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Biological responses of ultrafine grained pure titanium and their sand blasted surfaces

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ABSTRACT

The use of materials as implants has become vital for determining optimal product design to enhance the needs of usage and longevity in body. Ultrafine grained pure titanium offers advanced mechanical properties for medical applications for most adequate materials *meso*/micro scaled dental implants. Besides advanced mechanical properties, increased surface properties also offers enhance biocompatibility. In this experimental study, the effects of bulk structure on surface modification by sand blasting for coarse-grained and ultrafine-grained (UFG) commercially pure titanium reported. To determine the effects of bulk structure on the polished and modified surfaces the specimen groups are investigated using Optic Microscope (OM), Electron Back Scattering Diffraction (EBSD) and Confocal Laser-Scanning Microscope (CLSM). Surface roughness is determined with stylus profilometer (SP) and CLSM. Understanding the biocompatibility of titanium surfaces to cell-cell interactions and cell proliferation capacity of attached-cells were determined by cell viability assays and fluorescence microscopy techniques. According to our results, the titanium surfaces were highly available to cell attachment and cell proliferation. The ratios of cell proliferation of cells which are attached on different titanium surfaces were dependent on the grain size and the surface roughness. UFG and blasted surfaces are more suitable for cell proliferation of human gingival fibroblast cells.

1. Introduction

Severe plastic deformation methods have been widely investigated due to fine grain materials superior properties. Different kinds of technique are being used in order to decrease the grain size down to the low sub-micron range such as Equal Channel Angular Pressing, Accumulative Roll-Bonding, Hydrostatic Extrusion, High Pressure Torsion [1–5]. Despite of Al and V toxic effect to the body, titanium alloys with these element are being used due to their positive affect as alloying element on biocompatibility and mechanical properties [6,7]. Especially for biomedical materials SPD methods enables use of non-toxic materials such as pure titanium with increased mechanical strength. Previous studies highlighted that ultrafine grained materials positively increases the cell activation during the osseointegration period [8–14]. Besides grain size different surface modifications are widely being used to change surface topographies in order to increase hydrophilicity of surface, cell attachment and proliferation [15–22]. Implant surface geometries and chemistry interacts directly with the tissues and the cell proliferations starts instantly. As highlighted in [15] surfaces roughness is crucial for cytokine production that accelerates osseointegration. Moreover, dental implants early osseointegration period dominates implant success such as mechanical stability based on enough and homogenous cell attachment. Implant material and surface characteristics are known to be the most important parameters that effects implant success in the early stage of implantation [16,17]. Due to the different surface modification methods 2D surface roughness (R_a) are changeable between 1 and 3 μm [6,18–21]. Sand blasting procedure is one of the most popular method that being used to make modification of surface. In order to understand effect of grain size and surface modification on cell attachment, proliferation and osseointegration there are some surface studies they worked with polished [23], acid etched [24], SLA treated [25] and coating technique [26] on ultrafine grained materials. C. Nune and at. all investigated polished nano and coarse grained stainless steel surfaces in order to understand grain size effect on protein absorption that refers osteoblasts functions. In their study nano grained specimens showed better cellular activity [23].

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Valiev et al. investigated nano grained acid etched pure titanium osseointegration activity with fibroblast mice cells L929. Researchers used hydrofluoric acid for surface etching technique and the nano grained surfaces result with higher fibroblast colonization and cell-adhesion [24]. A.E. Medvedev et al. investigated polished and sand blasted acid etched surfaces (SLA) with mesenchymal stems and osteoblast cells activity. In their study, before surface modification procedure the surfaces prepared identically and afterwards surfaces were modified identically. Due to the effect of grain size on mechanical properties the roughness changed between 1.5 and 2.5 μm. SLA-UFG specimen group showed better cell viability and matrix mineralization [25]. In an another study [26] researchers studied Ca-P coating by means of Micro-Arc-3 technique in order to manufacture calcium phosphate coating on ultrafine grained pure titanium. The researchers made in vivo test and observed that ultrafine grain titanium provided homogeneous structure and high mechanical characteristics and CaP coating provided increased osteogenic properties [26]. As mentioned in the literature given above increased osseointegration performance based on ultrafine grain dimension are attributed to different grounds. When the underlying grounds investigated it is assumed that smaller grooves show better cell proliferation for similar surface roughness values. For example decreasing grain size also decreases grooves on the surfaces. Another important ground is the increased surface energy due to increased grain boundary length of ultrafine grained materials. Total lengths of grain boundaries in unit area increases with SPD methods. Also these grain boundaries typically are unsteady with high energy that tries to become stable by interacting with liquids, proteins and etc. in body environment [27]. An another ground found for increased better cell proliferation found to be more homogenous surface morphology and stable oxide layer of ultrafine grained materials than coarse grained materials [24].

In this experimental study pure titanium specimens were thermo-mechanically deformed and afterwards surface modification applied by means of TiO_2 sand blasting. Surface roughness differences among the specimen groups may mask ultra-fine grained structure effect on osseointegration. Thus, differently from previous studies surface roughness values kept between 1.8 and 2 μm in order to understand grain size effect on cell responses.

2. Materials method

Titanium samples were prepared from 99.5% pure titanium Grade 4. Mechanical properties are given in Table 1. In order to manufacture ultrafine grained material, equal angular channel pressing (ECAP) method used. Cylindrical samples with the dimension φ 5 mm × 80 mm were prepared from a titanium bar. Before extrusion, all materials were annealed in argon atmosphere for 2 h at 700 °C and air cooled to remove any residual stress that might remain. An ECAP die with an inner channel angle of $\varphi = 110^\circ$ and outer corner angle of $\Psi = 0^\circ$ was used. The specimen extruded at 450 °C for 8 passes. During pressing a high temperature lubricant material used with molybdenum disulfide ($MoSi_2$) content.

Optical techniques were used for coarse grained materials grain size measurements. Coarse grains were photographed with optical microscope and image processing programs were used to obtain average grain size. An average initial grain size was calculated as 58 μm optical microscope images and this value successful decreased to ultrafine grain size. For ultrafine grained specimen, Electron Back Scatter Diffraction (EBSD) used in order to measure average grain size (Fig. 1). Tescan Lyra scanning electron microscope equipped with NordlysNano EBSD detector operating at an accelerating voltage of 20 kV with specimen tilted at 70° was used for investigations. EBSD specimens were mechanically ground using SiC papers up to 4000 grit and polished using a solution of colloidal silica with 0.06 μm particle size (50 ml), 30% hydrogen peroxide (10 ml) and Kroll's reagent (5 ml, Kroll: 92 ml H_2O, 6 ml HNO_3, 2 ml HF). It was followed by 4–6 h of vibratory polishing with a colloidal-silica suspension to remove slight surface roughness. The specimens were then ultrasonically cleaned to remove the residual colloidal silica. The high-angle grain boundaries (HAGBs) were characterized as boundaries with misorientation angle $\theta > 15^\circ$ and low-angle grain boundaries (LAGBs) as boundaries with $\theta < 15^\circ$.

Bimodal grain sized microstructure was observed with EBSD and the grain size was calculated as 550 nm grain size. The maximum tensile strength for initial state was measured as 553.22 MPa and after ECAP procedure this value measured as 924.72 MPa. Hardness value for initial state was 179.41 $Hv_{0.2}$ and after severe plastic deformation this value increased to 309.35 $Hv_{0.2}$ hardness of Vickers (Table 1).

Table 1
Hardness and maximum tensile strength before and after severe plastic deformation.

Specimen groups	Hardness $Hv_{0.2}$	Strength (maximum) MPa
Ti initial	179.41 ± 0.25	553.22 ± 6.17
Ti ultrafine grain	309.35 ± 1.48	924.72 ± 8.81

2.1. Surface modification and characterization

Surface wettability of the all sample groups was determined with Attension Theta Lite. All sample surfaces were ultrasonically cleaned, autoclaved and sample groups were stabilized under measuring chamber for 15 min before and after measurements. Three repetitive measurements applied from three different specimen surfaces. Sessile drop experimental technique was used with Young-Laplace analysis mode. Approximately 4.2 μl water drop on the surfaces and image recorded for 10 s. Afterwards, the average value concluded.

Four specimen groups were prepared for tests. First group was coarse grained and its surface was polished. The second specimen group was coarse grained blasted. The chemical composition (Ti grade 4) and blasting procedure was same with commercially available implant. The third group was ultrafine grained with a polished surface. In this second group specimen were ultra-fine grain and size measured as ~520 nm with EBSD. Polished surface used to understand the bulk structure effect on osseointegration. Fourth group was ultra-fine grained and blasted specimens. Surface modification applied under same conditions with different durations to all specimen groups. Blasting torch was positioned 20 mm distance between them. During the blasting procedure 6 Bar blast pressure was applied for 30–70 s. For coarse grained specimen 30 s was enough to obtain 1.75 μm. In order to get similar surface roughness the modification procedure is applied 5 s intervals and roughness measured after each 5 s with a contact stylus profilometer. As blasting material titanium oxide (TiO_2) was used; particles of TiO_2 was approximately 100–250 μm. After polishing and surface modifications, all specimen groups were ultrasonically cleaned, with 96% ethanol, sonication, and followed by washing with H_2O, then sterilized by autoclave and stored in isolated containers before cell culture tests. To determine the biological response of titanium surfaces, all materials were sterilized identically.

After and during the sand blasting procedure mean roughness values were determined with a mechanical profilometer (Mitutoyo SJ-301) in three different points of the specimen surface. Topography of the modified samples was analyzed by scanning electron microscopy (SEM) in a microscope (Zeiss EVO LS 10) with an acceleration voltage of 20 kV.

2.2. Cell lines and reagents

The human gingival fibroblast (HGF) cell lines were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and