



PREVENTION OF CORROSION ON 316L STAINLESS STEEL BY NANO BIOCOMPOSITE COATING FOR BONE TISSUE APPLICATION

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Abstract-Nano biocomposite coating on 316L SS was developed by a simple electrophoretic deposition process (EPD) followed by vacuum sintering to enhance the corrosion resistance, biocompatibility and cell proliferation. The nano biocomposite coated surfaces were investigated by X-ray diffraction, FT-IR spectroscopy, Profilometer studies, Tape adhesion test, Vickers Microhardness and cell culture studies. Different electrochemical characterizations studies were employed to investigate the corrosion stability of the coated samples in Hank's solution. The electrochemical studies proved that nano biocomposite coated 316L SS samples have superior corrosion resistance than uncoated sample due to the stable nano biocomposite coating on metal surface. The higher values of Vickers microhardness support the enhancement in mechanical strength for coatings. In vitro studies with MC3T3-E1 osteoblast cell show non-toxicity with improved cell attachment and elevated proliferation of biocomposite coated samples compared to uncoated samples.

Keywords: Biocomposite. Coatings. Stainless steel. Corrosion resistance. Cell proliferation.

I. INTRODUCTION

Metallic implant materials and alloys are widely used for load-bearing applications due to excellent corrosion resistance and mechanical strength [1]. Medical grade 316L Stainless Steel (316L SS) has been used as an implant material for the past few decades owing to its biocompatibility, availability, ease of fabrication and corrosion resistance in the human body, Clinically a few cases of failures due to pitting, crevice and localized corrosion on implantation in the human body have been reported along with the release of metal ions into the tissues surrounding

the implants [2, 3]. Hence, there is an imperative need to modify the interactive surface to control the corrosion rate and to get a proper biological response from living tissue.

Many researchers have studied the surface modification of the metallic implants through the alloying, protective coating and chemical treatment [4]. Protective coatings are carried out using Hydroxyapatite (HAP), β -Tri Calcium Phosphate (β -TCP), Graphene oxide, TiO_2 , and ZrO_2 to reduce the corrosion rate of 316L SS [5,6]. Hydroxyapatite (HAP, $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$) is a most promising bioceramic material, used as a drug carrier, a bone graft and as a coating for metallic implants [7]. Moreover, HAP nano powder can interact easily with vitronectin and fibronectin, which having important roles in cell adhesion [8,9]. However, HAP applications are limited because of poor mechanical properties [10,11]. The mechanical limitations could be rectified by the addition of bioinert nano TiO_2 powder for improving chemical stability and adhesion property of the coating compared to pure HAP coating.

Many researchers have reported the effect of TiO_2 in composite coatings on implant material. Koike et al. have reported that TiO_2/HAP composite coatings on metallic implants produced by plasma spraying technique lead to improved corrosion resistance [12]. Webster et al. showed that nano TiO_2 promotes osteoblastic adhesion compared to conventional powders [13]. Further, Mohan et al. have reported that the addition of nano TiO_2 to nano-HAP is found to enhance the adhesion of the coating with the substrate and also with the bone [14]. Amaravathy et al. have suggested HAP/ TiO_2 coatings on metallic implants produced by sol-gel technique provide the implants with improved mechanical stability [15]. Nagarajan et al. have reported that $\text{TiO}_2/\text{ZrO}_2$ nano composite coatings on 316L SS provide the implant with enhanced biocompatibility [16]. Further, Chellappa et al. reported the composite ($\text{SiO}_2:\text{TiO}_2:\text{ZrO}_2$) coating on implant material by an electrophoretic deposition process (EPD) improves the corrosion-resistance [17]. Morteza et al. has reported HAP / TiO_2 nano composite coatings on 316L SS developed by EPD process to provide a crack-free coating with enhanced corrosion resistance [18].

So far, there are no reports on suitable surface roughness obtained through mechanically polishing the metal implants surface with different SiC grit paper starting from the sheet 320 to 1000 grit. Before coating, surface roughness on metal implants plays an important role in deposition of nano particles from suspension and their mechanical and biocompatibility properties. In this present work, metal surface was mechanically polished using different SiC grit paper starting from 320 to 1000 and nano biocomposite (nano HAP/ nano TiO_2) were fabricated on polished 316L SS. An attempt has been made to improve the adhesion strength, corrosion resistance and biocompatibility of the implant 316L SS by applying nano biocomposite coatings by EPD followed by vacuum sintering at 700°C for 1 h.

II. METHODOLOGY

A. Substrate Preparation

316L SS was obtained from SAIL, India (ASTM F138- 13a). In this experiment two electrodes are used, the anode is 314 SS with a dimension of $11 \times 8 \times 0.5$ mm and the cathode is 316L SS with dimensions of $10 \times 10 \times 2$ mm respectively. All the samples were polished mechanically with SiC paper starting from the sheet 320 to 1000 grit. After polishing, the samples were thoroughly washed with soap solution, rinsed with distilled water and then ultrasonically degreased in acetone for 30 min and dried at room temperature.

B. Electrophoretic deposition on 316L SS

2% suspension of nano biocomposite (nano HAP/ nano TiO_2) was prepared using isopropyl alcohol in a 100 mL beaker. The suspension kept for 24 h in undisturbed condition to get uniform dispersion of the particles. Then, the suspension was stirred for 30 min. Deposition on 316L SS was achieved by EPD at room temperature. Anode and cathode were immersed in suspension and distances between these two electrodes were less than 1cm in order to achieve optimum particle deposition on the exposed electrode surface area. Before deposition one edge of the sample masked with tape Teflon and deposition was carried out on other 1cm^2 surface areas. All the coatings were achieved at an applied voltage of 30 V and a time of 60 seconds. After the coating process, all the coated samples were dried at room temperature followed by vacuum sintering at the temperature of 700°C for 1 h.

C. Physical characterization

The functional, morphological, structural and elemental compositions of the nano biocomposite coatings were characterized by using various analytical techniques. The coated layer was observed by optical microscopy and crystalline phases were determined by X-ray diffraction (Bruker model D8) with $\text{Cu } \alpha$ radiation, and λ of 1.5406\AA . The functional groups were characterized by ATR-FTIR technique (Model Perkin Elmer-Spectrum two). The surface morphology of the coated samples was investigated using field emission scanning electron microscope (Model CARL ZEIS SUPRA 55) equipped with energy-dispersive X-ray spectroscopy (EDS).

The surface roughness of the coated samples was measured by non-contact optical surface profiler using BRUKER (model CONTOUR GT), Germany. Average surface roughness (R_a) measurements were taken at ten different locations on each coated samples to obtain an accurate assessment. All measurements were taken perpendicular to the machine markings.

D. Mechanical properties of coatings

The adhesion strength of coatings after vacuum sintering was measured by Tape adhesion test (American Society for Testing and Materials (ASTM D3359)). The first cross-cut was developed on both the direction using cross-hatch cutter and adhesive tape was applied to the cross-cut area and pulled back and then estimation was made according to the ASTM standard. Vickers Microhardness Tester (Ever One Enterprises Limited, India) was used to study the microhardness properties of the coated samples (ASTM E92-17). A loading force of 100 gram Force (gF) was applied for the duration of 5 seconds to record the measurements.

E. Electrochemical analysis

A conventional three-electrode cell was used for all the electrochemical measurements as per ASTM guidelines (G61–86). A saturated calomel electrode (SCE), graphite electrode and the nano biocomposite coated sample was used as a reference electrode; counter electrode and working electrode, respectively. Hank's solution with pH 7.4 was used as the electrolyte during the electrochemical analysis. Electrochemical corrosion tests are open circuit potential (OCP)-time measurements, cyclic potentiodynamic polarisation (CPP) and electrochemical impedance spectroscopic (EIS) studies were carried out on uncoated and nano biocomposite coated 316L SS samples using Biologic-SP 240 (EC- lab Version 10.37) connected to a corrosion cell.

Without applying any current to sample OCP values were recorded until reach the study state potential. After that, EIS was carried out under OCP condition by applying a frequency starting from 20.00 kHz to 0.100 Hz. The impedance spectra were evaluated by fitting the experimental results to equivalent circuits through the non-linear least-square fitting procedure. The quality of fitting to the equivalent circuit was judged by the χ^2 value (sum of the square of the difference between theoretical and experiment point) and by limiting the relative error in the value of each element in the equivalent circuit to 5%. All the measurements were repeated at least three times and good reproducibility of the results was observed. This was followed by CPP with an initial potential below 0.250 V of the corrosion potential at a scan rate of 10 mV min⁻¹. Breakdown potential (E_b) and repassivation potential (E_p) of all the coated and uncoated samples were calculated from the CPP curve.

F. Cell culture study

Osteoblast cells MC3T3-E1 were obtained from National Centre for Cell Sciences, Pune (NCCS). Using MTT assay in vitro cytotoxicity was carried out [19]. The cells are cultured in minimum essential medium (MEM). MEM contains 100 $\mu\text{g ml}^{-1}$ of penicillin, 10% fetal bovine serum (FBS) and streptomycin. At last, using Acridine Orange (AO) dye cells are stained and the images were captured with a confocal microscope in the emission rate of 525 nm (Model Carl Zeiss LSM 710). The number of cell attachments was determined by Trypan blue assay method.

A number of cells present on the coated samples were measured after 24, 48 and 72 h of cultures. The cells were detached by trypsinization and counted using hemocytometer.

III. RESULTS AND DISCUSSION

A. Optical microscopy

After sintering process nano biocomposite coated 316L SS samples were visualized using optical microscope to study the morphological characterization and the images of nano biocomposite coated 316L SS sample polished at different SiC grit is given in Fig. i.

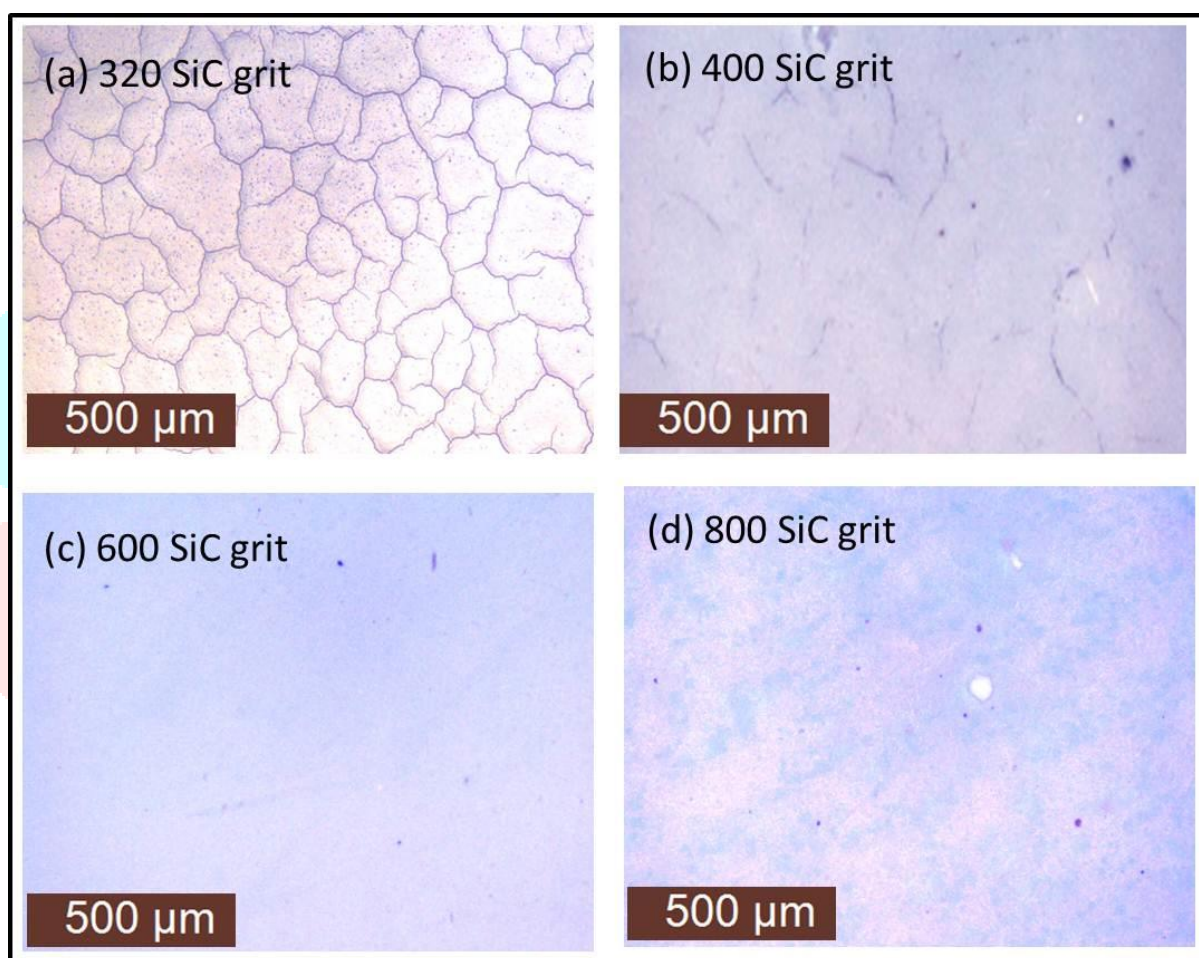


Fig. i Optical images of nano biocomposite coated 316L SS polished at different SiC grit after sintering.

Different SiC grit papers were used for mechanically polishing 316L SS are 320, 400, 600, 800 and 1000 respectively. Very dense coating was observed at using 320, Thick coating with hair line cracks was obtained at 400, mild coating observed at 800, no coating was observed on 1000 and thin, uniform and crack free coating was observed at 600 SiC grit. Metal implants polished using 320 SiC grit show the surface with more roughness, it allows more nano particle to deposit on the surface. So that after sintering the coated layer no longer found on the metal surface. Polished metal surface at 1000 to give smooth surface and will not receive the nano particles and deposit on the metal surface. Metal surface polished using 400 give rough surface, it receiving more nano particle

from suspension to produce thick coating. Similarly, coated surface at 600 produced thin layer due to sufficient roughness on metal surface. Finally, coating at 800 produce mild coating due to insufficient roughness on metal surface. Optical microscopy studies confirmed thin, uniform and crack free coating observed on 316L SS metal surface using 600 Sic grit. Hence, coated samples polished with 400, 600 and 800 SiC grit were consider for further characterization studies.

B. X-Ray diffraction studies (XRD)

Fig. ii shows that the XRD spectra for nano biocomposite coated 316L SS polished with 600 SiC grit after vacuum sintering at 700°C for 1 h. The peaks with maximum intensity for nano-HAP was detected at (002), (210), (211), (300), (221), (222), (213) and (004) reflection planes are assigned to nano-HAP. The obtained results were in good agreement with the JCPDS data (JCPDS Card No 09-0432). Generally, TiO₂ crystallites exist in three forms; brookite (orthorhombic), anatase (tetragonal), and rutile (tetragonal). At room temperature brookite and anatase phases are stable and they are changed to rutile phase above 700°C. The intensity peaks of rutile nano TiO₂ showed at (110), (200), (111) and (211), and compared with JCPDS file no.21-1276 [20]. No base metal peaks or their oxides were detected indicating that a strong interface between the base metal and bioceramics could be formed. The Figure also indicates that during the EPD process and after sintering there was no decomposition of the bioceramics materials. Thus, XRD spectra confirmed the presences of two crystalline phases are nano-HAP and nano TiO₂ on 316L SS after vacuum sintering.

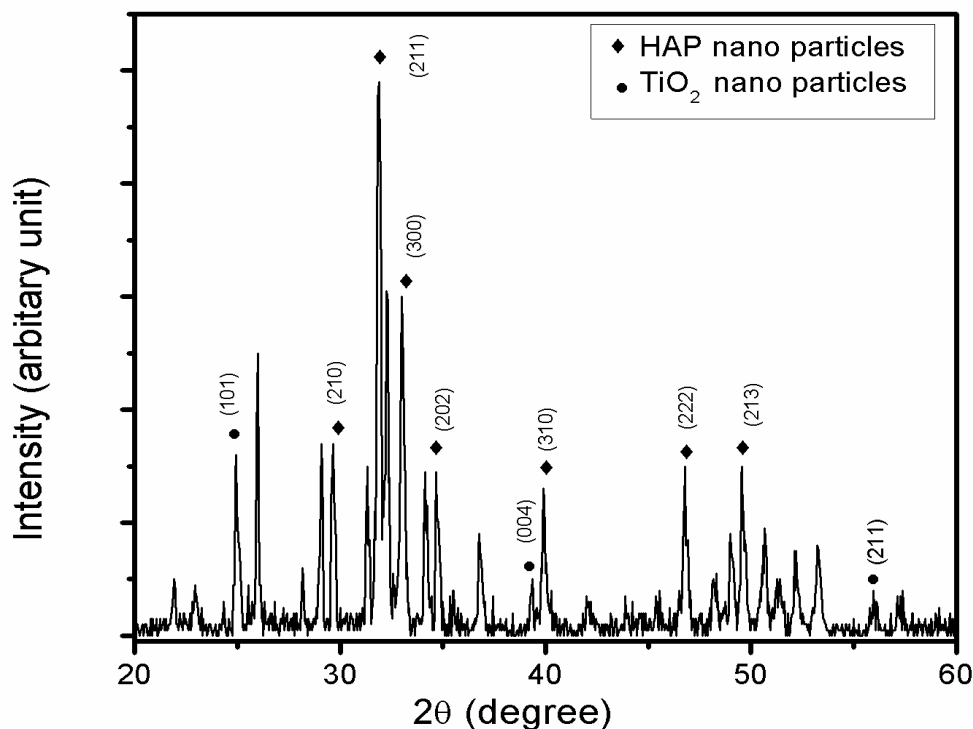


Fig. ii XRD spectra of nano biocomposite coated 316L SS at 600 SiC grit after sintering.

C. FTIR spectra analysis

FTIR spectrum of nano biocomposite coated 316L SS samples polished with 600 SiC grit after sintering is shown in Fig.iii. These spectra show characteristic bands for Phosphate (PO_4^{3-}) corresponding to the four different vibration modes are ν_1 , ν_2 , ν_3 , and ν_4 . The bands observed at $960\text{-}980\text{ cm}^{-1}$ and $470\text{-}473\text{ cm}^{-1}$ indicate the ν_1 and ν_2 vibration modes of phosphate respectively. The bands obtained at $1040\text{-}1094\text{ cm}^{-1}$ and $560\text{-}620\text{ cm}^{-1}$ correspond to ν_3 and ν_4 modes of phosphate ions respectively. The band at $1640\text{-}1645\text{ cm}^{-1}$ corresponds to the ν_2 bending mode of the H_2O molecules. The CO_3^{2-} bands were observed in the region of $1380\text{-}1430\text{ cm}^{-1}$. The bands in the region 3570 cm^{-1} could be attributed to the presence of the OH group in nano-HAP. The band at 449 cm^{-1} confirm the presence of TiO_2 in triphasic coatings [11]. FT-IR spectrum confirms the presence of characteristics functional group of nano-HAP, and nano TiO_2 in nano biocomposite coatings on 316L SS after vacuum sintering.

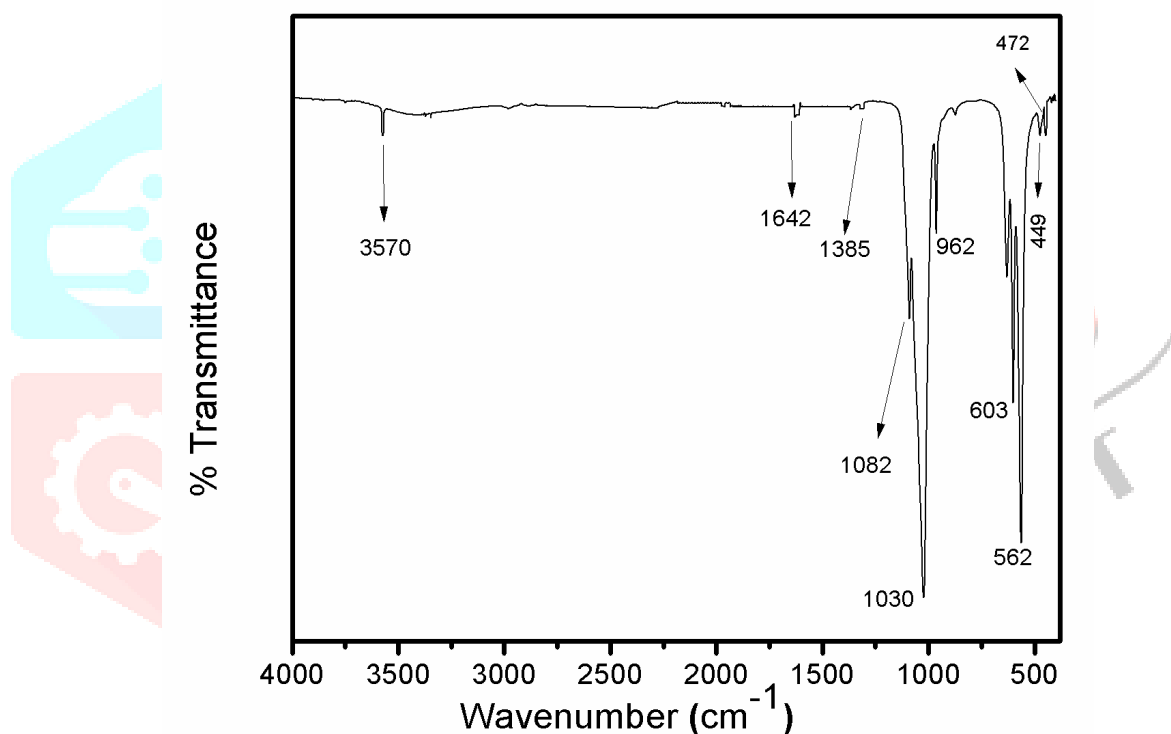


Fig.iii FT-IR spectrum of nano biocomposite coated 316L SS at 600 SiC grit after sintering.

D. Surface roughness measurements

Fig. iv shows that the surface profilometer images of nano biocomposite coated 316L SS mechanically polished with different SiC grit paper. Overall roughness of the coated surface was recorded using surface profilometer. The average surface roughness for the nano biocomposite coated 316L SS sample at polished with 400, 600 and 800 SiC grit were found to be $0.374 \pm 0.06\ \mu\text{m}$, $0.602 \pm 0.03\ \mu\text{m}$ and $0.497 \pm 0.01\ \mu\text{m}$ respectively. The increased surface roughness obtained at 600 SiC grit polished sample is due to interlocking of rough porous surface of nano-HAP with

nanoTiO₂ powder. At this condition coated sample shows enhanced average surface roughness when compared to other coated samples. Surface roughness on nano biocomposite coated sample is a one of the important characteristics for cell growth. Generally, the stability of the implant can be improved by increasing the surface roughness of the coatings. Amaravathy et al. have demonstrated that osteoblast cells tend to attach more rapidly on rough surfaces and increase the surface roughness that results in a fivefold increase in the contact area with the bone. This can provide a suitable environment for the bone cells to attach to the surface of the implant [15]. Thus, nano biocomposite coated samples mechanically polished at 600 SiC is suitable in bone-bonding ability than the other coated samples.

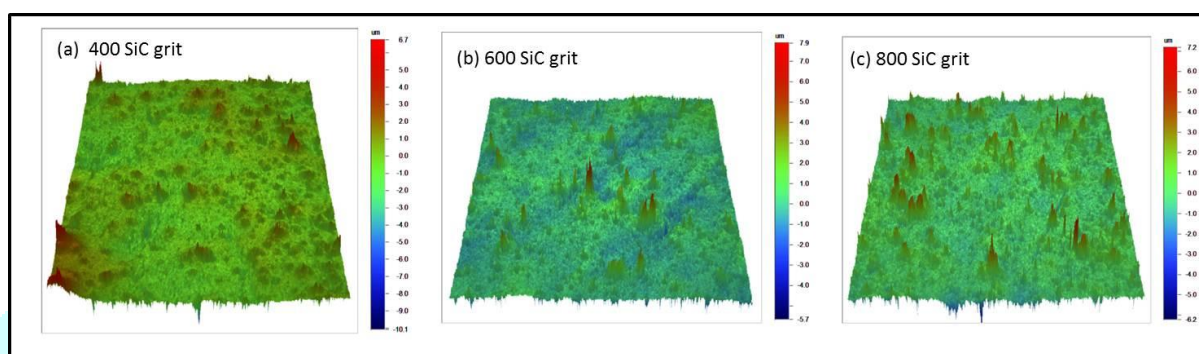


Fig. iv Surface profilometer image of nano biocomposite coated 316L SS polished at different SiC grit after sintering.

E. Electrochemical Studies

Open circuit potential-time measurements for uncoated and nano biocomposite coated 316L SS polished at different SiC grit paper are shown in Fig. v. For uncoated 316L SS sample OCP value decreases with respect to time (-0.318V), nano biocomposite coated 316L SS sample polished at 400, 600 and 800 show an increase in potential is -0.164 V, +0.029 V and -0.115 V respectively. For the entire coated samples OCP values shift in potential towards the nobler direction and it can prove better behaviour than uncoated samples. The highest shift was observed in 600 SiC grit polished coated sample (+0.029 V) than other coated samples. Enhanced behaviour of nano biocomposite coated sample at 600 SiC grit towards nobler direction because of the corrosion protective environment. The enhanced corrosion resistance behaviour of coating due to the formation of compact, uniform along with strong adhesion coating on the surface of the metal due to interlocking and diffusion bonding between nano-HAP and nano TiO₂.

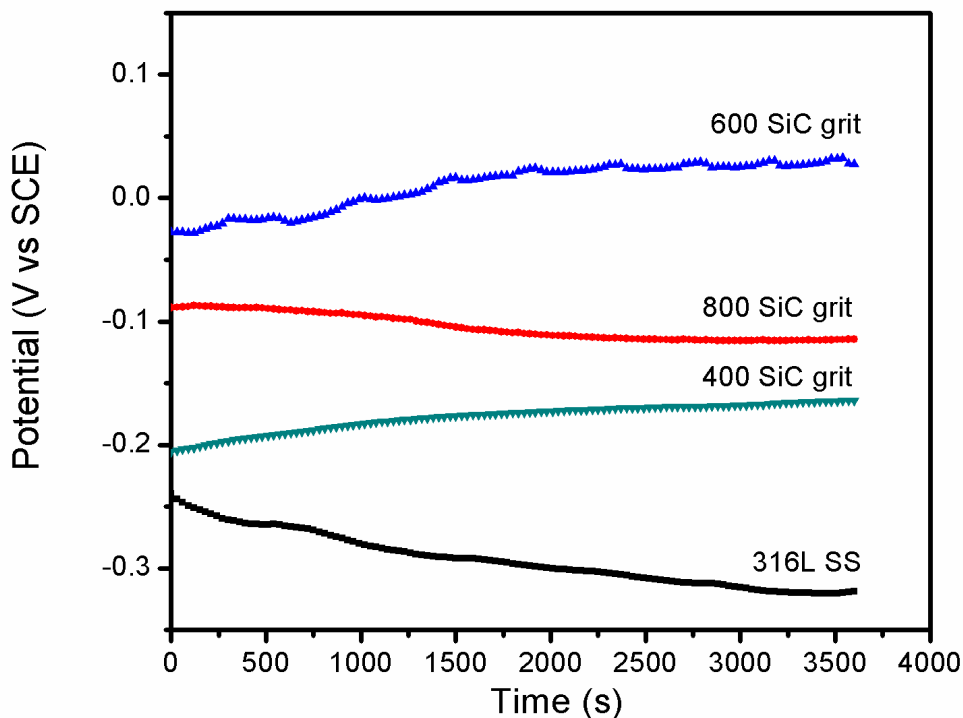


Fig.v OCP-Time measurements for uncoated and nano biocomposite coated 316L SS polished at different SiC grit after vacuum sintering at 700°C for 1 h in Hank's Solution.

An electrochemical impedance spectroscopic method is an important and effective tool to study the electrochemical behaviour of the samples [22, 23]. EIS studies of all the coated samples were evaluated in Hank's solution. Nyquist plots of uncoated and nano biocomposite coated 316L SS polished at different SiC grit after vacuum sintering at 700°C for 1 h in Hank's solution are shown in Fig.vi. Nyquist plots show that all the nano biocomposite coated samples were shifted towards maximum impedance than the uncoated 316L SS. Coated 316L SS polished at 600 SiC grit shows the highest impedance than the other coated 316L SS samples. Maximum impedance obtained at coated sample polished at 600 SiC grit due to the formation of a productive layer with good adhesion coating. Nan composite coatings prevent the interaction between metal surface and solution. Low impedance obtained on nano biocomposite coated sample polished at 400 and 800 SiC grit compared to coated sample polished at 600 SiC grit due to lack of adhesion strength on the metal surface. Consequently, a productive layer with a metal surface is formed on 316L SS polished with 600 SiC grit after vacuum sintering.

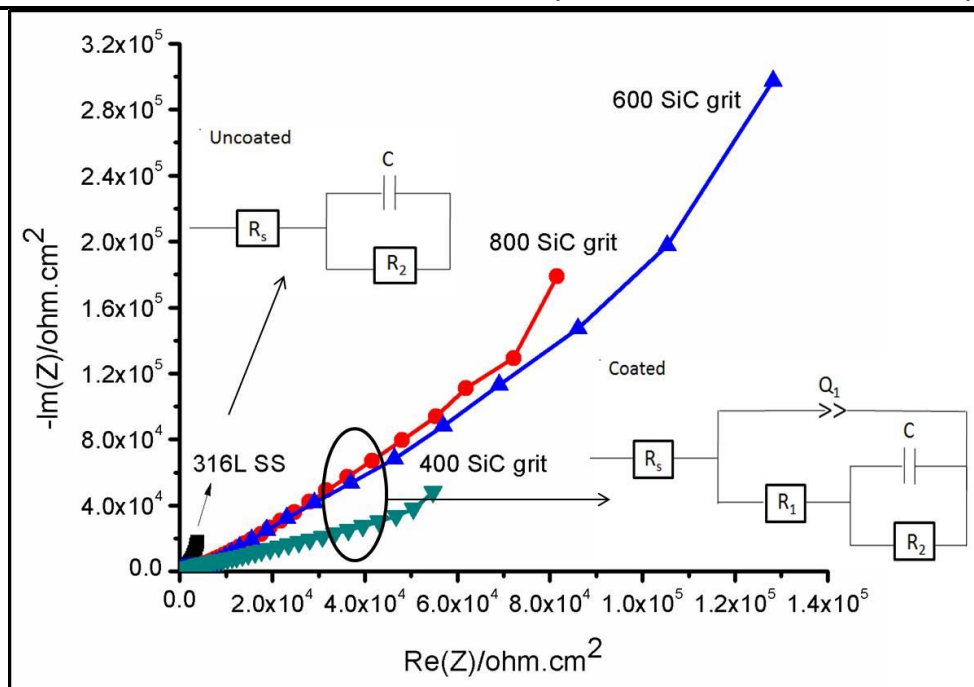


Fig.vi Nyquist Plots for uncoated and nano biocomposite coated 316L SS polished at different SiC grit after vacuum sintering at 700°C for 1 h in Hank's Solution.

The equivalent circuit model for uncoated and nano biocomposite coated 316L SS shown within the Fig.vi. The circuits were obtained by fitting the spectra of Nyquist plots for uncoated and coated samples. The fitting was done with ZSimp Win software and the fitted EIS results are shown in Table 1. The EIS spectra were fitted with the model $[R_s(R_2C)]$ for uncoated 316L SS. R_s , R_2 and C represents the solution resistance, polarization resistance of the metal surface and double-layer capacitance of metal surface respectively. The EIS spectra were fitted with the model $[R_s(R_1Q_1)(R_2C)]$ for coated 316L SS. R_s represents solution resistance, R_1 and Q_1 represents the polarization resistance of microporous coated layer and Constant Phase Element (CPE) of microporous coated layer, R_2 and C represents polarization resistance of metal surface and double-layer capacitance of metal surface respectively. The capacitance does not act as an ideal capacitor. It was introduced as CPE for the biocomposite coated layer. This confirms that the bioceramic coated layer avoids the release of metal ions into the solution than the uncoated 316L SS sample. The impedance of CPE expressed by equation (1) [24].

$$Z_{CPE} = 1/[(j\omega)^n Q] \quad (1)$$

In this equation, Q and ω is the constant phase element and angular frequency respectively. Where n is the exponent of CPE with non-uniform sharing of current and due to surface roughness with values between -1 and 1 ($0 < n < 1$). If $n = 0$, it behaves as a pure resistor and $n = 1$, it acts as an ideal capacitor. Nano biocomposite coated samples polished at 400 and 800 shows the n value at 0.69 and 0.62 respectively. Coated 316L SS sample polished at 600 shows the n value at 0.60 which indicates that the particles are uniformly distributed with less porous on the coated sample. It represents that the nano biocomposite coating at 600 SiC grit polishing avoids the interaction between

metal ions and the solution. This enhanced corrosion resistance due to good adhesion of nano biocomposite coating on the metal surface and interlocking diffusion bonding between nano-HAP and nano TiO₂ after vacuum sintering.

Table 1 shows coated samples at 600 SiC grit polishing has high impedance, low capacitance, and enhanced polarization resistance when compared to other coated and uncoated 316L SS sample. This enhanced protection on 316L SS metal surface achieved by the formation of nano biocomposite layer through strong adhesion properties of nano TiO₂ on the metal surface and diffusion bonding between nano-HAP and nano TiO₂ after vacuum sintering. The electrochemical studies proved that the enhanced corrosion resistance obtained on 316L SS sample polished with 600 SiC grit.

Table i EIS spectra fitted values for uncoated and nano biocomposite coated 316L SS polished at different SiC grit after vacuum sintering at 700°C for 1 h in Hank's Solution.

Coating condition	$ Z $ (ohm) $\times 10^4$	R ₁ (ohm)	R ₂ (ohm)	n _{coat}	Q ₁ (F s ⁿ)	R ₃ (ohm)	C ₃ (F)
Uncoated	1.7743	56.41	-	-	-	1.912×10 ³	6.763×10 ⁻⁵
400 SiC	4.7899	16.01	7.328×10 ¹²	0.69	8.092×10 ⁻¹⁰	7.025×10 ⁹	1.104×10 ⁻¹²
600 SiC	29.7711	10.13	9.998×10 ¹⁵	0.60	5.115×10 ⁻⁸	2.143×10 ¹³	7.254×10 ⁻¹⁵
800 SiC	18.0147	12.25	9.451×10 ¹⁴	0.62	6.349×10 ⁻⁹	4.178×10 ¹¹	3.161×10 ⁻¹⁴

Fig.vii shows that the cyclic potentiodynamic polarization studies of for uncoated and nano biocomposite coated 316L SS polished at different SiC grit after vacuum sintering at 700°C for 1 h in Hank's Solution. The breakdown potential (E_b) and repassivation potentials (E_p) for uncoated 316L SS sample were found to be at +0.413 and -0.245 (vs. SCE) respectively. The E_b values for nano biocomposite coated 316L SS polished at 400, 600 and 800 SiC grit were found to be +0.463, +0.668 V and +0.627 (vs. SCE) and corresponding E_p values are obtained at -0.164, +0.132 V and -0.032 (vs. SCE), respectively. All the coated samples showed a higher shift in E_b values towards the nobler direction indicating improved corrosion resistance performance of the coating. The breakdown and repassivation potentials indicate that enhanced corrosion resistance performance of all the bioceramic coated samples than the uncoated 316L SS sample. Because of bioceramic coating present on the metal surface, hinders the interaction between metal surfaces with Hank's solution.

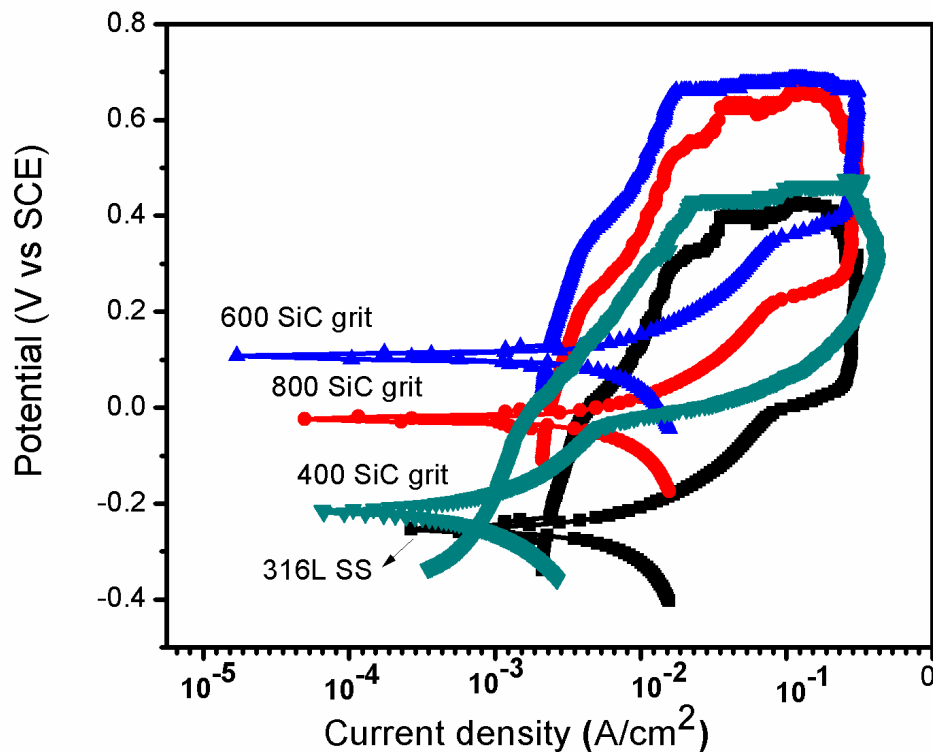


Fig.vii Cyclic potentiodynamic polarization study for uncoated and nano biocomposite coated 316L SS polished at different SiC grit after vacuum sintering at 700°C for 1 h in Hank's Solution.

The hysteresis loop area for all the coated samples are smaller than the uncoated 316L SS sample. The hysteresis loop formed for coated sample polished at 600 SiC grit was very small when compared with other coated sample. It indicates the formation of a stable coated layer with strong adhesion which prevents the breakdown of the coatings. Nano biocomposite coated sample polished at 600 SiC grit shows enhanced electrochemical parameters (both E_p and E_b) compared to other coated samples. It shows that improved corrosion resistance behaviour due to the formation of the protective and stable coated layer on the 316L SS sample.

All the result shows that nano biocomposite coated 316L SS sample polished at 600 SiC grit shifted to nobler potential than the uncoated sample. Biocomposite coated 316L SS sample polished at 600 SiC grit shows high E_{corr} value (0.029 V) when compared with all other coated and uncoated 316L SS samples. Hence, nano biocomposite coated 316L SS sample polished at 600 SiC grit shows that improved corrosion resistance behaviour due to the formation of protective coating on 316L SS sample.

F. Mechanical characterization of the coating

The adhesive strength of the nano biocomposite coated 316L SS samples polished at different SiC grit were tested by tape adhesion test (ASTM D3359). In the case of nano biocomposite coated 316L SS sample polished at 400 and 800 have less adhesion property with sample and delamination occurred after the test and it could be categorized by the 4B grade. The improved adhesive strength was obtained on biocomposite coated sample polished at 600 SiC grit as no delamination occurred after the test representing that the coating was stable and this could be

categorized using the maximum grade of 5B. In nano biocomposite coated sample polished at 600 SiC grit enhanced adhesion strength occurred due to the presence of suitable surface roughness. Then, nano HAP and nano TiO₂ forms a very good chemical bonding with metal surface, which was also enhanced by vacuum sintering. Increased adhesive strength is most important for clinical applications [25].

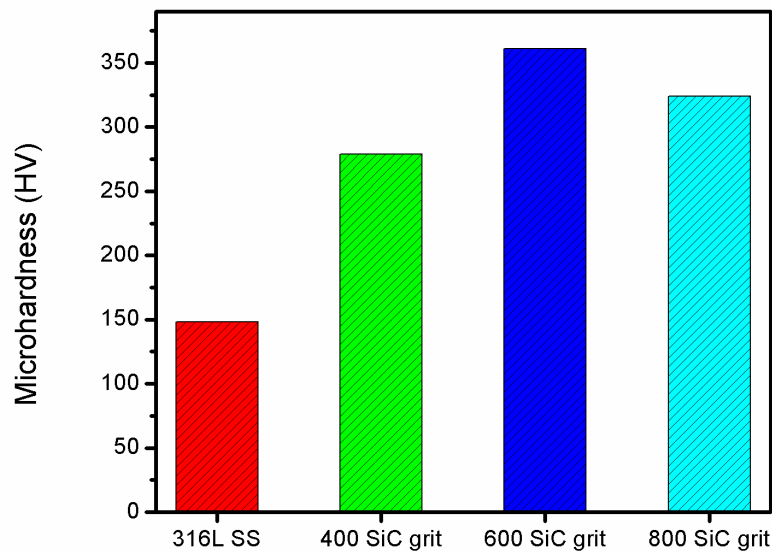


Fig.viii Vickers microhardness for uncoated and nano biocomposite coated 316L SS polished at different SiC grit after vacuum sintering at 700°C for 1 h.

Implants meet intricate physiological environment not only have good biological properties but also obtain adequate mechanical strength for durable performance [26]. Results from Vickers microhardness for uncoated and nano biocomposite coated 316L SS polished at different SiC grit polished shown in Fig.8. The obtained hardness value for uncoated and nano biocomposite coated 316L SS samples polished at 400, 600 and 800 SiC grit were found to be 148 HV, 279 HV, 361 HV and 324 HV respectively. The coated samples showed significantly ($P < 0.05$) enhanced hardness compared to uncoated 316L SS sample. Nano biocomposite coated 316L SS polished at 600 SiC grit shows higher mechanical strength ($P < 0.05$) than the other coated and uncoated samples. The higher mechanical strength obtained on coated samples polished at 600 SiC grit due to enhanced adhesion strength in the existence of suitable surface roughness along with nano HAP and nano TiO₂ after sintering.

G. Cell culture studies

Fig. ix shows the cell viability of uncoated and nano biocomposite coated 316L SS polished at different SiC grit. The cell viability for an uncoated sample is 65.92% which is less when compared to nano biocomposite coated sample. In contrast, an increase in cell viability for 316L SS coated sample polished at 600 SiC grit (98.05%) when compared to other coated samples. The obtained data shows that the cell viability on coated sample polished at 600 SiC grit was significantly higher ($p < 0.05$) compared to other coated samples, while no significant difference was

found between coated samples polished at 400 and 800 SiC grit. Enhanced cell viability obtained at 600 SiC grit polished coated 316L SS sample by very strong surface protection.

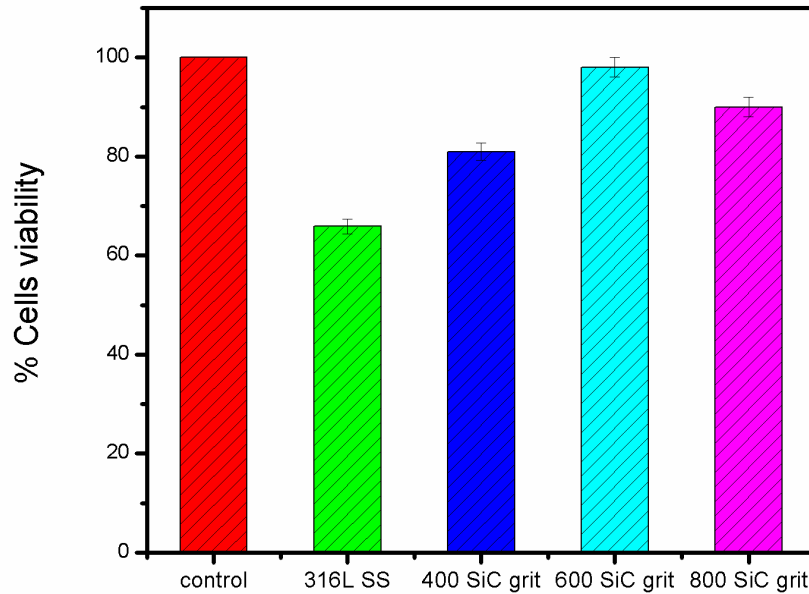


Fig. ix Cell viability by MTT assay for uncoated and nano biocomposite coated 316L SS polished at different SiC grit after vacuum sintering at 700°C for 1 h.

The mechanical strength of the coating was increased by presence of suitable surface roughness after polishing with 600 SiC grit and also presence of nano HAP with nano TiO₂ in nano biocomposite coating. Thus cells which were attached on coated sample polished at 600 SiC grit proliferated more actively and covered the entire surface area. This result indicates that the cell proliferation on coated sample polished at 600 SiC grit for all stages (24h, 48h and 72h) were significantly higher ($p < 0.05$) compared to other coated samples (Fig. x). The higher proliferation was due to higher mechanical strength by the presence of nano TiO₂ along with biocompatible nano-HAP on the metal surface. Another reason is the higher nano roughness created by coating which is in accordance with surface roughness. Wei et al. reported that the rough surface is favored for cell adhesion [27]. Hence, it can be concluded that the factors such as corrosion resistance, surface topography, surface roughness and mechanical strength of the coating influence the cell attachment and the proliferation process.

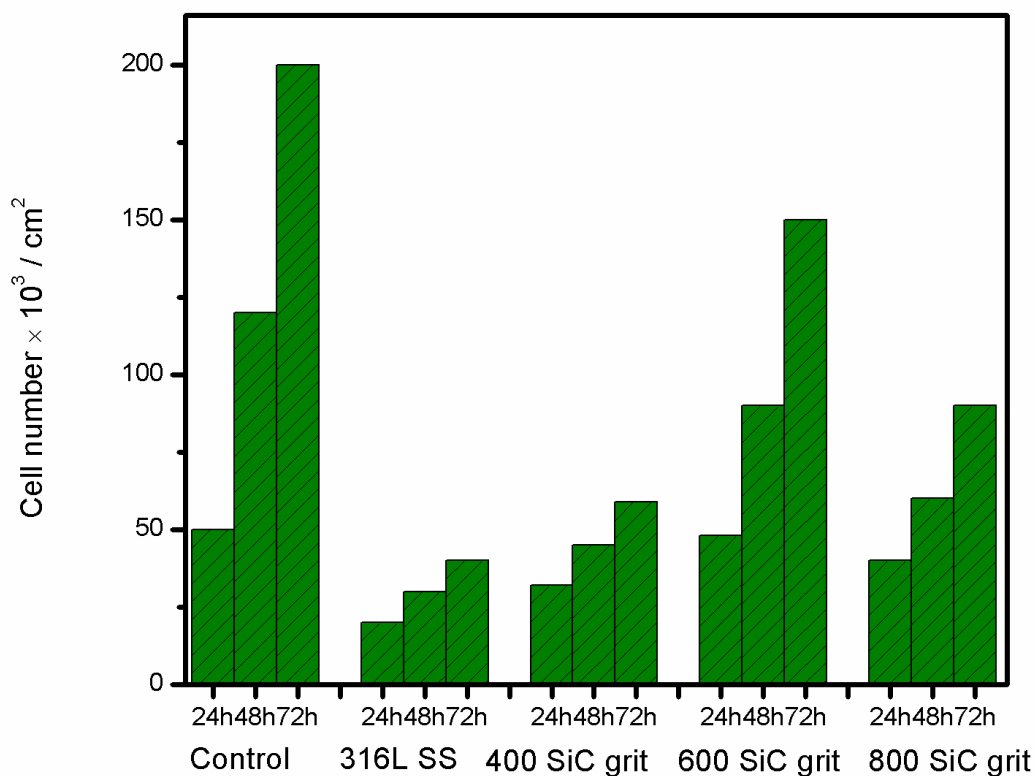


Fig.x MC3T3-E1 osteoblast cell proliferation after 24, 48 and 72 h of culture on uncoated and nano biocomposite coated 316L SS polished at different SiC grit.

IV. CONCLUSIONS

In this study, nano biocomposite coating on 316L SS polished by different SiC grit were successfully achieved by a simple and inexpensive electrophoretic deposition technique. Presence suitable surface roughness along with nano HAP and nano TiO₂ in the biocomposite improves the adhesion strength of the coating on 316L SS metal implant. The XRD and FT-IR results confirmed the structural and the functional groups of the coatings. Electrochemical characterizations studies revealed OCP and breakdown potentials of coated sample polished at 600 SiC grit shifted towards the nobler direction when compared to other coated and uncoated 316L SS sample. EIS studies confirmed an increase in impedance, low capacitance and enhanced polarization resistance for coated 316L SS sample polished at 600 SiC grit was electrochemically stable and prove improved corrosion resistance in Hank's solution. The nano biocomposite coated sample polished at optimized condition exhibited high adhesion and hardness strength than other coated samples. The in vitro cell culture studies at optimized condition shows that coated sample with interface facilitate high cell adhesion and enhanced cells proliferation compared with that of other coated and uncoated samples. Based on the above findings, nano biocomposite coated 316L SS implant polished at 600 SiC grit has prevents the corrosion and enhanced biocompatibility as well as faster bone growth which is considered as the potential candidate as a coating material for 316L SS bio-implants.

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