In-vitro biocompatibility and corrosion resistance of electrochemically assembled PPy/TNTA hybrid material for biomedical applications

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ABSTRACT

Nanostructured hybrid materials composed of inorganic and organic constituents of different chemistry and functionality have attracted wide range of biomedical applications. The uniform electrodeposition of polypyrrole into titania nanotube arrays was achieved by normal pulse voltammetry technique in lithium perchlorate electrolyte by varying the pulse period. The electrochemically assembled polypyrrole/titania nanotube arrays (PPy/TNTA) surface was characterized by structural characterizations including attenuated total reflectance -fourier transform infrared spectroscopy, Raman and X-ray photoelectron spectroscopy analysis. Morphological study carried out by high resolution scanning electron microscopy demonstrates the influence of varying pulse period in achieving the controlled deposition of polypyrrole into the nanotube framework. Cyclic voltammetry study reveals the electroactive nature of the hybrid material. The deposition of polypyrrole enhanced the corrosion resistance of TNTA as evidenced from the lower icorr value observed for PPy/TNTA. The corrosion protection behavior of the hybrid material revealed from the electrochemical impedance spectroscopic studies was clearly noticed from the increase in impedance and maximum phase angle values. Further in-vitro cell culture studies were carried out using MG63 osteoblast cells to evaluate the biocompatibility of the hybrid material. Noticeable improvement in corrosion protection and biocompatibility performance suggest the possible application of PPy/TNTA hybrid material for biomedical applications.

1. Introduction

Over the past decades, there is an increasing demand for biomedical implants and the researchers have been trying to develop a appropriate bioactive implant material for use in the human body [1,2]. Titanium and its alloys has become the material of choice in medical applications due to unique combination of favorable material properties like strength, biocompatibility and corrosion resistance compared to other metallic implants [3,4]. However, being bioinert in nature, it cannot bond directly to living bone after implantation. In order to solve the above stated issue, modifying the surface to improve the bio performance of titanium implants is essential [5]. Recent researches in this field have highlighted the importance of altering the surface morphology to nanoscale by the formation of TiO2 nanotubes to better mimic the surface features of natural bone in the nanoscale regime and to favor a positive cellular interaction with cells [6–8].

Polypyrrole (PPy), one of the promising conducting polymers has been highly investigated for biomedical applications because of its significant features like conductivity, ease of preparation, good biocompatibility and chemical stability [9–11]. PPy can be used for the development of efficient biomimetic systems such as artificial muscles [12], drug delivery [13], scaffold material for nerve regeneration [14] and compatible with wide range of cell types in-vitro [15] and in vivo [16,17]. PPy is being employed as a biocompatible and electroactive polymeric substrate for the manipulation of cell growth, adhesion and proliferation of numerous tissues like bone, skin, cartilage etc due to its capability to electronically control a wide range of physical and chemical properties [18]. Despite all the advantages, PPy has some inherent shortcomings such as poor mechanical strength, processability, which hinders its application in biomedicine. One of the known drawbacks of the polypyrrole is its poor adhesion to oxidizable metals.
(Al, Fe, Zn, Ti) [2]. Development of hybrid nanomaterial by combining inorganic and organic components into a single material has been one of the recent approaches in attempting to overcome the above limitations.

Hybrid assemblies with ordered structure, distinct morphologies and good electrical contact with substrate can be developed by infiltrating the polypyrrole into TiO2 nanotube framework. The properties of both oxide semiconductor and the organic counterpart can be combined through the formation of hybrid structures. Among the different approaches reported such as chemical [19] thermal [20] and UV photopolymerization [21] to develop homogenous hybrid materials, electropolymerization is a particular versatile method to deposit the conducting polymer as it can be directly electrodeposited on the inorganic nanostructure which acts as the working electrode. The fundamental understanding of the site selective filling of the nanotubes with polypyrrole using pulse current approach was reported by Kowalski et al. [22]. Ngaboyamahina et al. [23] reported the deposition of polypyrrole into titanium nanotube arrays in dark using lithium perchlorate electrolyte, signifying the influence of back ground salt in the rate of deposition of polypyrrole. However, controlled experimental conditions are required for the effective and homogenous electrodeposition of conducting polymer into TiO2 nanostructures. The electrochemically synthesized NTA/PPy hybrid system has been used for solar energy [24] and super capacitor [25] applications as revealed from literature. Despite the fact that titania nanostructures and polypyrrole were distinctly used for many implant applications, to the best of our knowledge, the use of polypyrrole/titania nanotube arrays (PPy/TNTA) hybrid system to modify the bioimplants in terms of electrochemical stability and biocompatibility is rather scarce.

Hence, the main goal of the present study is to fabricate PPy/TNTA hybrid material for biomedical application. Indeed, we demonstrate normal pulse voltammetry technique by varying the pulse potential to achieve a homogenous deposition of polypyrrole on titania nanotube frame work. The phase composition, morphology, surface roughness and wettability of the hybrid material were studied. Further the paper mainly extends the investigation to focus on the analysis of the electrochemical corrosion behavior of PPy/TNTA using potentiodynamic polarization and electrochemical impedance spectroscopy studies. We also evaluated the biocompatibility performance of PPy/TNTA on MG63 osteoblast cells via In-vitro cell culture studies.

2. Experimental section

2.1. Materials

Commercially pure titanium (Cp-Ti) of thickness (2 mm) was obtained from Ti Anode Fabricators, Chennai. Pyrrole (monomer), lithium perchlorate, glycerol and all other chemicals used in the present study were purchased from Sigma Aldrich chemical company. The pyrrole monomer was distilled before use and all other chemicals were used without any further treatment. Double distilled water was used to make all the solutions.

2.2. Fabrication of titania nanotube arrays (TNTA)

Prior to anodization, titanium sheets (1.5 × 2 × 0.2 cm3) were cut and mechanically polished using abrasive silicon carbide (SiC) paper up to 1000 grit to obtain scratch free smooth surface and then rinsed with acetone followed by distilled water in an ultrasonic bath. The specimens were then etched in a mixture of HNO3, HF and H2O (Kroll’s reagent) taken in the ratio of 6:2.5:1.5 for 10 s to remove the natural oxide layer. Titania nanotube arrays were formed on Ti substrate by potentiostatic anodization process using a mixed electrolyte solution of glycerol with 1 wt% NH4F and 20 vol% water [26]. The titanium and platinum were used as the anode and cathode respectively. A constant potential of 20 V was applied between the two electrodes using direct current (DC) voltage power source (M/S Aplab Model L1285) and allows the system to anodize for an hour. After anodization the specimens were washed well with distilled water and air dried. Annealing at 500 °C for 2 h at a heating rate of 2° per minute was done to acquire anatase crystalline phase titania nanotubes.

2.3. Formation of PPy/TNTA hybrid material

The deposition of polypyrrole in to titania nanotube structure was achieved by normal pulse voltammetry electrodeposition method in a three electrode system using TNTA as working electrode, platinum as counter electrode and saturated calomel electrode (SCE) as reference electrode. The aqueous electrolyte medium for electrodeposition was prepared by dissolving 0.1 M pyrrole and 0.2 M LiClO4 in water. The pulse potential was increased from 0.6 to 1.2 V with a pulse potential increment of 0.00105 V. The pulse width was 0.06 s and the pulse period was controlled at 0 and 6 s respectively to achieve the uniform deposition of polypyrrole. After the deposition process the specimen was removed from the medium, washed well with water and finally dried at room temperature. The anodized specimens before and after the deposition of polypyrrole are denoted as TNTA and PPy/TNTA respectively throughout the manuscript.

2.4. Characterization of TNTA and PPy/TNTA hybrid material

2.4.1. Cyclic voltammetry studies

To study the electroactivity of TNTA and PPy/TNTA cyclic voltammetry characterization was carried out in the same 0.2 M LiClO4 supporting electrolyte without pyrrole monomer at a scan rate of 0.05 V/s using three electrode system.

2.4.2. Morphology studies

The morphology of the TNTA and PPy/TNTA specimens were studied using high resolution scanning electron microscope (HR-SEM, Model FEI Quanta FEG 200) at an accelerating voltage of 20 kV. Energy dispersive X-ray spectrometer (EDS) coupled with HR-SEM was used to investigate the elemental composition of the hybrid material. Scanning probe image processor WSxM 4.0 beta software [27] was used to obtain the 3D images of the TNTA and PPy/TNTA. The cross sectional view of the nanotubes was carried out on mechanically cracked samples.

2.4.3. ATR-FTIR studies

To find out the functional groups present on the surface of TNTA and PPy/TNTA, attenuated total reflectance fourier transform infrared (ATR-FTIR) spectra were recorded on Perkin Elmer FT-IR spectrometer spectrum Two with UATR Two Accessory and KBr window in the 400–4000 cm−1 range.

2.4.4. XRD studies

The phase composition of the formed hydroxy apatite over PPy/TNTA surface after 7 days immersion in Hanks’ solution was analyzed using X-ray powder diffractometer (XRD, D8 DISCOVER, Bruker, USA) using Cu Kα radiation at 40 kV and 30 mA at a scan rate of 0.02°.

2.4.5. Wettability and roughness measurements

The water contact angles (CA) of the specimens were measured using contact angle analyzer model Phoenix 300 Plus instrument. Water droplet with a drop volume of 8 μl was positioned on the
sample surface with a syringe and the CA’s were obtained by mea-
suring at different positions on each sample and the average value
was reported. Surface roughness of TNTA and PPy/TNTA was mea-
sured from the 3D images. Image analysis and processing were
done using WSxM 4.0 beta software.

2.4.6. Raman analysis

The Raman spectra were measured using FT-RamA006E, Bruker
RFS 27 instrument at room temperature. The Nd: YAG laser (1064
nm) was used as the excitation source for the measurement.

2.4.7. Adhesion studies

The adhesion strength of PPy to the TNTA was determined by
Pull Off adhesion test using PosiTest Automatic Adhesion Tester
(DeFelsko Corporation) using ASTM D4541-02 procedure E. In
order to compare the results of the test on TNTA, identical PPy
coating at the same polymerization conditions were done on the
Ti substrate.

2.4.8. XPS analysis

The chemical state and elemental composition of the specimens
were analyzed by X-ray Photoelectron Spectroscopy (XPS) S-
ProbeTM 2803 instrument, JAPAN. The XPS spectra were recorded
using Al Kx monochromatized X-ray radiation with 1486.6 eV
energy as excitation source. The source was used to stimulate pho-
toemission of the inner shell electrons on the titanium surface. The
energy from these electrons was then recorded and analyzed for
identification purposes concerning chemical composition of the
specimens.

2.5. In-vitro immersion studies

To evaluate the ability of PPy/TNTA specimen to favor apatite
growth on its surface, immersion studies were carried out by immersing
the specimens for 7 days in freshly prepared Hanks’
physiological solution at room temperature. The ion concentration
of the Hanks’ solution is nearly equal to that of human blood plasma (Na+ = 42.0, K+ = 5.0, Mg2+ = 1.5, Ca2+ = 2.5, HCO3- = 4.2,
HPO4 2- = 1.0, SO4 2- = 0.50 and Cl- = 147.96 mM). The composition
of Hanks’ solution is as follows: 0.185 g CaCl2, 0.4 g KCl, 0.06 g
KH2PO4, 0.1 g MgCl2.6H2O, 0.1 g MgSO4.7H2O, 8.0 g NaCl, 0.33 g
NaHCO3, 0.48 g Na2HPO4 and 1.00 g d-glucose in 1 litre of double
distilled water [28]. While preparing the Hanks’ solution, intensive
care was taken in order to avoid the precipitation and deposition of
the ions at the bottom of the beaker. The solution was renewed at
two day intervals to minimize concentration changes. After soak-
ing for 7 days the specimens were removed from the solution,
washed with distilled water and then dried in a clean work surface.

2.6. Electrochemical characterization

Electrochemical measurements were performed using one com-
partment cell with three electrodes viz TNTA and PPy/TNTA as the
working electrode, platinum as the counter electrode and Satu-
rated Calomel Electrode (SCE) as the reference electrode using
Autolab PGSTAT 302 N Model controlled by personal computer
with dedicated Nova 2.1 software. The experiments were performed
in 250 ml of Hanks’ physiological solution by immersing
the specimens in solution for an hour to establish the steady state
potential. An AC sinusoid of ±10 mV and the frequency range of
100 kHz – 0.01 Hz were employed. The impedance data obtained
after each experiment was represented in the form of Bode plots.
The Bode plot is a plot of log |Z| vs log f and –phase angle (0) vs
log f, where |Z| is the absolute impedance and f is the frequency.

A suitable equivalent circuit was assigned for the acquired data
and the data were curve fitted using Zsimpwin software to get
the equivalent circuit parameters. After EIS measurements, poten-
tiodynamic polarization studies were carried out in a potential
window from –1.0 to 1.5 V at a scan rate of 0.001 V/s. The poten-
tials recorded are with respect to SCE. The corrosion potential
(Ecorr) and corrosion current density (i_corr) were obtained from
the polarization curves. The corrosion current is obtained using
Stern - Geary equation [29].

\[
\text{i}_\text{corr} = \frac{\beta_a \times \beta_c}{2.3 \ R_p (\beta_a + \beta_c)} \tag{1}
\]

where \(\beta_a\) and \(\beta_c\) are the Tafel slopes of the anodic and cathodic
parts of the Tafel plot, respectively, and \(R_p\) is the polarization resis-
tance. Triplicate measurements were made to check the repro-
ducibility of the results.

2.7. Cell culture studies

2.7.1. Cell culture

MG63 (Human Osteosarcoma) cells were obtained from the
National Centre for Cell Science (NCCS), Pune, India. In order to
maintain the cells healthy, the osteoblast cells were cultured in
Dulbecco’s minimal essential medium (DMEM) supplemented with
10% fetal bovine serum and antibiotics in an incubator maintained
at 37 °C in a humidified atmosphere of 5% CO₂.

2.7.2. MTT assay

MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium
bromide) assay was employed to evaluate the viability and prolif-
eration of MG 63 cells on the surface of both substrate, TNTA and
PPy/TNTA. MTT assay is a colorimetric assay for assessing meta-
bolic activities of cell. The selective viable cells are able to reduce
tetrazolium component of MTT in to purple colored formazan crys-
tals [30]. The 2 x 10⁴ cells/well was seeded over substrate, TNTA
and PPy/TNTA specimens placed in a 6 well plate containing
DMEM. After 24 h time point incubation in CO₂ incubator, the wells
were treated with MTT (0.5 mg/ml in PBS) and incubated for 3 h at
37 °C. DMSO was used to dissolve the formazan complex formed
by the live cells. Then the plates were read on a microplate reader
(Bio-Rad) to measure the color developed using a test wavelength
of 570 nm and a reference wavelength of 630 nm.

2.7.3. Cell morphology

The substrate, TNTA and PPy/TNTA specimens were pre-
sterilized and placed in 6 well tissue culture plates. The osteoblast
cells were trypsinised from a 25 cm² tissue culture flask after a
thorough wash and were counted. A count of 2 x 10⁴ cells were
seeded over each sample in a well plate containing DMEM. The
well plates were incubated for 24 h time point in a humidified
CO₂ incubator. The specimens were then washed with phosphate
buffer solution (PBS) and fixed with 4% paraformaldehyde for 20
min. The fixed cells were treated with a series of graded ethanol
(50, 70, 80, 95 and finally 100%) and then with hexamethyldi-
zasane (3:1 Ethanol: HMDS, 1:1 Ethanol: HMDS, 1:3 Ethanol:
HMDS and 100% HMDS) for 15 min each step and the final treat-
ment with 100% HMDS was left for drying. The morphology of
the fixed cells on the specimens was examined by HR-SEM.

2.8. Statistical analysis

All experiments reported were carried out in triplicate and the
values were expressed as average ± standard deviation. P-Values
were obtained from the Student’s t-test and the significance
indicated.
3. Results and discussion

3.1. Preparation of PPY/TNTA hybrid

PPy/TNTA hybrid was prepared by normal pulse voltammetry electrodeposition process. After a series of optimization experiments, electrolyte solution containing 0.2 M LiClO4 and 0.1 M pyrrole were chosen for the deposition process. Fig. 1(a) shows the applied potential curve in the normal pulse voltammetry electrodeposition process. The pulse width was 0.06 s and the pulse period was controlled at 4 s and 6 s respectively to benefit pyrrole monomer deposition. The current curve obtained during the synthesis of PPY/TNTA hybrid was shown in Fig. 1(b). When the electrode potential increase from 0.6 V to 0.7 V, the corresponding current density slightly decreases. Further, when the potential raise from 0.7 V to 1.2 V, a sudden increase in the current density value was observed, indicating that the specific resistance of as formed PPY/TNTA gradually decreases due to the deposition of polypyrrole on titania nanotube array.

The self-organized and independent nanotube structures formed on the titanium substrate is a good platform for the electrodeposition process. However the formation of polymer on the surface of wide band gap inorganic TiO2 nanotube structured is more challenging. The deposition of polymer depends upon the geometrical structure of the formed nanotubes. The electropolymorization of pyrrole is an anodic process and it occurs via reactions shown in Fig. S1(a) [31] given in Electronic Supplementary Information (ESI) part. Initially the pyrrole monomer oxidizes at the surface of TiO2 nanotubes and forms the cation radical. The cation radicals then coupled and forms dimers followed by the formation of PPY chain after deprotonation. Finally the polymer chain carries a positive charge at every 3–4 pyrrole units, which was compensated by the anions (ClO4−/CO32−) present in the electrolyte to maintain the oxidative state of the pyrrole. The electrochemical resistance inside the TiO2 nanotubes was high leads to low conductivity at the sealed nanotube bottom layer. The better wetting behavior at the interspaces compared to the inner spaces of the nanotubes favors the filling of the polymer initially at the spaces between the tube walls until the PPY was fully filled [25].

3.2. Cyclic voltammetry studies

The electrochemical activity of TNTA and PPY/TNTA were characterized using cyclic voltammetry in the same supporting electrolyte in the absence of monomer and the corresponding cyclic voltammograms obtained are shown in Fig. S2(b) in the ESI part. As can be observed from the figure, the current density of TNTA slightly increases at the negative end of the potential window and remains constant thereafter from −0.4 to 0.6 V, showing no electroactivity. However, PPY/TNTA specimen shows better electroactivity at the positive end than the TNTA by displaying an increase in current density from the beginning of the experiment throughout the forward scan. The anodic peak observed at 0.2 V/SCE and the two cathodic peaks obtained at −0.2 V/SCE and −0.78 V/SCE during the reverse scan exhibits the features of a reversible PPY/TNTA junction. The pyrrole monomer can inject electrons to the polarized titania nanotube surface via surface states enabling electropolymerization without light excitation during deposition and thereby enhances the electroactivity of the TNTA specimen [23].

Bones are the integral part of the human body. It exhibits natural piezoelectric property. Piezoelectric property is the property of the material to produce current in response to strain or stress. Materials that are piezoelectric can similarly exhibit the reverse piezoelectric effect, meaning that when current is applied, the material compresses on itself. This property is assumed to play a role in bone healing application. By applying current to a fractured bone, it will promote compression of the bone to allow it to heal faster [32]. Bone healing can significantly expedited by applying electrical stimuli in the injured region. Electrical stimulation of bone has been touted as an effective and non-invasive method for enhancing bone healing and treating fracture non-union [33]. Also electrical stimulation has been studied as a physical cue for promoting bone regeneration in various osseous defects [34].

Yasuda et al. [35] demonstrated the new bone formation in the vicinity of cathode by applying continuous current to a rabbit femur for three weeks. Electrical stimulation has shown to be effective in aiding bone healing in a variety of orthopedic conditions such as aiding internal and external fixation, enhancing delayed or nonunion fractures, improving the efficacy of bone
grafts, treating fresh fractures, and aiding femoral osteonecrosis [36–40]. The electrical stimulations consequently makes the use of electrically conducting polymers. It has also been reported that, conductive polymers with electroactive surfaces may increase intercellular communication to promote cell proliferation and adhesion [34,41].

3.3. Morphology characterization

Upon comparing the micrographs of TNTA and the PPy/TNTA surface, the morphological differences became apparent. The change in surface morphology after the deposition of polypyrrole on the TNTA surface was observed under high resolution scanning electron microscope. The HR-SEM images for TNTA and PPy deposited on TNTA at different pulse potential 4 s and 6 s, the 3D images generated from the HR-SEM images and the cross sectional view of the nanotubes are shown in Fig. 2(a–i). It is clear from the top view and cross sectional images that, the nanotubes formed are self-organized, ordered and are aligned vertically on the titanium surface. The diameter of the nanotubes was 40–45 nm and the wall thickness was approximately 8–10 nm. The formed nanotubes were grown to a length of about 580 nm. The intertubular space between the nanotubes was found to be 12 nm.

As can be seen from the images the in-filtered PPy initially fills the intertubular space between the nanotubes and then partially covered at the surface of the nanotubes at a pulse potential 4 s. Further increase in pulse potential to 6 s, the pores of the TNTA was increasingly filled with PPy and a densely packed polymer film with spherical morphology was observed on the nanotube surface. The top surface image does not aid to differentiate well the morphology of the substrate. The cross sectional images (Fig. 2(f) and (i)) shows that the deposition of PPy has occurred throughout the length of the nanotubes. The disappearance of spaces between the tubes can be observed after the deposition of PPy. Further, 3D analysis of the specimens was carried out to validate the filling of polypyrrole. Fig. 2(j–l) showed the 3D images obtained from HR-SEM images for a pair of nanotubes of TNTA and PPy/TNTA at pulse potential of 4 s and 6 s respectively. Electropolymerization under controlled pulse potential conditions led to the deposition of polypyrrole initially in to the spaces between the tubes, inside the tubes and if prolonged on the surface of the nanotubes. The elemental composition of TNTA and PPy/TNTA (6s) obtained from EDS analysis was shown in Fig. S2(a and b) respectively in the ESI part. The EDS analysis of TNTA (Fig. S2(a)) shows that the nanotubes have higher concentration of titanium and oxygen whereas after the deposition of polypyrrole (Fig. S2(b)) a substantial amount of carbon and nitrogen were also observed. In order to find out the even distribution of carbon and nitrogen, elemental mapping of PPy/TNTA specimen was carried out and the images are shown in Fig. 3. The presence of carbon (C), Nitrogen (N) along with Titanium (Ti) and Oxygen (O) confirms the uniform distribution of polypyrrole on the surface of TNTA.

3.4. ATR-FTIR studies

The ATR-FTIR spectra recorded for TNTA and PPy/TNTA (6 s) are represented in Fig. 4(a). The presence of broad brand in the range of 450 cm\(^{-1}\)–800 cm\(^{-1}\) observed in TNTA could be due to the formation of Ti–O–Ti and Ti–O bonds originated from the oxide layer of the titanium dioxide [26]. In the case of PPy/TNTA, the
A characteristic peak observed at 1036 cm$^{-1}$ and 1064 cm$^{-1}$ is attributed to the N–H deformation band and C–H stretching vibration respectively in the pyrrole ring [42]. The peak observed at 1407 cm$^{-1}$ and 1444 cm$^{-1}$ corresponds to C–N stretching vibration in the pyrrole ring. The band at 1554 cm$^{-1}$ is attributed to the fundamental C=C stretching vibration in the pyrrole ring [43,44]. The broad band at 1166 cm$^{-1}$ related to the C–N in plane ring deformation and bending modes [45]. All the characteristic peak
observed for polypyrrole confirms the deposition of PPy on TNTA through electrodeposition process.

3.5. Raman analysis

Raman spectroscopy measurements were carried out for the confirmation of PPy deposition on TNTA. The spectra recorded for both TNTA and PPy/TNTA (6 s) in the range 50 – 2000 cm⁻¹ is shown in Fig. 4(b). In the case of TNTA the two characteristics peaks observed at 147 cm⁻¹ and 637 cm⁻¹ were ascribed to O–Ti–O symmetric deformation vibration and Ti–O stretching vibration respectively. The peak observed at 398 cm⁻¹ is attributed to the formation of TiO₂ oxide in the anatase form [33]. Regarding the Raman spectrum of PPy/TNTA, the peaks observed at 939 cm⁻¹ and 1088 cm⁻¹ is due to the C–H ring deformation vibration [46]. The peaks observed at 1371 cm⁻¹ and 1584 cm⁻¹ was ascribed to C–N stretching vibration and C=C stretching vibration respectively [47]. The absorption peak observed at 1254 cm⁻¹ was caused by C–H plane bending vibration. It could be concluded from the characteristics peaks observed for PPy/TNTA that the polypyrrole has been fully deposited on titania nanotube arrays by pulse voltammetry deposition process to form PPy/TNTA hybrid.

3.6. Adhesion studies

Adhesive strength is the measure of the pull off force required for the retraction of the coating from the Ti substrate. The result of the adhesion studies showed that, the force required to pull PPy from Ti substrate and TNTA with hydraulic pressure were found to be 0.78 MPa and 1.28 MPa respectively and are shown in Fig. S4 in the ESI part. It was evident from the result that PPy adheres so well into the nanotubes. The adhesion strength of PPy/TNTA was higher, which could be due to the electrochemical deposition of polypyrrole in to the nanotubular framework. Since the PPy is electrodeposited on the self-organized TNTA, the delamination tendency was greatly reduced. This fact was supported from the improved adhesion strength value obtained for PPy/TNTA compared to PPy/Ti.

3.7. XPS analysis

The polypyrrole film deposited on TNTA surface was analyzed by XPS. The survey spectra, core level spectra of Ti2p, O1s, N1s and C1s are given in Fig. 5(a–e) respectively. The survey spectrum shows the presence of Titanium, Carbon, Nitrogen and Oxygen. Prior to the deconvolution of elements, the measured peaks were corrected with the true value 284.6 eV of carbon. The Ti2p spectrum showed well resolved doublet peaks of Ti2p corresponding to Ti2p3/2 at binding energy at 458.54 eV and Ti2p1/2 at binding energy 464.25 eV respectively, which were ascribed to the Ti–O bond [48]. Similarly the three deconvoluted peaks of O1s obtained at binding energies 529.78 eV, 531.87 eV and 533.59 eV are assigned to oxide, hydroxide and C–OH groups respectively [49]. The C1s spectrum obtained for PPy/TNTA specimen could be fitted into four component peaks with binding energies of 284.46 eV (C–C in aromatic rings), 284.92 eV (C–N), 286.23 eV (C=O) in C–OH/C–O–C and 288.06 eV (C=O) [50,51]. The high resolution N1s XPS spectrum displays a strong peak at binding energy 399.77 eV, which is ascribed to neutral pyrrole nitrogen — NH —, in the PPy chain [52].

3.8. Wettability and roughness analysis

Wettability is the ability of a liquid to maintain contact with the solid surface. It can be determined from the contact angle that is formed between the liquid drop and the implant surface [53]. The wettability of substrate, TNTA and PPy/TNTA (6 s) specimens were determined from the contact angle (CA) measurements and the obtained results are shown in Fig. S3(a) in the ESI part. The optical images of the water droplets are compared in the inset. The CA of the TNTA specimen was found to be 29.4° which was lower compared to the substrate value of 60.8°, shows the hydrophilic nature of the titanium after the formation of nanotubes. The contact angle obtained for PPy/TNTA specimen was found to be 37.9°. This slight increase in CA value could be explained by the structural modification of TNTA due to the partial filling of the PPy into and between the nanotube arrays as revealed by HRSEM studies. However, the PPy/TNTA specimen still exhibits hydrophilic nature and it could be due to the presence of amine groups in the polymer matrix. It is well known that the functional groups like amine, carboxyl and hydroxyl groups have hydrophilic nature [54]. It has been reported that the hydrophilic nature promotes the growth of apatite like layer over implant material by improving the ion exchange behavior from the physiological Hanks' solution. The wetting ability of surface also promotes the adhesion of host cells and interaction between the biological fluids and the implant surface [55].

The root mean square roughness (Rrms) of the specimens was determined using WSxM 4.0 jeta software. Histogram analysis was used to determine the height of the maximum available peak and the surface height distribution diagrams obtained from the 3D images for substrate, TNTA and PPy/TNTA at 4 s and 6 s are represented in Fig. S3(b). The polished substrate showed roughness value of 18 nm, whereas the anodized specimen showed roughness value of 45.60 nm. The polypyrrole deposited on TNTA at pulse potential of 4 s showed roughness value of 34.69 nm. The decrease in roughness value is due to the deposition of polypyrrole resulting in uniform layer. It was also observed that PPy deposited on TNTA at pulse potential 6 s showed lower surface roughness value of 25.49 nm when compared to both TNTA and PPy/TNTA deposited at 4 s. It may be due to the smoothing of the TNTA rough surface due to the filling of the PPy during the electropolymerization process. However, this slight decrease in roughness value of the PPy/TNTA specimen does not showed any significant influence on the wettability of the specimens.

3.9. In-vitro immersion studies

The bioactivity of any substrate material is associated with its ability to form apatite like layer, when kept in contact with biological fluid. The PPy/TNTA specimen was immersed in Hanks’ solution for 7 days to evaluate the bioactivity, as the apatite formation is considered to be an essential step for the bone bonding ability of biomaterials.

X-ray diffraction analysis was performed to identify the phase composition of the formed apatite over PPy/TNTA surface after 7 days immersion in Hanks’ solution and the result was shown in Fig. 6(a). Typical diffraction peaks of pure titanium at 2θ values of 35.08°, 38.50°, 40.1°, 53.04°, 62.95° and 76.1° (JCPDS No.44-1294) corresponding to (100), (002), (101), (102), (110) and (112) phases respectively were observed. Besides the peaks of titanium, the peaks detected at 2θ values of 21.5°, 31.78° and 77.24° (JCPDS No. 89.6440) corresponding to (200), (211) and (513) respectively after immersion in Hanks’ solution were ascribed to the preferential planes of apatite, which are in consistent with the standard XRD peaks of apatite. There were also some HAp peaks at 2θ values of 25.4°, 53.04° and 70.5° (JCPDS No. 89.6440) which coincided with the substrate peaks. The observed sharp HAp peaks endorse the crystalline nature of the formed apatite. The presence of apatite peaks confirms that the PPy/TNTA specimen possess bioactivity and can induce the formation of hydroxy apatite after implantation.
Fig. 6(b) shows the ATR-FTIR spectrum of PPy/TNTA specimen after immersion in Hanks’ solution for 7 days. The broad band observed between 3600–3450 cm\(^{-1}\) corresponds to O-H stretching. The peak at 561 cm\(^{-1}\) results from the \(v_4\) mode of O-P-O bending [56]. The small bands observed at 1402 cm\(^{-1}\) and 871 cm\(^{-1}\) correspond to the CO\(^{3-}\) group in the \(\beta\)-carbonated HAp. A strong band
observed at 1028 cm\(^{-1}\) is attributed to the \(v_3\) mode of PO\(_4^{3-}\) group [57]. The high intensity of the phosphate peak observed in the PPy/TNTA specimen demonstrates the formation of dense apatite layer over the surface. Hence the presence of carbonate and phosphate bands obtained from the ATR-FTIR results confirms the growth of hydroxy apatite over the PPy/TNTA surface. The HRSEM image and EDS profile of substrate, TNTA and PPy/TNTA after in-vitro immersion test was given in Fig. 7. It could be observed from the Fig. 7(a) that, few white apatite like particles are formed randomly on the substrate surface. The growth of apatite on the TNTA surface was more compared to the substrate as revealed from the HR-SEM image given in Fig. 7(c). Higher magnification image Fig. 7(d) reveals that the formed apatite appears to be in the form of globular shaped particles. The chemical nature of the apatite was identified by EDS analysis and confirms the formation of apatite by the presence of characteristics peaks of Ca and P in the EDS profile for substrate (Fig. 7(b)) and TNTA (Fig. 7(e)). The intensity of Ca and P peaks obtained for TNTA specimen was high compared to the substrate. In the case of PPy/TNTA (Fig. 7(f)), it was observed from the SEM image that the surface was covered by a new layer of material that appeared to be constituted by newly nucleated mineral layer of HAp. The apatite appears to be white clustered deposit, covering the entire surface of PPy/TNTA was apparent from the corresponding higher magnification image (Fig. 7(g)). The newly formed apatite layer on the PPy/TNTA surface was confirmed by EDS analysis, showing the characteristic peak of Ca and P along with metal peak. The incorporation of polypyrrole in to the nanotubes accelerated the growth of apatite from Hanks’ solution, which was further substantiated from the intense peaks of calcium and phosphate observed from ATR-FTIR results discussed above in Fig. 6(b). It should be worth mentioning here that the higher growth of hydroxy apatite on the PPy/TNTA surface could be the synergistic influence of both TNTA morphology and deposited polypyrrole. The deposition of PPy induces the formation of semi crystalline hydroxy apatite rich layer on the TNTA surface as a result of the chemical reaction of the particles with Hanks’ solution. Additionally the presence of amine groups in the polymer matrix has hydrophilic nature which promotes the growth of HAp [44]. Initially the positively charged calcium ions are attracted to the surface and then combines with negatively charged phosphate ions to form metastable amorphous calcium phosphate which in turn transforms to crystalline bone-like hydroxy apatite [58].

The above results indicated that deposition of polypyrrole could enhance the growth of hydroxy apatite on the TNTA surface and there by enhances the bioactivity of the implant material.

Fig. 8(a) showed the 3D image derived from the HR-SEM image for PPy/TNTA specimen. As evidenced from SEM images, the apatite deposit is clear from the 3D images. Also the surface height distribution diagram taken from the 3D images are presented in Fig. 8(b). The apatite deposit with 50 nm height is observed in PPy/TNTA. It has been reported that the formation of bone like hydroxy apatite layer on the surface of implant is essential for osseointegration between the implant and living bone tissue. It is also effective in attracting cells and allows osteoblast to form new tissue [59,60].

### 3.10. Potentiodynamic polarization studies

The potentiodynamic polarization curves of substrate, TNTA and PPy/TNTA deposited at a pulse potential of 6 s in Hanks’ solution was recorded in the potential range \(-1.0\) to \(1.5\) V at a scan rate of 0.001 V/S to study the corrosion behavior and the representative plots are shown in Fig. 9(a). The polarization parameters, corrosion potential (\(E_{corr}\)) and corrosion current density (icorr) values obtained from the polarization studies are presented in Table 1. The corrosion potential of PPy/TNTA (-0.272 V) showed a significant shift towards nobler direction when compared the \(E_{corr}\) values of TNTA (-0.309 V) and substrate (-0.452 V). This nobler shift indicates the stable nature of the PPy/TNTA specimen in Hanks’ solution. The icorr was obtained by extrapolation of the cathodic and anodic branches of the polarization curves to the corrosion potential. The icorr value for substrate and TNTA was found to be 0.188 \(\mu A/cm^2\) and 0.031 \(\mu A/cm^2\) respectively. The icorr value of TNTA was reduced to 0.0013 \(\mu A/cm^2\) after the deposition of PPy. This may be attributed to the possible filling and compact pore walls of the titania nanotubes due to the deposition of polypyrrole. The deposited polypyrrole will restrict the flow of electrolyte to reach the interface making the nanotubes more corrosion resistant. Furthermore, the current density corresponding to the passive region for both TNTA and PPy/TNTA was lower than that of the bare substrate, which exemplifies the stability of the nanotubes. The nobler shift in corrosion potential and decreased corrosion current density for the PPy coated TNTA, indicates the higher corrosion resistive behavior. Moreover, the release of metal ions or particles from the PPy/TNTA surface could be effectively blocked.
by the deposited polypyrrole layer, which in turn enhances the corrosion resistance of the surface.

3.11. Electrochemical impedance spectroscopic analysis

For a deeper insight into the electrochemical behavior of TNTA and PPy/TNTA hybrid deposited at a pulse potential of 6 s, EIS measurements were performed in Hanks', solution and the results are presented in Fig. 9(b–d) in the form of Nyquist and Bode plots. The Nyquist plots (Fig. 9(b)) showed the presence of a single unfinished semicircle arc. An increase in diameter of the semicircle can be observed for PPy/TNTA specimen, indicates an increase in corrosion resistance behavior. It could be due to the formation of compact polymer film deposited initially on the tube walls and then on the top surface, which in turn hinders the ingress of aggressive corrosive ions from the electrolyte solution into the metal surface.

In Bode plot (Fig. 9(c)) the substrate showed one time constant, indicating a single and compact passive oxide layer whereas the anodized specimen (TNTA) showed two time constant, corresponds to the presence of compact barrier oxide and a porous layer in the nanotube structure. The limiting impedance at the high frequency region corresponds to the response of the electrolyte resistance. For substrate, the Bode phase angle started around $-2^\circ$ in the high frequency region, reached to $-80^\circ$ in the mid frequency region and decreased to $-40^\circ$ in the low frequency region, indicates the less protective nature of titanium oxide layer. The phase angle of TNTA in the high frequency region was close to $-10^\circ$ and increased to $-63^\circ$ and remains stable in the frequency range (10 Hz – 100 Hz) and attains a maximum value of $-80^\circ$ in the low frequency region, which is less than the value for an ideal capacitor($-90^\circ$). Similarly in the case of PPy/TNTA, as the frequency reduced from 100 Hz to 10 Hz, there was an increase in the phase angle from $-9^\circ$ to $-70^\circ$ in this frequency range. The phase angle increased to $-80^\circ$ in the low frequency region, shows a considerable shift in the phase angle when compared to the substrate. This higher phase angle observed for PPy/TNTA may be attributed to the physical changes happened

Fig. 7. HR-SEM/EDS images of (a and b) Substrate, (c and e) TNTA, (f and h) PPy/TNTA, (d) TNTA and (g) PPy/TNTA at higher magnification after immersion in Hanks' solution for 7 days.
in the nanotubes due to the deposition of the polypyrrole and less interaction of corrosive ions from the Hanks, solution. The Bode magnitude plot (Fig. 9(d)) also showed that both TNTA and PPy/TNTA specimens are stable in Hanks’ solution. The high impedance observed for PPy/TNTA in the low frequency range showed the better corrosion resistant nature.
To gain further insight into the interrelationship between the material interfaces and corrosion resistance behavior, impedance results were analyzed using equivalent circuit models. The analysis of the impedance spectra obtained for substrate, TNTA and PPy/TNTA was done by fitting the data using the nonlinear least square fitting procedure and the corresponding equivalent circuit models used for fitting the experimental data are presented in Fig. 10. In the equivalent circuit constant phase elements (CPE) are used as substitute for pure capacitor representing the deviation from the ideal capacitive behavior. This signifies the probability of non-ideal capacitance, whose impedance is defined as $Z_{CPE} = \frac{1}{Q(j\omega)}$ with \( n \) less than 1; for ideal capacitance \( n = 1 \) \cite{28}. The \( n \) values observed for substrate, TNTA and PPy/TNTA was less than 1, confirms the existence of a CPE. The EIS parameters obtained by best fitting the equivalent circuit are enlisted in Table 2 The substrate is fitted with simple randles circuit $R_s(R_1Q_1)$, which is typical for passive oxide layer. The resistive component $R_s$ is related to the solution resistance whereas $Q_1$ and $R_1$ were assigned for capacitance and resistance of the oxide layer. The resistance ($R_1$) $5.56 \times 10^5$ value is due to the presence of passive oxide layer formed on the titanium surface. The \( n \) value for CPE representing passive oxide film is 0.91. The equivalent circuit used to fit for TNTA is $R_s(Q_1(R_1(Q_2(Q_3R_3))))$ which consist of two oxide layer, namely an inner barrier and outer porous layer. The $R_2$ and $Q_2$ are associated with resistance and capacitance of the inner barrier layer. The fitting parameters given in Table 2 for TNTA specimen showed that the resistance $R_1$ is due to the presence of outer porous layer of the nanotubes ($1.62 \times 10^4 \ \Omega \ \text{cm}^2$) and $R_2$ ($1.58 \times 10^6 \ \Omega \ \text{cm}^2$) obtained is associated with the corrosion resistance of inner barrier layer. The \( n \) value for constant phase element representing $Q_2$ and $Q_3$ is 0.89 and 0.82 respectively. Warburg impedance observed in the TNTA specimen is due to the diffusional process through the oxide layer \cite{61}.

In the case of PPy/TNTA deposited at a pulse potential of 6 s, the equivalent circuit has three time constant. The equivalent circuit used to fit for PPy/TNTA is $R_2(Q_1(R_1(Q_2(Q_3R_3))))$. From the fitted values given in Table 2, the resistance ($R_1$) of the outer porous layer is $64 \ \Omega \ \text{cm}^2$. The lower resistance explains that the nanotube surface has not fully covered with polypyrrole. The low value of \( n' \) (0.78) corresponding for the CPE, $Q_1$ implies that surface is still porous in nature and are in good accordance with the SEM results substantiated above. The $Q_2$ and $R_2$ are due to the capacitance and resistance of nanotube filled with polypyrrole. The high $R_2$ ($1.27 \times 10^7 \ \Omega \ \text{cm}^2$) value endorses that the deposited PPy layer implies an efficient blocking of the passage of ions from the Hanks' electrolyte. The $Q_3$ and $R_2$ are associated with the capacitance and resistance of the tube bottom (barrier oxide layer) close to the substrate. The \( n' \) value for CPE representing $Q_2$ and $Q_3$ is 0.89 and 0.82 respectively. In the present case the value of $R_2$ is $8449 \ \Omega \ \text{cm}^2$.

It is concluded from the stimulated values that the corrosion protection of PPy/TNTA specimen is dominated by the $R_2$ and $R_3$ and the increase in resistance can be explained in terms of the deposition of polypyrrole on the tubular surface and also due to the presence of oxide layer at the tube bottom close to the substrate.

### 3.12. Cell culture studies

The initial attachment of cells to the titanium surface is very important when it is implanted in to the human body conditions. Improving osseointegration on the implant surface will reduce the implant failure.

#### 3.12.1. Cell morphology

The cell morphology of MG 63 cells on the surface of substrate, TNTA and PPy/TNTA after 24 h incubation was observed by HR-SEM and the images are shown in Fig. 11. As the metal is not transparent, the understanding of cell interaction and morphology was done through Scanning Electron Microscopy. The HR-SEM image gives a representation of uniform distribution of cells over a small area on the metal surface, which is indicative of the overall cell distribution over the metal surfaces.

The result of MG63 cell adhesion after 1 day was given in Fig. S5 in the ESI part. The result indicates an approximate 80% increase in the number of cells present on the PPy/TNTA surface compared to TNTA. Adhesion of cells on the substrate, TNTA and PPy/TNTA was found to be $50 \pm 2.5\%$, $116 \pm 3.5\%$ and $200 \pm 7.4\%$ (\( p < 0.002 \)) respectively based on quantification of cells adhered over the samples on day1. The cells adhered over coverslips were considered as 100% for the calculation.

Osteoblast cells are mature adult bone cells responsible for bone growth, metabolism and depositing minerals to make up bone matrix components \cite{62}. It can be seen from the images that the osteoblast cells possessed typical spindle shape with prominent filopodial extensions. The spreading of osteoblast cells on the smooth surface of the substrate was relatively poor, whereas both TNTA and PPy/TNTA promotes cell adhesion and proliferation.

The cell bodies attach closely on the nanotube surface which was apparent from the SEM images. The nanopatterned surfaces provide greater reactive surface area and higher number of sites for the adsorption of proteins. Also, the presence of nanoscale holes allowing the penetration of cell filopodia and are better suited for the attachment of osteoblast cells \cite{63}.

Surface roughness of the specimens also plays important role in the cell spreading and proliferation. When compared to the substrate both TNTA and PPy/TNTA specimens provides more surface roughness, which improves the cell attachment to the surface and also helps in cell proliferation. Moreover there was a signifi-

### Table 1

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample</th>
<th>$E_{corr}$ (V)</th>
<th>$i_{corr}$ ($\mu$A/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Substrate</td>
<td>-0.452</td>
<td>0.188</td>
</tr>
<tr>
<td>2</td>
<td>TNTA</td>
<td>-0.309</td>
<td>0.031</td>
</tr>
<tr>
<td>3</td>
<td>PPy/TNTA</td>
<td>-0.272</td>
<td>0.001</td>
</tr>
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Fig. 10. Equivalent circuits used to fit the substrate, TNTA and PPy/TNTA specimens.
cant increase in the adhesion of cells on polypyrrole coated titania nanotube surface with better cell spreading, which could be ascribed to the synergistic effect of both active functional groups present in the polypyrrole and nanostructured inorganic semiconductor titanium substrate. The existence of polypyrrole inside and on the surface of nanotubes is responsible for the increase in biocompatibility of PPy/TNTA. Thus, it could be inferred that the cells had an enhanced propensity to attach to the PPy/TNTA compared to TNTA and substrate as evident from the HR-SEM images.

3.12.2. MTT assay

For better understanding the cell surface interaction on the nanoscale topography, MG63 osteoblast cells were allowed to proliferate on the nanotube surface before and after the deposition of polypyrrole. The initial cell response of implants is very important to establish stable bioactive interface for further cell proliferation, differentiation and attachment [64,65].

The metabolic activity of the osteoblast cells on the substrate, TNTA and PPy/TNTA were analyzed using MTT assay. The MTT was performed based on the cellular density on the sample surface with the same initial cell implant density 2 x 10^4 cells/well and the corresponding absorbance values were recorded. The increase in absorbance value observed for TNTA and PPy/TNTA specimens when compared to the substrate and control indicates an increase in the number of living cells. The percentage of cell proliferation obtained for substrate, TNTA and PPy/TNTA (assuming the proliferation for control are 100%) from the absorbance values are converted to the bar plots as shown in Fig. 12. It can be clearly observed from the figure that the cell proliferation on both the substrate and anodized specimens increases when compared to the control with values: Blank – 100% ± 0.001, Substrate – 120% ± 0.008, TNTA – 131% ± 0.038 and PPy/TNTA – 142 ± 0.001. The titanium substrate shows 20% and TNTA specimen shows 31% more growth, whereas the PPy/TNTA specimen showed 42% more proliferation than the control, which shows that polypyrrole grown on the nanotube surface promotes cell spreading and proliferation when compared to the other specimens. The p-value was calculated using Students t-test and was determined to be p < 0.01 for PPy/TNTA when compared to the blank sample.

<table>
<thead>
<tr>
<th>Samples</th>
<th>R_1 (Ω cm²)</th>
<th>Q_1 (S cm⁻⁶)</th>
<th>n_1</th>
<th>R_2 (Ω cm²)</th>
<th>Q_2 (S cm⁻⁶)</th>
<th>n_2</th>
<th>R_3 (Ω cm²)</th>
<th>W (μΩ⁻¹ s⁰⁵ cm⁻⁵)</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>69.85</td>
<td>–</td>
<td>–</td>
<td>2.18 x 10⁻¹</td>
<td>0.91</td>
<td>5.56 x 10⁻¹</td>
<td>–</td>
<td>–</td>
<td>1.11 x 10⁻³</td>
</tr>
<tr>
<td>TNTA</td>
<td>76.79</td>
<td>1.02 x 10⁻⁵</td>
<td>0.81</td>
<td>1.62 x 10⁴</td>
<td>0.93</td>
<td>1.58 x 10⁵</td>
<td>–</td>
<td>–</td>
<td>2.73 x 10⁻⁵</td>
</tr>
<tr>
<td>PPy/TNTA</td>
<td>20</td>
<td>2.38 x 10⁻⁸</td>
<td>0.78</td>
<td>64</td>
<td>1.27 x 10⁻³</td>
<td>0.89</td>
<td>1.27 x 10⁵</td>
<td>2.63 x 10⁻³</td>
<td>8449</td>
</tr>
</tbody>
</table>

Fig. 11. HR-SEM images of osteoblast like cells (MG 63) for Substrate, TNTA and PPy/TNTA specimens.
Fabrication of PPy/TNTA hybrid material was successfully achieved by normal pulse voltammetry electrodeposition method. The morphology, chemical identity and interaction between PPy and TNTA were studied using HR-SEM, XPS, ATR-FTIR and Raman spectroscopy studies respectively. TNTA retained the nanotubular morphology even after the deposition of polypyrrole as evidenced from the HR-SEM images. Controlled filling of PPy initially in to the spaces between the nanotubes at potential of 4 s and then gradually covers the wall surface of the nanotubes at potential of 6 s was apparent from the 3D images. The cyclic voltammetry study showed the electroactive nature of PPy/TNTA. The contact angle value obtained for PPy/TNTA (37.9°) from the wettability studies showed the hydrophilic nature of the specimen. The synergetic influence of TNTA morphology and the deposited PPy facilitated the higher growth of hydroxyapatite over PPy/TNTA surface in Hanks’ solution. The enhanced adhesion strength of PPy/TNTA (1.28 MPa) examined using PosiTest pull-Off adhesion test revealed the beneficial role of TNTA framework in improving the adhesion of PPy. The nobler shift in corrosion potential (−0.272 V) and lower icorr value (0.0013 μA/cm²) obtained from the potentiodynamic polarization studies demonstrated the enhanced corrosion resistance nature of PPy/TNTA compared to TNTA and substrate in Hanks’ solution. The healthy morphology and increased growth rate of MG 63 cells on PPy/TNTA revealed from the In-vitro cell culture studies confirmed the biocompatible nature of the specimen. The above results indicated that the enhanced surface properties, corrosion resistance and biocompatibility of PPy/TNTA pave the way towards the employment of the hybrid material as a viable alternative in biomedical field, especially in orthopedic implants.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.apusc.2018.03.151.

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