Cell attachment on ion implanted titanium surface

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Properties

ABSTRACT

Purpose: Of outmost importance for the successful use of an implant is a good adhesion of the surrounding tissue to the biomaterial. In addition to the surface composition of the implant, the surface topography also influences the properties of the adherent cells. In the present investigation, ion implanted and untreated surfaces were compared for cell adhesion and spreading.

Design/methodology/approach: The surface topography of the surfaces were analyzed using AFM and the cell studies with SEM.

Findings: The results of our present investigation is indicative of the fact that ion implanted titanium surface offer better cell binding affinity compared to untreated/polished surface.

Practical implications: Success of non-biodegradable implants will first and foremost depend on biocompatibility, followed by the capacity of the surface topography of the implants to evince desired cell matrix, surface cell matrix interactions. In the present study, the cell growth on ion implanted Ti material is analyzed and discussed.

Originality/value: In this paper, we have utilized ion implantation technique, which will produce nano-texturing of the surface without producing any detrimental effects to both the dimensions and properties of the implants.

Keywords: Ion implantation; Surface topography; Osteoblasts; Roughness; Extra cellular matrix

1. Introduction

In the past decade, significant scientific advances have been made in establishing therapeutic methods for treatment of diseases that require reconstructive surgery or organ replacement. Soft and hard tissue engineering has emerged as one of the most exciting areas of research in healthcare product engineering [1-3]. These approaches have been effective in regenerating functional tissues or organs ranging from bioartificial skin to functional urinary bladder and blood vessels using cell-scaffold-based approaches.

Scaffold-guided tissue engineering (TE) has been developed to regenerate specific and functional human tissues or organs [4, 5]. As the scaffolds form the platform for cells to develop and to be organized into tissues and organs, TE scaffolds should facilitate the colonization of cells and possess properties and characteristics that enhance cell attachment, proliferation, migration and expression of native phenotypes. Scaffold characteristics and properties such as porosity, surface area to volume ratio, pore size, pore interconnectivity, structural strength, shape (or overall geometry) and biocompatibility [6, 7] are often considered to be critical factors in their design and fabrication.

The outstanding biocompatibility of titanium (Ti) was already recognized by many researchers [8-10]. The mechanical properties of Ti compare favorably with those of other implantable metals and alloys. The yield strength is approximately the same as that of surgical quality 316L

Short paper

stainless steel and almost twice that of the familiar cast Co-Cr-Mo alloy used in orthopedic implants. The elastic modulus is approximately half that of the other common metal alloys used in surgery. This low modulus results in a material that is less rigid and deforms elastically under applied loads. This is important in the development of orthopedic products where a close match is desired between the elastic properties of long bone and the surgical implant. The fatigue strength is about twice that of stainless steel [8-12].

Ti has an extreme low toxicity and is well tolerated by both bone and soft tissue. Animal experiments have revealed that the materials may be implanted for an extensive length of time; fibrous encapsulation of the implants is minimal to nonexistent. Histopathological examinations have failed to reveal any cellular changes adjacent to titanium implants. Careful examination of tissues adjacent to titanium has revealed neither giant cells nor macrophages, nor any other signs of inflammation. The material has been found to be safe in intravascular applications, owing to its high electro negativity and passive surface. For the same reason titanium does not cause hypersensitivity, which makes it the metal of choice in patients suspected of being sensitive to metals [13 -18]. For several decades, special Ti implants have been used with outstanding success in patients with histories of severe allergic reactions. Ti implants are extensively used in cardiovascular, spinal surgery, orthopedic and dental surgery as well as in reconstructive and plastic surgery.

Several in vitro experiments and animal studies have demonstrated the importance of the implant surface to host response [19-20]. Surface topography influences the rate at which bone is formed next to the surface. Variations in surface texture can affect the cellular response to an implant. It has been shown that a higher percentage of osteoblast like cells attached to a rougher surface [18-20]. In vivo studies demonstrated that bone contact to Ti implants was different, depending on whether the surface was smooth or rough, even though the surfaces were of similar oxide thickness [19]. In this study, the synthesis of extra cellular matrix (ECM) and subsequent mineralization were substantially enhanced on rough or porous coated Ti. The topography and chemistry of the surface on which cells are cultured can profoundly affect their shape and function. Davies et al [19] and Lowenberg et al [20] demonstrated that differentiation osteoblasts were capable of laying down a mineralized collagen-free matrix in direct contact with the metal oxide surface of titanium. A major consideration to be done certainly relates to the surface topography of the implants, in that cell behavior around implants is modified by it.

In recent years it has been understood that tissue reactions are determined mainly by surface parameters of the biomaterials used [12-15]. A detailed understanding of these reactions is the basis for targeted approaches towards implants improvement. Various studies have demonstrated that it may be possible to enhance the performance of an implant by designing the texture of the surface. Success of non-biodegradable implants will first and foremost depend on biocompatibility, followed by the capacity of the surface topography of the implants to evince desired cell matrix, surface cell matrix interactions. In the present study, the cell growth on ion implanted Ti material is analyzed and discussed.

2. Materials and methods

2.1. Materials

Samples and Sample Preparation: Commercially available Ti (grade 2) in the form of rods (5/8"ϕ) was used for the experiments. 6 mm thickness discs were cut from the rod. The discs were molded in Bakelite and one side of the discs was then polished to a high degree similar to that for metallographic sample preparation. Silicon carbide paper having grit size 180, 280, 400 and 600 were used for initial polishing. The final polishing was carried out with a mixture of colloidal silica and hydrogen peroxide (30%). During this chemical mechanical polishing, the reaction product of the hydrogen peroxide with titanium is continuously removed from the sample surface with the silica suspension, which leaves the surface free of mechanical deformation. The samples were then removed from Bakelite, cleaned with acetone in an ultrasonic vibrating chamber. Then the samples were washed and dried in alcohol.

2.2. Ion implantation technology

Since the 1970's, ion implantation has been used to increase the surface hardness and to improve the wear resistance in applications such as bearings and turbine blades. This process results in near surface modification, leaving bulk properties virtually unchanged, and has beneficial effects on the fatigue strength and corrosive wear resistance.

A template was designed for fixing the specimens for implantation (Fig. 1). Specimens were masked with aluminium sheets and foils, so that half of the surface area of each specimen was implanted leaving one-half the surface untreated.

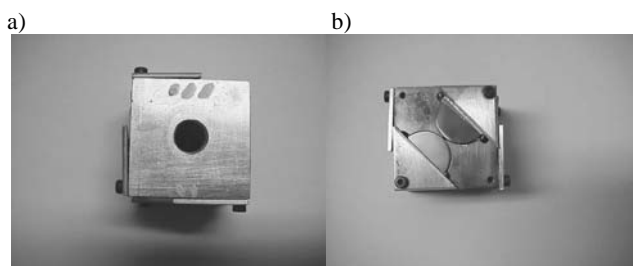a)   b)

Fig. 1. (a) Photograph of the template in which the specimens were fixed for implantation (b) Photograph after implantation

Ion implantation was carried out on half the polished side of discs with an implanter developed by Lucas Heights Research Laboratory. The specimens were implanted with argon ions using a Varian/Extrion 200-1000 Ion Accelerator. All Ti discs were ultrasonically degreased and cleaned prior to ion implantation treatments. Dosimetry was by charge integration in a well-calibrated end station, which provided for rastering of beam and suppression of secondary electrons. The samples were secured to a massive cooled heat sink during ion implantation, so as to limit temperatures to about room temperature. Ion fluences of 1.6×10^{15}, 10^{16} and 10^{17} ions/cm^2 were used with a constant energy of 30 KeV.

2.3. Cell culture

The implanted samples were then subjected to cell culturing. Human osteoblasts used for this study were isolated from alveolar bone. The alveolar bone specimens were obtained from healthy young patients and were first treated by collagenase digestion and then used as explants for establishment of cell culture. The cells were maintained in culture in 75 cm^2 flasks containing Dulbecco's modified Eagle's medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 1% penicillin/streptomycin. Confluent cultures of osteoblasts between 4 to 8 passages were trypsinized and the released cells were suspended in culture medium containing 10% FCS. Aliquots (1ml) of cells at a density of 5×10^3/ml were seeded on the implanted and untreated sides for 24 hrs.

2.4. Surface topography

Surface roughness measurement (Ra), root mean squares (RMS) were measured using Surtronic 3+ portable, self contained instrument and the surface topography images were taken using a Solver Atomic Force Microscope (AFM), NT-MDT Co., Zelonograd Research Institute of Physical Problems 124460, Moscow Russia. High-resolution "Golden" silicon cantilevers (CSG11 Series) with a cantilever length of 250 μm (± 5 μm), width of 35 μm (± 3 μm) and a thickness of 1.0 μm were used in the contact mode. A zeroth order flattening algorithm was used to remove scan line anomalies, and a second order plane fit was then used to remove image bow in the different directions. A low pass filter-smoothing algorithm was used to remove excessive noise.

2.5. SEM

Scanning Electron microscopy (SEM, FEI Quanta, Oregon, USA) was used to view the cell cultured surfaces-both implanted and untreated surfaces. For cell analyses, cells on Ti discs were fixed with aldehyde fixative solution (glutaraldehyde). The fixed specimens were then placed in a buffer solution (0.1M cacodylate buffer) with 2 changes of 10 minutes each. Further to it, the following procedure was followed.

70% ethanol	2 changes of 10 minutes each
90% ethanol	2 changes of 10 minutes each
100% ethanol	2 changes of 15 minutes each
100% amyl acetate	2 changes of 15 minutes each with the change of container.

The samples were then dried using a critical point drying apparatus. The dried samples were then coated with gold using the sputter coater for viewing at the SEM.

2.6. Statistical analysis

Means and standard deviations were calculated for descriptive statistical documentation. The unpaired students *t*-tests was applied for analytical statistics. A value of *p<0.05* was considered significant.

3. Results

3.1. Surface topography and roughness

Fig. 2. shows an AFM image of the surface topography obtained for 1.6×10^{17} fluence. Texturing of the implanted surface is clearly visible from the figure. The AFM image shows the presence of mound-like features (nodules) on the implanted surface. The formation of nodules will contribute to the surface texturing.
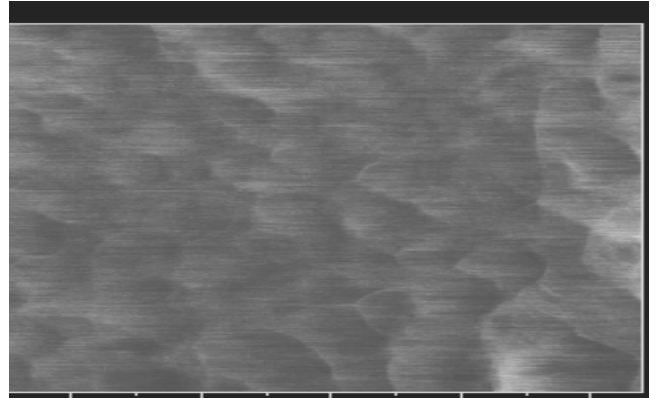

Fig. 2. AFM image for 1.6×10^{17} fluence

Table 1 indicates the roughness average, Ra and RMS, for the implanted and untreated regions of the surface. For a fluence level of 1.6×10^{17} ions/cm^2, the Ra and RMS were about 8 and 14 nm for implanted surface and about 2 and 4 nm respectively for untreated surface. Similar values corresponding to the other fluences suggest similar texturing at the lower fluence level.

Table 1.
Roughness Measures

Fluence	Ra (nm)		RMS (nm)	
	Implanted	Untreated	Implanted	Untreated
1.6×10^{15}	5.0 ± 0.2	2.0 ± 0.3	9.0 ± 0.3	3.7 ± 0.2
1.6×10^{16}	7.0 ± 0.2	2.1 ± 0.2	11.0 ± 0.2	3.5 ± 0.2
1.6×10^{17}	8.0 ± 0.2	2.1 ± 0.3	14.0 ± 0.2	3.7 ± 0.2

3.2. Cell attachment

Cell adhesion is directly involved with cell growth, migration and proliferation. It is now clearly established that surface properties of biomaterials play a critical role in the establishment of cell biomaterial interface. The purpose of the study was to evaluate the influence of ion implanted titanium surface characteristics on cell attachment.

Fig. 3 represent the scanning electron micrographs of cells on ion implanted and untreated Ti surface after 24 hrs with a fluence of 1.6×10^{17} ions/cm^2. Cell attachment and spreading was much higher on the implanted surface as can be seen from the SEM figures and also the graph.
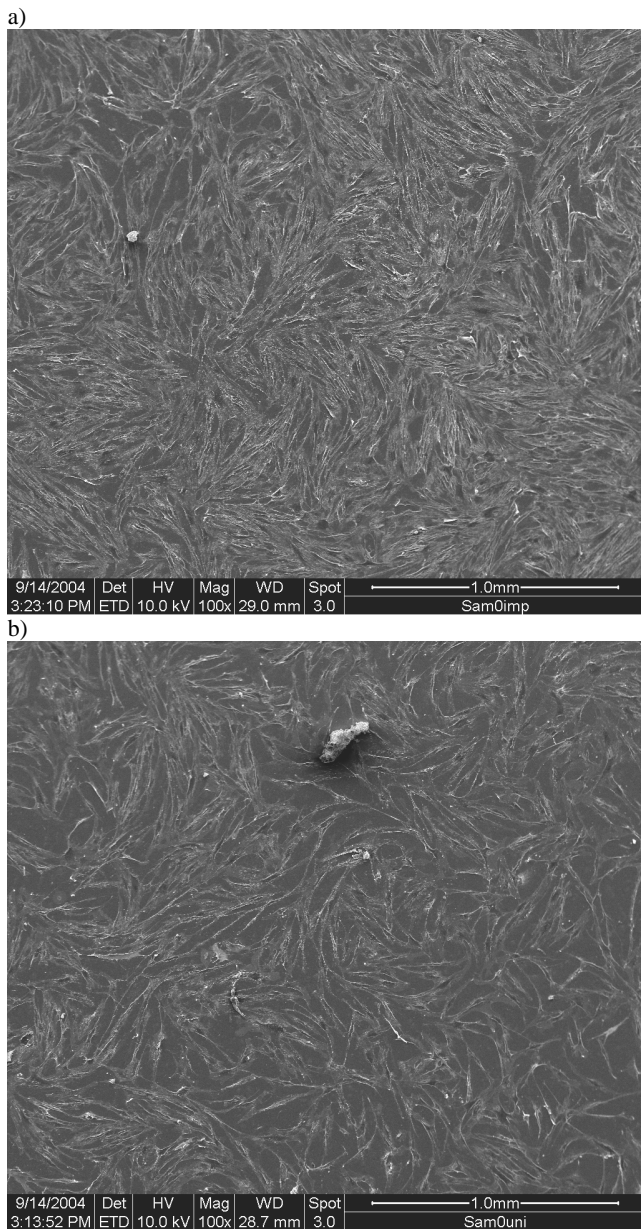
a)

b)

Fig. 3. SEM photograph of cell attachment a) on implanted surface; b) on untreated surface

4. Discussion

The interaction between cells and implants are governed by a number of physical and chemical processes, among which a major factor is implant topography as reported by many investigators [16-20]. The interaction between the bone matrix and osteoblasts with the biomaterial determines the development of the bone-implant interface. For bone-biomaterial interaction, osteoblastic adhesion is an essential requirement. Adhesion of cells is essential for embryogenesis, tissue integrity and wound healing. Numerous proteins are involved in adhesion to the ECM proteins (fibronectin, collagen, laminin, vitronectin), cytoskeletal proteins (actin, talin, vinculin), and membrane receptors (integrins). Interactions between these proteins and their specific receptors induce signal transduction, which influence cell growth and proliferation.

Some of the recent studies [9, 10] were carried out on surface roughened implants using different other techniques. The disadvantage of such techniques is that they will change the dimensions and properties of the implant materials. Therefore in this study, we have utilised ion implantation technique, which will produce nano-texturing of the surface without producing any detrimental effects to both the dimensions and properties of the implants. It has also been reported that cell shape and cyto-skeleton alignment was with respect to surface topography of the implants, which also seem to have a profound influence on osteogenesis. This work investigated the influence of surface topography of ion implanted titanium surface and polished surface on cell adhesion and proliferation. It is seen that the growth ratio on implanted surface is statistically greater than untreated surfaces. The cells were very densely packed and confluent in the implanted region as seen from the figure. The general shape of the cells is the same for both implanted and untreated surface.

Depending on the ion fluences, the effect on the behaviour of cells cultured on the surfaces varies. Within the three fluence level tested, it can be seen that the cell attachment increases as the fluence level increases. In all the three cases, cell attachment was higher with implanted surfaces. It is obvious that from the data, the difference on the titanium surface roughness affects biological responses such as cellular attachment and spreading. The highest percentage of cell attachment was obtained on the surface which has been treated with 1.6×10^{17} ions/cm^2.

5. Conclusions

The findings indicate that the attachment and spreading of osteoblasts are influenced by the surface texture of the titanium implants. Cells spread and grow effectively on nano textured ion-implanted surfaces compared with polished (untreated) surface. However, more investigations are required to determine the optimal ion implanted surface for cell attachment and proliferation.

The cells attached on the ion-implanted surface indicate that these cells adhere in better conditions to the surface, increasing the possibility of greater bone integration. Ultimately, the difference in the cell behavior on the implanted titanium surface is due to the changes originated by the ion implantation treatment both in the physical, chemical surface properties and topography, which is modified at nano-scale providing better anchorage points to the cells.

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