Evaluation of corrosion resistance and cytocompatibility of graded metal carbon film on Ti and NiTi prepared by hybrid cathodic arc/glow discharge plasma-assisted chemical vapor deposition

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Hybrid cathodic arc/glow discharge plasma-assisted chemical vapor deposition is used to prepare graded films on Ti and NiTi. XPS reveals the formation of a Ti and carbon graded interface between the substrate and carbon surface layer. The graded film significantly improves the corrosion resistance of Ti and NiTi in 154 mM NaCl as demonstrated by the smaller corrosion current density, larger resistance, and smaller capacitance. The graded film on NiTi shows near-capacitive impedance spectra and 14 times larger phase angle maxima at 105 Hz. In addition, the osteoblasts cells spread well on the graded film surface suggesting good cytocompatibility.

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1. Introduction

Immobilizing protein molecules by covalent binding on surfaces while retaining their conformation for a long period of time is crucial to the success of biomedical implants and biosensors [1–6] because weak immobilization leads to protein detachment and undesirable conformational changes which affect the bioactivity of the implant surface resulting in adverse response such as inflammation, cellular immune response activation, clotting, excessive fibrosis, and rejection. Ti and Ti alloys are extensively used in hard tissue replacements such as dental implants, bone joints, and artificial bones as well as cardiovascular implants such as artificial hearts, protective cases of pacemakers, and artificial heart valves [7,8]. In particular, NiTi (Nitinol) shape-memory alloys have applications in intra-vascular devices such as occlusion coils and stents [7]. Unfortunately, inflammatory response has been observed from Ti6Al4V alloy (Ti with 6 wt.% of Al and 4 wt.% of V) under in vivo conditions [9]. Harmankaya et al. [10] studied the inflammatory response between immunoglobulin-coated Ti implants and ozone/ultraviolet illuminated Ti in the rat tibia model after 1, 7, and 28 days. Stronger inflammatory response was observed from the immunoglobulin-coated Ti implant than ozone/UV illuminated Ti during the first week of healing and the former exhibited enhanced expression of inflammatory markers, TNF-α, and IL-1β during the entire observation period. Significantly higher inflammatory activity of RAW264.7 macrophages with respect to chemokine and cytokine gene expression/protein secretion was observed from the untreated Ti compared to TiO2 coated Ti [11]. Higher inflammation and larger CD68+ macrophage distributions were observed from the untreated Ti surface than the anodized/ultraviolet treated Ti in vivo [12]. Furthermore, higher inflammatory response, lower alkaline phosphate activity, and lower calcium deposition by MG-63 cells were observed from the pristine Ti than protein-immobilized heparin-grafted Ti [13]. Inflammation with subsequent discharge of chemotactic and growth factors after arterial injury accompanying restenosis formation is one of a significant clinical problems for stents [14–16]. In order to diminish the inflammatory response, studies have been conducted to mask the surface exposed to protein molecules in blood but success has heretofore been limited [17]. It may be due to the difficulty in attaining strong binding with proteins or conformational changes (denaturation) of protein molecules. Immobilization of an biomolecule while preserving its function is very important and changing of conformational state of a molecule can severely influence the bio-recognition process [18].

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Amorphous carbon films are generally regarded to be biocompatible [19–26] and have attracted research interest from the perspective of covalent immobilization of protein molecules [1,3,27,28]. For instance, Wang et al. [19] showed that amorphous diamond-like carbon films exhibited good biocompatibility by enhancing osteoblast attachment, proliferation, and differentiation and Huang et al. [20] observed enhanced L-929 fibroblast-like cell attachment, proliferation, and differentiation from Nb-incorporated graphite-like carbon films. Rodil et al. [21] observed that amorphous carbon films enhanced attachment and proliferation of human osteoblasts and fibroblasts and Linder et al. [22] studied the adhesion, cyto-architecture, and activation of primary human monocytes on amorphous diamond-like carbon films. By determining the generation of three osteoblast-specific marker proteins, the effects of the amorphous diamond-like carbon film on cellular metabolism was examined and no undesirable effects on the three osteoblast-specific marker proteins, osteocalcin, type I collagen, and alkaline phosphatase were observed [22]. Bruinink et al. [24] showed that amorphous hydrogenated carbon films had no toxic effects against bone marrow cells and Liu et al. [25] observed that amorphous carbon films had lower inflammatory activity of RAW264.7 macrophages and higher primary mouse osteoblasts viability than amorphous hydrogenated carbon films.

Bilek et al. [1] demonstrated immobilization of protein molecules by strong covalent binding while retaining the function on a hydrophilic carbon polymer due to the generation of mobile unpaired electrons on the hydrophilic plasma-modified surface, and protein conformation could be preserved by the hydrophilic surface [29]. Nevertheless, the protein binding ability of hydrophilic surfaces is low [30]. Covalent binding of tropoelastin protein molecules on plasma modified amorphous carbon films was studied by Yin et al. [27] and the thrombogenicity on plasma-modified amorphous carbon films was studied by Waterhouse et al. [3]. Strong adhesion between the carbon film and implant surface is desirable because of the mechanical function in orthopedic/dental applications, for example, strong deformation during surgical insertion in cardiac and cardiovascular applications [7]. Nevertheless, strong adhesion between the carbon film and metallic materials can be difficult to achieve due to the difference in the thermal expansion coefficients of different materials. Nevertheless, strong adhesion between 18 and 26 MPa was achieved by Yin et al. [28] between the carbon film and stainless steel substrate by means of a graded interface having both the metal and carbon polymer components and strong covalent binding of protein molecules was observed on the plasma modified carbon film [28]. Strong adhesion was also achieved by Jamesh et al. [2] between the carbon film and stainless steel metallic substrate using metal and plasma carbon polymer components containing a graded interface. Neither cracks nor delamination was observed from the graded film even after being subjected to strong mechanical deformation by bending the coating outward by 64° and bending inward by 50° [2] and strong covalent binding of protein molecules was observed as well [2].

Under in vivo conditions, the implant surface is susceptible to corrosion, for example, when exposed to blood plasma having 113 mM of Cl⁻ and interstitial fluid having 117 mM of Cl⁻ [7]. Moreover, the protein molecules and amino acids in the body fluid accelerate corrosion [31]. The normal body fluid pH of about 7.4 diminishes to about 5.2 during the initial period (about 2 weeks) after hard tissue implantation [7] and Ti and Ti alloys corrode differently depending on the environment and conditions. The in vivo corrosion behavior of Ti and released Ti ions accumulated in the tissues near the implant surface have been studied [32] and electrochemical techniques such as electrochemical impedance spectroscopy and potentiodynamic polarization are useful in monitoring the corrosion process and elucidate the mechanism [33]. Formation of a surface oxide layer such as TiO₂ on Ti by thermal oxidation, chemical oxidation, electrochemical oxidation, and arc process can improve the corrosion resistance. Jamesh et al. [34] observed that TiO₂ film prepared by thermal oxidation exhibited significant improvement in the corrosion resistance of Ti in 154 mM NaCl. The TiO₂ film (~1 μm thick) thermally oxidized at 650 °C/14 h showed about 80 times smaller passive current density (at 2000 mV vs. SCE) than the TiO₂ film (~20 μm thick) thermally oxidized at 850 °C/6 h, revealing that the thinner TiO₂ film was more compact and had better corrosion resistance. Chu et al. [35] observed that nano-structured TiO₂ films prepared by Fenton’s oxidation improved the corrosion resistance of NiTi in simulated body fluids and Park et al. [36] showed that TiO₂ film prepared by anodic oxidation followed by heat treatment showed improvement in the corrosion resistance as well. Duarte et al. [37] observed that the oxide layer prepared by micro-arc oxidation at 300 V improved the corrosion resistance of Ti–13Nb–13Zr alloy in a phosphate buffer saline solution. Formation of a nitride layer such as a TiN film on the surface of the Ti can improve the corrosion resistance. Razavi et al. [38] observed that TiN, O₂, films prepared by laser surface nitriding improved the corrosion resistance of Ti6Al4V alloy in 2 M HCl solution. In addition, formation of an apatite layer can improve the corrosion resistance of Ti. Wang et al. [39] observed that a bone-like apatite film prepared by NaOH pretreatment followed by simulated body fluid immersion showed improvement in the corrosion resistance of Ti in simulated body fluids. However, apatite deposition can be influenced by surface topography. In this respect, Jamesh et al. [40] observed apatite deposition on the Ti after surface mechanical attrition treatment and immersion in simulated body fluids due to the formation of nano-structured grains in the near surface, higher surface roughness, surface wettability, and higher surface energy. Flamini et al. [41] demonstrated that a polypyrrole film containing molibdate ions prepared by electrodeposition enhanced the corrosion resistance of NiTi in 0.15 M NaCl and Manhabosco et al. [42] observed that a diamond-like carbon film prepared improved the corrosion resistance of Ti in the phosphate buffer saline solution. All in all, although TiO₂, TiN, O₂, bone-like apatite, polypyrrole, and DLC films prepared by various techniques have been shown to improve the corrosion resistance of Ti and its alloys, it is still necessary to optimize all the related properties including adhesion, biocompatibility, corrosion resistance, and covalent binding of protein molecules for biomedical applications.

Plasma surface modification is widely adopted to improve surface properties [7,18] such as adhesion [2,28], corrosion resistance [43–45], covalent binding of protein molecules [1–3,46–48], biocompatibility, and bacterial resistance [49]. For instance, formation of a ZrO₂ layer by Zr plasma immersion ion implantation (PIII) enhances the corrosion resistance of ZK60 Mg alloy in simulated body fluids showing 5 times smaller corrosion current density whereas dual Zr and O PIII enhances oxidation of Zr on the surface and corrosion resistance of Mg alloy as demonstrated by the 37-folds smaller corrosion current density [43]. Zr and O PIII was found to enhance the corrosion resistance of Mg–Sr and Mg–Ca alloys in simulated body fluids, cell culture, and tryptic soy broth medium due to the formation of the ZrO₂ layer on the surface [49] and improve both the in vitro cytocompatibility with MC3T3-E1 cells and antibacterial activity against Staphylococcus aureus. The SiO₂ layer formed by Si PIII enhanced the corrosion resistance of WE43 Mg alloy in simulated body fluids (23 times smaller corrosion current density) [50] and the Ti/TiN multilayer prepared by pulse-biased arc ion plating improved the corrosion resistance and Ni release from NiTi orthodontic brackets in artificial saliva [51]. The ZrN layer prepared by Zr and N PIII enhanced the corrosion resistance of WE43 Mg alloy in simulated body fluids (12 times smaller corrosion current density) and complete cell
between the anode and cathode. A steel disk substrate film. A LabRam HR spectrometer for 7 min and for G2 the C topcoat cytompatibility has seldom been reported and the objective of this work is to use hybrid cathodic arc/glow discharge plasma and cytocompatible and graded coating on Ti and NiTi to satisfy both of the mechanical and biological requirements.

2. Methods and materials

2.1. Plasma surface modification processes

The Ti and C graded films were deposited using a Ti cathode (99.998% pure) by cathodic arc deposition. A curved magnetic sole-sion filter was used to filter macro-particles and neutral atoms from the plasma stream [54–59]. A current of 60 A and pulsed voltage power source was used in substrate plasma. The pulsed voltage power source was used to power a cathodic arc to produce the Ti cathode and anode, respectively. An 2.5 m coaxial cable was used to connect the pulsed power source with the substrate and mass flow controllers were employed to regulate the C2/H2 gas flow. Deposition was carried out at about 10−6 Torr and Ti and NiTi samples with dimensions of 1 × 1 × 0.2 cm3 were used as the substrates.

The experimental parameters in the plasma deposition of G1 and G2 Ti/C graded film with a carbon coat on Ti and NiTi are listed in Table 1. Prior to deposition, the substrates were cleaned ultra-sonically in alcohol for 5 min and distilled water for 5 min followed by drying with compressed air. Pulsed voltages of −1.9 kV for Ti and −2 kV for NiTi were applied to the cathodic arc to produce the Ti and NiTi samples were dipped into a mixture of 35 vol% HNO3 and 5 vol% HF for about 1 min. The samples were ultrasonically cleaned for 5 min with distilled water and then dried by compressed air. The untreated Ti, G1 graded film, untreated NiTi, or G2 graded film formed the working electrode while the platinum and reference electrodes, respectively. Only 0.28 cm3 of the working electrode was exposed to the 154 mM NaCl solution. The impedance spectra were acquired in the frequency range between 100 kHz and 0.1 Hz by applying a sinusoidal perturbation signal of 10 mV (root mean square) at their respective open circuit poten-tials (OCPs) after stabilizing in the NaCl solution for 600 s. The EIS parameters were derived using ZSimpWin 3.21 software from the Nyquist plots. The Bode impedance and Bode phase angle plots were acquired. Potentiodynamic polarization was performed followed by impedance measurements at a scanning rate of 1 mV/s in the potential range between −250 mV vs. SCE in the cathodic direction and +350 mV vs. SCE in the anodic direction from their

Table 1

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample name</th>
<th>Type of substrate material</th>
<th>Film type</th>
<th>Flow rate of acetylene during Ti cathodic arc deposition (scm/min)</th>
<th>CVD of acetylene</th>
<th>Pressure (mTorr)</th>
<th>Duration (min)</th>
<th>Bias volt (kV)</th>
<th>Total duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G1</td>
<td>Ti</td>
<td>Ti and C Graded film with a carbon topcoat</td>
<td>0, 10, 20, 40, 60 and 80</td>
<td>Pressure (mTorr)</td>
<td>85</td>
<td>7</td>
<td>−1.9</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>G2</td>
<td>NiTi</td>
<td>Ti and C Graded film with a carbon topcoat</td>
<td>0, 10, 20, 40, 60 and 80</td>
<td>Pressure (mTorr)</td>
<td>90</td>
<td>8</td>
<td>−2</td>
<td>14</td>
</tr>
</tbody>
</table>

2.2. Materials characterization

A field-emission scanning electron microscope was used to examine the surface morphology and cross sections and X-ray photoelectron spectroscopy (XPS) with Al Kα irradiation was used to determine the chemical states and elemental depth profiles. The sputtering rate was calculated to be about 45.87 nm/2 min for 0–14 min, 22.98 nm/min for 15–112 min, and 45.87 nm/min for 113–140 min based on the rates determined under similar conditions from a standard SiO2 film. A LabRam HR spectrometer (Horiba Jobin Yvon) with an argon laser (green line at 514.5 nm) and an Olympus BX41 microscope were used to acquire the Raman scattering spectra. The water wettability was measured using the sessile drop method on the Kruss D10 contact angle equipment [60].

2.3. Electrochemical characterization

The corrosion behavior was evaluated in 154 mM NaCl at 20 °C by electrochemical impedance spectroscopy (EIS) and potentiody-namic polarization conducted on the Zahner Zennium electrochemical work station. Additional details about the electrochemical experiments can be found from our earlier articles [33,34,45,61]. The NaCl solution having inorganic Na+ and Cl− ions concentrations (154 mM Na+ and 154 mM Cl−) similar to those in human body fluids was used as the electrolyte in this study [62]. The 154 mM NaCl solution is one of the common test solution in electrochemical experiments [34,44,63]. The electrochemical experiments were performed on a conventional three-electrode cell. To remove the naturally formed oxide layer, the untreated Ti and NiTi samples were dipped into a mixture of 35 vol% HNO3 and 5 vol% HF for about 1 min. The samples were ultrasonically cleaned for 5 min with distilled water and then dried by compressed air. The untreated Ti, G1 graded film, untreated NiTi, or G2 graded film formed the working electrode while the platinum sheet and saturated calomel electrode (SCE) served as the counter and reference electrodes, respectively. Only 0.28 cm2 of the working electrode was exposed to the 154 mM NaCl solution. The impedance spectra were acquired in the frequency range between 100 kHz and 0.1 Hz by applying a sinusoidal perturbation signal of 10 mV (root mean square) at their respective open circuit poten-tials (OCPs) after stabilizing in the NaCl solution for 600 s. The EIS parameters were derived using ZSimpWin 3.21 software from the Nyquist plots. The Bode impedance and Bode phase angle plots were acquired. Potentiodynamic polarization was performed followed by impedance measurements at a scanning rate of 1 mV/s in the potential range between −250 mV vs. SCE in the cathodic direction and +350 mV vs. SCE in the anodic direction from their
respective OCPs. The same samples were used in the impedance and potentiodynamic polarization measurements. By using the Tafel extrapolation method, the corrosion potential ($E_{corr}$) and corrosion current density ($i_{corr}$) were derived from the polarization curves. The EIS and potentiodynamic polarization studies were repeated twice to improve the statistics and confirm reproducibility.

2.4. Cell adhesion

In vitro cell adhesion tests were carried out using mouse MC3T3-E1 pre-osteoblasts. The MC3T3-E1 cells were cultured in Dulbecco’s modified eagle medium (DMEM) along with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere with 5% CO$_2$. Amino acids, vitamins, inorganic salts, d-Glucose (Dextrose), and phenol red were the constituents in the DMEM powder and more details about the composition can be found elsewhere [45]. All the samples were sterilized by 70 vol% ethanol for 1800 s and washed with sterile phosphate-buffered saline three times. The MC3T3-E1 cells were seeded at a density of $1.5 \times 10^4$ cells per well on each sample on 24-well tissue culture plates and cultured for 5 h. The seeded samples were then fixed with 2% polyoxymethylene after rinsing twice with sterile phosphate-buffered saline. The nuclei were stained with Hoechst 33342 (Sigma) and the cytoskeleton protein F-actin were counterstained with phalloidin–fluorescein isothiocyanate (Sigma). Fluorescence microscopy (Carl Zeiss Axioplan 2) was used to obtain the cell images.

3. Results

The surface and cross-sectionals view of the G1 Ti/C graded film with the C topcoat on the Ti and G2 Ti/C graded film with the C coat...
on NiTi are shown in Fig. 1. Many bud-like protrusions are observed from Fig. 1b and d and the surface of G1 is rougher than that of G2 but both films appear uniform. Both G1 and G2 show a clear layer with an interface (Fig. 1e and f) and the thickness of the layer on G2 is larger than that on G1. In addition, a coating interface underneath is observed from G2 (Fig. 1f) on NiTi but it is not observed from G1 (Fig. 1e) on Ti. The XPS depth profile of G1 in Fig. 2 shows a 0.53 μm thick carbon rich layer followed by gradual

Fig. 3. High-resolution XPS spectra of (a–d) C 1s and (e–h) Ti 2p obtained from G1.
decrease in the carbon concentration. No Ti is detected from the top 0.53 μm and the Ti concentration increases gradually until reaching a plateau indicative of the bulk materials. The high-resolution C 1s and Ti 2p spectra of G1 are depicted in Fig. 3a–d and e–h, respectively. A strong peak at 285.62 eV is observed from the C 1s spectra from the graded film (about 0.53) of G1 (Fig. 3a) and the intensity diminishes afterward. Another peak at 282.87 eV is observed from the C 1s spectra after sputtering for 0.53 μm from G1 (Fig. 3a) reaching a plateau and decreasing (disappearing) eventually. Two peaks at 454.88 eV and 460.89 eV are observed from the Ti 2p spectra after sputtering 0.53 μm from the G1 graded film (Fig. 3e). The binding energies decreases with sputtering time reaching 454.0 eV and 460.14 eV after sputtering for 140 min (Fig. 3e–h). The Raman spectrum in Fig. 4 reveals two sub-bands: C5/C7 odd ring and Csp²–Csp² cluster bands at 1527 cm⁻¹ and 1339 cm⁻¹, respectively.

The Nyquist plots of the untreated Ti, G1, untreated NiTi, and G2 in 154 mM NaCl are depicted in Fig. 5a–d. The diameter of the capacitive loop of G1 is larger than that of the untreated Ti (Fig. 5a and b) and that of the capacitive loop of G2 is also larger than that of the untreated NiTi (Fig. 5c and d). The equivalent circuits obtained after fitting the EIS spectra are shown in Fig. 5a–d and the EIS parameters are shown in Table 2. The Bode impedance and Bode phase angle plots are depicted in Fig. 5e–h. At all frequencies, a larger impedance is observed from G1 than the untreated Ti, G1 (Fig. 5e). A larger impedance of 3.78 ± 1.81 x 10⁵ Ω cm² at 0.1 Hz is observed from G1 but 2.60 ± 0.47 x 10⁴ Ω cm² is observed from untreated Ti (Fig. 5e). Moreover, a steady increase in the impedance is observed from the untreated Ti at 166 Hz whereas a similar observation is made from G1 at a higher frequency of 10⁵ Hz (Fig. 5e). G1 shows larger phase angles over a wide range of frequencies than the untreated Ti. A phase angle maximum of −6 ± 0° at 10⁵ Hz is observed from the untreated Ti but −27 ± 1° is obtained from G1 (Fig. 5f). A larger impedance is also observed from G2 than the untreated NiTi (Fig. 5g). A larger impedance of 2.38 ± 0.24 x 10⁵ Ω cm² at 0.1 Hz is obtained from G2 but 4.63 ± 0.96 x 10⁴ Ω cm² is observed from untreated NiTi (Fig. 5g). A steady increase in the impedance is observed from untreated NiTi at 342 Hz whereas a similar observation is made from G2 at a higher frequency of 10⁵ Hz (Fig. 5g). G2 shows larger phase angles over a wide range of frequencies than the untreated NiTi. A phase angle maximum of −5 ± 1° at 10⁵ Hz is seen from untreated NiTi whereas −71 ± 3° is observed from G2 (Fig. 5h).

The potentiodynamic polarization curves and corresponding Tafel fittings are depicted in Fig. 6a and b and the corrosion potential (Ecorr), corrosion current density (icorr), and passive current density (ipass) derived from potentiodynamic polarization curves are shown in Table 3. As shown in Fig. 6a, Ecorr of G1 shifts toward the positive direction from −458 ± 43 to −314 ± 54 mV vs. SCE and the cathodic branch of the polarization curve shifts toward a smaller current density compared to the untreated Ti in 154 mM NaCl (Fig. 6a). The anodic branch of the polarization curves of both the untreated Ti and G1 exhibits an active passive transition. The active and passive regions of the polarization curve of G1 shifts toward smaller current density compared to the untreated Ti (Fig. 6a) and G1 shows smaller kcorr and icorr than the untreated Ti (Table 3). At 350 mV vs. SCE, G2 shows an icorr of 0.46 ± 0.29 μA/cm² which is about 16 times smaller than that of the untreated Ti (7.46 ± 0.58 μA/cm²). As shown in Fig. 6b, Ecorr of G2 shifts toward the positive direction from −295 ± 49 to −215 ± 51 mV vs. SCE and the cathodic branch of the polarization curve of G2 shifts toward lower current density (Fig. 6b). The anodic branches of the polarization curves of both the untreated NiTi and G2 show an active passive transition. Nevertheless, the active and passive regions of the polarization curve of G2 shift toward smaller current density (Fig. 6b). G2 shows smaller kcorr and icorr (Table 3). At 350 mV vs. SCE, G2 has an icorr of 0.22 ± 0.15 μA/cm² that is about 27 times smaller than that of the untreated NiTi (6.09 ± 2.23 μA/cm²).

The contact angles are shown in Table 4. They are 84° on the untreated Ti, 76° on G1, and 75° on G2. There is no considerable difference between the untreated NiTi and G2.

The morphology of the mouse MC3T3-E1 pre-osteoblasts after culturing for 5 h is presented in Fig. 7. The untreated Ti and G1 show good cell spreading and osteoblast attachment (Fig. 7a and b). The attached cells also exhibit ordered F-actin, apparent filopodia, and flattened membranes on untreated Ti and G1. The same is true on the untreated NiTi and G2 (Fig. 7c and d).

4. Discussion

The surface of G1 is rougher than that of G2 while both are uniform and show some bud-like protrusions (Fig. 1b and d) possibly due to the generation of plasma clusters [64]. The cross sectional views in Fig. 1e and f disclose an interface between the substrate and topcoat and its thickness is larger on G2 than G1. This may be due to the higher C₄H₂ pressure and longer deposition time. Besides the topcoat and interface, a coating interface underneath is observed from G2 (Fig. 1f) on NiTi due to the graded interface formed during deposition.

XPS (Fig. 2) reveals a C layer thickness of 0.53 μm on G1 and a graded interface of about 1.67 μm. The strong peak at 285.62 eV in the C 1s spectra (Fig. 3a) suggests that C–C bonding is formed on G1 to about 0.53 μm. The C 1s peak is composed of 2 sub-peaks with binding energies of 285.2 eV and 285.9 eV. The former represents sp² bonding and the latter indicates sp³ hybridization in the amorphous carbon phase with sp³ (64.18%) hybridization more pronounced than sp³ (35.82%) in G1. Another peak with a binding energy of 282.87 eV is observed from the C 1s spectra of G1 after sputtering for 24 min (Fig. 3a) suggesting that Ti–C bonding is formed to about 1.67 μm but there is no Ti–C bonding in the near surface (0.53 μm) of G1. Two peaks with binding energies at 454.88 eV and 460.89 eV are observed from the Ti 2p spectra after a depth of 0.53 μm in G1 (Fig. 3e) indicative of Ti–C [65–67]. These two Ti 2p peaks appear after sputtering for 24 min of G1 graded film. Their binding energies decrease with sputtering time becoming 454.0 eV and 460.14 eV after sputtering for 140 min (Fig. 3e–h). Ti–C bonding is formed in the near surface (after a depth of 0.53 μm) of the G1 graded film but decreases with depth eventually becoming the metallic state (Ti³⁺) after sputtering for
Fig. 5. (a–d) Nyquist plots and corresponding equivalent electrical circuits of (a) untreated Ti, (b) G1, (c) untreated NiTi and (d) G2 in 154 mM NaCl solution (*R*: solution resistance, *R*: charge transfer resistance, CPE: capacitance, *R*: resistances of the inner layer, CPE: capacitance of the inner layer, *R*: resistance of the outer layer and CPE: capacitance of the outer layer). (e, g) Bode impedance and (f, h) Bode phase angle plots of (e, f) untreated Ti and G1, and (g, h) untreated NiTi and G2 in 154 mM NaCl solution.
140 min. The intensity of C 1s corresponding to Ti–C shows the following trend: gradually increasing from 24 to 44 min, reaching a plateau at 48 min, decreasing gradually from 52 to 96 min, and disappearing (Fig. 3a–d). The results indicate that a Ti and C graded interface is formed between the Ti substrate and C topcoat and there is a Ti and C graded interface in between.

The Raman spectra in Fig. 4 can be deconvoluted into two sub-bands: C5/C7 odd ring and Csp2–Csp3 cluster bands at 1527 cm\(^{-1}\) and 1339 cm\(^{-1}\), respectively [68]. Normally, the band at 1527 cm\(^{-1}\) can be assigned to the G band and that at 1339 cm\(^{-1}\) to the D band. However, stress in a film can alter the Raman spectra. Usually, carbon films and graded films prepared by cathodic arc deposition and PACVD are not very soft and stress is present [2]. Hence, the observed bands at 1527 cm\(^{-1}\) and 1339 cm\(^{-1}\) may be up-shifted. The broader band at 1527 cm\(^{-1}\) can be assigned to the odd ring C5/C7 band induced by ion irradiation and that at 1527 cm\(^{-1}\) cannot be assigned to a G band. The lower side band at 1527 cm\(^{-1}\) of the C5/C7 band may be assigned to dangling Csp2–Csp3 bonds which usually exist at 1470 cm\(^{-1}\) when there is no stress. Considerable A-edge graphicen band can be observed and subsequently, only few hexagonal cortical sp\(^2\) rings are destroyed. The Raman spectra (Fig. 4) disclose the Csp2–Csp3 cluster band and the C5/C7 odd ring band confirms the sp\(^2\)/sp\(^3\) bonding in both G1 and G2 as consistent with the XPS results (Fig. 3a).

The Nyquist plots (Fig. 5a and b) show that the diameter of the capacitive loop of G1 is larger than that of the untreated Ti suggesting that the corrosion resistance is better on G1. Similar results are obtained from G2 (Fig. 5c and d). The equivalent circuits of the untreated Ti (Fig. 5a) and untreated NiTi (Fig. 5c) contain \(R_C^{CPE}(R_P)\) whereas G1 (Fig. 5b) and G2 (Fig. 5d) show \(R_C^{CPE}(R_{OL}R_{OL})(R_{PE}(R_P))\). The EIS spectra of the untreated Ti and NiTi are characteristic of a single time constant whereas those of G1 and G2 have three time constants. Here, in \(R_C^{CPE}(R_P)\) (untreated Ti in Fig. 5a and untreated NiTi in Fig. 5c), \(R_C\) is in series with \(CPE\), where \(R_C\) is the solution resistance and \(CPE\) indicate the charge transfer resistance and capacitance, respectively. The Nyquist plots of the untreated Ti, G1, untreated NiTi, and G2 exhibit depressed semicircles in 154 mM NaCl. Hence, instead of the double layer capacitance (\(C_{DL}\)), the constant phase element (\(CPE\)) is used. The EIS spectra of the untreated Ti and NiTi have a single time constant, \(R_C^{CPE}(R_P)\) because at the metal surface and electrolyte (154 mM NaCl solution) interface, the resistance to electron transfer during the faradic process (charge transfer resistance) in parallel with the double layer capacitance. A single time constant was observed from Ti and NiTi in 154 mM NaCl by Jamesh et al. [34], Li et al. [52], and Shanaghi et al. [69]. In this equivalent circuit, \(R_C^{CPE}(R_{OL}R_{OL})(R_{PE}(R_P))\) (G1 in Fig. 5b and G2 in Fig. 5d), \(R_{OL}\) is in series with \(CPE\), \(CPE\), and \(CPE\), where \(R_C\) indicates the solution resistance and \(R_P\) and \(CPE\) represent the charge transfer resistance and capacitance, respectively. \(R_{OL}\), \(R_{OL}\), \(CPE\), and \(CPE\) indicate the resistance of the inner layer, resistance of the outer layer, capacitance of the inner layer, and capacitance of the outer layer, respectively. The EIS spectra of G1 and G2 show three time constant, \(R_C^{CPE}(R_{OL}R_{OL})(R_{PE}(R_P))\), suggesting that there are two additional layers besides the surface one on the untreated Ti and NiTi. These results are in agreement with the cross sections of the graded films shown in Fig. 1e and f as well as XPS results in Figs. 2 and 3. In fact, 3 time constants were observed from TiO2 coated 316 stainless steel in NaCl solution by Shen et al. [70]. The equivalent circuits are chosen based on the non-linear least square fit of the experimental data with an error of less than 5%. The EIS parameters are displayed in Table 2 and good agreement between the fitted and experimental data can be obtained with \(X^2\) being 10^-3. Changes in \(R_C\) are observed between the untreated Ti and G1 as well as between the untreated NiTi and G2 (Table 2). However, they do not necessarily explain the corrosion resistance [71]. \(R_C\) is the solution resistance between the working electrode and reference electrode and changes with the geometry of the cell as well as conductivity of the electrolyte. Here, \(n_P\), \(n_{OL}\), and \(n_{OL}\) are indices of the dispersion effect of the CPE components representing their deviations from the ideal capacitance due to surface roughness and inhomogeneity of the electrode on the micro-scale. The values of \(n_P\), \(n_{OL}\), and \(n_{OL}\) are always between 0 and 1 [72] and are in this range in this study. The untreated Ti has an \(n_P\) of 0.85 ± 0.01 whereas G1 shows an \(n_P\) of 0.83 ± 0.12 along with \(n_{OL}\) of 0.77 ± 0.09 and \(n_{OL}\) of 0.81 ± 0.19 (Table 2). The untreated NiTi shows an \(n_P\) of 0.84 ± 0 and G2 has an \(n_P\) of 0.72 ± 0 with \(n_{OL}\) being 0.87 ± 0 and \(n_{OL}\) being 1 ± 0 (Table 2). The \(n\) indices of the untreated Ti and G1 are in the similar range whereas the higher \(n\) index of the outer layer of G2 reveals its near capacitive behavior. G1 and G2 show higher resistance and lower capacitance than the untreated Ti and NiTi (Table 2) and it is reflected by the Nyquist plots (Fig. 5a–d) in which the diameter of the capacitive loop of G1 and G2 is larger. In general, materials that exhibit lower capacitance and higher resistance offer better corrosion resistance and vice versa [45,61]. The untreated Ti shows \(R_P\) of 7.68 ± 0.74 × 10^4 Ω cm^2 and G1 has \(R_P\) of 1.42 ± 0.69 × 10^5 Ω cm^2 along with \(R_{OL}\) of 1.56 ± 0.42 × 10^5 Ω cm^2 and \(R_{OL}\) of 6.25 ± 3.64 × 10^5 Ω cm^2 (Table 2). Moreover, the untreated Ti shows \(CPE\) of 5.03 ± 1.76 × 10^-3 cm^2 S^-1 and G1 shows \(CPE\) of 2.84 ± 1.46 × 10^-6 cm^2 S^-1 along with \(CPE\) of 2.67 ± 2.67 × 10^-10 cm^2 S^-1 and \(CPE\) of 2.16 ± 2.10 × 10^-14 cm^2 S^-1 (Table 2). The higher resistance and lower capacitance of G1 reveals better corrosion resistance. Similarly, the untreated NiTi shows \(R_P\) of 6.94 ± 0 × 10^4 Ω cm^2 and G2 shows \(R_P\) of 2.11 ± 0 × 10^5 Ω cm^2 along with \(R_{OL}\) of 1.15 ± 0.210 Ω cm^2.
and $R_{OL}$ of $9.88 \pm 0 \times 10^2 \Omega \cdot \text{cm}^2$ (Table 2). The CPE$_{OL}$ of the untreated NiTi is $3.26 \pm 0 \times 10^{-5} \Omega^{-2} \cdot \text{cm}^{-2} \cdot \text{S}^{-1}$ where G2 shows CPE$_{OL}$ of $4.30 \pm 0 \times 10^{-5} \Omega^{-2} \cdot \text{cm}^{-2} \cdot \text{S}^{-1}$ along with CPE$_{OL}$ of $3.98 \pm 0 \times 10^{-8} \Omega^{-2} \cdot \text{cm}^{-2} \cdot \text{S}^{-1}$ and CPE$_{OL}$ of $5.20 \pm 0 \times 10^{-8} \Omega^{-2} \cdot \text{cm}^{-2} \cdot \text{S}^{-1}$ (Table 2). Hence, a similar conclusion can be drawn on G2.

### Table 3
Mean and standard deviation of corrosion potential ($E_{corr}$), corrosion current density ($i_{corr}$) and passive current density ($i_{pass}$) calculated from potentiodynamic polarization data of untreated Ti, G1 graded film on Ti, untreated NiTi and G2 graded film on NiTi in 154 mM NaCl solution (SCE: saturated calomel electrode).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$E_{corr}$ (mV vs. SCE)</th>
<th>$i_{corr}$ (µA/cm²)</th>
<th>$i_{pass}$ (µA/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Ti</td>
<td>$-458 \pm 43$</td>
<td>$1.06 \pm 0.26$</td>
<td>$7.46 \pm 0.38$</td>
</tr>
<tr>
<td>G1 graded film on Ti</td>
<td>$-314 \pm 54$</td>
<td>$0.07 \pm 0.05$</td>
<td>$0.46 \pm 0.29$</td>
</tr>
<tr>
<td>Untreated NiTi</td>
<td>$-295 \pm 49$</td>
<td>$0.31 \pm 0.03$</td>
<td>$6.09 \pm 2.23$</td>
</tr>
<tr>
<td>G2 graded film on NiTi</td>
<td>$-215 \pm 51$</td>
<td>$0.06 \pm 0.01$</td>
<td>$0.22 \pm 0.15$</td>
</tr>
</tbody>
</table>

The Bode impedance and Bode phase angle plots show that G1 has larger impedance (Fig. 5e). A steady increase in the impedance is observed from the untreated Ti at 166 Hz and similar effects are observed from G1 at a higher frequency of $10^5$ Hz (Fig. 5e). G1 shows larger phase angles over a wide range of frequencies than the untreated Ti. The larger impedance and phase angle maxima suggest that G1 has a near-capacitive behavior and better corrosion resistance. Larger impedance is also observed from the G2
graded film than the untreated NiTi (Fig. 5g) and the larger impedance and phase angle maxima also indicate that G2 has a near-capacitive behavior and better corrosion resistance.

The potentiodynamic polarization curves provide more information about the corrosion mechanism. As shown in Fig. 6a, $E_{\text{corr}}$ of G1 shifts toward the positive direction from $-458 \pm 43$ to $-314 \pm 54$ mV vs. SCE suggesting better corrosion resistance. Moreover, the cathodic and anodic branch of the polarization curve of G1 graded film shift toward lower current density (Fig. 6a). In general, the cathodic region of the polarization curve describes the cathodic reduction reactions. That is, addition of released electrons from the anodic oxidation reaction and the anodic part of the polarization curve indicate oxidation reactions taking place at an elevated potential, i.e. dissolution of metals/elements. The cathodic branch of the polarization curve of G1 shifts toward smaller current density (Fig. 6a) suggesting that the cathodic reduction reactions are suppressed. The anodic branch of the polarization curves of both the untreated Ti and G1 show an active passive transition. The active and passive regions of G1 shift toward lower current density (Fig. 6a) suggesting that the anodic oxidation reactions at the applied higher potential are suppressed on G1. As a result, the corrosion resistance of G1 is improved. Furthermore, G1 shows smaller $i_{\text{corr}}$ and $i_{\text{pass}}$ than the untreated Ti (Table 3) suggesting that G1 is more resistant against corrosion [34]. The potentiodynamic polarization curves impart more information about the corrosion mechanism. As shown in the polarization curves (Fig. 6b), $E_{\text{corr}}$ of G2 shifts toward the positive direction from $-295 \pm 49$ to $-215 \pm 51$ mV vs. SCE and the cathodic and anodic branches of the polarization curve shift toward smaller current density compared to the untreated NiTi in 154 mM NaCl (Fig. 6b) suggesting that the cathodic reduction reactions on G2 are suppressed. The anodic branches of the polarization curves of both the untreated NiTi and G2 show an active passive transition and the active and passive regions of the polarization curve shift toward smaller current density (Fig. 6b) suggesting that the anodic oxidation reactions at the applied higher potential are suppressed. G2 shows smaller $i_{\text{corr}}$ and $i_{\text{pass}}$ than the untreated NiTi (Table 3) again suggesting better corrosion resistance [34]. The potentiodynamic polarization studies further substantiate the EIS studies confirming that G1 and G2 have much better corrosion resistance than the untreated Ti and NiTi, respectively.

When Ti is exposed to air, a few nm thick oxide film grows spontaneously on its surface. Studies suggesting that the titanium oxide surface has both acidic type and basic type hydroxide groups when titanium is immersed in an aqueous solution give the following two reactions [7].

$$\text{Ti} - \text{OH} + \text{H}_2\text{O} \rightarrow [\text{Ti} - \text{O}]^- + \text{H}_3\text{O}^+ \quad (1)$$

$$\text{Ti} - \text{OH} + \text{H}_2\text{O} \rightarrow [\text{Ti} - \text{OH}_2]^+ + \text{OH}^- \quad (2)$$

Thus, the oxide film of Ti possesses amphiporic properties that is, acidic (positive charge) due to reaction (2) and basic (negative charge) due to reaction (1). Generally, the native oxide film on Ti is protective and plays an important role in its corrosion resistance. However, body fluid having protein molecules and amino acids, blood plasma having 113 mM of Cl$^-$ and interstitial fluid having 117 mM of Cl$^-$ accelerate corrosion on implant surface under in vivo conditions. Consequently, metal ion dissolution can be possible at the metal/solution interface [32] and a barrier layer is necessary to improve the corrosion resistance of the implant surface by preventing metal ion dissolution. Here, EIS and potentiodynamic polarization studies revealing that G1 and G2 have much better corrosion resistance than the untreated Ti and NiTi, respectively and it can be inferred that the G1 and G2 graded films act as barrier layers on Ti and NiTi, respectively, to improve the corrosion resistance in 154 mM NaCl solution. A schematic model of the corrosion mechanism of the untreated Ti/NiTi and G1/G2 graded films in 154 mM NaCl solution stipulated based on the EIS and potentiodynamic polarization studies is illustrated in Fig. 6c.

The contact angles in Table 4 show that the plasma modified G1 is more hydrophilic ($76^\circ$) than the bare Ti substrate ($84^\circ$) but there is no appreciable difference between the untreated NiTi and G2 although G2 is also hydrophilic ($75^\circ$). A more hydrophilic surface bodes well for biocompatibility and the surface can better immobilize protein molecules while preserving the native conformation [1]. Although the SEM images in Fig. 1 reveals a rougher surface.

![Fig. 7. Fluorescent images of MC3T3-E1 pre-osteoblasts cultured for 5 h: (a) untreated Ti, (b) G1, (c) untreated NiTi, and (d) G2.](image-url)
on G1 than G2, there is no significant difference in the contact angles. Protein adhesion and biocompatibility depend not only on the contact angle/surface energy of the surface, but also the geometric topography and stoichiometric distribution. The surface energy, topography, and composition affect surface passivation. Water molecules can be preferentially adsorbed on a hydrophilic surface and may generate a hydrated interfacial complex facilitating subsequent protein adsorption. Therefore, the hydrophilic surface on G1 and G2 can better retain the conformation of immobilized protein. Nevertheless, it should be noted that the hydrophilic/hydrophobic characteristics are not the whole factors. A rough surface consisting of different dangling bonds, defects, and non-homogeneous mixed networks of weaker and stronger bonds tends to provide less surface passivation than a smooth surface consisting of a homogeneous dense network with strong bonds. Hence, G1 and G2 may exhibit different surface passivation leading to variable biocompatibility and ability to preserve the conformation of immobilized protein molecules.

The untreated Ti and G1 show good cell spreading and osteoblast attachment (Fig. 7a and b) with the attached cells exhibiting ordered F-actin, apparent filopodia, and flattened membranes, suggesting that the cyto-compatibility is not affected by the plasma treatment. Similar effects are observed from the untreated NiTi and G2 (Fig. 7c and d). In general, ordered F-actin, apparent filopodia, and flattened membranes indicate good cyto-compatibility [7,8,21]. In general, the quantity of osteoblasts attached to amorphous carbon and Ti is similar because both possess good biocompatibility [7,8,21] and our study confirms it suggesting that the good interface can enhance bone formation. G1 and G2 show good interactions with cells. However, the nature of the protein layers on the surface can influence the cell interactions affecting cell attachment and activation. Moreover, adsorption of protein molecules is faster than cell transportation to the implant surfaces. Hence, the nature of the protein molecules plays an important role in improving the interactions between the cells and implant surface. The plasma-modified surface can generate covalent binding sites for protein molecules and the ability to immobilize protein molecules covalently on a hydrophilic surface is an important aspect to preserve the conformation of protein molecules and bioactivity [73]. Bilek et al. [1] have observed that the formation of about $6 \times 10^{25}$ m$^{-2}$ free radicals on the plasma-modified surface is responsible for the covalent immobilization of protein molecules. The unpaired electrons on the surface provide a universal platform for protein binding and adequate electron mobility within the layer. The free radicals having unpaired electrons can link covalently with residues of amino acids in the protein molecules. However, the mobility of the unpaired electrons is reduced by inclusion of elements that hamper $\pi$ conjugation in the carbon structures. Addition of hydrogen, oxygen, or stainless steel inclusions diminishes the ability of covalent binding on the plasma-modified samples. For example, the ability of covalent binding is not observed from some polymers such as polydimethylsiloxane (PDMS) due to the presence of large concentrations of Si. Therefore, the density of mobile unpaired electrons in the carbon sites reflects the ability of covalent binding. Strong covalent immobilization on a dense monolayer of an extra-cellular protein matrix on a plasma-modified surface was observed by Yin et al. [27] and the proteins on the plasma treated surface also enhanced human umbilical vein endothelial cell adhesion and growth. Bax et al. [4] observed robust covalent immobilization of collagen molecules with retained conformation on the plasma modified surface. The collagen adsorbed on the plasma-treated surface also enhanced human dermal fibroblast adhesion and spreading. Similar effects were reported by Nosworthy et al. [46]. Covalent immobilization of enzyme horseradish peroxidase with 100% protein coverage on the plasma-modified surface was observed by Gan et al. [74] and rapid covalent immobilization of protein molecules on the plasma-modified surface with a larger cell density was observed by Tran et al. [60]. Ho et al. [75] also observed improved binding of protein molecules with retained conformation and Waterhouse et al. [3] observed robust covalent immobilization of protein molecules having low thrombogenicity on the plasma-modified stainless steel coronary stent. A high degree of covalent binding of protein molecules was observed by James et al. as well [2]. Hence, the graded films with C topcoats described in this paper provide covalent binding of protein molecules while retaining the functional state as well as enhanced corrosion resistance rendering the materials attractive to many biomedical applications.

5. Conclusion

Protective Ti and carbon graded composite films with carbon topcoats are prepared on Ti and NiTi by hybrid cathodic arc–glow discharge plasma assisted chemical vapor deposition. The graded films significantly improve the corrosion resistance in 154 mM NaCl solution and good cyto-compatibility is also observed in vitro.

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