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Electrodeposition of HAP/TiO_2 on type 316L stainless steel for orthopedic application

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The effect of nano HAP/TiO_2 coating on type 316L stainless steel was investigated. Type 316L stainless steel substrates were electrophoretically coated for various coating time spans and voltages. The coated samples were sintered in an air furnace at 700°C for 1 h. The electrochemical corrosion test was performed in simulated body fluid to evaluate the coating impact on the corrosion behavior of the substrates. The results showed that the coated substrate at 30 V for 60 sec provides a uniform, crack-free coating with enhanced adhesion strength, improving the corrosion resistance of the substrate. *In vitro* study with MC3T3-E1 osteoblast cells showed improved cell attachment and better cells proliferation compared to uncoated sample, by controlling the release of metal ions.

Keywords: Nano biocomposite, Corrosion resistance, Electrophoretic deposition, Type 316L stainless steel.

INTRODUCTION

Metallic implants such as titanium, type 316L stainless steel (316L SS) and magnesium alloy are extensively utilized for load bearing applications, which can provide many solutions to problems such as osteoarthritis, damaged hard tissues and bone fractures in dental and orthopedic applications [1-3]. However, the corrosion behavior of metallic implants such as pitting corrosion, crevice corrosion and fretting corrosion has been observed in the physiological body fluid [4, 5]. It can lead to the release of ions into the tissue environment. Hence, the metal that is to be used as implant must possess high corrosion resistance and better biocompatibility. Till today type 316L SS is the most commonly used biomaterial to orthopedic prostheses due to its excellent corrosion resistance and biocompatibility [6].

In order to enhance the cell-implant material interaction and to increase the longevity of the material, bioactive ceramic-based coating has been applied to type 316L SS. Among the protective and biocompatible coatings, nano hydroxyapatite (HAP) is an attractive biomaterial for human hard tissue implants since it contains a similar chemical composition to that of natural bones and teeth [7, 8]. Nano HAP plays an excellent role in biomedical applications owing to its excellent biocompatible, osteoconductive and bioactive properties, and its close resemblance to the mineral component of bone tissue [9, 10]. Though nano HAP can bond directly to natural bones, its brittle nature and poor strength limit its clinical applications under load-bearing conditions.

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To overcome these problems and provide better corrosion resistance, incorporation of reinforcing materials like nano TiO_2 is used [11, 12]. Nano TiO_2 is a biocompatible, bioinert material and it was added as a matrix to produce composite coatings possessing higher chemical stability. It has also been reported that TiO_2 coating is biocompatible and improves the calcium and phosphate precipitation onto the metal surface [13].

Many researchers have reported different methods for obtaining of bioceramic coatings on implants, such as sol-gel, micro-arc oxidation, ion beam sputtering, air brush spraying, electrophoretic deposition (EPD), plasma spraying, etc. [14, 15]. Among many techniques, electrophoretic deposition has been found to be an efficient technique to fabricate nano triphasic bioceramic coatings from suspensions and it is an easier process for obtaining nano structural deposits [16, 17]. Thus, nano biocomposite (nano HAP/nano TiO_2) coatings provide the implants with bioactivity, better chemical stability and enhanced adhesion property. So far, there are no reports on nano hydroxyapatite/nano TiO_2 composite coating on type 316L SS. The present work investigates the development of nano biocomposite coating on type 316L SS by electrophoretic deposition process (EPD). EPD was carried out at different voltages from 10 to 40 V for different time intervals (30 to 90 sec) to find out the optimum voltage and its respective time using corrosion studies.

EXPERİMENTAL

Substrate preparation

Type 316L SS substrate was obtained from Steel Authority of India Limited (ASTM F138- 13a).

Type 316L SS substrate was used as a cathode with a size of 10 mm × 10 mm × 2 mm and 314 SS was used as an anode with a size of 11 mm × 8 mm × 0.5 mm. The substrates were abraded using grit emery paper starting by ranges from 120 to 1000 followed by soap solution treatment, ultrasonically cleaned with acetone and dried at room temperature.

Suspension preparation

A 2% suspension of nano biocomposite in isopropyl alcohol (IPA) was taken in a 100-ml beaker. Iodine (0.1 g) was added to the suspension. Iodine acts as a dispersant to stabilize the suspension and also prevents the agglomeration of the nano particles in the suspension. The suspension was kept for one day in undisturbed condition to get uniform dispersion of the particles. Then, the suspension was sonicated for 30 min to get uniform suspension of particles for the EPD coating process.

Deposition of nano biocomposite

Deposition of nano biocomposite on type 316L SS substrate was achieved by EPD process at room temperature. Two electrodes (type 316L SS and 314 SS) were immersed in a suspension and the distance between these two electrodes was less than 1 cm in order to achieve optimum particle deposition on the exposed electrode surface area. Before deposition one edge of the sample was masked with a Teflon tape and deposition was carried out on other 1 cm^2 surface areas. After the coating process, the coated substrates were dried at room temperature. The dried substrates were subjected to a sintering process at 700°C in an air furnace at a heating rate of 10°C/min for 1 h. The samples were removed after cooling from the furnace and were stored in vacuum desiccators. The coating parameters as voltage and time were optimized by means of electrochemical studies.

Preparation of SBF

Simulated body fluid (SBF) with ion concentration nearly equal to that of human blood plasma, modified fluids with different ion concentrations shown in Table 1, were prepared by dissolving reagent grade NaCl, $CaCl_2$, KCl, $NaHCO_3$, NaH_2PO_4, glucose, $MgSO_4.7H_2O$, $MgCl_2.6H_2O$, $Na_2HPO_4.2H_2O$ in distilled water. In this study, the SBF solution does not contain organic materials which would be appropriate to systems *in vivo*.

Characterization of coated substrate

Electrochemical impedance spectroscopic (EIS) and potentiodynamic polarization studies were performed using a Biologic-SP 240 (EC-lab Version 10.37) interfaced with a computer. A conventional three-electrode cell was used for electrochemical measurements according to ASTM guidelines (G61–86). A graphite electrode, saturated calomel electrode (SCE) and the nano biocomposite coated 316L SS sample were used as a counter electrode, reference electrode and working electrode, respectively. Electrochemical measurements were carried out using an SBF solution with pH of 7.4 at room temperature. EIS was carried out by applying a frequency range from 100 kHz to 10 mHz. Using Nyquist plot, the polarization resistance (R_p) was calculated. Potentiodynamic polarization study was initiated with a starting potential less than 0.250 V of OCP at a scan rate (dE/dt) of 10 mV/min and the parameters like corrosion potential (E_{corr}) and corrosion current density (I_{corr}) were measured. The microhardness properties of the composite coated samples (ASTM E92-17) were measured using Vickers microhardness tester (Ever One Enterprises Limited, India). A loading force of 100 gram Force (gF) was applied for 5 sec to record the measurements.

Table 1. Chemicals used for preparing SBF

Order	Chemicals	Purity (%)	Weight (g/ L)
1	NaCl	99.5	8.00
2	$CaCl_2$	96.0	0.14
3	KCl	99.5	0.40
4	$NaHCO_3$	99.5	0.35
5	NaH_2PO_4	99.5	0.10
6	Glucose	99.5	1.00
7	$MgSO_4.7H_2O$	99.0	0.06
8	$MgCl_2.6H_2O$	96.0	0.10
9	$Na_2HPO_4.2H_2O$	96.0	0.06

The X-ray diffraction (Bruker model D8, Germany) analysis was conducted using Cu kα radiation, and λ of 1.5406Å. The 2θ angles were swept from 10 to 70° in steps of one degree. The surface roughness of the composite coated 316L SS samples was measured by a non-contact optical surface profiler (BRUKER model CONTOUR GT, Germany). Average surface roughness (R_a) measurements were taken at ten various locations on the coated samples to get an accurate assessment. All the measurements were taken perpendicular to the machine markings.

MC3T3-E1 osteoblast cells were obtained from the National Centre for Cell Sciences, Pune (NCCS) and used to assess cell viability on nano biocomposite coated and uncoated 316L SS substrate. *In vitro* cytotoxicity study was carried out using MTT assay [18].

RESULTS AND DISCUSSION

Electrochemical studies

EIS technique was used to characterize the electrochemical nature of uncoated 316L SS and nano biocomposite coated type 316L SS substrates. Figs. 1a and 1b show the Nyquist plots of uncoated 316L SS and nano biocomposite coated type 316L SS substrates at different coating voltages and time spans. The polarization resistance (R_p) and impedance (|Z|) values were higher for coated substrate than for the uncoated type 316L SS.

The presence of nano biocomposite outer coating acts as a barrier layer which prevents the metal ions from corrosive attack due to SBF fluids. Thus, coatings provide protection to metallic substrates in the corrosive body fluid atmosphere mainly due to the presence of chloride ions. Table 2 shows the electrochemical parameters for uncoated and nano biocomposite coated type 316L SS substrate at a coating time of 60 sec and different coating voltages.

From Table 2 it can be observed that the maximum resistance and impedance values obtained for 30 V are due to formation of uniform, crack free coating with strong adhesion on the metal surface. In the case of 20 V a thin coating is produced, which can easily cleave and let the SBF solution enter into the metal surface and stimulate a release of metal ions into the solution. Similarly, coating at 40 V yields a thick layer with hair crack formation, which provides a pathway for body fluids to attack the metal ions. This leads to a decrease in polarization resistance and impedance values at higher voltages. Thick coating with more crack formation was observed on the nano biocomposite coated layer on type 316L SS above 40 V. Thus, the best protective coating was formed on type 316L SS at 30 V.

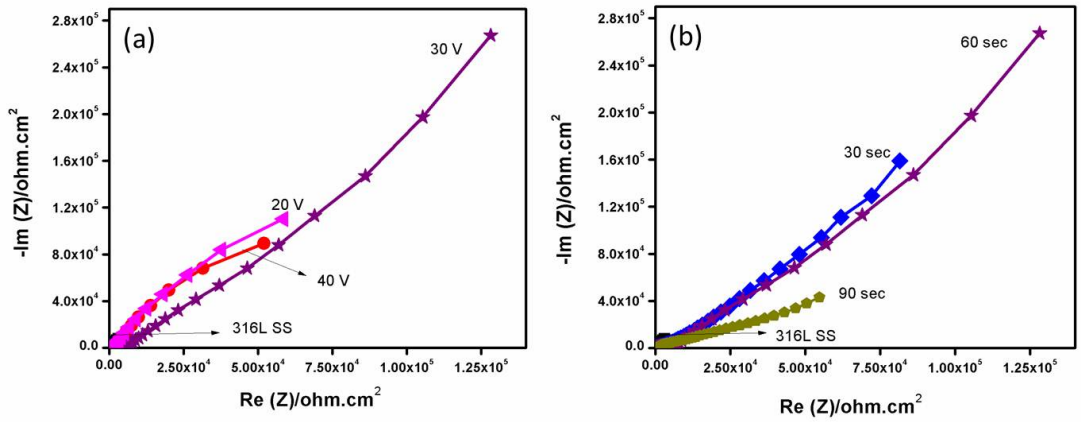

Fig. 1. Nyquist plots of uncoated and nano biocomposite-coated type 316L SS

Table 2. Electrochemical parameters for coated substrates with coating time of 60 sec at different coating voltages

Coating voltage	E_{corr} (mv) *vs* SCE	I_{corr} (mA/cm^2)	R_p (KΩcm^2)	|Z| Ωcm^2
316L SS	-246	-4.33	19.72	17443
20V	-163	-4.91	38.77	123037
30V	-138	-5.81	90.17	266790
40V	-223	-4.76	31.28	91332

Table 3 shows the electrochemical parameters for uncoated and nano biocomposite coated type 316L SS substrate at a coating voltage of 30 V for various coating times. Maximum polarization resistance and impedance was observed at a coating time of 60 sec compared to other coated and uncoated type 316L SS substrates. At coating time spans of 30 sec and 90 sec a thin, respectively, thick coating is produced with crack formation. So, at these coating time spans the SBF solution contacts with the metal surface and initiates a corrosion reaction. Hence, poor resistance and impedance was observed at this condition. With coating times above 90 sec, thick coating with more crack formation was observed on the nano biocomposite coated layer on type 316L SS. However, a highly protective bioceramic coating on type 316L SS substrate was observed at a coating time of 60 sec. Fig. 2a shows the potentiodynamic polarization curves of uncoated 316L SS and nano biocomposite coated type 316L SS substrates at a coating time of 60 sec at different coating voltages. Fig. 2b shows the potentiodynamic polarization curves of uncoated 316L SS and nano biocomposite coated type 316L SS substrates at a coating voltage of 30 V with different coating times. Potentiodynamic polarization curves can be seen in Fig. 2 and the electrochemical parameters including E_{corr} and I_{corr} obtained from the Tafel curves are shown in Tables 2 and 3. It can be noted that all coated substrates showed higher values of I_{corr} and E_{corr} than uncoated ones. It also can be observed that the passivation curves of the coating at 30 V and 60 sec show higher electrochemical stability on the dynamic nature of the corrosion process, when compared with other coated and uncoated substrates. Hence, the best protective coating is obtained on type 316L SS substrate at 30 V for 60 sec.

Mechanical characterization

Implants meeting an intricate physiological environment not only should have good biological properties but also adequate mechanical strength for durable performance [19]. The mechanical strength of nano biocomposite coated and uncoated type 316L SS samples were measured by Vickers microhardness test. The hardness values for bioceramic coated and uncoated type 316L SS substrates are shown in Fig. 3.

Table 3. Electrochemical parameters for coated substrates with coating voltage of 30 V at different coating times.

Coating time	E_{corr} (mv) *vs* SCE	I_{corr} (mA/cm^2)	R_p (KΩcm^2)	|Z| Ωcm^2
316L SS	-246	-4.33	19.72	17443
0 sec	-182	-4.85	47.93	159218
60 sec	-138	-5.81	90.17	266790
90 sec	-234	-4.53	41.01	43458

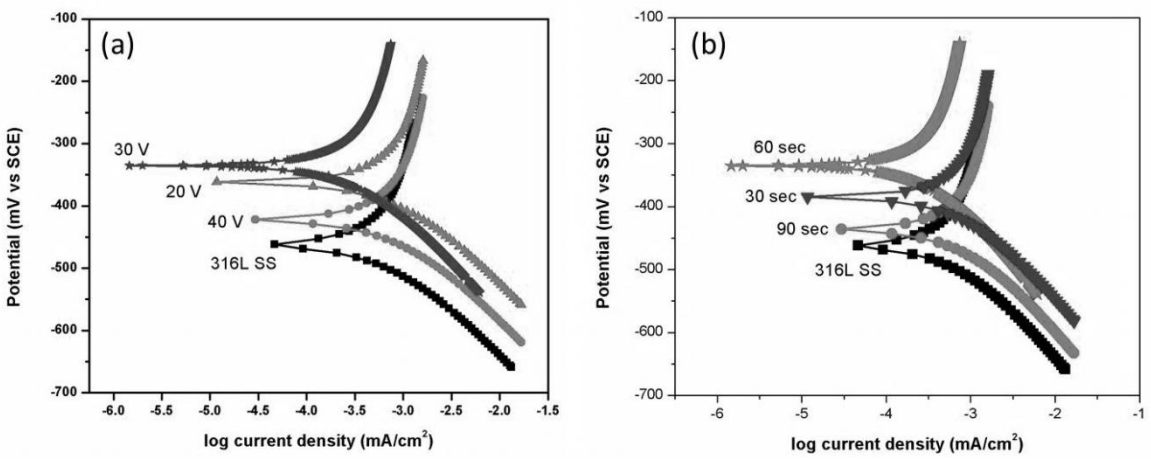

Fig. 2. Potentiodynamic polarization curves

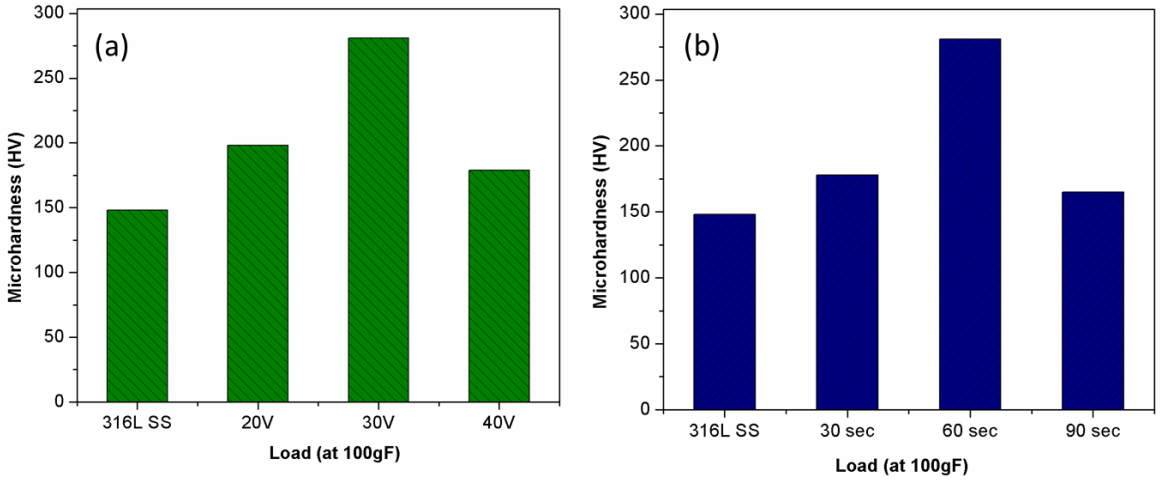

Fig. 3. Vickers microhardness for uncoated and nano biocomposite coated type 316L SS

Fig. 3a shows the Vickers microhardness for uncoated 316L SS and nano biocomposite coated type 316L SS substrates at a coating time of 60 sec with various coating voltages. Fig. 3b shows the Vickers microhardness for uncoated 316L SS and nano biocomposite coated type 316L SS substrates at a coating voltage of 30 V with various coating times. Vickers microhardness test was carried out on nano biocomposite coated type 316L SS after sintering at 700°C in an air furnace. From Fig. 3 it can be observed that coated substrates showed significantly enhanced hardness compared to uncoated 316L SS sample. The higher mechanical strength observed on nano biocomposite coated type 316L SS at 30 V and 60 sec is due to the enhanced adhesion strength of the uniform crack-free coating after sintering. Thus, the coated sample at 30 V and 60 sec was highly suitable for biomedical application.

XRD analysis

Fig. 4 shows the XRD spectra of nano biocomposite coated type 316L SS substrate obtained at 30 V and 60 sec after sintering. The peaks with maximum intensity detected at (002), (210), (211), (300), (202), (310), (222), (213) and (004) reflection planes, were assigned to nano HAP. The obtained results were in good agreement with the JCPDS file card No. 09-0432.

Generally, TiO_2 crystallites are present in three forms: brookite (orthorhombic), anatase (tetragonal), and rutile (tetragonal). Brookite and anatase phases are stable at room temperature and they are transformed to rutile phase at 700°C [20]. The remaining peaks corresponding to rutile nano TiO_2 were observed at (101), (004), (200) and (211) (JCPDS file no.21-1276) [21]. This rutile phase of nanoTiO_2 enhances the bioactivity and cell proliferation when compared to anatase and brookite. No other peak was observed after sintering of nano biocomposite coated type 316L SS substrate. Thus, the XRD spectra confirmed the presence of bioactive nano HAP and bioinert nanoTiO_2 crystalline phases in nano biocomposite coated type 316L SS substrate.

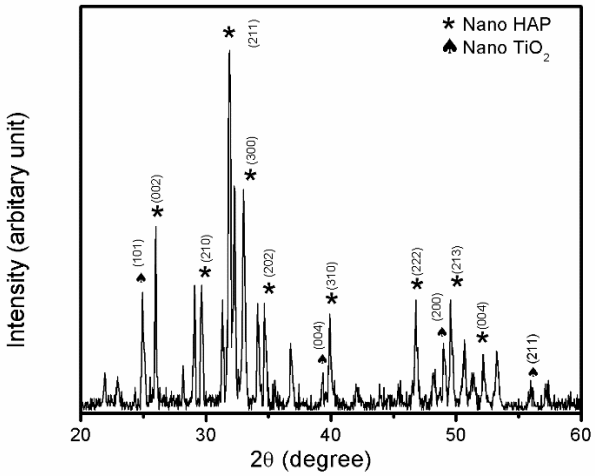

Fig. 4. XRD spectra of nano biocomposite-coated type 316L SS

Surface roughness analysis

Surface profilometer image of nano biocomposite coated type 316L SS substrate obtained at 30 V and 60 sec after sintering is shown in Fig. 5. Overall surface roughness of the coated substrate was recorded using a surface profilometer. The average surface roughness for the nano biocomposite coated substrate was found to be 0.497 ± 0.06 µm. The enhanced surface roughness obtained for the nano biocomposite coated substrate is due to interlocking of the rough porous surface of nano HAP with nano TiO_2 powder. Surface roughness is one of the important characteristics for cell attachment and proliferation.

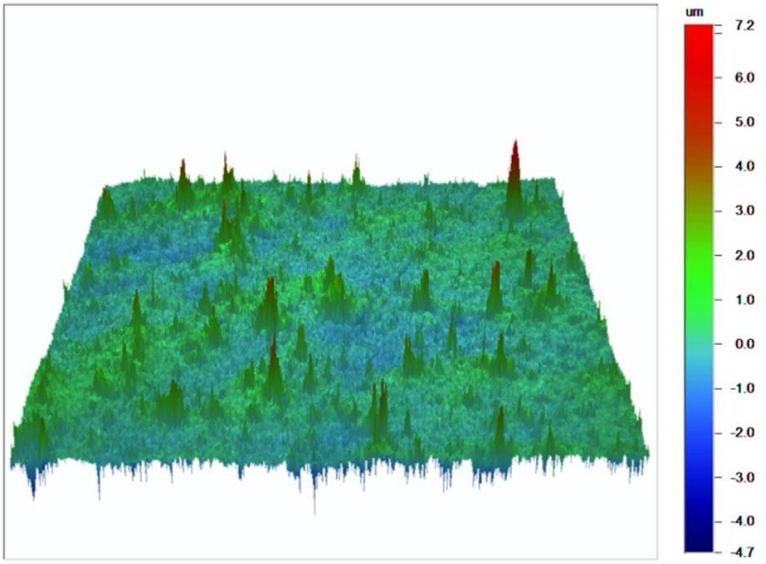

Fig. 5. Surface profilometer image of nano biocomposite coated type 316L SS substrate

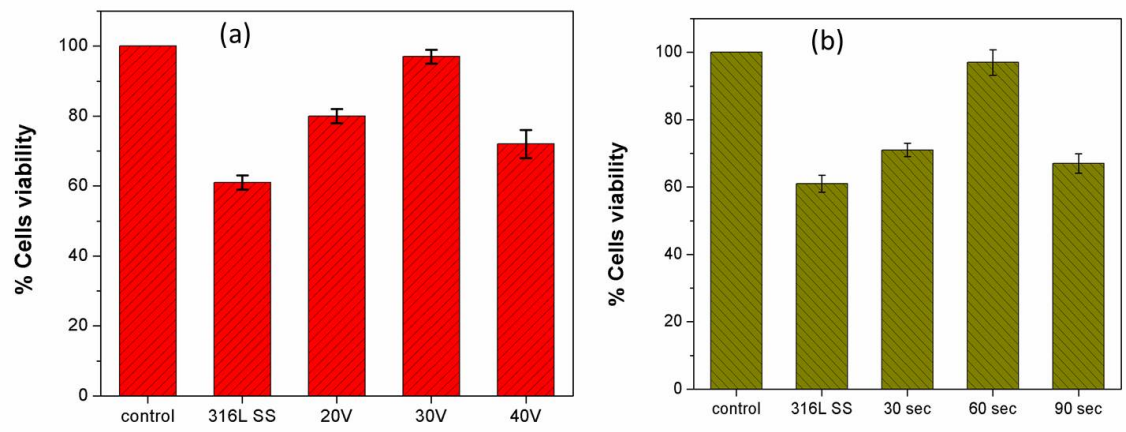

Fig. 6. Cell viability with MC3T3-E1 osteoblast

The stability of the implant can be improved by increasing the surface roughness of the coatings [22]. This can provide a suitable environment for the bone cells to attach on the surface of the implant. Thus, nano biocomposite coated type 316L SS substrate at optimized voltage of 30 V and time of 60 sec is suitable for bone bonding.

Cell culture studies

Surface roughness, corrosion resistance and mechanical property of the coated layer are playing very important roles in cell attachment, proliferation, and diversity of MC3T3-E1 osteoblast cells. In addition to that the greater surface area on the coated substrate can capture more cells [23]. Fig. 6a shows the cell viability of MC3T3-E1 osteoblast cells for uncoated 316L SS and nano biocomposite coated type 316L SS substrates at a coating time of 60 sec with various coating voltages. Fig. 6b shows the cell viability of MC3T3-E1osteoblast cells for uncoated 316L SS and nano biocomposite coated type 316L SS substrates at a coating voltage of 30 V with various coating times. Cell viability was measured using MC3T3-E1 osteoblast cells for uncoated and nano biocomposite coated type 316L SS samples after culturing for 72 h.

The cell viability of the coated substrate was higher than that of the uncoated one. Uncoated substrate easily releases metal ions and affects the cells viability due to non-protected surface. Better performance of the coated substrate was observed when compared with uncoated substrate due to the protective layer of the coating. Crack free coating with a very good protective layer and rougher surface along with superior mechanical strength were achieved at 30 V and 60 sec compared with other coated substrates. It was found that better cell viability (98%) was obtained for the coated type 316L SS substrate at 30 V and 60 sec than for other coated and uncoated substrates. All the above factors lead to improved osseointegration properties of the coated substrate at 30 V and 60 sec.

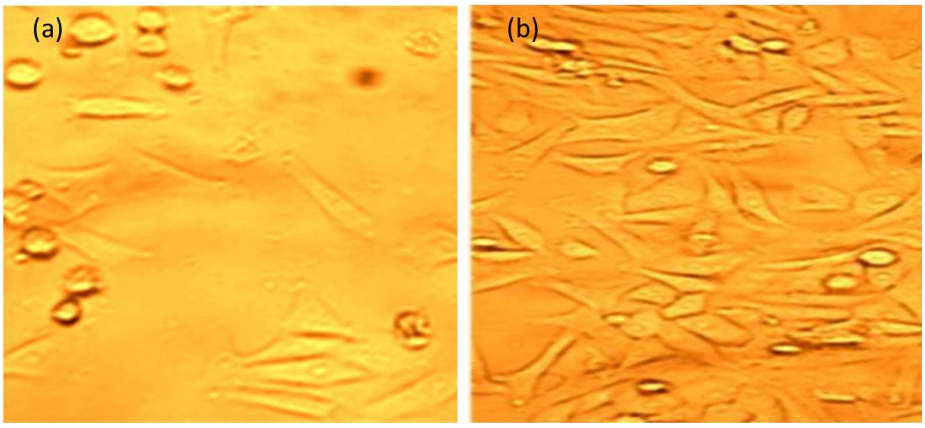

Fig. 7. Histomorphology cell image for nano biocomposite coated type 316L SS

Osteoblast MC3T3-E1 cells exposed to uncoated and coated type 316L SS implants are shown in Fig. 7. The histomorphology cell image for nano biocomposite coated type 316L SS substrate at 30 V and 60 sec is seen. Histomorphology was taken for cell growth using osteoblast MC3T3-E1 cells after 72 h. This histomorphology shows that more cells are present on the coated substrate than on uncoated 316L SS.

The presence of a good number of live cells which proliferate on the coated substrate because bioactive nano HAP is a natural component which is plentifully found in bone matrix, favors osseointegration of cells. The presence of biocompatible nano TiO_2 provides strong adhesion of the bioceramic through the coating on type 316L SS substrate. The number of cells per unit area of the coated substrate increased with surface roughness. Nano biocomposite coating provides uniform crack free coating with strong adhesion and improved corrosion resistance with good surface roughness at 30 V and 60 sec.

Thus, cells which were attached on coated substrate proliferated more actively and covered the entire surface area at the optimized conditions. Thus, higher cell viability and proliferation was achieved on coated type 316L SS substrate at 30 V and 60 sec. Hence, nano biocomposite coated substrate at 30 V and 60 sec could be considered as a better coating to improve the life span of implants in orthopedic applications.

CONCLUSIONS

Nano biocomposite was coated by EPD on type 316L SS substrates using different voltages and coating time spans and was subsequently sintered at 700°C in an air furnace. The electrochemical studies in an SBF solution revealed that coated substrates at 30 V and 60 sec exhibit higher corrosion resistance when compared to other coated and uncoated type 316L SS substrates. The XRD analysis confirms the presence of two bioceramics: nano HAP and nano TiO_2 in the coating. The composite coating at optimized conditions exhibited enhanced microhardness strength. *In vitro* cell culture studies showed that the coated substrate at the optimized conditions facilitated cell viability and enhanced cells proliferation. Hence, nano biocomposite coated type 316L SS substrate at 30 V and 60 sec could improve the life time of implants by enhanced bioactivity and faster bone growth, as well as enhanced corrosion resistance.

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