tube-shaped nanostructure of TiO_2 and its effects on creating profound impacts on cell behavior. We will also shed light on the effects of changing the nanotube diameter size and optimizing the geometry for enhanced cell behavior. This work focuses on the tissue specific areas of cartilage and bone. Specifically, we will discuss how the desired cell behavior and functionality are enhanced on surfaces with TiO_2 nanotube surface structuring. Here we reveal how the TiO_2 surface nano-configurations are advantageous in various tissue engineering and regenerative medicine applications, for osteo-chondral, orthopedic, and osteo-progenitor implant applications discussed here and beyond. This chapter will also shed light on future applications and the direction of nanotube surface structuring.

2. Electrochemical anodization

In general, the mechanism of TiO_2 nanotube formation in fluorine-ion based electrolytes is said to occur as a result of three simultaneous processes: the field assisted oxidation of Ti metal to form titanium dioxide, the field assisted dissolution of Ti metal ions in the electrolyte, and the chemical dissolution of Ti and TiO_2 due to etching by fluoride ions, which is enhanced by the presence of H^+ ions (Shankar et al. 2007). TiO_2 nanotubes are not formed on the pure Ti surface but on the thin TiO_2 oxide layer naturally present on the Ti surface. Therefore, the mechanism of TiO_2 nanotubes formation is related to oxidation and dissolution kinetics. Schematic diagram of the formation of TiO_2 nanotubes by anodization process is shown in Figure 1. For a description of the process displayed in Figure 1, the anodization mechanism for creating the nanotube structure is as follows:

a. Before anodization, a nano scale TiO_2 passivation layer is on the Ti surface.
b. When constant voltage is applied, a pit is formed on the TiO2 layer.
c. As anodization time increases, the pit grows longer and larger, and then it becomes a nanopore.
d. Nanopores and small pits undergo continuous barrier layer formation. (e) After specific anodization time, completely developed nanotubes are formed on the Ti surface.

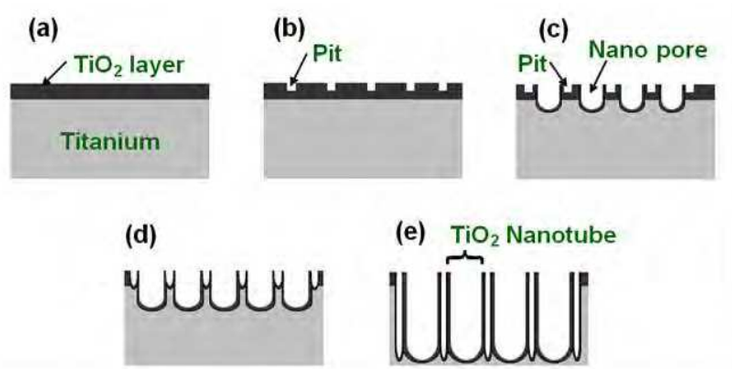


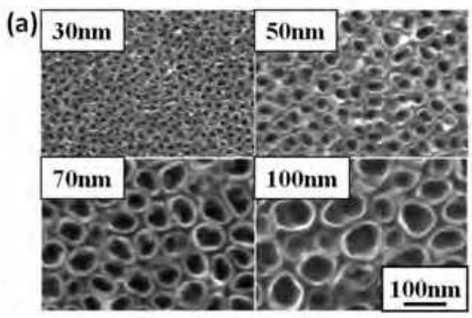
Fig. 1. Schematic illustration of TiO_2 nanotube formation.

Furthermore, based on the mechanism of nanotube formation, it is inherent that the nanotubular structure formation depends on both the intensity of applied voltage and the concentration of fluorine ions in the electrolyte solution. It is well-known that by increasing the applied voltage, larger diameter nanotubes can be formed. This aspect of diameter manipulation using applied voltage will be further emphasized and the effects on cell function and fate is also discussed.

3. Nanotube size effects

The Jin lab was the first to demonstrate that TiO_2 nanotubes can significantly accelerate osteoblast (bone cell) adhesion and proliferation at the biomaterial/tissue interface and enhance bone mineral formation. The TiO_2 nanotubes are formed as vertically aligned configuration, with an average diameter of ~100 nm, a height of ~300 nm, and a wall thickness of ~10 nm. According to published research (Oh S 2006), nanotube arrays on titanium surfaces induced proliferation of osteoblasts by as much as 300 – 400% compared to non-modified titanium surfaces. In other research groups studying nanoporous materials,

major accomplishments have been made in the generation of geometrically defined surfaces with the fabrication of Al and Si nanostructured surfaces. There is rapidly increasing evidence that the lateral spacing of features on the nanoscale can impact and change cell behaviour (Boyen et al. 2002; Cavalcanti-Adam et al. 2006; Popat et al. 2006). Therefore, in order to optimize the lateral spacing of the TiO_2 nanotube system, by changing the geometry of the nanotubes, four different pore sizes (30, 50 70, and 100nm in diameter) were created (Figure 2) for examination of cartilage chondrocyte cells, bone osteoblast cells, and osteo-progenitor mesenchymal stem cells.



(b)

Sample	Applied Voltage (V)	Estimated Inner Pore Size (nm)	Roughness Ra (nm)	Contact Angle (°)
Ti	NA	NA	9.7	54
30nm	5	27.0	13.0	11
50nm	10	46.4	12.7	9
70nm	15	69.5	13.5	7
100nm	20	99.6	13.2	4

Fig. 2. Physical characterization of different size nanotube surfaces. (a) SEM micrographs of self-aligned TiO_2 nanotubes with different diameters. The images show highly ordered nanotubes with four different pore sizes between 30-100nm created by controlling the voltage from 5-20V. (b) Table with the applied voltage parameter, estimated inner pore size from SEM images, average roughness (Ra) and surface contact angle measurements for Ti and 30-100nm TiO_2 nanotube surfaces.

In terms of current biologically active implants, enhanced surface roughness is one of the important factors in providing the proper cues for a positive cell response to implanted materials. However, much of the research related to the effect of macro and micro-roughness on cellular responses and tissue formation are inconclusive due to the non-uniformity of macro and micro-roughness stemming from crude fabrication methods like polishing, sand blasting, chemical etching and so on. An important aspect of our nanotube system shown in the SEM images (Figure 2) is that the nano-topography can feature a more defined, reproducible and reliable roughness than micro and macro-topography for enhanced bone cell function *in vivo*. Although, the heights of the nanotube walls increase proportionally to the increasing diameter, there is no evidence of changes in surface roughness between the different sized nanotubes based on atomic force microscopy (AFM) data (Figure 2 (b)). As expected, the nanotube surfaces have a slightly higher roughness over flat Ti, but between the nanotubes, there appears to be no difference. The AFM data was performed because it is a somewhat standard surface analysis technique as it is useful for coarser or microscale roughness measurements, say for other convential coatings, but for the nanotube dimensions it may not always represent the true roughness when the probe tip radius is not substantially finer than the nanotube dimensions such as in the TiO_2 nanotube case. The wall thickness, pore diameter, nanotube spacing, etc can be as small as ~10 nm, while the AFM probe tip diameter can be as large as 30 - 50 nm.

Furthermore, it can be assumed that the surface area on the nano-scale may be affected based on the various sizes and the surface area probably increases proportionally with

increasing nanotube size. It is expected that the surface area to be 3 times higher on the 100nm diameter nanotubes compared to the 30nm diameter nanotubes, respectively. Additionally, the contact angle describing the wettability of the surface is enhanced, more hydrophilic, on the nanotube surfaces (showing contact angles between 4-11°), which can been advantageous for enhancing protein adsorption and cell adhesion.

3.1 Protein adhesion properties based on pore size

Cells respond to the amount and area of proteins that are available for binding. In fact, cells do not see a naked material, *in vivo* or in *in vitro* culture. At all times, the material is conditioned by the components of the fluid in which the material is immersed, whether it is serum, saliva, cervicular fluid or cell culture media. As the cell begins to adhere and spread on the nanotubes, there will be a dissimilar protein density and extra cellular configuration based on the nanotube diameter. The behaviour of protein adsorption on the nanotube surfaces are shown in Figure 3. On the 30nm diameter nanotubes there is a large number and thorough distribution of protein nanoparticles covering the whole surface of the nanotubes after just 2 hours of incubation in culture media. However, proteins on 100 nm TiO_2 nanotubes can only adhered sparsely at the top wall surface owing to the presence of large empty nanotube pore spaces. This inherent protein adsorption property of the nanotubes based on poresize is hypothesized to influence cell shape and fate. It is shown in the next sections that the changes in poresize even in such a small range of dimensions (30-100nm) will have huge impacts on downstream cell morphology and behavior.

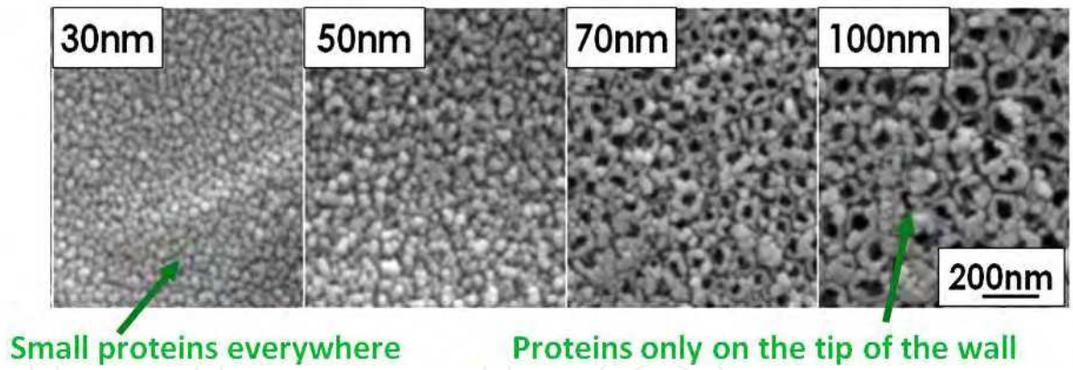


Fig. 3. SEM micrographs of flat Ti and 30, 50, 70, 100nm diameter TiO_2 nanotube surfaces after 2 hours of culture showing protein adsorption from media.

4. Osteo-chondral applications of TiO_2 nanotube constructs

Artificial cartilage prepared from cultured chondrocytes offers promise as a treatment for cartilage defects (Fedewa et al. 1998), but connecting this artificial soft tissue to bone in the attempts to restore the defected cartilage is difficult. One strategy employed in this section is to develop a dually functional substrate that supports the growth and attachment of cartilage tissue on one extremity and encourages osseointegration, a direct structural and functional connection to living bone, on the other. This substrate should be an engineered interface between artificial cartilage and native bone (Zhang, Ma, and Francis 2002).

In recent studies, Ti has emerged as a candidate material in cartilage tissue formation as well. It has been demonstrated that a micrometer porous substrate of Ti-6Al-4V provided

conditions that favored cartilage tissue formation by influencing cell attachment, spreading and the amount and composition of cartilaginous tissue that forms (Spiteri, Pilliar, and Kandel 2006; Bhardwaj et al. 2001; Ciolfi et al. 2003). Not only porosity, but also surface geometry and topography have been found to have positive effects on the behaviour of chondrocytes (Bhardwaj et al. 2001).

Nanoscale topographic effects have been illustrated in nanostructured poly-lactic-co-glycolic acid (PLGA)/nanophase Titania (TiO_2) composites, which have elicited an enhanced chondrocyte response compared to surfaces with a conventional or micrometer topography (Savaiano and Webster 2004). We have recently reported on our hypothesis that the nanotopographical cues, from porous nanotubular structured substrates made of TiO_2, already being an osseointegrating biomaterial (Bjursten 2009; Oh et al. 2006), may also be a candidate for providing an alternative way to positively influence cartilage formation and the cellular behaviour of cartilage chondrocytes.

4.1 Up-regulated chondrocyte synthesis of extracellular matrix components

The dimensions of the nanotubes were varied in order to determine if the size of the nanotube diameters would play a role in the chondrocyte behaviour. For this comparative chondrocyte cell culture study, a commercially pure Ti surface, without surface modification was used as a control, as it commonly used as implant material.

It is well known that chondrocytes, the primary cells of cartilage, are extremely active cells. They produce a large amount of extracellular matrix (ECM) that is critical for the mechanical properties and joint lubrication characteristics of cartilage. In the SEM micrographs in Figure 4, the nanotubes substrates appear that they are inducing a positive response from the chondrocytes because the cells begin synthesizing abundant ECM deposition and fibril organization. In the SEM observations of chondrocytes a striking difference in the production of ECM fibrils between the flat Ti without a nanostructure vs. TiO_2 nanotube surfaces is revealed. Fibrils are abundant and extending from all areas of the chondrocyte cell creating a dense network of ECM on the nanotube substrates.The flat Ti most likely lacks surface structuring cues for signaling ECM fibril production and organization. One possibility is that ECM protein formation into dense fibrils on the surface may be "nano-inspired" to form on the nanotube structure because of the precise dimensions or fine scale cues of the top surface (tip of the vertical wall) of TiO_2 nanotubes having a physically confined geometry which could aid in fibril formation. It was demonstrated previously that the nanotubes produced bio-active nanostructured formations of sodium titanate nanofibers directly on the top of TiO_2 nanotube walls when the nanotubes were exposed to NaOH solution (Oh S 2005). ECM proteins once secreted, in this study, may also self-assemble according to the top-wall surface geometric nanocues.

In the lower panel of Figure 4, immunofluorescent images for collagen Type II (red color) are illustrated for flat Ti vs. TiO_2 nanotubes. Both surfaces stained positive for collagen type II, but there was large networks of connected bundles expressed across the surface of the nanostructure.

When a morphological analysis was conducted, it was determined that nanotubes induce a more spherical chondrocyte cell shape (data not shown). Fibroblastic shaped cells were observed on the flat controls. The percentage of round shaped, spherical cells was significantly lower for chondrocytes on the polystyrene, Ti, and the smallest diameter (30nm) nanotube substrates compared to the larger diameter 50, 70, and 100nm TiO_2

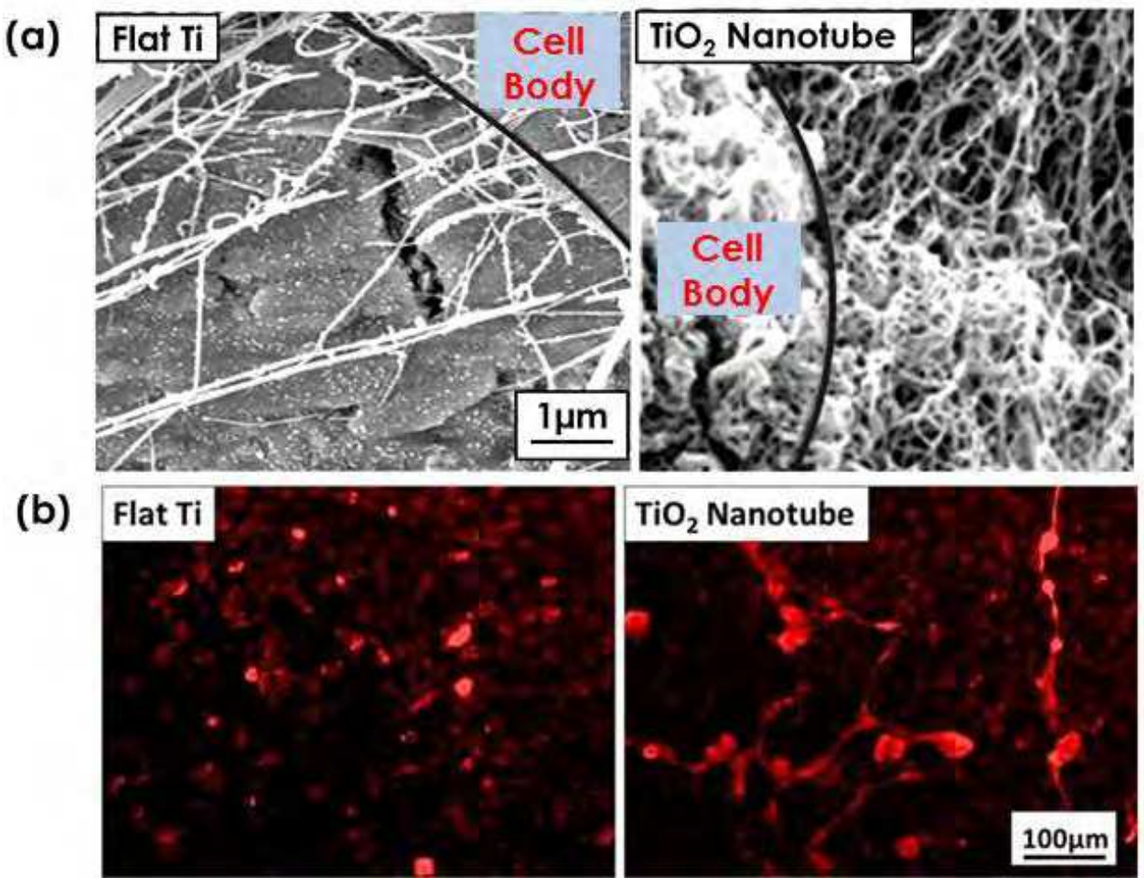


Fig. 4. Extracellular matrix (ECM) production on experimental surfaces Ti vs. TiO_2 nanotubes. High magnification SEM observations of chondrocytes reveal a striking difference in the production of ECM fibrils between the flat Ti without a nanostructure vs. TiO_2 nanotube surfaces. Fibrils are abundant and extending from all areas of the chondrocyte cell creating a dense network of ECM on the nanotube substrates. (b) Immunofluorescent images of collagen type II (red) ECM fibrils produced by chondrocytes on flat Ti and nanotube surfaces (100nm diameter shown in this image).

nanotube surfaces respectively. It was also determined that all diameter nanotube surfaces were significantly higher than flat Ti which probably indicates that the cell shape was influenced by the presence of the nanostructure itself. The nanotube geometry seen in Figure 2 most likely aids in preserving the chondrocyte spherical morphology because of the distinct structure of the surface. Cells may be localized atop the pores, anchored possibly at the tip of the nanotube walls and confined by the tube contour. The chondrocytes on the flat Ti seem to be spread along the surface probably because the necessary structuring cues and nanopores needed for shape confinement are absent. To further describe the chondrocyte shape phenomenon found the experimental surfaces, a schematic is shown in Figure 5.

It was formerly assumed that focal contacts should be a specific length in order to promote adhesion and that the maximum overall contact of the cell with its substrate was most favourable (Ohara and Buck 1979). Yet, more recent studies suggest that cell-flattening or

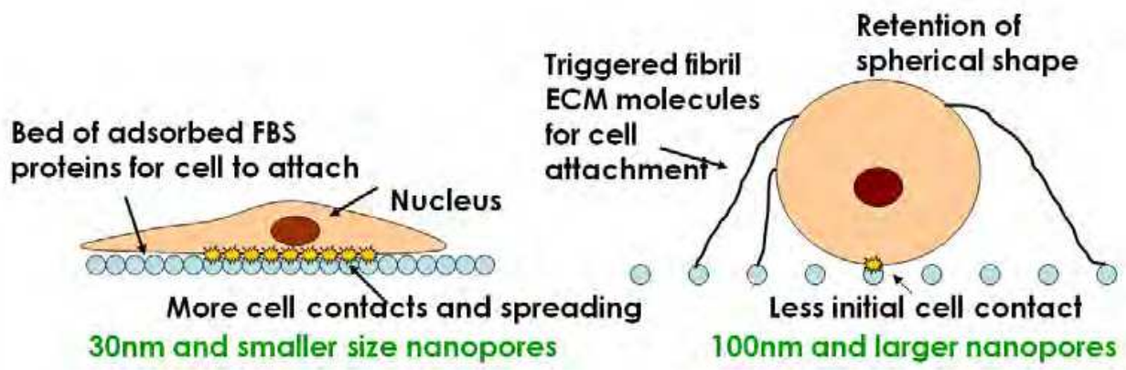


Fig. 5. Chondrocyte cell adhesion and spreading schematic determined by the size of the nanotube diameter and focal attachment sites.

spreading is not always compatible with differing cell types, particularly in the case of chondrogenesis (Solursh 1989; Benya and Shaffer 1982; Solursh 1982; Zanetti and Solursh 1984). Thus, the type of focal adhesion and its geometry can influence the shape the cell assumes, ultimately influencing the phenotypic expression. It has been reported that the dedifferentiation of chondrocytes in culture is usually associated with changes in cell morphology, from a rounded to a spread one (Costa Martinez et al. 2008). The results reported here suggest that creating pores by fabricating nanotubes on Ti surfaces provides a more favorable environment for the retention of the rounded morphology and the prevention of chondrocyte spreading, reducing the risk of a loss of phenotype. It should be noted that although chondrocyte cells retain this type of spherical morphology in response to the nanotube pores, different cell types will differ in size, shape, function, and how they operate on the nanotube surfaces. It is well known that different cell types elicit their own unique responses to environmental cues. The chondrocyte cells with their spherical morphology are much different than mesenchymal stem cells and osteoblast cells, described in later sections, and therefore will adhere differently to the topography and form different morphologies.

Because chondrocytes are very dynamic cells that produce and maintain the cartilaginous matrix, which consists mainly of collagen and proteoglycans, it is important to test the biochemical ECM production on the different experimental surfaces. Therefore, to further evaluate the response of BCCs for this comparative report, the glycosaminoglycan (GAG) secretion in the media was also studied and shown in Figure 6.

Naturally, aggrecan draws water into the tissue and swells against the collagen network, thereby resisting compression and allowing for proper joint movement (Muir 1995). An interesting concept worthy to note is that the up-regulation of GAG chains indicative of increased aggrecan production observed on the larger sized nanotube pores could imply that because there are increased storage volume capabilities as pore size increases it triggers a higher rate of production because the molecule retention ability of the cellular environment has been inflated.

It was determined that there was a correlation in the increased GAG secretion in the media and the reduction of fibroblastic shaped cells. Specifically, TiO_2 nanotubes with diameters

in the range of 50-100nm had significantly higher levels of both round, spherical shaped cells (more phenotypic) and GAG secretion over flat Ti and small 30nm TiO_2 nanotube surfaces.

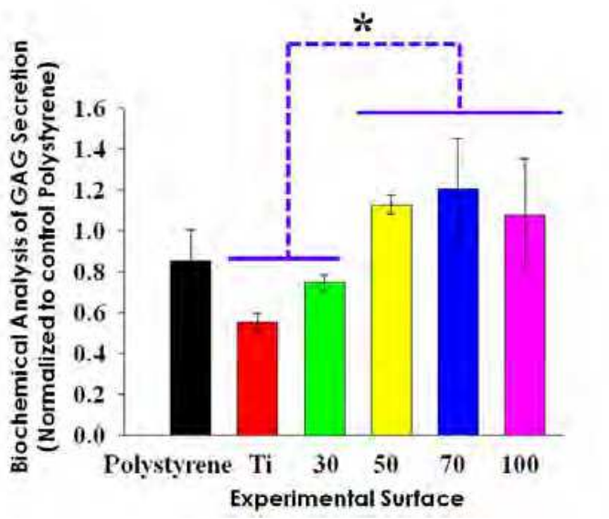


Fig. 6. Glycosaminoglycan (GAG) secretion in the media.

In this study, the larger diameters (50nm-100nm) nanotubes were revealed to be most suitable for chondrocyte culture *in vitro*. It would be interesting to investigate nanotube diameter sizes larger than 100nm by other fabrication means so as to elucidate the possible effect of large pore size and find an ultimate productivity saturation limit; this would certainly allow more light to be shed on the beneficial nature of nanotopography.

4.2 Conclusions and considerations for further osteo-chondral development

As Ti is the well accepted orthopaedic implant material, the results obtained are very encouraging and suggest that the use of nanotube structures could up-regulate production of extracellular matrix by chondrocytes. In clinical applications the TiO_2 nanotube surface can be utilized as an *in-vitro* culture surface to enhance chondrocyte cell behavior and extracellular matrix production during patient-specific *in-vitro* chondrocyte expansion, which can then be transplanted to the defective cartilage areas. In addition, the TiO_2 nanotube surface exhibits significantly augmented, mechanically and chemically strong osseo-integration with existing bones with a minimal chance of bone loosening evidenced by *in vitro* data [22], and our preliminary *in vivo* animal data indicating a strong new bone integration on the nanotube surface with reduced soft tissue trapping (data not shown). Therefore, a Ti implant with all surfaces covered with the nanotubes can be potentially utilized, for some specific types of articular cartilage injuries, to serve with dual function of accelerated osseointegration to the existing articular bone surface at the bone-facing contact interface while the exposed nanotube surface can accelerate the cartilage tissue regeneration by providing positive surface nanostructuring effects on chondrocytes, as illustrated in Figure 7.

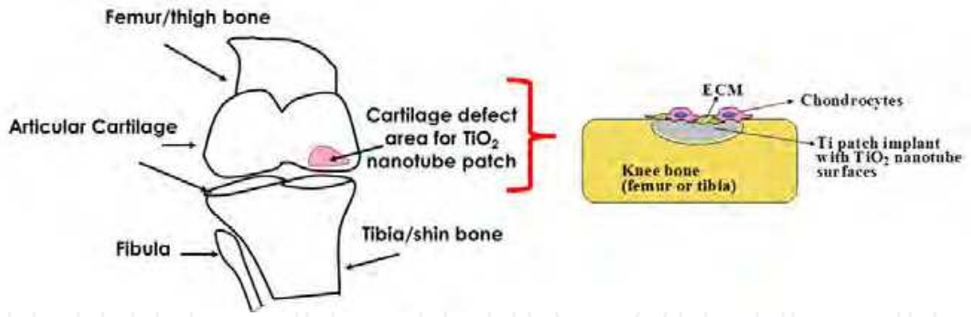


Fig. 7. Schematic illustration of TiO_2 nanotubes for dual function of osseointegration and enhanced chondrocyte function and ECM production.

5. Orthopedic implant applications of TiO_2 nanotube constructs

As mentioned earlier, while a thin TiO_2 passivation layer on the Ti surface can impart improved bioactivity and better chemical bonding to the bone (Feng et al. 2003), other techniques have been developed to further enhance the bioactivity of a pure Ti surface, such as direct coating of bioactive materials like hydroxyapatite and calcium phosphate (Puleo et al. 1991; Salata 2004; Satsangi et al. 2003). However, even though these surface modified layers have good bioactivity and high surface area, they tend to delaminate at the interface between the implant and the bone due to the relatively large, micrometer-regime thickness of the coated layer on Ti (Ong et al. 1992), presumably due to the stress accumulation commonly seen in a thick coating of foreign material. This ultimately leads to implant failure. In order to overcome this problem, some plasma spray Ca-Si based ceramic coatings have been developed but still have roughness and layer thickness in the micrometer range. For the purposes of this study, the focus is on nanoscale thickness surface coatings. Therefore, developing an implant bioactive surface layer having high surface area for enhanced bonding yet thin enough, in the nanometer range per se, to minimize delamination would be desirable.

Recent reports indicate that modifying Ti surfaces with TiO_2 nanotubes for orthopedic applications significantly enhances the mineral formation (Oh et al. 2005), adhesion of osteoblasts *in vitro* (Oh et al. 2006), and strongly adherent bone growth *in vivo* (Bjursten 2009), showing better bone bonding characteristics than conventional micro-roughened Ti surfaces by sandblasting. One physical advantage of the TiO_2 nanotube surface system is that it is composed of and created directly from the native underlying Ti constituent, unlike the foreign ceramic and spray coatings on Ti or Ti alloyed surfaces mentioned previously. As well, the nanotube layer is at most ~300nm tall (for the purposes of this work) which in the scheme of things is a much thinner layer and this nanometer length scale eliminates the tendency of delamination prevalent in thick micrometer layers.

Because orthopedic implants encounter two types of cells, osteoblast cells in bone tissue and osteo-progenitor cells also know as mesenchymal stem cells (MSCs) present in bone marrow, it is advantageous to look at the differentiation potential of orthepeadic implant surfaces in order to initiate a mature population of bone building cells for enhanced osseointegration. One key principle in terms of orthepaedic implant technologies, to initiate the differentiation of bone in the absence of chemical factors, hormones, or any other synthetic, possibly toxic chemicals traditionally used by biochemists in *in vitro* differentiation. The two cell types are illustrated and described in Figure 8.

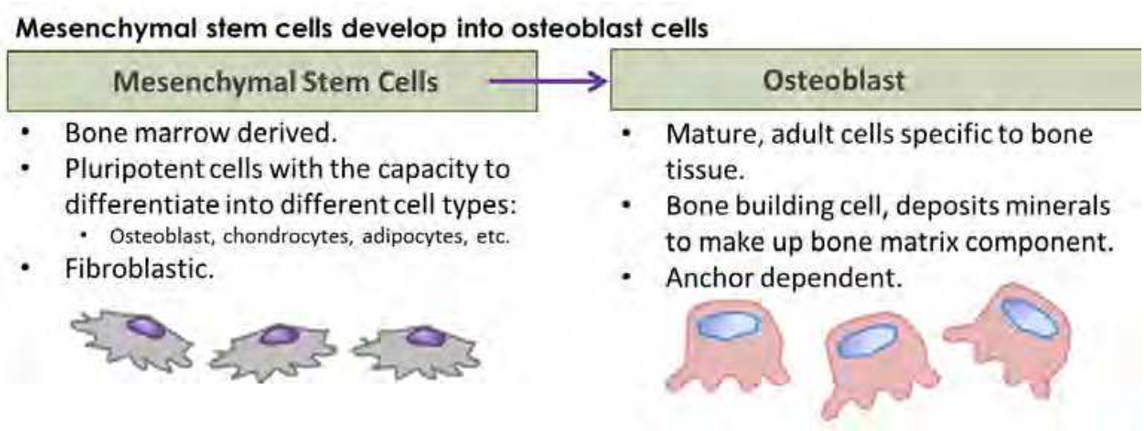


Fig. 8. Schematic illustration showing osteo-progenitor cells developing into osteoblast cells. A description of the two cell types is also portrayed in list format.

Stem cells, which have the potential to differentiate into multiple cell types, provide great promises in advances in regenerate medicine. The differentiation of stem cells into the appropriated lineages is temporal- and spatial-specific, with the surrounding microenvironment playing critical roles in governing the stem cell fate. For generation of osteoblasts, the MSCs need to be guided to selectively differentiate to osteoblasts, rather than differentiating into other types of cells. The use of nanostructures and surface topographical features have recently been shown to have positive effects on specific differentiation of mesenchymal stem cells (Dalby, Andar et al. 2008), illustrating that the surface topography alone can stimulated osteogenic differentiation.

5.1 Osteogenic functionality depends on nanotube diameter

In terms of the dimensions of the nanotubes in our osteoblast (bone cell) and mesenchymal stem cell (osteo-progenitor cell) studies, we have reported a unique variation in cell behavior even within a narrow range of nanotube diameters from 30-100nm (Brammer et al. 2009; Oh et al. 2009) and it seems that a similar trend is established for both cell types.

When cells were grown on four different diameter nanotubes, shown in Figure 2, osteoblast functionality in terms of bone forming ability, or alkaline phosphatase activity (ALP), and mesenchymal stem cell (MSC) osteogenesis (bone cell differentiation) in terms of osteogenic gene expression (osteopontin (OPN), osteocalcin (OCN), and alkaline phosphatase (ALP)) were most prominent on the large 100 nm diameter nanotubes, Figure 9 (a and b). During periods of active bone growth, ALP activity levels are elevated in osteoblast cells so it is beneficial to design an implant surface that would enhance the ALP activity to initiate the formation of new bone. As well, it is critical to design a surface that is capable of allowing the attachment of MSCs and promote osteogenic differentiation of cells for delivering a mature osteoblastic cell population capable of rapidly forming bone. On the nanotube surfaces, a reoccurring trend was revealed that as we increased the diameter of the nanotubes, there was an increase in osteogenic biochemical activity and relative gene expression, Figure 9 (c).

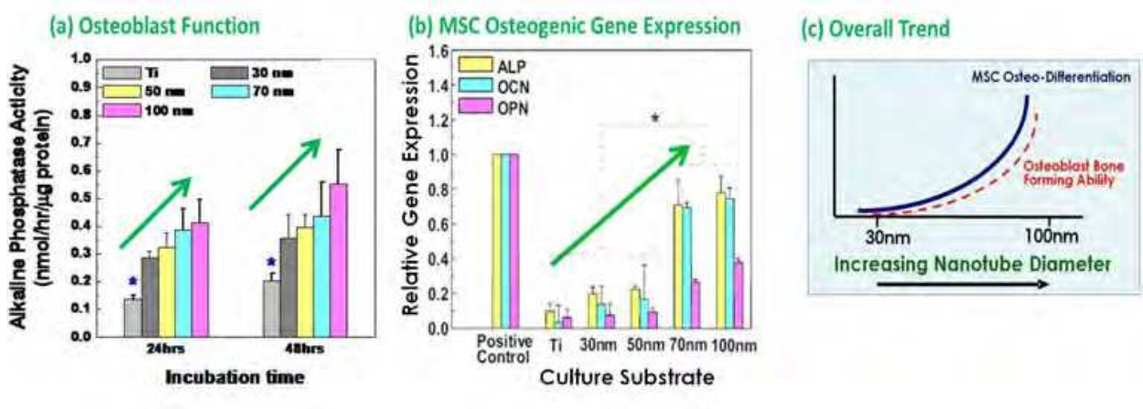


Fig. 9. Comparative graphs showing the influence of TiO_2 nanotube diameter on osteogenic cell behavior. (a) Osteoblast functionality in terms of alkaline phosphatase activity or bone forming ability. (b) Osteogenic gene expression. RNA levels of alkaline phosphatase (ALP), osteocalcin (OCN), and osteopontin (OPN) are given for mesenchymal stem cells grown on different size diameter nanotubes. (c) Overall all trend for diameter effect on osteogenic cell behavior. As the nanotube diameter increases the osteogenic cell behavior is enhanced.

In a recent review article, Bettinger et al. claimed that the most palpable effect of nanotopography on cells is the distinct changes in cell geometry or shape (Bettinger, Langer, and Borenstein 2009). In fact, on the nanotube substrates with varying diameters, it was revealed that the osteoblast and mesenchymal stem cells have reacted to the nanostructures by changing shape. As the nanotube diameter increases we found a clear trend of increasing cell elongation, Figure 10. The stretching aspect ratio was as great as 12:1 (length:width) on the 100 nm diameter nanotubes. It can be assumed that the initial cell stretching and elongated shape of the adhering cells on the large nanotubes impacted the cytoskeletal (actin) stress. This is supported by the general notion that nanostructures (and the adhered protein configuration for example, Figure 3) act as an extracellular matrix which imposes physical forces and morphological changes to the cell (Chen 2008). It is probable that cells must elongate their bodies to find a protein deposited surface, extending across larger areas and thus eventually forming an exceedingly elongated shape on the 100nm diameter nanotubes (because of the sparse distribution, Figure 3). Thus altering the density of extracellular matrix (ECM) attachment sites or initial protein adhesion density affects the shape of adhered cells. It has been reported that the focal attachments made by the cells with their substrate determine cell shape which, when transduced via the cytoskeleton to the nucleus, result in expression of specific phenotypes (Boyan et al. 1996). In our results, we hypothesize that increasing nanotube diameters, changes the focal adhesion sites of the osteoblast and mesenchymal stem cells, increases the cell elongation, and increases osteogenic potential.

Interestingly, the cell nuclei also exhibit a somewhat similar trend of increased elongation with increasing nanotube diameter, with the 100nm TiO_2 nanotubes showing the most significantly elongated nuclear shape (by ~20-25%) (data not shown). It can be hypothesized that the nucleus organelle elongation on the TiO_2 nanotube surfaces is in part due to the

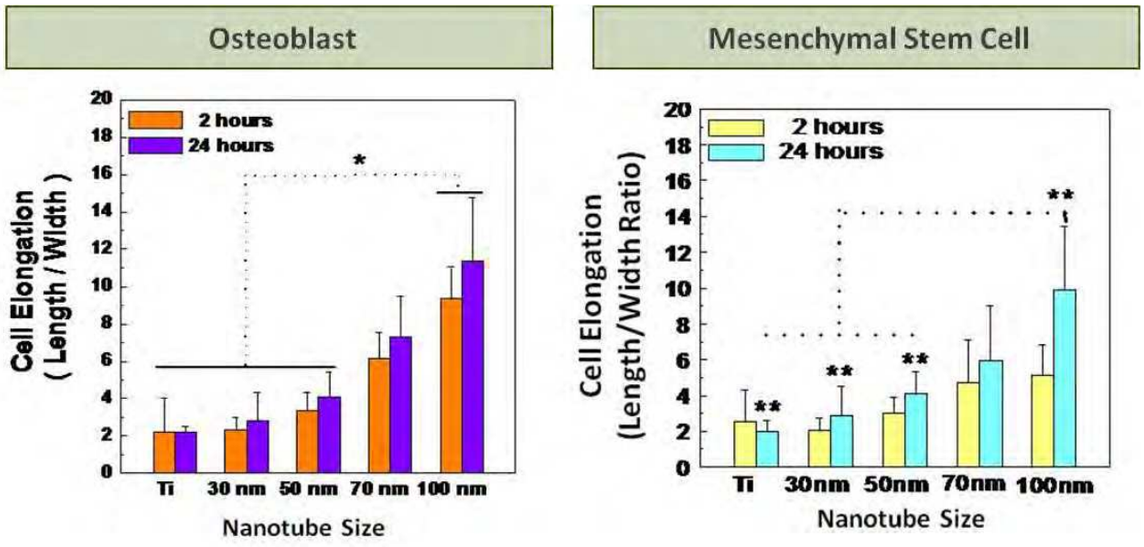


Fig. 10. Quantification of cell elongation for osteoblast and mesenchymal stem cells on nanotubes with different diameters ranging from 30-100nm. There is a trend of increased elongation with increasing nanotube diameter.

gross elongated cytoskeletal morphology of the cell. It has been reported that cell shape maintained by the cytoskeletal assembly may also facilitate nuclear shape distortion which may promote DNA synthesis by releasing mechanical restraints to DNA unfolding, changing nucleocytoplasmic transport rates, or alternating the distribution and function of DNA regulatory proteins that are associated with the nuclear protein matrix (Maniotis, Chen, and Ingber 1997). A change in the nuclear structure has an effect on the 3-dimensional internal organization (Getzenberg et al. 1991). It appears that the osteoblasts and mesenchymal stem cells are adapting to the nanotube substrate nanotopography by organizing both external and internal shapes.

A concept developed by Dalby et al. (Dalby et al. 2008) suggests that MSC osteo-differentiation is determined by mechanotransductive pathways that were stimulated because the cell was under tension caused by way the cell was adhering and the shape it assumed due to the underlying nanostructure surface. Cell morphology/spreading dominates cell fate. McBeath et al. showed that commitment of stem cell differentiation to specific lineages is dependent upon cell shape (McBeath et al. 2004). In a single cell experiment with micropatterned surfaces the critical role of cell spread/shape in regulating cell fate was determined.

Nonetheless there is a need to better understand the mechanism by which such nanosurfaces direct MSC osteogenesis, and to optimize the culture conditions in order to maximize MSC expansion and differentiation. For bone growth, it requires cell proliferation and selective differentiation, and these processes are found to occur at different but discrete nanosurface topography conditions such as variations in nanotube diameter.

5.2 Conclusions and considerations for further orthopaedic considerations

Establishing possible connections between mechanical properties of MSCs in differentiation is valuable to the field of mechanobiology. It can be speculated that natural forces that MSCs

encounter in a physical environment does not need a strong cytoskeleton, however upon osteogenic differentiation, in which differentiation of MSCs become part of a larger bone structure that functions to provide both form and strength, the supporting structure of the cells are enhanced to enable function and withstand load bearing wear that bones endure. Understanding the physical characteristics of MSCs during differentiation may aid in the development of new biomaterials, which can potentiate the necessary mechanics of the cells for the advancement of tissue engineering.

In terms of the dimensions of the nanotubes in the osteoblast (bone cell) and mesenchymal stem cell (osteo-progenitor cell) studies, it was reported that a unique variation in cell behaviour even within a narrow range of nanotube diameters (Brammer et al. 2009; Oh 2009).The results of the previous research can be simply summarized: osteogenic functionality, both biochemical activity in osteoblasts and internal gene regulation of osteo-progenitor cells, were altered by the size/diameter of TiO_2 nanotubes, as the nanotube diameter increased, the osteogenic function also increased. Such a trend can be utilized for improvement and control of the bone forming functionality for advanced orthopaedic implant technologies.

In these studies however, TiO_2 nanotubes having a 1: 3 diameter: height aspect ratio was used, which was determined by the electrochemical anodization conditions including electrolyte solution, voltage, time, etc. While this current-state-of-the-art self assembly process of TiO_2 anodization does not easily allow fabrication of TiO_2 nanotubes with the diameter larger than ~100nm with the electrolyte used in this study, it would be interesting to study the effect of even larger diameter TiO_2 nanotubes, possibly using a modified chemical process, on osteogenic cells.

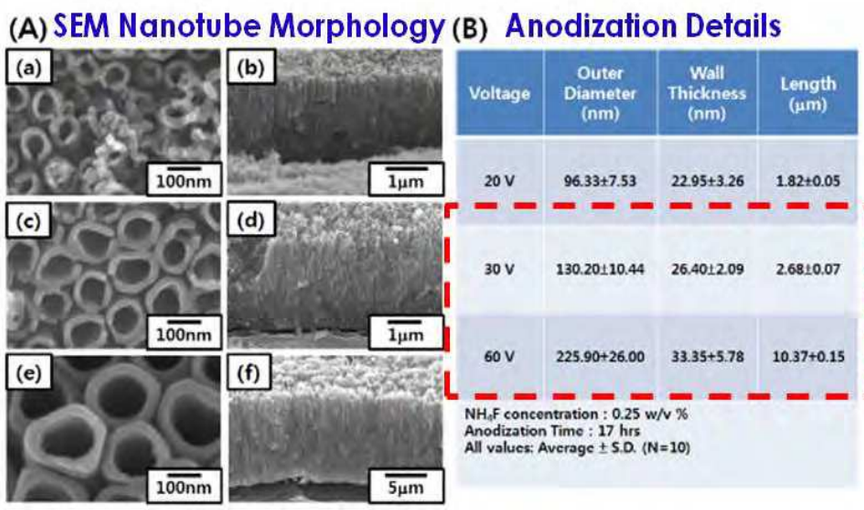


Fig. 11. Large diameter nanotubes prepared in 0.25 w/v% NH_4F with various applied voltages. (A) SEM micrographs showing nanotube morphology by top view (a, c, e) and cross-sectional view (b, d, f). (B) Chart describing the effect of applied voltage on the physical nature of the nanotube dimensions.

Other methods for making large diameter (>100nm) TiO_2 nanotubes using aqueous organic electrolytes with a future potential use as orthopaedic implant surfaces have been explored. Previously the anodization electrolyte method included aqueous dilute hydrofluoric acid. In

an alternative fabrication method ammonium fluoride (NH_4F) in an ethylene glycol solution as an electrolyte can be investigated. Figure 11 illustrates the SEM figures of TiO_2 nanotubes prepared by 0.25 w/v% NH_4F electrolyte at various anodization voltage for 17 hrs. The table in Figure 11 indicates the variations in applied voltage and the resultant physical dimensions of the fabricated nanotubes. By controlling the applied voltage, the diameter of the nanotubes could also be controlled. Nanotubes with diameters from ~130-225nm have been successfully prepared. Future work should include the use of large size nanotubes to find the most optimal stem cell osteogenesis and bone cell/tissue function.

6. Future direction and applications

In the future, nanostructured ceramics will be created that demonstrate even higher degrees of integration between materials and biology (Narayan et al. 2004). Future ceramic nanostructures may possess even more precisely tailored grain and/or pore sizes in order to obtain specific tissue interactions. For instance, loading nanoporous structures can provide an implant with biological functionalities (Cowan et al. 2003) are key prospects in deriving therapeutic based nanostructures that integrate and for wound healing, bone repair, and cardio vascular restoration, to name just a few. For example, silver and zinc-containing zeolites, marketed under the trade name AgION®, are currently being assessed for use in wound dressings. Figure 12 shows the idea of a "nanodepot" using the nanopores of the TiO_2 nanotubes for loading biological agents.

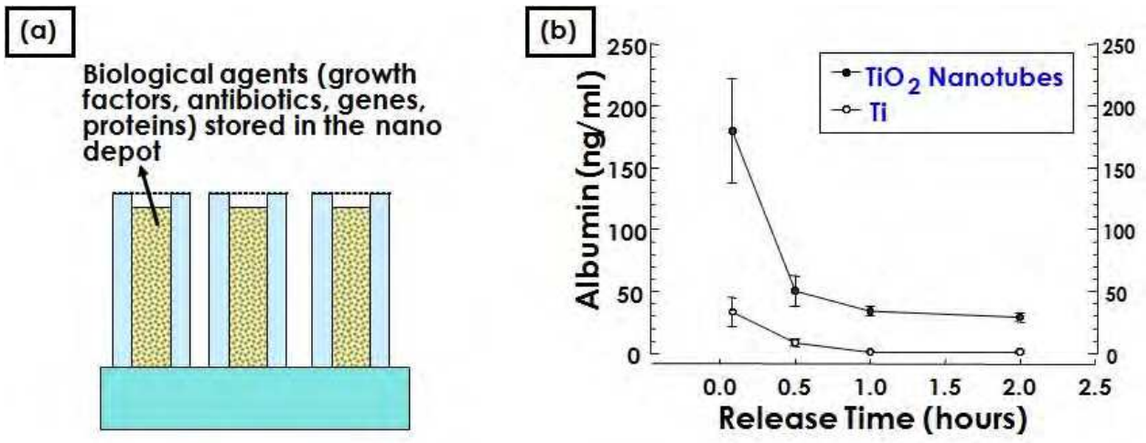


Fig. 12. (a) Schematic illustration of "nanodepot" concept in TiO_2 nanotubes. (b) Example of albumin protein elution from inner pores of TiO_2 nanotube structures.

In the previous sections it has been shown that the physical environment of nanotopography has positive effects on cell behaviour, yet direct comparisons of nanotopographic surface chemistry has not been fully explored. For instance the possibility of nanotubes made of different materials, i.e. carbon, gold-Pd, zirconia, or tantalum for instance.

To date, a large part of the interest has remained on titanium oxide (TiO_2) nanotubes because it is well known that titanium (Ti) is a biocompatible orthopedic material which provides an excellent osseointegrative surface. However, little notice has been given to zirconium oxide (ZrO_2) nanotubes, which are formed via the similar self-assembled

mechanism as TiO_2 nanotubes, through an electrochemical anodization process (Berger et al. 2008). Zirconium (Zr) is similar to titanium in that it possesses a thin passivation oxide layer which makes it highly resistant to corrosion in bodily fluids (Oliveira et al. 2005). In fact, while the corrosion resistance and biocompatibility of certain Zr alloys are as good as those of Ti alloys, the mechanical properties have been found to be superior to those of the commonly used Ti-6Al-4V alloy (Kobayashi et al. 1995). Furthermore, a recent study by Bauer and co-workers demonstrated that mesenchymal stem cells react in the same manner to ZrO_2 nanotubes, AuPd-coated TiO_2 nanotubes, and as-formed TiO_2 nanotubes (Bauer et al. 2009). Their results indicate that the cell response is chiefly due to nanotopographical cues instead of a specific surface chemistry pertaining only to TiO_2.
There is a vast parametric space in which to explore novel nanostructures, nanopore dimensions, and material compositions for optimizing and advancing implant designs for desired tissue interactions.

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These contribution books collect reviews and original articles from eminent experts working in the interdisciplinary arena of biomaterial development and use. From their direct and recent experience, the readers can achieve a wide vision on the new and ongoing potentials of different synthetic and engineered biomaterials. Contributions were not selected based on a direct market or clinical interest, than on results coming from very fundamental studies which have been mainly gathered for this book. This fact will also allow to gain a more general view of what and how the various biomaterials can do and work for, along with the methodologies necessary to design, develop and characterize them, without the restrictions necessarily imposed by industrial or profit concerns. The book collects 22 chapters related to recent researches on new materials, particularly dealing with their potential and different applications in biomedicine and clinics: from tissue engineering to polymeric scaffolds, from bone mimetic products to prostheses, up to strategies to manage their interaction with living cells.

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