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Inducing apatite pre-layer on titanium surface through hydrothermal processing for osseointegration

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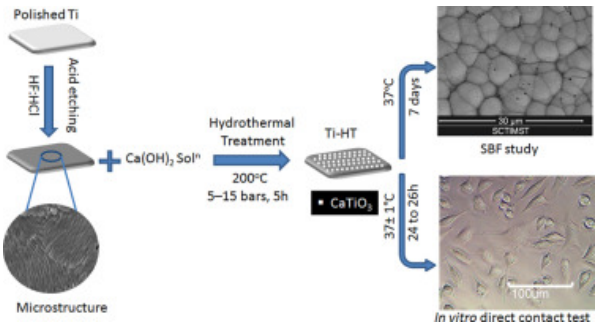
Highlights

- Surface engineered titanium implants for biomedical application is proposed.
- Nano coated surfaces of titanium were fabricated via hydrothermal treatment.
- Surface modified Titanium showed excellent bioactivity than conventional one.
- This technique can use to develop large number of samples economically in a short duration.

Abstract

Commercially available titanium (Ti) having high mechanical strength and a low area of cross-section can be adequately exploited for minimally invasive dental implantation. Current directions in clinical dental implant therapy focus on endosseous dental implant surfaces with nanoscale topographies using easy and economical processing approaches. The present study describes the generation of a novel nanolayer nucleating agent on the surface of Ti implant for early endosseous after implantation. The strategy is to modify the surface of Ti implant using $Ca(OH)_2$ via hydrothermal technique (Ti-HT). The X-ray photoelectron spectroscopy analysis confirmed the presence of chemically bonded Ca ions on the Ti surface in the form of $CaTiO_3$. In vitro studies are carried out to confirm the bone bonding ability of calcium enriched Ti surface. The apatite deposition on the surface after exposure to SBF for 7 days is confirmed via scanning electron microscopy, X-ray powder diffraction, Fourier-transform infrared spectroscopy and energy-dispersive X-ray spectroscopy techniques. The cell viability of Ti-HT was evaluated using direct contact method and MTT assay. The potential of Ca^{2+} ion on Ti surface via hydrothermal pre-treatment to enhance osseointegration of Ti has been proposed for achieving early stability for dental implants.

Graphical abstract



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Keywords

Titanium implant; Endosseous; Hydrothermal reaction; Calcium titanate; Bioactivity

1. Introduction

Nowadays, endosseous dental implants are widely used for single tooth replacement. Titanium is preferred for making the screw-abutment assembly for a standard endosseous implants system due to its matching mechanical performance compared to that of bone. The implant mechanical property as well as the anchorage into bone is crucial, as they are continuously subjected to heavy and repeated masticatory forces [1,2].

Generally, a screw part of the implant system is introduced in to the healed bone at the extracted site followed by a period of recovery and integration for the titanium implant to be firmly anchored into alveolar bone. This period should be short and is crucial for the success of the implant. The process of time dependent integration of the surface onto the surrounding bone is termed osseointegration, which is the deciding factor for acceptance of the implant [3].

There are several approaches to improve the osseointegration of endosseous implants both at micron and nanometer length scales. The micro level surface modification can enhance the contact between the bone and implant and thereby achieving improved biomechanical compatibility, which provides a conducive environment for accessible contact osteogenesis and signalling for the implant-tissue interactions. Such a microscale surface modification was reported by Park et al. using acid etching method to improve osseointegration Ti surfaces, which was widely accepted in the endosseous dental implant market [4]. Sandblasting with alumina particles is another approach to generate surface roughness in titanium implants to increase the implant-tissue contact area and interaction. However the success of such techniques is based on the processing parameters such as size, shape and kinetic energy of the particles used for blasting [5,6].

Plasma spray and laser coating using calcium phosphate are relatively novel and popular techniques for surface modification of Ti [7,8]. The plasma sprayed hydroxyapatite (HA) coated Ti implants are available in the market for dental and orthopaedic applications. However, such modification processes involve high temperature which causes dehydroxylation and decomposition of HA to form amorphous crystals reducing the mechanical integrity of the coating [9]. Non-uniform coating thickness, poor adhesion between coating and substrate, poor resistance to delamination and

micro cracks on the coated surface are other reported problems of these techniques limiting the efficient use of such techniques [10,11].

Recent advances in processing techniques and nanoengineering offer several approaches to carry out atomic level manipulations on surfaces with fine control on the composition and thickness of the deposited layer. The primary advantage of nanolayer modification is minimum tolerance of dental implants, which leads to a close fit between the coupling surfaces of the abutment and the implant to avoid both mechanical and biological complications. It can improves surface properties (biochemical bonding capability, hydrophilicity and roughness) to regulate cell behaviour on the implant surface such as adhesion, proliferation and differentiation of cells as well as the mineralization of the extracellular matrix [12]. There are several physical and chemical techniques reported for generation of nanolayer surface modification such as particle compaction, ion beam deposition, acid/alkali etching, peroxidation, sol gel, electrochemical, hydrothermal, crystalline deposition etc. [13]. Such techniques can impose surface roughness and bioactivity on various implant surfaces in nanometer length scales. Most of them are complicated, expensive, unstable and prone to damage under harsh in vivo conditions encountered by the implant [14,15]. Till date, there is no reported technique that can be considered as an ideal process for implant surface modification. However, it is reported extensively in the medical literature that moderate surface roughness has a positive effect in the healing and implant stability [16].

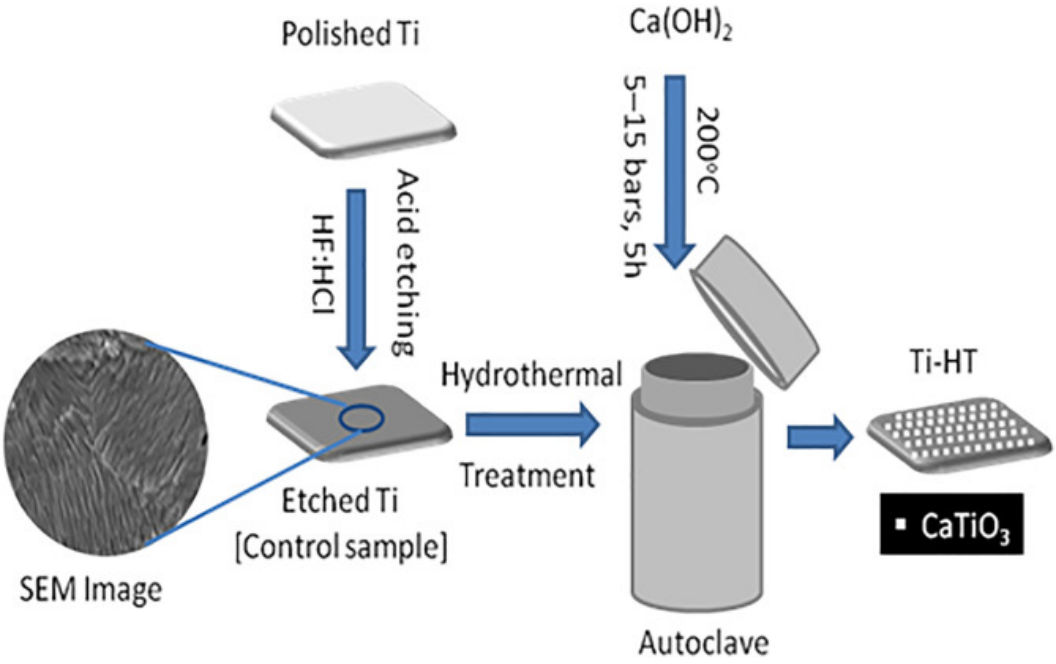
In the present work, a calcium rich layer has been deposited on titanium metal surface to improve osseointegration. Among the reported surface engineering methods, hydrothermal technique (HT) was considered as the most suitable one for this particular case [17]. In this technique, the implant surface is subjected to homogenous reaction atmosphere to promote uniform nanolayer formation over the surface [18]. Moreover, HT has the capability for the large scale production of surface modified implants economically. Earlier studies using this technique reported that highly crystalline $BaTiO_3$ could be synthesised on titanium surfaces in an aqueous $Ba(OH)_2$ solution at 160 °C under 800 kPa for 1 h [19]. Such reports motivated the authors to hypothesize that a calcium enriched surface can be built-up using aqueous solutions containing Ca^{2+} ions at high temperature and pressure. Furthermore, this approach could help achieve early osseointegration of Ti implants used in orthopaedic and dental applications.

2. Materials and methods

Commercially available titanium (Ti, Manhar Metal Supply Corporation, Mumbai, India), hydrochloric acid (HCl, SD Fine Chemicals, Mumbai, India), Hydrofluoric acid (Merck, Darmstadt, Germany), calcium hydroxide ($Ca(OH)_2$, Merck) were purchased and used as such without further purification. Silicon carbide papers of various grit sizes were used to polish the Ti surface.

2.1. Pre-treatment of Ti surface

Pre-treatment of implant surface is generally used to increase the surface area and to alter microtopography [20]. Surgical grade titanium (Ti) was used as substrate with the dimensions of 4 mm in thickness and 12 mm in diameter. The disc were polished Buehler-Ecomet 250 with silicon carbide papers of 100, 250, 600 grit sizes in succession emery paper to remove surface defect and finally with 0.5 μm alumina slurry. Further the polished discs were ultrasonically cleaned in soap solution and distilled water in succession to remove any loosely bound particles/impurities on the surface. The cleaned discs were etched by a mixture of HF and HCl [20:8 ml] in the ratio at 27 °C. The substrates were subsequently sonicated in distilled water and subjected to hydrothermal process (Scheme 1).



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Scheme 1. Schematic of the Ti-HT process.

2.2. Hydrothermal treatment of Ti surface

The hydrothermal reaction was executed in a Teflon lined authoclave (Amar Equipments, India) which has temperature and pressure ratings at 200 °C and 200 bars respectively. Ti discs were treated in $Ca(OH)_2$ aqueous solutions (Ti-HT) at 200 °C, pressure range of 5 bars and basic pH conditions in the autoclave for over 5 h (Scheme 1). The Ti disc substrate were taken out, rinsed with deionised water and dried slowly in vacuum desiccators and used for further characterization and studies.

2.3. In vitro bioactivity studies - evaluation of apatite deposition on Ti

The biomineralization behavior (apatite deposition) on the surface modified titanium was evaluated in Kokubo's simulated body fluid (SBF) solution [21]. The reagents used for preparing 1.5 SBF as per literature [21]. The surface-modified discs were immersed in Kokubo's solution at 37 ± 1 °C in a polypropylene vials for 7 days with the solutions being changed every 2 days to retain the concentrations of the solutions after chemical reactions between titanium surface and solutions. The solution volume was 15 ml per tube and one disc was immersed in an each tubes. After immersion for 1, 5 and 7 days, the discs were rinsed in distilled water and dried in a vacuum desiccator. The deposition of apatite crystals over Ti discs morphology; crystallinity and chemical bonding were analyzed.

2.4. Physicochemical characterization

Pre-treated surface morphology of Ti was analyzed using light microscope and scanning electron microscopy (SEM, FEI Quanta 200, Netherlands). The surface chemistry of Ti discs after the hydrothermal treatment was examined using XPS (Thermo Scientific, model – Multilab 2000). The measurement was performed using Al-Kα radiation (1486.6 eV). The binding energies were calibrated with respect to C1s at 284.8 eV (adventitious carbon) with a precision of ±0.1 eV. XPS results were analyzed on the basis of binding energies of Ti2p, O1s and Ca2p peaks to evaluate the chemical environment and oxidation states of the respective elements on the surface. The apatite deposition or biomimetic

growth via SBF immersion was evaluated by SEM. The composition of the precipitated crystals was analyzed with an energy dispersive spectroscopy (EDS) associated with the SEM.

The crystal phases of the deposited CaP were analyzed using X-ray diffraction (Bruker, D8 Advance, Karlsruhe, Germany). The different compounds on the surface was determined by analyzing the position and intensities of the diffraction peaks typically observed in the range of diffraction angle $2\theta = 20–60°$. FTIR analysis was performed to confirm the functional groups in the specimens by Thermo Nicolet 5700 spectrometer (USA). The sample for FTIR was collected by scratching the deposited CaP from the Ti-HT surface after SBF immersion. The spectra were collected at a resolution of 4 cm^{-1} and scanned in the range of 400–4000 cm^{-1}.

2.5. In vitro cytocompatibility: direct contact test and MTT assay

An in vitro cytotoxicity test of Ti and Ti-HT using direct contact followed by MTT assay method was performed using test samples Ti and Ti-HT as per ISO 10993-5. The culture medium from the L-929 monolayer was replaced with fresh medium. Test samples, negative control [Ultra high molecular weight poly ethylene] and positive control [Stabilized PVC disc] in triplicate were placed on the cells. After incubation at 37 ± 1 °C for 24 to 26 h, cell monolayer was examined microscopically for the response around the test samples. Further MTT assay was performed after the evaluation of direct contact to measure the metabolic activity of cells to reduce yellow colored tetrazolium salt 3-(4, 5-Dimethyl thiazol -2-yl)-2,5-diphenyltetrazolium bromide to purple colored formazan. After removing the test samples from the cell monolayer, 400 µl MTT (1 mg/ml in medium without supplements) solution was added to test sample and controls, and then the plates were wrapped with aluminium foil and were incubated at 37 ± 2 °C for 2 h. After discarding the MTT solution, 300 µl of isopropanol was added to all wells and mixed thoroughly. The colour developed was quantified by measuring absorbance at 570 nm using a spectrophotometer. The data obtained for the test samples was compared with cell control.

3. Result and discussion

Surface pre-treatment of substrate is an essential step in the functionalization. In the present study a combination HF-HCl etchant was used as roughening agent. HF is reported to be highly effective in dissolving the dense and stable surface of native TiO_2 layer and HCl act as a good cleaning agent for removing salt deposits [22,23]. The Ti surface pre-treated with HF/HCl. The SEM images of pre-treated surface are presented in Fig. 1. The micrographs revealed that polishing produce smooth and flat surface, though some abrasion scratches and small pits are present over Ti surfaces. The process effectively generates rough surface by exposing the grains with sharp boundaries and edges. Also, it increases surface area of the substrate by creating pits. High surface area and roughness provide nucleation centers for growing calcium titanium reactive sites, thereby improving both coverage and adhesion of coating due to chemical bonding between calcium ion and Ti surface [24].

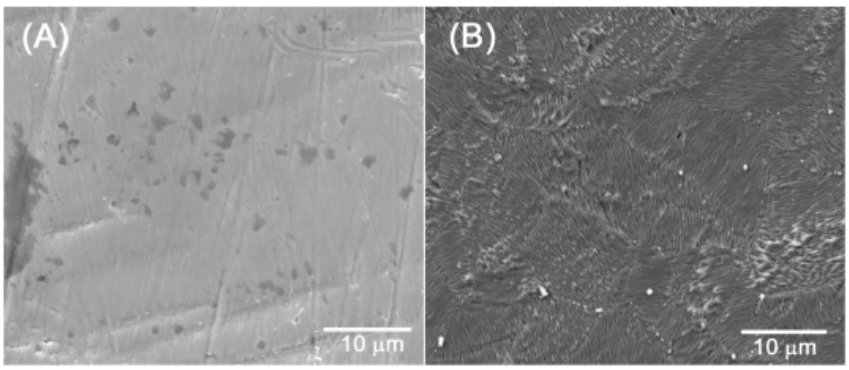

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Fig. 1. Secondary electron images of (A) polished surface and (B) HF/HCl pre-treated surface of Ti sample.

The pre-treated Ti samples were hydrothermally modified using $Ca(OH)_2$ solution. Different from solid state reactions, the solution phase reaction can be manipulated by the alteration of precursor concentration. Also this technique can be used for different shape of implant surface with uniform reaction condition. The hydrothermally treated Ti surface was evaluated using XPS technique. The survey spectrum on the surfaces of Ti-HT is represented in Fig. 2 gives the binding energy peak positions for Ca, Ti and O [[25], [26], [27]].

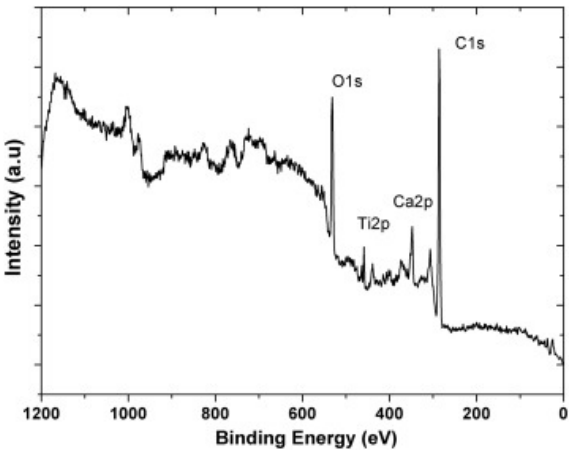

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Fig. 2. The binding energy survey spectrum of the Ti-HT surface.

The regions corresponding to O1s, Ca2p and Ti2p were scanned at a higher resolution, compared with standard value represented in Table 1 and are given in Fig. 3(A), (B) and (C) respectively. The data obtained by the high resolution scans were analyzed using Fityk, a peak fitting program [28]. Initially the data for O1s was examined, it was observed from the fitting that O existed in two different forms O^{2-} (529.5 eV) and OH^- (531.5 eV) on the surface of the sample. The intensity of the OH^- was higher compared to O^{2-} indicating that the content of oxygen in $Ca(OH)_2$ is higher on the surface of the sample, which is expected due to the hydrothermal treatment with $Ca(OH)_2$. Similar analysis on Ca2p data revealed that apart from $Ca(OH)_2$, Ca also exists in the form of CaO (346.7 eV) and $CaTiO_3$ (346.7) [29]. However, from the relative areas of the respective peaks, it can be seen that the amount of Ca in CaO is higher than Ca in $CaTiO_3$. Since the atom % of Ca in both the compounds is the same, it can be inferred that the amount of CaO is higher than $CaTiO_3$ on the sample surface. The presence of $CaTiO_3$ is further confirmed by the Ti2p peak data. The binding energy peak 458.87 eV [30] corresponding to $CaTiO_3$ was observed (see Fig. 3C) and other peaks of Ti were absent indicating the surface of the sample has Ti in the form of $CaTiO_3$ as opposed to TiO_2 or unreacted Ti.

Table 1. High resolution XPS measurement standard binding energies for Ca, Ti and O in different chemical environments.

Element	BE (standard value, eV)
Ca $2p_{3/2}$ (CaO)	347.2
$2p_{1/2}$ (CaO)	349.8
Ca 2p3/2 ($CaTiO_3$)	346.7
Ti $2p_{3/2}$ ($CaTiO_3$)	458.87

Element	BE (standard value, eV)
$2p_{1/2}$ ($CaTiO_3$)	464.70
O1s (O^{2-})	529.5
O1s (OH^-)	531.5

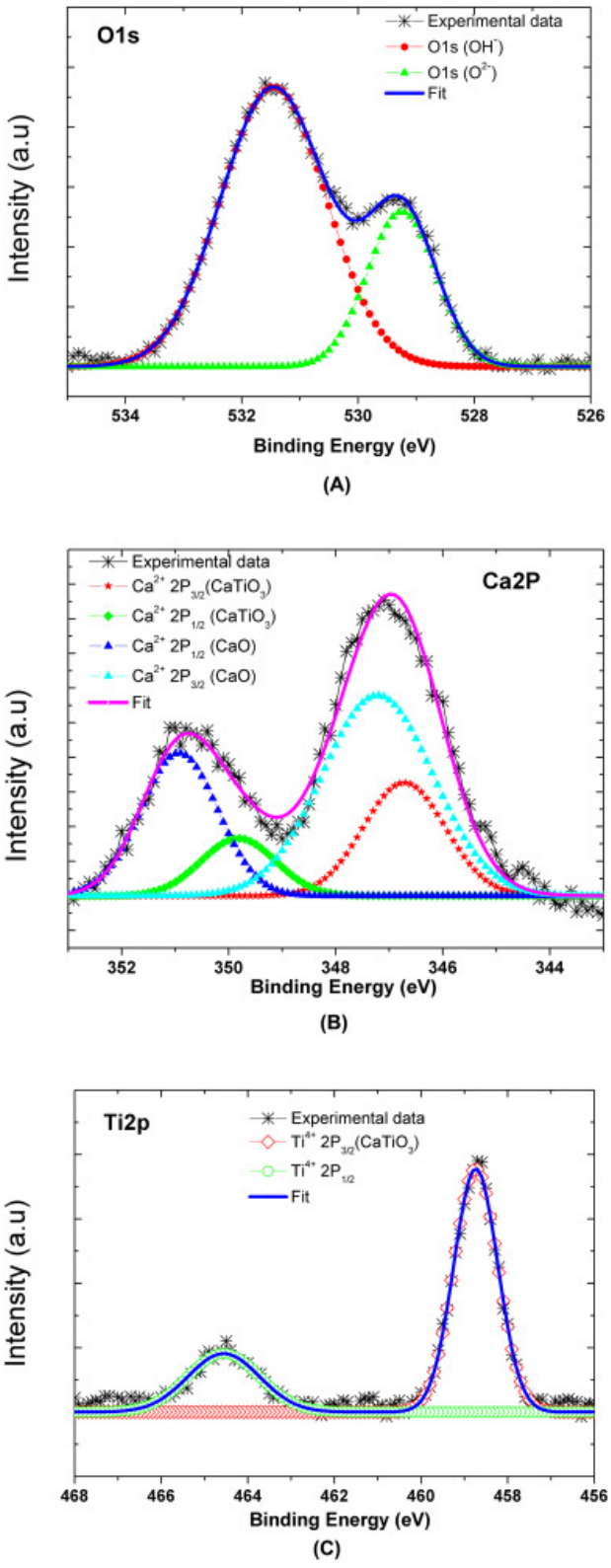

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Fig. 3. High resolution XPS spectra from Ti-HT surface of (A) O1s, (B) Ca2p and (C) Ti2p showing the deconvolution of the experimental peaks corresponding to contribution from different chemical states of each element.

During the hydrothermal reaction, both the dense oxide (TiO_2) surface layer and part of Ti in the bulk which is close to the outer oxide layer are attacked by OH^- ions, dissolved into the $Ca(OH)_2$ solution. This increases the concentration of Ti^{4+}, which is followed by re-crystallization in the vicinity of Ti surface due to the lower energy of Ti—O bond. The dissolution-re-crystallization process produces large number of negative charges on the Ti surfaces which leads to the migration of Ca^{2+} ions from the solution to Ti surface and precipitated [18]. Also the surface of Ti-HT analyzed via XRD technique (Fig. S1), which did not give any significant peak intensities of $CaTiO_3$ pattern due to the thin layer. The $CaTiO_3$ on Ti surfaces is expected to increase apatite deposition and achieve a higher bonding strength. The presence of $CaTiO_3$ has also been shown to improve the integration and acceptance of the bone implant [31,32]. The reaction scheme for the formation of $CaTiO_3$ is as follows-

$$\mathbf{Ca\,(OH)_2\ (s) \rightarrow Ca^{2+} + 2OH^-}$$

$$\mathbf{Ca^{2+} + Ti(OH) \rightarrow CaTiO_3\ (s) + 2H^+ + H_2O}$$

The in vitro apatite formation in SBF was assessed via SEM imaging and is showed in Fig. 4. After 1 day of SBF soaking, it was difficult to detect any CaP spheroids on Ti-HT, but several small grains with size less than 1 μm in diameter were observed. After 5 days of immersion, no uniform and continuous CaP coating had formed, but spherical CaP crystals were uniformly distributed on Ti-HT surface. The surface coverage of CaP increased in subsequent days. After seven days of soaking the apatite layer coating over Ti-HT surface was thick and with larger granules. The higher magnification of SEM image after 7 days of SBF socking was represented in Fig. 5(A) confirmed the close contact of granules and typical apatite morphology. The entire surface was covered by dense and compact coating of CaP. The elemental composition of corresponding surface assessed via EDS spectra (Fig. 5(B)) showed the presence of peaks of only Ca and P, while no trace of from Ti substrate was detected as the higher thickness of CaP coating and that of Ca/P ratio is approximately 1.67. The phase purity and chemical bonding of CaP precipitated surface was assessed using XRD and FTIR techniques and is represented in Fig. 6.

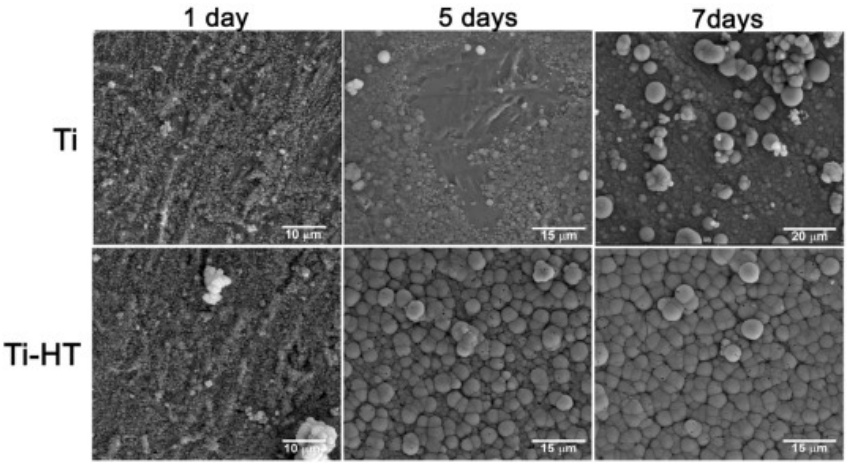

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Fig. 4. SEM images of Ti and Ti-HT surfaces after SBF studies of 1 day, 5 days and 7 days.

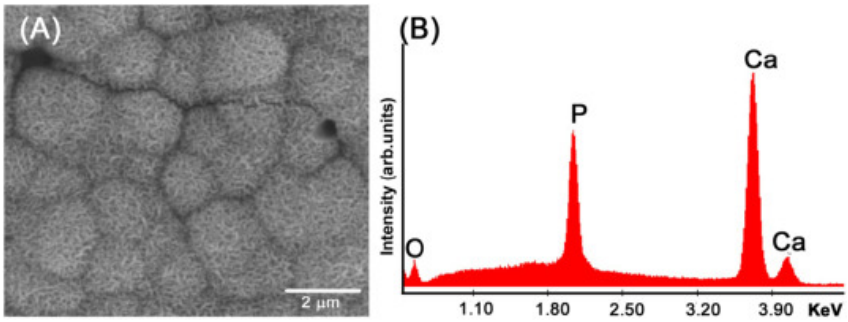

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Fig. 5. (A) Higher magnification of SEM images of Ti-HT surfaces after 7 days of SBF socking and (B) corresponding surface EDS spectrum.

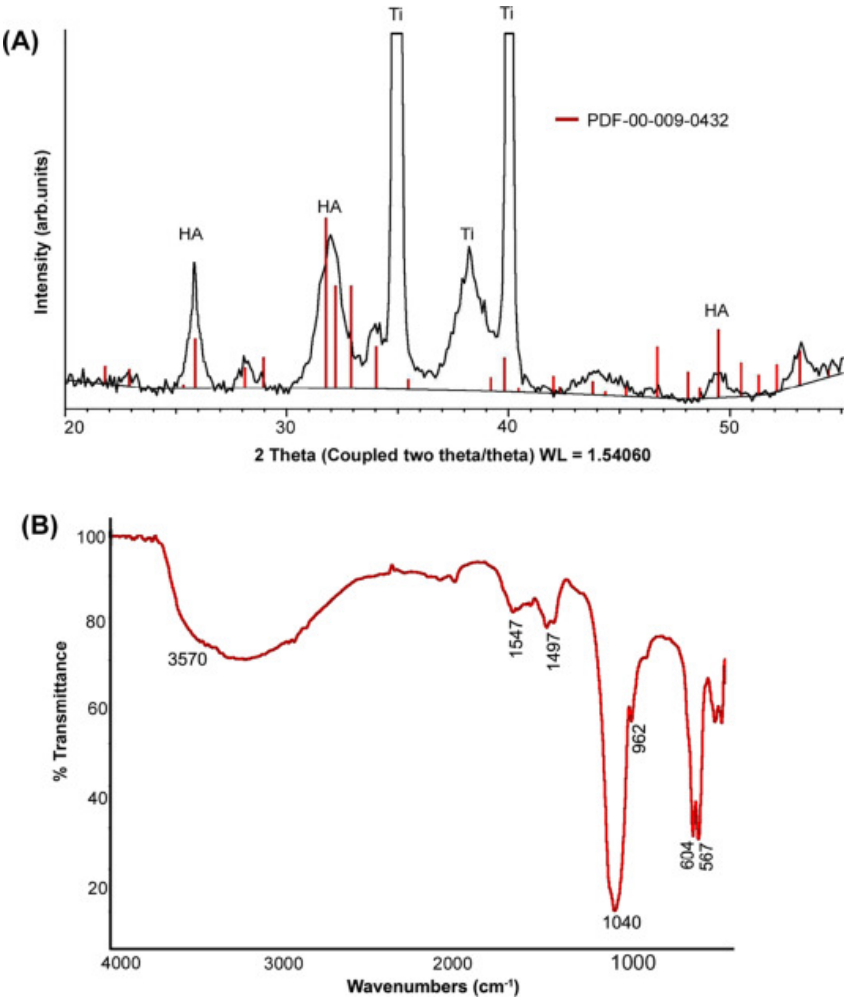

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Fig. 6. The 7 days of SBF soaked Ti-HT surfaces XRD pattern (A) and FTIR spectra (B).

The XRD patterns (Fig. 6(A)) revealed the formation of apatite on Ti-HT soaked in SBF for 7 days. The peak at 2θ of 32–33° corresponded to the overlapping of the (211), (112), (300) and (202) diffraction peaks and the peak at 2θ of ~25.5° was attributed to the (002) diffraction plane. Both peaks matched with those of PDF pattern (00-009-0432) of HA. FTIR

spectrum of sample in Fig. 6(B) demonstrated the characteristic peaks of apatite formation and the vibrational bands of PO_4^{3-} group observed at 1061, 597 and 563 cm^{-1}. Also the bands assigned to O—H stretching and bending were detected at 3443 and 1641 cm^{-1}, respectively. Finally, three strong CO_2^{3-} group bands, at 873, 1423 and1471 cm^{-1}, were detected. Based on the above results, it can be resolved that the CaP coating on Ti-HT is characteristic carbonated apatite [33].

The direct contact test and MTT assay was carried out with Ti-HT samples and is depicted in Fig. 7. The data demonstrated that cells were well attached and spread in the presence of Ti-HT and there were no significant difference between the cells morphology, proliferation and metabolic activity with respect to the controls.

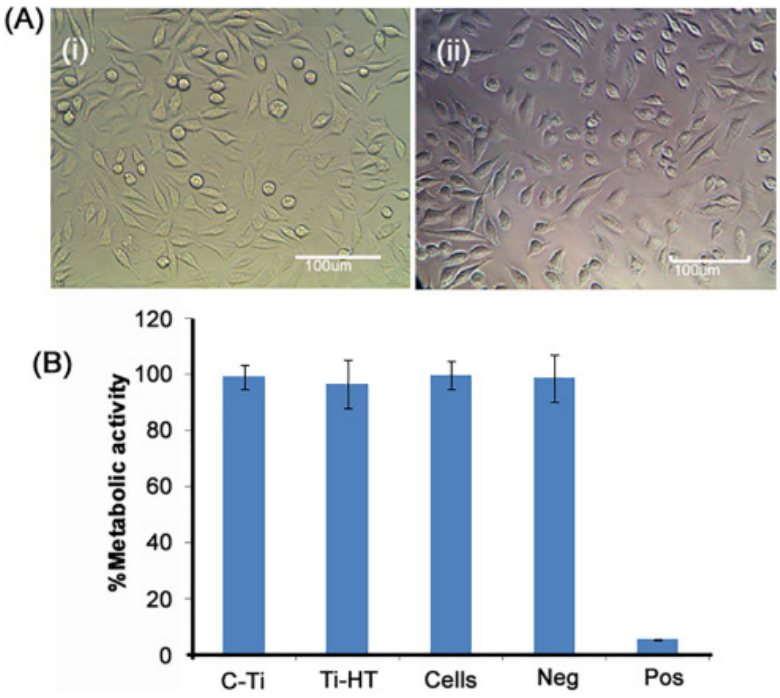

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Fig. 7. Cytocompatibility evaluation of titanium samples represents in 'A' direct contact method [(i) Ti and (ii) Ti-HT] and 'B' MTT assay using L929 cells.

These results indicated that the surface modification via hydrothermal treatment created calcium rich nanolayer formation on Ti-HT discs facilitated apatite formation in SBF. Supplementary material in Figs. S2 & S3 indicates material to uncompromisingly support L929 as well as osseous tissue derived HOS cells across various time points maintaining proliferation and morphology (Figs. S4 & S5). Also the biological studies such as direct contact test and MTT assay revealed the cell friendly nature of material surfaces.

4. Conclusion

The present study demonstrates the enhanced in vitro bioactivity of the hydrothermally modified surface of Ti implant. XPS of Ti-HT surface strongly indicates the presence of surface bound calcium in the form of calcium titanate. It was observed that the Ti-HT surface promote apatite precipitation in in vitro bioactivity test, which in turn a confirmation of the presence of calcium layer. These conclusions are supported by SEM, XRD and FTIR analysis. Ti-HT surface showed high levels of cell compatibility. The hydrothermal surface modification of implantable Ti to enhance the bioactivity

could be easily and viably done. This will be more advisable than thick plasma spray coating of hydroxyapatite on dental implant surface to achieve osseointegration.

Declaration of Competing Interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

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Supplementary material

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...Thus, the critical analysis of the studies included in this systematic review allows us to infer that codependent and simultaneous biomineralization and osteogenesis events are indeed influenced by the electrical charge, but not dependent on a positive biomaterial for the attraction of osteoblastic cells, due to the driving force of biomineralization induced by Ca+2 to induce osteoblastic attraction and differentiation. Since in the studies by Hu et al., 2012 and Tovani et al., 2019, the more negative surface of the biomaterial from surface treatment applied immobilization of VEGF on Ti via either covalent binding of heparin-VEGF (Hu et al., 2012) or immobilization of Col and CaCO3 by LbL3 (Tovani et al., 2019), allowed greater mineralization and osteoblastic viability, respectively, a fact that can be explained by the electrical attraction between the negative surface and the Ca+2 ion, providing the formation of an electrostatic bridge between the Ca+2 ions deposited on the surface with the negatively charged bone cells (Sul, 2007; Bodhak et al., 2010; Hu et al., 2012; Saffarian Tousi et al., 2013; Anitua et al., 2017; Ansar et al., 2019; Dai et al., 2019; Tovani et al., 2019; Canepa et al., 2020; Lin et al., 2020; Krenek et al., 2021). This corroborates with the study of Sunarso et al., 2016, who reported that the presence of Ca+2 induces osteoblastic differentiation....

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