

Bioresorbable whitlockite coatings on titanium by EPD for biomedical applications

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We have developed biodegradable tri calcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$, β -TCP (whitlockite)] coatings on titanium by surface modification to improve the corrosion resistance and biocompatibility. It is a bioresorbable biomaterial and is absorbed for the formation of new bone tissues. Nano whitlockite powder was synthesized by wet chemical precipitation method and coated on titanium surfaces by electrophoretic deposition (EPD) from a 1.5% suspension in ethanol. The coatings were carried out at various potentials ranging from 10-50 V and were followed by sintering in vacuum at 800 °C for 1 hr. The crystallinity, vibrational states, surface morphology and composition of the coatings were characterized by XRD, FTIR, and FE-SEM with EDAX respectively. The corrosion resistance of the coating was further evaluated electrochemically to study the stability of the coatings using open circuit potential-time measurements, potentiodynamic polarization studies and impedance behavior in Ringer's solution. The electrochemical corrosion parameters evaluated indicate a shift towards noble direction for the whitlockite coated samples in comparison with uncoated titanium metal. Electrochemical impedance spectroscopic investigations revealed the stable nature of the coatings formed. In vitro cytotoxicity studies of the coated material were carried out by MTT assay. The stable nature of the cells confirms the biocompatibility of whitlockite coatings on the titanium substrate.

Key words: Whitlockite, Electrophoretic deposition, Titanium, Electrochemical studies, Biocompatibility.

Introduction

The improved corrosion resistance, mechanical strength coupled with biocompatibility and light weight of titanium and its alloys make it a preferred candidate as an implant material in orthopedic and dentistry. These properties are far more superior to stainless steel and Co-Cr alloys [1-3]. The service life of metallic orthopedic implant is affected by corrosion and wear resistance, tissue reactions, surface conditions, and osseointegration. This emphasizes the need for surface modification of these materials and also to enhance their bioactivity and osseointegration capabilities [4]. Bioceramic coatings are the most viable options to improve the performance of implants. Bioactive properties of calcium phosphate minerals are exhibited by compounds containing Ca/P ratio ranging from 1.5 to 2. β -tricalcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$ which is commonly known as β -TCP, (whitlockite)] has Ca/P ratio of 1.5 [5]. β -TCP is a bioresorbable bioceramics, which will get resorbed and helps in the formation of new bone tissue, thereby replacing the implanted β -TCP [6]. It exists in two allotropic forms, α and β tricalcium phosphate. Generally β -TCP is formed at low temperature around 700 °C and it is converted into α -TCP at 1125 °C [7, 8]. Beyond this temperature, α -TCP phase is stable up to 1430 °C. Usually, the crystal

forms of β -TCP are called as Whitlockite [9]. A resorbable β -TCP is the best implant, which starts to degrade and helps in regeneration of bone tissues that leads to better interfacial stability [10]. The rate of solubility of β -TCP is less and it is stable at room temperature when compared to α -TCP. But it shows higher solubility than hydroxyapatite (HAP) which is a bioactive ceramic. This leads to fast degradation inside the body and there will be a moderate resorption rate for the formation of new bone tissues [11]. Hence β -TCP is considered as the most fascinating material in biomedical applications. The low mechanical properties prevent us from using it directly as an implant material. Many techniques such as electrodeposition [12], metal-organic chemical vapor deposition [13], Plasma spraying [14], Micro-arc oxidation [15], electrochemical [16] and electrophoretic deposition (EPD) [17] are available for the development of bioceramic coatings on titanium. Among these techniques EPD method was chosen to achieve a fine accumulation of the particles on the substrate from alcoholic suspension. EPD includes several advantages such as simple equipment, rate of deposition can be controlled by varying applied voltage, low cost and coating process can be completed in a few minutes [18].

Over the past few years several studies were reported on calcium phosphate coatings on titanium and its alloys. Both HAP and TCP were the most biocompatible materials and plays a vital role in biomedical applications. Kivrak et al. [19] synthesized both HAP/ TCP powder and studied their sintering behavior. Matthew Stewart

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et al. [14] studied the effect of HAP/TCP coatings on titanium alloys. Albayraktet et al. [17] has produced the TCP coating surface and formed TiO_2 layer on titanium. Hafezi et al. [9] have prepared the whitlockite/merwinite nanocomposite and studied its characterization. Even the sintering effect on whitlockite compound was reported by Mehdikhani et al. [10]. Recently Fathi et al. [7] has prepared high purity β and α -tricalcium phosphate by wet chemical method. The above reports are related with synthesis, characterization and production of TCP on titanium. But, synthesis and deposition of whitlockite as a pure phase on titanium by EPD and its electrochemical evaluation is rarely found. Since whitlockite is a bioresorbable ceramic, a few layers would uniformly dissolved after a few months and this would be deposited on the coating thus enabling bone growth. Also the whitlockite gets resorbed easily when compared to other bioactive and bioinert bioceramics on the metal surface. This process prevents the bare metal from undergoing degradation after a few years. Hence to take advantage of the dual properties of the ceramic and base metal we have developed the coatings using EPD.

The present work describes the synthesis of n- β -TCP (whitlockite) by wet chemical precipitation method and to deposit it on titanium metal by EPD. The coated samples were sintered at 800 °C for 1 h in a vacuum furnace (10^{-5} Torr). These coated samples were optimized by means of electrochemical studies in Ringer's solution.

Materials and Methods

Synthesis of n- β -TCP powder

Synthesis of n- β -TCP was carried out by wet chemical precipitation method [6]. β -TCP nano powders were prepared by the reaction of calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, Alfa Aesar) and ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$, Alfa Aesar), where calcium and phosphorous were taken as a precursor. About 50 ml of 0.4 M $\text{NH}_4\text{H}_2\text{PO}_4$ solution is taken and its pH is initially maintained at 4. The phosphate solution was vigorously stirred at room temperature. Then 50 ml of 0.6 M $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ with pH = 7.2 was slowly added to the phosphate solution to get a white precipitate. During the mixing of calcium solution into phosphate solution, the pH should be maintained at 8 by adding 0.1 M Sodium hydroxide (Alfa Aesar). The obtained precipitate was centrifuged, washed with absolute ethanol and finally washed with double distilled water. Then the filtrate was dried in an oven at 80 °C for 12 hrs. The dried powder was crushed by using agate mortar and pestle and this crushed powder was sintered at 650 °C for 2 hrs in air atmosphere.

Development of metallic substrate

The dimension of titanium electrode used was of $10 \times 10 \times 3$ mm in size. The samples were mechanically polished by using silicon carbide grit papers from

120-1000. The polished sample was used as cathode in the EPD process. The 314SS sheet of dimensions $11 \times 8 \times 0.5$ mm in size was used as anode. Then the polished samples were washed thoroughly with soap solution, followed by running distilled water, rinsed and ultrasonically degreased with acetone and dried at room temperature.

Suspension preparation

About 1.5 g of n- β -TCP powder was dispersed in 100 ml of ethanol to obtain 1.5% suspension. After dispersing, the suspension was let undisturbed for 24 hrs to get uniform dispersion of the particles. The suspension was further ultrasonicated for 15 min followed by stirring for 30 min.

Electrophoretic deposition

EPD of whitlockite on titanium metal was carried out at room temperature. A 100 ml beaker was taken with 1.5% suspension of nano powder and it was closed with teflon covering. The two electrodes were dipped in the suspension and the distance to be maintained between two specimens was 10 mm. Deposition was carried out on 1cm^2 surface area and the other edges and sides of titanium were masked with non-conducting Teflon tape. EPD was achieved with varying applied potentials. Samples were coated at potential of 10-50 V and at a constant time of 3 minutes. The coated samples were sintered at 800 °C in Vacuum for 1 h.

Characterization of whitlockite coatings

The crystalline phase of the coatings were investigated by X-ray diffractometer (Bruker model D8) using Cu K_α radiation λ -1.5406 Å. The nano size of whitlockite was calculated from XRD broadening line measurement from the Scherrer equation [20].

$$D = \frac{0.94\lambda}{\beta_{1/2} \cos \theta} \quad (1)$$

Where λ is the wavelength of used Cu K_α radiation, $\beta_{1/2}$ is the full width at the half maximum of the whitlockite line and θ is the diffraction angle. FTIR was recorded in the ATR mode using UATR Perkin Elmer spectrum Two. The morphology of the coatings was taken with FE-SEM (Model CARL ZEIS SUPRA 55) and the compositional analysis was carried out with the EDAX system attached to FE-SEM.

Potentiodynamic polarisation and electrochemical impedance studies

The corrosion studies of both uncoated and n- β -TCP coated on titanium were carried out using Ringer's solution (NaCl -8.60, CaCl_2 -0.33, KCl -0.30 and NaHCO_3 -0.2 g/l respectively). The functioning of the electrochemical measurements were done by a conventional three electrode system at 37 ± 1 °C. Saturated calomel electrode (SCE)

was used as reference electrode and graphite was used as auxiliary electrode respectively. The electrochemical measurements were carried out on both uncoated and TCP coated on titanium are open circuit potential (OCP)-time measurements, potentiodynamic polarization studies and electrochemical impedance studies. These studies were carried out using Biologic SP-240. A flat corrosion cell was used to measure the corrosion behavior of uncoated and TCP coated sample. The exposure of the sample in the solution was 1 cm². OCP-time measurement with respect to SCE was carried out in equilibrium condition for one hour. Followed by electrochemical impedance measurement at equilibrium condition with frequency range from 10 kHz to mHz. Potentiodynamic anodic polarisation was initiated after the samples attained a constant potential. The potential was increased from 0.500 V below the E_{corr} and reaches the passivation region at a scan rate of 10 mV/min. The corrosion potential (E_{corr}) and current density (I_{corr}) were measured from polarisation curve by using Tafel fit.

Cell line and culture

Osteoblast cell line was procured from National Centre for Cell Sciences, Pune. The cells were maintained in minimal essential medium (MEM) supplemented with 10% FBS, penicillin (100 µl/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO₂ at 37 °C.

Reagents

MEM was purchased from Hi Media Lab. Fetal bovine serum (FBS) was purchased from Cistron Lab., Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from Sisco Research Lab. Chemicals Mumbai. All the other chemicals and reagents were obtained from Sigma Aldrich.

In Vitro assay for Cytotoxicity activity (MTT assay) (Mosmann, 1983)[21]

Cells (1×10^5 /well) were plated in 24-well plates and incubated in 37 °C with 5% CO₂ condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100 µl/well (5 mg/ml) of 0.5% MTT was added and incubated for 4 hrs. Yellow MTT is reduced to purple formazan in the mitochondria of living cells. After incubation, the resultant purple formazan was dissolved by adding 1 ml of DMSO in all the wells followed by incubation for 10 mins at room temperature. The absorbance at 570 nm was measured with UV-Spectrophotometer-Shimadzu RF-5301 using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC₅₀) was determined graphically. The % cell viability was

calculated using the following formula;

$$\% \text{ cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100 \quad (1)$$

Results and Discussion

The rate of deposition of suspended particles increases with the applied potential [3]. The quality of deposits on the substrate were unaffected by the applied potential ranging from 10-50 V. But, the applied potential above 50 V results in changes of coating morphology and formation of non-uniform coating. This is due to rapid movement of the particles which leads to collision and non-uniformity [22]. Fig. 1 shows the coating weight of whitlockite on titanium at various potential (10-50 V) at a constant time of 3 min. It was observed that the coating weight of whitlockite increases with increasing in applied voltage up to 30 V and the weight gain starts to decrease above 40 V. A uniform distribution of the particles with maximum weight gain is obtained at 30 V.

The formation of crystalline phase in the whitlockite powder coated on Ti at an optimum potential of 30 V and 3 minutes at 800 °C in vacuum at 10^{-5} Torr in comparison with un-sintered sample were investigated by XRD and the patterns recorded are given in Fig. 2(a) and Fig. 2(b), respectively. The coatings have the corresponding hkl indices of 210, 220, 214, 300, 1010, 4010 and 2020 [11] of whitlockite. These values are indexed with JCPDS file No. 090169, which confirms the presence of whitlockite alone in the compound with sharp intense peak. The XRD patterns of both vacuum sintered and un-sintered whitlockite were similar. Hence, there is no change in patterns after vacuum sintering. This shows the stable nature of whitlockite coatings. The size of nano whitlockite powder calculated using Scherrer's equation are 47.87 nm, 48.26 nm and 50.29 nm respectively. HAP and other phases of calcium phosphate peaks are not detected implying

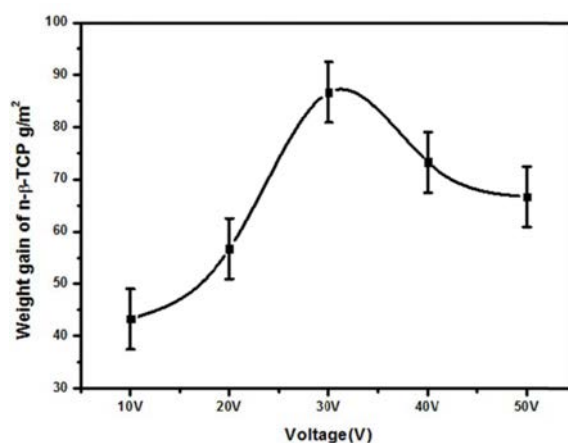


Fig. 1. Change in weight gain of whitlockite coatings on titanium at different voltages (10-50 V) at a constant time of 3 min.

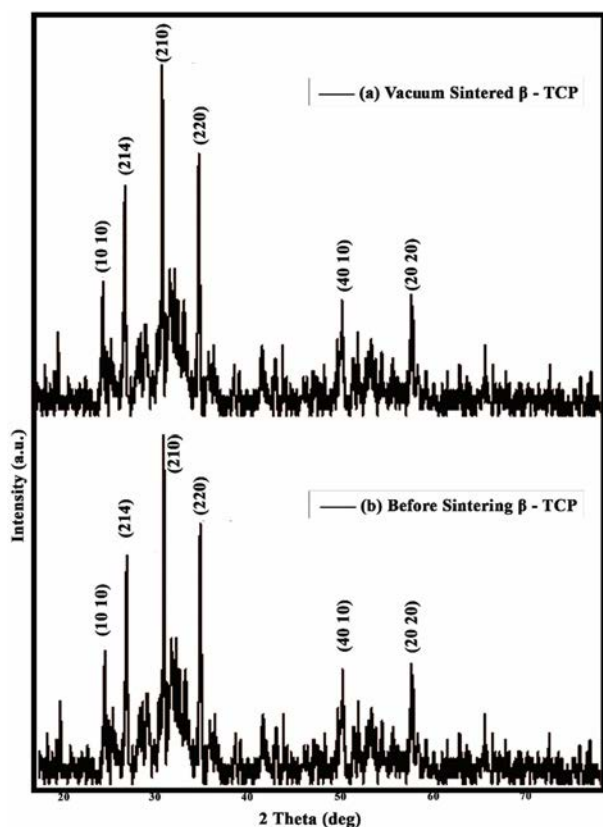


Fig. 2. XRD patterns recorded for whitlockite coated Ti obtained at the optimum coating potential of 30 V and 3 min. (a) vacuum sintering at 800 °C and (b) Before sintering

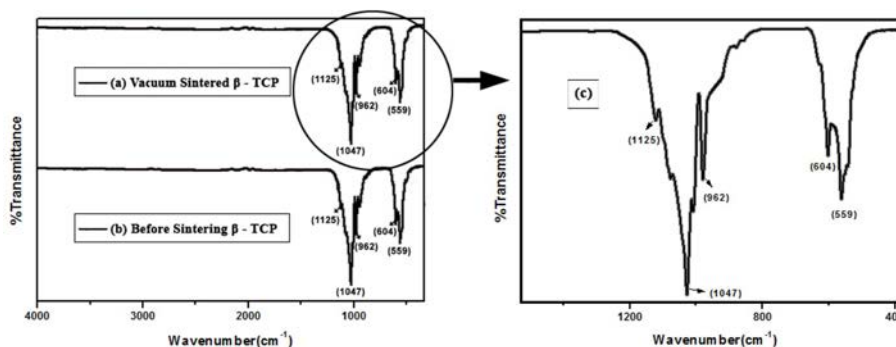


Fig. 3. FTIR of whitlockite coated Ti obtained at the optimum coating potential of 30 V and 3 min. (a) vacuum sintering at 800 °C (b) before sintering and (c) zoomed image of vacuum sintered whitlockite.

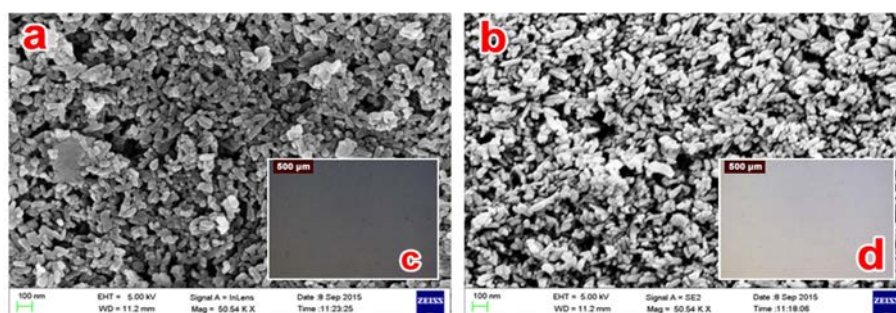


Fig. 4. Surface morphologies of whitlockite coatings on Ti obtained at 30 V at 3 min. (a) before sintering and (b) after sintering. Inset shows Optical images of (c) before sintering and (d) after sintering.

pure phase of the synthesized powder.

The spectra of whitlockite powder coated on titanium at an optimum potential of 30 V and 3 mins at 800 °C in vacuum at 10^{-5} Torr in comparison with un-sintered whitlockite sample are shown in Fig. 3-a and Fig. 3-b respectively. The expanded view of the image of vacuum sintered whitlockite sample is shown in Fig. 3-c. The characteristic peaks of whitlockite are present at 1125 and 1047 (ν_3), 559 and 604 (ν_4) corresponding to the phosphate band. Hence, the bands at 900-1200 cm^{-1} correspond to the stretching mode of phosphate group and 559 and 604 cm^{-1} is vibrational mode of phosphate group [23]. The sintered and un-sintered whitlockite peaks were similar. This confirms the presence of whitlockite as a single phase and is in agreement with XRD and no other phases were detected. The results indicate the presence of stoichiometric whitlockite with a Ca/P ratio of 1.5.

Fig. 4(a) shows the formation of microporous layers of whitlockite coating on titanium for the un-sintered sample as observed by FESEM. The particles on the substrate were uniform and no crack was observed on the surface. Fig. 4(b) shows the micrographs of sintered whitlockite on titanium. The coatings are denser and the particles are tightly packed after sintering. No cracks are observed after sintering. Fig. 5 shows the elemental composition of the coating surface observed by FESEM-EDAX. This analysis confirms the presence of the elements Ca (21.21 at. %), P (14.10 at. %) and O

Table 1. EDAX analysis of whitlockite coated on titanium.

Element	Weight%	Atomic%
O	41.46	61.43
P	22.68	14.10
Ca	35.86	21.21
Total	100.00	

(61.43 at. %) corresponding to whitlockite compound respectively. Further the Ca/P ratio of 1.5 confirms that the calcium phosphate formed is whitlockite. Table 1 shows the composition of elements present in the synthesized compound.

Figs. 4(c) and 4(d) shows the optical microscopic images of whitlockite powder coated on titanium before sintering and after sintering in vacuum at 800 °C

for 1 hr. For both sintered and un-sintered whitlockite samples, a uniform coating without crack was observed on the surface. The effect of sintering results in the formation of dense coatings when compared to un-sintered nano whitlockite coating and can be observed in Fig. (d). This could be due to the tight packing of particles after sintering.

Fig. 6 shows the OCP-time measurements of uncoated titanium and whitlockite deposited on titanium metal in Ringer's solution. The OCP of whitlockite deposited samples move towards positive direction and attains stable condition soon as compared to uncoated titanium metal. The potential of uncoated titanium sample shifts slightly towards the noble direction due to the presence of single passive layer on the metal surface. It reaches the potential of -378.9 mV after 60 min. Even though

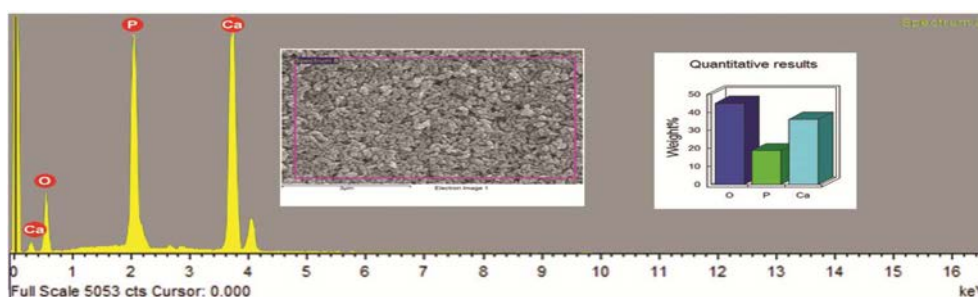
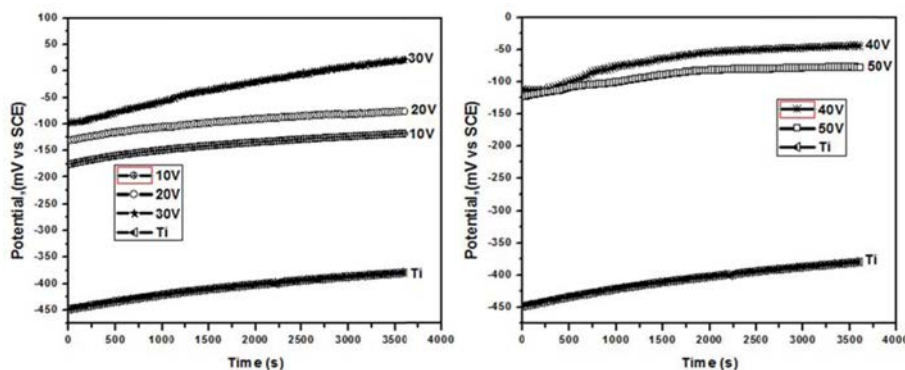
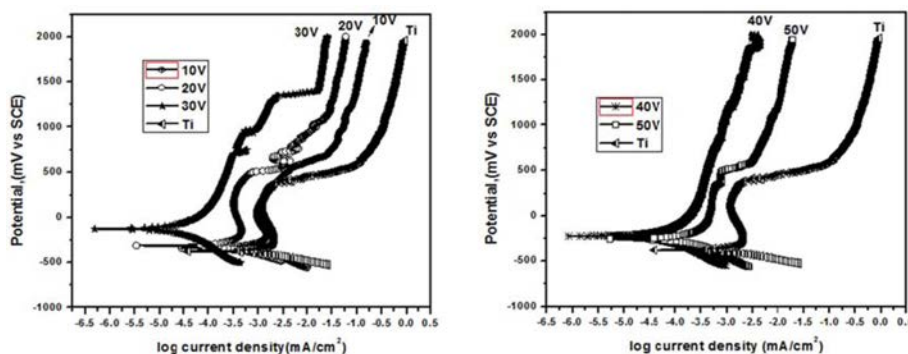
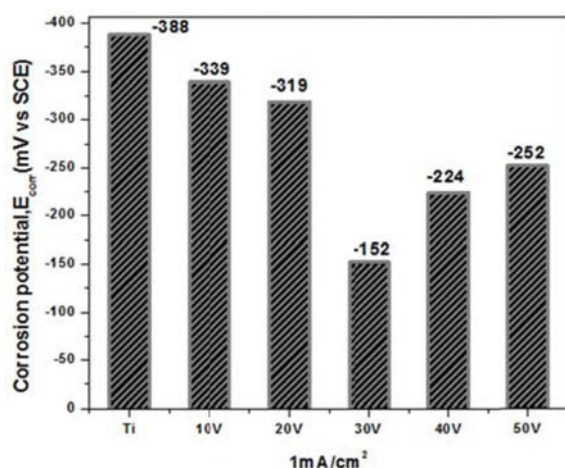

Fig. 5. EDAX spectra for whitlockite coated on titanium after sintering in vacuum at 800 °C and their Quantitative results.

Fig. 6. OCP-time measurements in Ringer's solution for uncoated and whitlockite coated on Ti at different voltages (10-50 V) at a constant time of 3 min.

Fig. 7. Potentiodynamic curves of uncoated Ti and those with whitlockite coated Ti in Ringer's solution at various coating potentials (10-50 V) at a constant time of 3 min.

Table 2. Polarization parameters of uncoated and whitlockite coated Ti in Ringer's solution (10-50 V).

Specimen	E_{corr} (mV) Vs SCE	I_{corr} ($\mu\text{A}/\text{cm}^2$)	R_p (k cm^2)
Titanium	-388	1.325	0.163
10 V	-339	0.921	0.420
20 V	-319	0.291	1.092
30 V	-152	0.076	5.274
40 V	-224	0.101	3.267
50 V	-252	0.160	2.203

**Fig. 8.** Comparison of the corrosion potential (E_{corr}) of uncoated Ti and those with whitlockite coated Ti in Ringer's solution at various coating potentials (10-50 V) at 1 mA/cm²

the OCP of metals moved slightly towards the noble direction, its potential is low when compared to the whitlockite deposited samples. The OCP of coating potential at 30 V and 3 min shifts towards nobler direction and attains the potential of +21.05 mV after 60 min. The OCP of all the coated samples shifted towards nobler direction and attains steady state earlier as compared to uncoated sample. These results indicate the protective nature of the bioceramic coatings. The potential increases from 10 V to 30 V and it tends to decrease above 40 V. This is due to a mild increase in porosity as a result of non-uniform distribution of the particles. The maximum potential was reached after 1 h at 30 V and 3 min

compared to all the coatings tested.

Fig. 7 shows the polarization curve of both uncoated titanium and n- β -TCP coated on titanium metal in Ringer's solution. E_{corr} , I_{corr} and polarization resistance (R_p) values for uncoated and coated titanium samples are presented in Table 2. Fig. 8 shows the graphical representation of the E_{corr} values for whitlockite deposited and uncoated titanium samples. The E_{corr} and I_{corr} values of uncoated titanium sample were found to be -388 mV and 1.325 $\mu\text{A}/\text{cm}^2$ and the optimized potential of 30 V was found to be -152.7 mV and 0.076 $\mu\text{A}/\text{cm}^2$. E_{corr} and I_{corr} values of 10 V, 20 V, 40 V and 50 V are -339 mV and 0.921 $\mu\text{A}/\text{cm}^2$, -319 mV and 0.291 $\mu\text{A}/\text{cm}^2$, -224 and 0.101 $\mu\text{A}/\text{cm}^2$, -252 and 0.160 $\mu\text{A}/\text{cm}^2$. From these results we can observe lower E_{corr} and higher I_{corr} values for uncoated sample. Whereas higher E_{corr} and lower I_{corr} values for coated samples. Comparatively the potential of 30 V has high E_{corr} and lower I_{corr} values than other coated samples. The coated sample has high polarization resistance value than uncoated titanium sample. This confirms the corrosion resistant property of the coated samples when compared to uncoated titanium sample. These results suggest that the optimized coating is 30 V and 3 min and shows enhanced corrosion performance. Hence, whitlockite deposited titanium samples exhibit stable and corrosion resistant surface than uncoated titanium.

Electrochemical impedance measurements were operated at equilibrium conditions to check the corrosion behavior of uncoated titanium metal and as well as whitlockite deposited titanium metal in Ringer's solution. Nyquist plots of both uncoated sample and coated sample are shown in Fig. 9. Equivalent circuit model of uncoated and coated sample is shown in inset of Fig. 9. This equivalent circuit model is obtained after fitting the spectra of uncoated and whitlockite deposited titanium metal by using EC-Lab V10.37 software (BioLogic-SP240). The best fitting is chosen by considering small chi square values as shown in Table 4. When the chi square values are very small, it is considered as suitable spectra. The resistance and capacitance values of uncoated and coated samples are shown in Table 3.

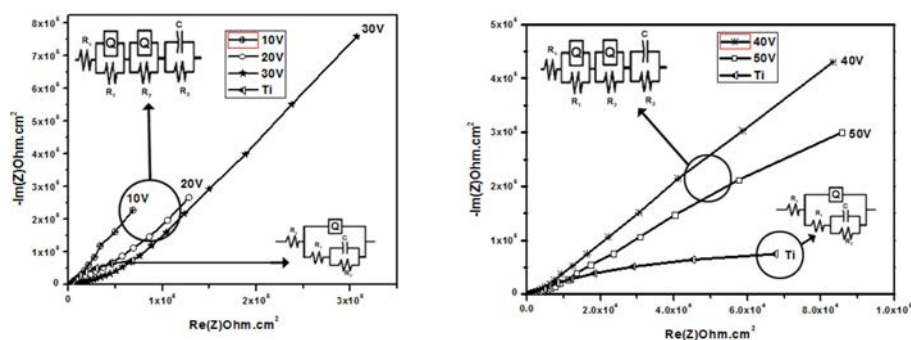
**Fig. 9.** Nyquist plots in Ringer's solution for uncoated and whitlockite coated Ti at different voltages (10-50 V) at a constant time of 3 min. Inset shows equivalent circuit model of uncoated and whitlockite coated titanium.

Table 3. EIS spectra fitted values for uncoated and whitlockite coated on Ti in Ringer's solution.

Coating Potential	$ Z $	R_s	Q_{Coat}	n_{Coat}	$R_1(_{Coat})$	Q_b	n_b	R_2	C_{dl}	R_3
Uncoated	100632	90.9	82.3×10^{-6}	0.88	224657	—	—	—	47.25×10^{-3}	15265
10V	238786	60	56.03×10^{-6}	0.77	44.82×10^4	60.47×10^{-6}	0.70	12.57×10^3	0.122×10^{-3}	20395
20V	296469	50	22.12×10^{-6}	0.73	4.18×10^6	26.21×10^{-6}	0.63	5.89×10^5	2.175×10^{-5}	24101
30V	818709	15.7	0.27×10^{-6}	0.62	2.014×10^{11}	0.139×10^{-6}	0.50	7.56×10^8	85.54×10^{-9}	78354
40V	438449	19.2	3.26×10^{-6}	0.66	3.58×10^9	10.67×10^{-6}	0.54	9.23×10^7	52.6×10^{-7}	52134
50V	311745	35.9	11.34×10^{-6}	0.71	5.235×10^8	21.5×10^{-6}	0.61	2.87×10^6	0.257×10^{-6}	9411

Table 4. Chi square values for whitlockite coated and uncoated titanium sample.

Samples	Chi square value
10 V	0.00084
20 V	0.00016
30 V	0.00022
40 V	0.00029
50 V	0.00018
Uncoated titanium	0.0021

The EIS spectra model for uncoated titanium metal is $[R_s(R_1Q_1)(R_2C)]$, as shown in Fig. 9. R_s represent solution resistance, R_1 and Q_1 represents polarization resistance and constant phase element of barrier oxide layer present on the titanium surface. R_2 and C indicate polarization resistance and double layer capacitance of metal surface. The EIS spectra model for whitlockite deposited titanium is shown in Fig. 9 is $[(R_s(R_1Q_1)(R_2Q_2)(R_3C))]$. R_1 and Q_1 indicate polarization resistance

and constant phase element of coated layer. R_2 and Q_2 indicate polarization resistance and constant phase element of passivation layer present in between metal surface and coated layer. R_3 and C represent polarization resistance and double layer capacitance of metal surface. A constant phase element (CPE) is an imperfect capacitor which is introduced in the fitting for barrier and coated layer alone. This indicates that the coated layer controls the release of metal ions in to the solution, thus prevents the corrosion. CPE can be expressed by the following equation [24].

$$Z_{CPE} = \frac{1}{(j\omega)^n Q} \quad (3)$$

Q is the constant phase element, ω is the angular frequency and n is the exponent of CPE. Generally n value arises due to non-uniform distribution of current by means of surface roughness [25]. It should be in the range of -1 and 1 ($0 < n < 1$). If n is equal to one, it acts as a perfect capacitor. From Table 3, the n_b values for

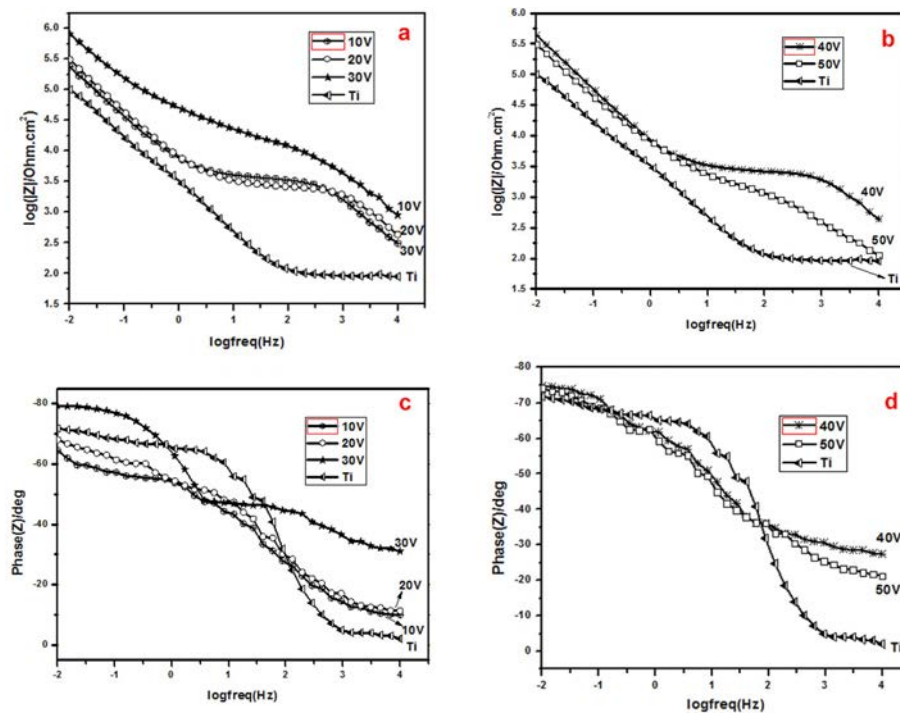

Fig. 10. Bode impedance (a) & (b) and (c) & (d) Bode phase plots in Ringer's solution for uncoated and whitlockite coated Ti at different voltages (10-50 V) at a constant time of 3 min.

Table 5. Cytotoxicity effect of whitlockite sample on osteoblast Cell line.

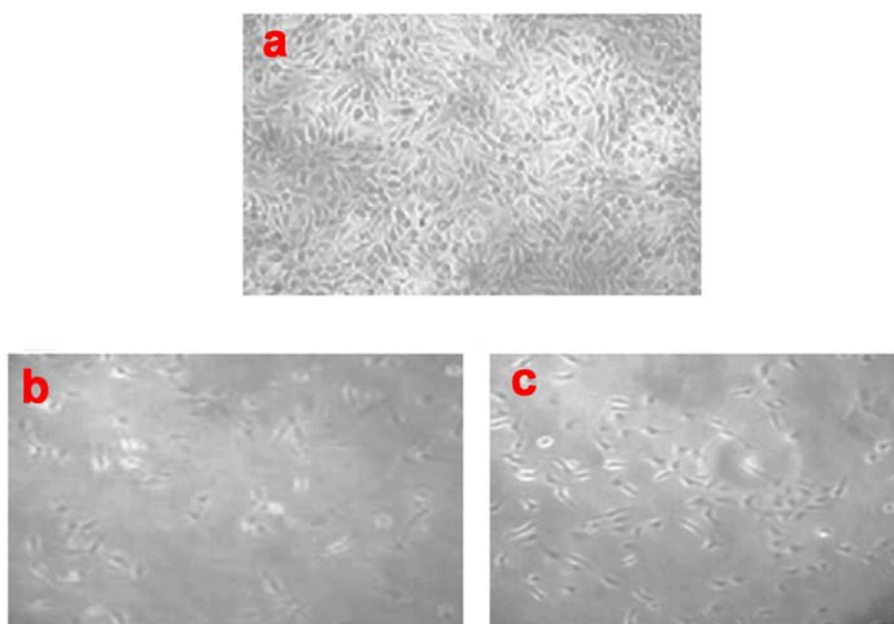
Sample No.	Concentration ($\mu\text{g/ml}$)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.36	73.4
2	500	1 : 1	0.37	75.5
3	250	1 : 2	0.39	79.5
4	125	1 : 4	0.41	83.6
5	62.5	1 : 8	0.43	87.7
6	31.2	1 : 16	0.44	89.7
7	15.6	1 : 32	0.46	93.8
8	7.8	1 : 64	0.48	97.9
9	Cell control	—	0.49	100

uncoated titanium are 0.88 which is nearly equal to one. Hence, it indicates a capacitive behavior due to the presence of single oxide layer on titanium [26]. The value of coated layer ranges from 0.62 to 0.77, which indicates that it is free from electrolytic attack and it is more protective in nature. For barrier layer, the n_b value starts from 0.50-0.70 which indicates that it is free of ions from the solution. The coated sample has high polarization resistance and very low capacitance values when compared to uncoated samples. These values show the stable nature of whitlockite coatings.

Figs. 10(a) and 10(b) show the bode impedance plots ($\log|Z|/\text{Ohm.cm}^2$ vs. $\log(\text{frequency})$) of uncoated sample and whitlockite coated sample in Ringer's solution. The coated sample shows maximum impedance while compared to uncoated sample. The optimized potential of 30 V has maximum impedance than other coated samples.

Fig. 10(c) and d show the bode phase angle plots ($\text{phase}(z)/\text{deg}$ vs. $\log(\text{frequency})$) of uncoated sample and coated titanium sample immersed in Ringer's solution. The uncoated sample has phase angle value around -75° and it is constant for a wide range of frequencies (10^{-2} to 10^2 Hz). This indicates the capacitive behavior of single oxide film present on the metal surface [25]. The coated sample has maximum phase angle value and higher resistance compared to the uncoated sample. These results show the presence of uniform coatings on the surface of the implant.

Biocompatibility of whitlockite can be evaluated under in vivo or in vitro. We have carried out in vitro experimentation for initial screening of the material's cytotoxicity, so that further experimentation with higher animals can be carried out in vivo. In this study, MTT Assay was carried out to check the viability of cells by using different dilutions of the sample based to minimize the toxicity levels. The samples were prepared for different dilutions (Table 5) from 1000 $\mu\text{g/ml}$ i.e. neat sample and also from 500 $\mu\text{g/ml}$ (1 : 1 ratio) to 7.8 $\mu\text{g/ml}$ (1 : 64 ratios). The MTT assay was carried out on Osteoblast cell lines. The absorbance after the results showed that the cell viability on neat sample i.e. the 1000 $\mu\text{g/ml}$ itself showed maximum viability of 73.4%. Hence the sample has no toxic effects on the cells. The maximum cell viability was obtained when the dilution in the ratio was 1 : 64. The Histo morphology of Osteoblast cell line (Fig. 11(a)) compared with sintered whitlockite powder with concentrations (1000 & 31.2 $\mu\text{g/ml}$) is shown in Fig. 11(b) and 11(c). More viability of cells can be observed in Fig. 11(c). Whitlockite bioceramic used on the MTT assay is more biocompatible and can be used as an implant for regeneration of bone tissues.

**Fig. 11.** Histomorphology of (a) Osteoblast cell line. Histomorphology of sintered whitlockite exposed to Osteoblast cells at concentration of (b) 1000 and (c) 31.2 $\mu\text{g/ml}$.

Conclusions

Nano bioceramic coatings have been successfully developed on Ti by EPD with enhanced corrosion resistance and biocompatibility for bone tissue engineering applications and the optimum potential was obtained at 30 V and 3 mins. The presence of α -TCP (whitlockite) in the coated samples after sintering was confirmed by XRD and FTIR. The crystalline phase and nano structure morphology of the samples were detected using XRD and FE-SEM. No HAP or other calcium phosphates were detected for both sintered and unsintered coatings. The OCP of coated sample shifts towards the passive direction and reaches steady state with in short duration when compared to uncoated sample. E_{corr} and I_{corr} values of coated sample confirmed the protective nature of the whitlockite coatings. Electrochemical impedance measurements results revealed high polarization resistance values and low capacitance values than the uncoated titanium sample. The MTT assay results showed that the sample is biocompatible and non-toxic in nature. It showed maximum cell viability in 31.2 μ g/ml. These results indicate the stable nature of whitlockite coating and hence can be used as orthopaedic implants.

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