Bioactivity of Chemically Modified Porous Titanium

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Abstract. The aim of the present investigation is to enhance in-vitro bioactivity and protein adsorption of porous titanium with 3D interconnected pores by various chemical treatments (alkali, dual acid, citric acid and fluoride treatments). The untreated and treated samples were characterized using X-ray powder diffraction (XRD), optical microscopy and scanning electron microscopy (SEM). The protein adsorption study was carried out with Bradford's reagent using Bovine Serum Albumin (BSA). The optical microscopy reveals that untreated Ti sample exhibited 41.36% surface porosity. The in-vitro bioactivity of the treated and untreated Ti sample was evaluated by immersing them in simulated body fluid (SBF) for different time intervals. The immersed samples were characterized using XRD and SEM to confirm the growth and morphology of apatite. It was observed that apatite deposition of fluoride treated sample was denser than other treated samples for the same period immersed in SBF. All the surface treated samples showed good protein adsorption. The alkali treated sample showed maximum protein adsorption amongst other chemically treated samples which may be due to enhanced micro-roughness and strong electrostatic affinity between the protein and the surface. The enhanced in vitro bioactivity in the surface treated porous titanium indicates that the healing time of the bone and implant in patients can be reduced with good osseointegration.

Introduction

Pure titanium (Ti) and its alloys are biologically inert materials capable of self-passivation are the best implant option for load bearing sites due to high strength to weight ratio, low density, low modulus and excellent biocompatibility. Implants exhibit direct contact with bone on implantation and smooth surface titanium implants show weaker bonding and stress shielding at bone due to higher stiffness. The low modulus of elasticity is helpful in reducing implant loosening, bone resorption and porous structure facilitates bone tissue interaction, desirable fixation at the bone-implant interface. Tissue ingrowth in the porous structure of the implant enhances the implant bone bonding. Porous titanium is capable to function as endo fixator and 3D matrix for osteogenic tissue. Higher porosity induces bone formation even at non-osseous sites in the absence of any osteoinductive agent [1-3]. Surface modification of porous titanium plays an important role in further enhancing the formation of hydroxyapatite (HA), the mineral phase of bone, during integration with tissues. The alteration in surface topology of titanium by chemical and thermal treatment shows better in vitro apatite forming ability and in vivo osteoconductive ability. In addition to this, chemically and thermally treated porous titanium implants have also proved to have osteoinductive abilities even without any bone morphogenetic protein [4, 5]. Bioactive porous titanium represents a preferable alternative for orthopedic implants. The aim of the present investigation is to enhance in-vitro bioactivity and protein adsorption of porous titanium with 3D interconnected pores by chemical treatments.

Materials and Methods

Titanium sponge (Fe 0.024%, Cl 0.046%, N 0.006%, O 0.026%, Si 0.002, Ni 0.013%, C 0.005%, Mg 0.018%, balance Ti) obtained from MIDHANI, Hyderabad were subjected to chemical modifications as shown in Table 1 and coded accordingly. The surface porosity was studied using optical microscope (Metscope I, Chennai). The untreated and surface treated samples were characterized using scanning electron microscope, SEM (NOVA NANO SEM_450 field emission microscope) and X-ray powder diffraction, XRD (Rigaku Ultima IV diffractometer, Japan) techniques. For XRD, CuKα radiation was used and the scanning range was kept between 20°-80°. The protein adsorption study of the surface treated samples were carried out by soaking it in Bovine Serum Albumin (BSA) protein solution (1:1w/v in phosphate buffer saline, PBS) and incubated at 37° C for 24 h. The adsorbed protein was quantified using Bradford reagent in a UV-visible spectrophotometer at 595 nm wavelength. The in-vitro bioactivity of the untreated and treated Ti samples was evaluated by immersing them in simulated body fluid (SBF) for four weeks in a constant temperature water bath maintained at 37° C [6]. The SBF immersed samples were characterized using SEM to confirm the growth and morphology of the apatite.

Table 1: Different chemical modification employed on titanium sponge

Sample Code	Modification Condition	Treatment details
UnTis	Untreated	Nil
AlkTis	NaOH (Alkaline) treated	5 M NaOH, 60° C, 24 h
DaTis	Dual acid treated	HCl: H_2SO_4: H_2O (3:3:9) ml, 20 min
CaTis	Citric Acid treated	0.2 M citric acid, 5 h
FlTis	Fluoride Treated	0.1M CaF_2 and 0.1M H_2SO_4 at 120ºC for 1 hour

Results and Discussions

The XRD pattern of untreated and surface treated Ti-samples are shown in Fig.1. All the treated and untreated samples showed titanium peaks (JCPDS#44-1294). In the AlkTis sample (Fig.1b), sodium titanate (Na2Ti5O11) peaks were observed (JCPDS#11-0289). The DaTis sample (Fig.1c) showed high-intensity peaks due to relative etching of close-packed planes. The FlTis sample (Fig.1e) treated at 120°C shows highly metastable calcium sulfate hemihydrate (JCPDS#41-0224) precipitation on its surface as the governing equation for this reaction was Eq. (1).

$$CaF_2\,(s) + H_2SO_4\,(l) \rightarrow CaSO_4\,(s) + 2HF\,(g) \qquad (1)$$

The surface porosity of UnTis (Fig.2) was evaluated using ENVISION-5.0 software and was found to be around 41.36%. The SEM images in Fig.3 reveals different surface morphologies of untreated and differently treated the samples. UnTis sample (Fig.3a) shows smooth pore-wall with 3D interconnected pores. AlkTis sample (Fig.3b) has sodium titanate on the surface which was confirmed by XRD and EDX studies. This sodium titanate layer is bioactive and induces micro-roughness on the sample surface. DaTis sample (Fig.3c) was found to have rough surface orientations due to the removal of entire passive oxide layer causing micro-roughness increasing the surface area of the sample. Surface morphology of CaTis sample (Fig.3d) does not exhibit significant difference due to the inertness of citric acid in titanium although minor rough orientations are found. SEM image of FlTis (Fig.3e) reveals the needle-like structure that may be due to the reaction that resulted in calcium sulfate hemihydrate crystals.

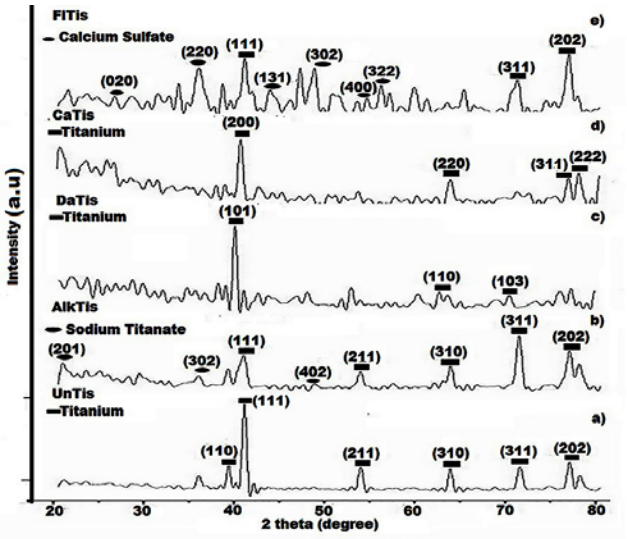

Fig: 1. XRD pattern of (a) UnTis (b) AlkTis (c) DaTis (d) CaTis (e) FlTis

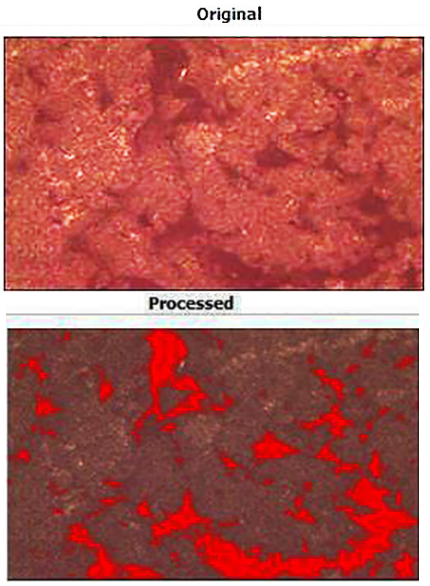

Fig: 2. Surface porosity analysis of UnTis

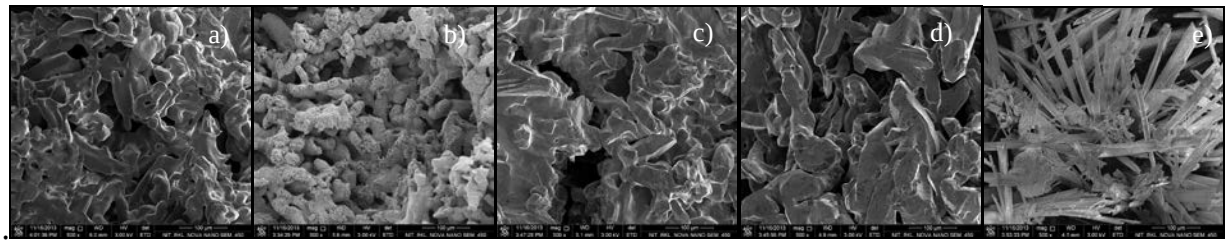

Fig: 3. SEM micrograph of (a) UnTis (b) AlkTis (c) DaTis (d) CaTis (e) FlTis

BSA adsorption is favorable for cell attachment and proliferation which is prominent from the protein adsorption on the surface treated Ti-samples. Table 2 gives the protein adsorption data for various surface treated porous samples. AlkTis sample shows maximum protein adsorption due to enhanced micro-roughness and strong electrostatic affinity between the protein and the surface [7].

Table 2: Protein adsorption in various treated samples

Sl. No.	Sample Name	Adsorbed Protein [µg/ml]
1.	UnTis	860.14
2.	AlkTis	933.37
3.	DaTis	821.53
4.	CaTis	710.11
5.	FlTis	808.22

The in vitro bioactivity of the untreated and treated titanium samples were evaluated using SEM. The morphology of apatite formation after four weeks of SBF immersion is shown in Fig.4. It was observed that The deposit of spherical particles on UnTis sample (Fig.4a) was seen as few apatite (HA) was deposited on the surface of UnTis sample (Fig. 4a). The AlkTis sample (Fig.4b) showed an increase in the HA patches due to an increase in the surface area. Na+ released forming sodium titanate act as nucleating sites for apatite. The DaTis sample (Fig.4c) shows surface bumps on the surface promoting roughness and surface area that turn to be the preferred sites for HA deposition. It was observed that CaTis sample (Fig.4d) showed less apatite formation on the surface and found to be scattered. Amongst all the surface treated

samples, the maximum apatite deposition was found on FlTis sample surface (Fig.4e). The maximum apatite is due to the calcium sulfate present on the sample surface which increases the surface energy thus facilitating apatite nucleation and growth. The Ca/P ratio of the apatite formed was found to be 1.65 from EDX, which is close to the stoichiometric of human bone.

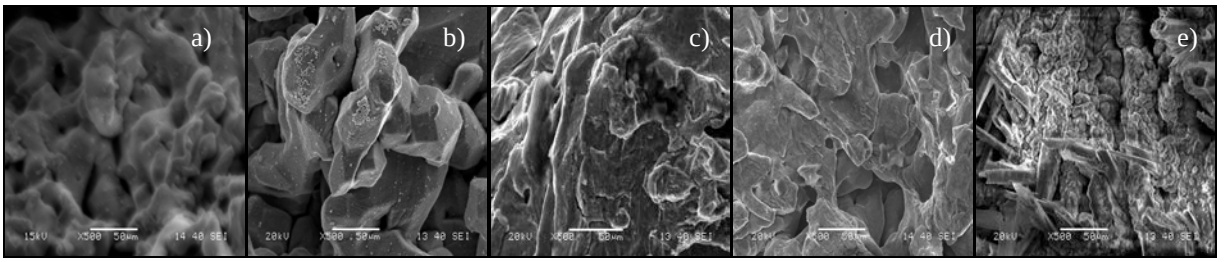

Fig: 4. SEM micrographs of in vitro bioactivity study in SBF for 4 weeks of (a) *UnTis* (b) *AlkTis* (c) *DaTis* (d) *CaTis* (e) *FlTis*

Conclusion

The in-vitro bioactivity and protein adsorption of porous titanium with 3D interconnected pores by various chemical treatments (alkali, dual acid, citric acid and fluoride) showed enhancement when compared to the untreated sample. The AlkTis sample showed maximum protein adsorption and homogenous apatite formation due to the combination of micro-roughness and strong electrostatic affinity. The enhanced in vitro bioactivity and protein adsorption on the surface treated porous titanium indicates that the healing time of the bone and implant in patients can be reduced with better osseointegration.

References

[1] S. Thelen, F. Barthelat, L.C. Brinson, Mechanics considerations for microporous titanium as an orthopedic implant material, Journal of Biomedical Materials Research Part A. **69**(4) (2004) 601-610.

[2] G. Ryan, A. Pandit, D.P. Apatsidis, Fabrication methods of porous metals for use in orthopaedic applications, Biomaterials. **27**(13) (2006) 2651-2670.

[3] S. Fujibayashi, M. Neo, H. M. Kim, T. Kokubo, T. Nakamura, Osteoinduction of porous bioactive titanium metal, Biomaterials. **25**(3) (2004) 443-450

[4] H. M. Kim, F. Miyaji, T. Kokubo, T. Nakamura, Preparation of bioactive Ti and its alloys via simple chemical surface treatment, Journal of biomedical materials research. **32**(3) (1996) 409-417.

[5] Stefano Tugulu, Konrad Lowe, Dieter Scharnweber, Falko Schlottig, Preparation of superhydrophilic microrough titanium implant surfaces by alkali treatment, J Mater Sci: Mater Med **21**(2010) 2751–2763.

[6] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity?, Biomaterials. **27**(15) (2006) 2907-2915.

[7] J. Ueta, N. Ohtsu, T. Kanno, K. Tada, J. Horiuchi, Protein adsorption properties on titanium with and without calcium titanate-coating, Materials Science and Engineering **18** (2011) 192023