Effect of carbon nanotube and aluminum oxide addition on plasma-sprayed hydroxyapatite coating’s mechanical properties and biocompatibility

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\textbf{A B S T R A C T}

This study reports on the synthesis of novel bioceramic composite coating of hydroxyapatite (HA) reinforced with carbon nanotubes (CNTs) and aluminum oxide (\(\text{Al}_2\text{O}_3\)) using plasma spray technique. Fracture toughness of HA–20 wt.% \(\text{Al}_2\text{O}_3\) improved by 158\% as compared to HA coating whereas HA–18.4 wt.% \(\text{Al}_2\text{O}_3–1.6\) wt.% CNT showed an improvement of 300\%. Carbon nanotubes provided reinforcement via rebar mechanism. Human fiber osteoblast cell-growth studies showed that biocompatibility of the coating remained unaltered, as \(\text{Al}_2\text{O}_3\) retained its bio-inertness and CNT, its bioactivity, within the composite coatings. Composite coating showed lower attachment, but higher proliferation rate, for the osteoblast cells, which has been attributed to the surface roughness. An optimized relation between coating composition, its biocompatibility and mechanical properties was established to predict the most suited coating material for orthopedic implants. HA–\(\text{Al}_2\text{O}_3–\text{CNT}\) composite coating displayed most improved mechanical properties while retaining its biocompatibility.

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\textbf{1. Introduction}

Hydroxyapatite (HA) is a calcium phosphate based ceramic (Ca/P ratio of 1.66) with a chemical composition \(\text{Ca}_{10}(\text{PO}_4)_{6}(\text{OH})_2\) similar to that of bone. HA has been widely accepted in the biomedical industry for orthopedics, maxillofacial surgery and dental implants because of its biocompatibility and osseointegration [1]. One of the main applications of HA is that as coatings on metallic medical implants. Despite the advantages that HA presents, the brittle nature, and low fracture toughness of HA coating often result in rapid wear, and premature fracture of the coated layer. Hence, there is a need to improve the mechanical properties of the HA coatings without compromising the biocompatibility.

As a response to such need, many efforts have attempted to enhance the mechanical properties of HA via the addition of secondary ceramic reinforcement materials such as \(\text{Al}_2\text{O}_3\), \(\text{ZrO}_2\), \(\text{TiO}_2\) [1–10]. Gautier and co-workers have reported four times increase in the fracture toughness with 20 vol.% of \(\text{Al}_2\text{O}_3\) addition in the form of platelets [8]. Li et al. also agreed to the fact that \(\text{Al}_2\text{O}_3\) addition helps increasing the bending strength of HA composite [4]. Evis and Doremus [7] studied HA–\(\text{ZrO}_2\) composites and reported the increase in hardness and fracture toughness of the composite with \(\text{ZrO}_2\) addition. Findings of Fu et al. also follow the same trend, and in addition they reported the significant reduction in decomposition of HA during high temperature plasma processing [2]. Wang et al. [1] has studied the wear property as an effect of partially stabilized \(\text{ZrO}_2\) addition to HA against ultra high molecular weight polyethylene in human plasma lubrication (a simulated bioimplant environment) and has reported improvement in the wear resistance due to the second phase particle addition.

A few researchers have attempted carbon nanotubes (CNTs) as the reinforcement in HA-based composites to improve its mechanical strength [11–15]. Chen and co-workers [13] were first to report such an effort where they have shown improvement in wear resistance with addition of up to 20 wt.% CNTs in HA-composites. Meng et al. reported an increase in flexural strength of HA–CNT composite by ~10 vol.% CNT addition [11]. Kaya has observed the increase in bond strength of coating with Ti–6Al–4V implant substrate with addition of 2% CNT to HA [12]. Theses coatings also demonstrated increase in the elastic modulus, hardness and fracture toughness [12,13]. Balani et al. from our research group reported 56% increase in the fracture toughness [14] and 60% decrease in the wear volume [15] of the plasma-sprayed composite coating of HA with 4 wt.% CNT. The biocompatibility of the CNT reinforced HA coating was established by cell growth and proliferation [14].

In general, addition of ceramics or second phase particles helps in increasing the mechanical properties of HA-based composite coatings for biomedical implants. Recently an attempt was made by Kaleem et al. [16] to overcome ceramic brittleness by dual strengthening via addition
of 1 vol.% SiC nanoparticles as second phase with higher mechanical properties than parent Al2O3 matrix. CNTs (7 vol.%) were also incorporated to achieve toughening via fiber reinforcement mechanism. Hence, it must be very interesting to study the effect of both these type of secondary phase reinforcements together on HA-composites and that should ideally produce the material with best mechanical properties without sacrificing biocompatibility. Therefore, the focus of the present study is to understand the toughening mechanism of HA-matrix composite coating by addition of CNT and Al2O3 simultaneously as the reinforcement. Biocompatibility of the HA coatings with dual reinforce-

### Table 1

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Composition</th>
<th>Powder processing</th>
<th>Agglomerate Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>100 wt.% HA</td>
<td>HA (spray dried)</td>
<td>HA (15–55 μm)</td>
</tr>
<tr>
<td>HA-A</td>
<td>80 wt.% HA and 20 wt.% Al2O3</td>
<td>1. HA (spray dried)</td>
<td>HA (15–55 μm)</td>
</tr>
<tr>
<td>HA-A16C</td>
<td>80 wt.% HA, 18.4 wt.% Al2O3 and 1.6 wt.% CNT</td>
<td>1. HA (spray dried)</td>
<td>HA (15–55 μm)</td>
</tr>
</tbody>
</table>

#### 2. Materials and methods

2.1. Processing

Precursor powders consisted of three different compositions viz. HA, HA–A and HA–A16C. The details of composition, processing techniques and particle size of the starting powders are given in detail in Table 1. Multiwalled carbon nanotubes (CNTs) used in this study are of 95% purity with OD of 40–70 nm, which was spray dried with 150 nm Al2O3 particles for preparing the spherical agglomerates.

Fig. 1. Low and high magnification (inset) SEM micrographs of spray dried powders: (a) HA, (b) Al2O3, (c) Al2O3-CNT composite and (d) Al2O3-CNT composite agglomerate at high magnification showing CNT distribution.
Fig. 1a, b, and c shows the SEM images of spray dried powders of HA, Al₂O₃ and Al₂O₃–CNT composite powders, respectively in as-received condition before blending. The features are magnified as inset. All the three powders show spherical shape in agglomerated condition. It is clearly evident from the micrographs that the average particle size for all compositions is same. The high magnification image of the composite powder (Fig. 1d) shows uniform CNT distribution in the agglomerates. CNTs are seen to be nicely attached to the Al₂O₃ nanoparticles in the agglomerate. As-received powders were blended in a jar mill (US Stoneware, East Palestine, OH) for 1 h as per the ratios described in Table 1. Plasma spraying of the precursor powders to synthesize coatings was carried out using Praxair SG-100 gun on Ti–6Al–4V substrates (100 mm×25 mm×2 mm) which are widely used for biomedical implants. Plasma processing parameters utilized are presented in Table 2.

2.2. Phase and microstructural characterization

Siemens 500D X-ray Diffractometer (XRD) with CuKα radiation (wavelength of 1.54 Å) operating at 40 kV and 20 mA was utilized to identify the phase constituents of the precursor powders and plasma-sprayed coatings. DIFFRACplus EVA software (Bruker, Madison, WI, USA) was utilized to analyze the XRD spectra. Fraction of crystalline HA phase present in the coatings was calculated using Eq. (1).

\[
\%\text{ Crystalline HA Phase} = \frac{\sum A_{HA}}{\sum A_{all\_peaks}}
\]

(1)

where A_{HA} is the area under the HA peaks, and A_{all\_peaks} is the area under all the peaks. Field emission JEOL JSM 6330 F scanning electron microscope was utilized to characterize the microstructural details of the coatings. Elemental analysis of the coating was performed using energy dispersive spectroscopy (EDS) at different morphological features. X-ray mapping was performed using a JEOL JSM 5910LV scanning electron microscope operating at 15 kV and 32 frames in a matrix of 1024×800 pixels taking 50 μs per square pixel, each pixel of dimensions 0.336 μm in x and y equally. Surface morphology of the polished cross-section of the coatings was evaluated using a 100 nm Berkovich tip in the scanning probe mode, using Hysitron Triboindenter. Triboindenter measures the topographic features with a piezoelectric scanner with a height resolution capacity of 0.2 nm. Surface roughness was obtained by image processing through SPIP-4.5.1 software.

2.3. Mechanical characterization

Relative density of free standing plasma-sprayed coatings was calculated using Archimedes principle and water as immersion medium. Fracture toughness of the coatings was estimated using Vickers microhardness tester (HXD-100 TMC, Shanghai Taiming Optical Instruments, China) with a load of 300 g and dwell time of 5 s. Fracture toughness was evaluated utilizing Anstis equation (Eq. [2]) that is based on the length of the radial crack generated.

\[
K_{IC} = 0.016 \left( \frac{E}{H} \right)^{0.5} \left( \frac{P}{c^{3/2}} \right)
\]

(2)

E is the elastic modulus, H is hardness, P is load utilized in N and c is radial crack length in m. Elastic modulus is taken as a 100 GPa for HA coating [17,18]. For the composite coatings, the elastic modulus has been calculated using rule of mixture considering the weight % of reinforcement addition to the HA matrix and elastic modulus of Al₂O₃ and CNT as 390 GPa and 1000 GPa respectively. The calculated elastic moduli for HA–A and HA–A16C coatings were 150 GPa and 160 GPa respectively.

2.4. Biocompatibility and cell culture

Human osteoblast cells (ATCC, CRL-11372) were cultured in a 1:1 mixture of Ham’s F12 Medium Dulbecco’s Modified Eagle’s Medium, with 2.5 mM l-glutamine without phenol red (GIBCO-11098). The base media was supplemented with 0.3 mg/mL of G418 sulfate (ATCC, 30-2305); fetal bovine serum (FBS, Hyclone, SH30071) to a final concentration of 10% and 1% PG (penicillin/streptomycin/glutamine). Osteoblasts were seeded on the plasma-sprayed coatings at a density of 30,000 cells/cm². Cells were grown in an incubator at a temperature of 34 °C, 5% CO₂–95% air.

2.4.1. Qualitative analysis

After cell culturing the cells were fixed and prepared for SEM observations. Preparation consisted of washing the samples with PBS (GIBCO, 14040-133) to remove the presence of any residual serum, followed by incubation in a 25% formalin solution (1:3 formalin and PBS, Thermo-Shandon, 990244) for 30 min. Afterwards samples were washed three times with PBS and the incubation on formalin solution for 30 min was then repeated. Finally samples were washed three times with PBS and then placed in a lyophilizer (LABCONCO, FREEZE DRY SYSTEM, FREEZONE 6) to dry for 24 h.

2.4.2. Quantitative analysis

Cell quantification was performed by carrying cell cultures on the samples in the same manner as explained previously. After each of the desired culturing times was achieved, coatings were moved from the culturing well to an empty well and then incubated at 34 °C, 5% CO₂–95% with trypsin EDTA (ATCC, 30-2101) for 15 min to detach the cells from the coating. Cells were then suspended in 1 ml of media and counted on a bright line hemocytometer. Experiment was repeated four times (n = 4) to achieve statistical significance, student t-test was performed to confirm any significant statistical difference at a 95% confidence level.

3. Results and discussion

3.1. Microstructure of plasma-sprayed coatings

Coatings were successfully deposited on Ti–6Al–4V substrate. Fig. 2a, c, and e shows cross sectional SEM micrographs of HA, HA–A and HA–A16C coatings, exhibiting uniform thickness of ~300 μm, 400 μm and 450 μm respectively. Low magnification images of the coatings reveal the presence of some porosity and microcracks in the coatings. High magnification view (Fig. 2b) of HA coating reveals the presence of smooth lamellar structure which is typical of plasma spray process. On the other hand, in HA–A coating, regions of Al₂O₃ present a rough surface with partially melted regions (Fig. 2d). High magnification image of HA–A16C coating (Fig. 2f) also reveals lamellar HA microstructure combined with uniformly distributed regions of rough morphologies attributed to the second phase. A distinct difference is observed in the distribution of second phase particle in HA–A and HA–A16C coatings. In case of HA–A coating, the second phase remains as larger particles, whereas in case of HA–A16C, it is present as uniformly dispersed smaller particles throughout the coating. These morphologies can be attributed to the bonding strength of the spray dried second phase powders. Spray dried Al₂O₃ agglomerates have better strength due to the bonding between similar interfaces. But, in case of spray dried CNT–Al₂O₃ agglomerate, the interparticle strength is lower owing to the presence of dissimilar interfaces. Hence, second phase particles in HA–A16C easily explode into smaller particulates during plasma spraying resulting in uniform dispersion in HA coating. These microstructural observations are in agreement with the measured surface roughness of the three coatings. The Ra values, measured to be 25.9 and 34.8 nm for HA–A and HA–A16C coatings respectively, show clearly that they are rough than HA coatings with Ra of 5.4 nm. It has been reported that morphological roughness is beneficial for biocompatibility as it promotes the protein absorption on the surface favoring cell attachment [14,19–21].
Features like microcracks and pullout regions are also visible in the high magnification micrographs of the coatings. Microcracks are generated from the interface of (i) secondary phases and (ii) unmelted particles with the matrix. The interface between fully molten HA splats and secondary phases experiences stresses due to the thermal mismatch caused by the difference in thermal conductivity of the second phase reinforcement particle with the matrix. CNT has the thermal conductivity of 2890 W/m K [22], whereas for HA and Al₂O₃ it is 0.7 W/m K and 36.16 W/m K respectively [23]. Al₂O₃, having more than 30 times thermal conductivity than HA, creates a thermal gradient across the HA/Al₂O₃ boundary in HA–A coating. This effect is significant in case of plasma spraying due to its inherent nature of rapid cooling rates that generates thermal stresses in the coating to nucleate microcracks at the interfaces. In case of HA–A₁₆C coating, the presence of CNTs that has thermal conductivity ~4000 times higher than HA matrix causes steeper temperature gradient. Hence, the probability of formation of microcracks should also be higher in HA–A₁₆C coating. On the contrary, fewer and shorter microcracks are visible in HA–A₁₆C coating which is attributed to the increase in the fracture toughness by CNT addition through rebar mechanism. Strengthening mechanism produced by CNTs has been discussed in details in the later part of this study.

3.2. X-ray analysis of coatings for phase distribution and quantification

The distribution of second phase should be uniform in the coating because it is important to possess homogenous mechanical properties and biocompatibility throughout the coating. So, it becomes necessary to understand the elemental distribution in the coatings to visualize the overall distribution of Al₂O₃ in the HA matrix. Fig. 3 shows elemental distribution of Calcium, Phosphorus and Aluminum in HA–A₁₆C coating analyzed using Energy Dispersive X-ray mapping. Al₂O₃ is homogeneously distributed throughout the HA coating as evidenced by uniform aluminum distribution in Fig. 3. When compared with the respective back scattered image, the smooth region in the images corresponds to the regions rich in Ca and P, which indicates the presence of HA, whereas the rough regions are rich in Al indicating the presence of second phase particles. This also emphasizes the earlier hypothesis of formation of rough morphologies in secondary phase region. CNTs are largely present in the Al rich region because they were integrated with Al₂O₃ using spray drying (Fig. 1c and d). X-ray signals of carbon were too weak for their presence in very small quantity.

X-ray diffraction pattern of coatings in Fig. 4 reveals the presence of HA, Tri-Calcium-Phosphate (TCP) and Al₂O₃ peaks. Carbon peaks were
not significant for their very low weight fraction in the composite. The generation of TCP phases is generally undesired due to the mismatch in degradation rates of HA and TCP which may lead to mechanical weakening of the implant [24,25]. On the other hand, degradation of the TCP phases lead to generation of pores in the coating allowing better integration with surrounding tissue as time progresses. Hence, it is beneficial to have higher fraction of crystalline HA phase in the coatings. Table 3 represents the fraction of crystalline HA phase present in the coatings from the XRD data. The comparison of the data for three coatings shows clearly that crystalline HA phase fraction has increased with the addition of second phase particles.

All coatings show a small hump in the XRD pattern (Fig. 4) at the lower 2θ values in the range of 30°–35°, indicating the presence of some amorphous phases in the coating. A qualitative comparison of the hump size reduction clearly shows that the overall crystallinity increases with the secondary phase reinforcement. It should be noted here that Al2O3 has ~30 times higher thermal conductivity than HA. Hence it allows lower cooling rate to the neighboring HA splats, resulting in increased crystallinity of HA in the HA–A coating. But, at the same time, microcracks generated at the second phase particle–matrix interfaces resists the heat flow to the neighboring HA region, causing them to cool faster and reducing crystallinity. Hence, the crystallinity of HA–A sample is due to these two competing phenomena. The presence of much sharper peaks in HA–A16C coating suggests that the increase in crystallinity is significant with the CNT addition. The reason for even increased crystallinity is attributed to higher thermal conductivity of CNTs (~100 times to Al2O3). The absence of severe microcracking also helps in improving the crystallinity of the material [13].

Higher thermal conductivity of CNTs also attributes towards a significant increase (~100%) in the crystallite size of Al2O3 in HA–A16C coating as compared to HA–A (Table 3). Al2O3 particles are subjected to the slower cooling rate being the nearest neighbors to CNTs. This inference becomes more evident when compared with the crystallite size of HA phase in both the coatings, which shows no significant change. The effect of strain was assumed to be negligible during the analysis of XRD peaks, as ceramic materials do not deform plastically.

### Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Crystalline HA phase</th>
<th>Crystallite size in coatings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HA (nm)</td>
</tr>
<tr>
<td>HA</td>
<td>47.6</td>
<td>21.9</td>
</tr>
<tr>
<td>HA–A</td>
<td>51.4</td>
<td>22.1</td>
</tr>
<tr>
<td>HA–A16C</td>
<td>53.9</td>
<td>25.2</td>
</tr>
</tbody>
</table>

Fig. 3. Back scattered SEM micrograph of HA–16C coating and corresponding X-ray elemental maps for Al, P and Ca.

Fig. 4. XRD patterns of HA, HA–A and HA–A16C coatings.
Table 4
Density, fracture toughness and surface roughness of plasma-sprayed coatings.

<table>
<thead>
<tr>
<th>Coating</th>
<th>Density g/cc (% theoretical)</th>
<th>Fracture toughness (MPa m^{1/2})</th>
<th>Surface roughness, Ra (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>2.96 (94%)</td>
<td>0.7±0.2</td>
<td>5.4</td>
</tr>
<tr>
<td>HA–A</td>
<td>3.04 (92%)</td>
<td>18±0.1</td>
<td>25.9</td>
</tr>
<tr>
<td>HA–A16C</td>
<td>3.01 (92%)</td>
<td>2.9±0.2</td>
<td>34.8</td>
</tr>
</tbody>
</table>

3.3. Mechanical characterization

Fracture toughness (K_c) of the plasma-sprayed coatings is listed in Table 4. The fracture toughness of HA coating was computed to be 0.71 ± 0.19 MPa m^{0.5}. The theoretical value of fracture toughness of fully dense HA is reported as 1 MPa m^{0.5} [26]. Addition of 20 wt.% Al_2O_3 to the HA matrix increased the fracture toughness to 1.83 ± 0.11 MPa m^{0.5} and HA–A16C yielded a fracture toughness of 2.92 ± 0.23 MPa m^{0.5}. Addition of 20 wt.% Al_2O_3 results in 158% improvement in the fracture toughness of HA–A coating as compared to HA coating. However, addition of 1.6 wt.% CNT resulted in 300% improvement in fracture toughness of HA–A16C coating as compared to HA coating. Increase in the fracture toughness is attributed to addition of (i) Al_2O_3 and (ii) CNT reinforcement. Theoretical fracture toughness of Al_2O_3 is 3.2 MPa m^{0.5} which is significantly higher than that of pure HA [27–28].

The increase of ~300% of fracture toughness of HA compared to HA–A16C invited for a deeper analysis of the role of CNTs in fracture toughening which is dependent on dispersion of the CNTs in the matrix and their toughening mechanisms. A new toughening mechanism is proposed which is “rebar reinforcement” by CNTs. Rebar toughening mechanisms are evidenced by SEM micrographs in Fig. 5. This concept is similar to the building construction that consists of the use of carbon steel bars as concrete reinforcement. CNT acts similar to the reinforcing rebars with the HA–A16C matrix. Their good thermal conductivity allows for ceramic melting and covering of the CNTs with the molten ceramic layer, creating good interfacial bonding of CNT–ceramic matrix as has been proven by Balani and Agarwal [29]. Fig. 5a and b shows the fracture surface of the HA–A16C coatings where substantial portions of the CNT tips have been exposed from the upper splat removal. Fig. 5c illustrates the concept schematically in which upper splats have been removed by fracture surface exposing the CNT tips or rebars.

3.4. Biocompatibility of the coatings

After ascertaining that the mechanical properties had been enhanced, the biocompatibility of the coatings was determined. HA was elected as the control coating since it is the widely accepted coating for orthopedic implant since 1980 [9,30–32], due to its excellent biocompatibility and bioactivity properties that allow biological fixation [2,4,6,23,33,34]. Human fetal osteoblast cells (ATCC, CRL-11372) were cultured on the three coatings as the substrates to find out their biocompatibility and bioactivity properties based on their composition. Osteoblast cells were cultured on coatings of HA, HA–A and HA–A16C at seeding density 30,000 cells/cm² to quantify the growth trends on each of the coatings.

Fig. 6 shows the osteoblast cell morphology on HA, HA–A and HA–A16C coatings. Overall morphology of the hFOB cells in all the coatings is observed to be globular and flat which is an indicator of normal cell attachment and growth process [20,21,35–37], proving the coating compositions to be biocompatible. All coatings have network of fibrous cell extensions coming out from the cell body, for anchoring to the surface. Globular and flatten cells observed in HA coating after 1-day, Fig. 6a, indicate normal hFOB cell attachment and growth. Inset of Fig. 6a elicits overall spread of globular osteoblasts on HA surface. Protruding hFOB fibers initiate contacts with surrounding cells to obtain a confluent layer on top of the HA coating. Complete confluence was observed after 3-day culture on HA coating, Fig. 6b, showing flattened cells. Growth of cells in HA–A coating changes from the two dimensional cellular network (in 1-day culture, not shown here) to a three dimensional cellular network (in 3-day culture, Fig. 6c). This results owing to higher roughness of the HA–A coating surface (Ra=25.9 nm) that offers different surface levels for the cells to attach. Inset of Fig. 6c shows spread of globular osteoblasts on HA–A coating surface. Undeterred cell growth throughout the matrix implies that hFOB cells are not affected by the presence of Al_2O_3 particles in the HA–A matrix, Fig. 6c, thus indicating non-cytotoxicity of Al_2O_3 nanoparticles. Fig. 6d reveals progression of inhomogeneous irregular cell growth to a smooth cellular surface after 3-day cell-culture on HA–A16C coating. Specifically, protrusions present on the globular cells, Fig. 6d, extend arms as network of fibrils, which eventually achieve flattened structures. Global confluence on the HA–A16C coating is observed in the inset of Fig. 6d. Rapid total confluence is achieved in HA–A16C coating surface due to enhanced roughness (~34 nm) and excellent bioactivity of CNTs as discussed later.

Table 5 displays the quantitative data of cell proliferation on the coatings after 1, 3 and 7 days. After one day of culture, the highest cell attachment level was found in HA coating with 23,750±3862 cells/cm² (79% attachment). Other coatings exhibited lower attachment rates like HA–A (49.5%) and HA–A16C (51%) after one day of culture. The initially

![Fig. 5.](image-url)
delayed and then prolonged cell proliferation is in agreement with the observations made by other researchers [38,39]. Ko et al. [39] has shown an initial delayed cell growth on ceramic (ZrO$_2$/Al$_2$O$_3$) surface with respect to metal (Ti) surface, but did not explain their finding. We attribute the delayed cell growth in HA–A and HA–A16C coatings to two reasons: (i) the presence of bioinert Al$_2$O$_3$ in HA matrix and (ii) increased surface roughness of coatings with the addition of secondary phase. Quan et al. [38] also observed delayed cell growth on HA/ZrO$_2$ composite which was attributed to the increased surface roughness as compared to smooth ZrO$_2$ surface. It has been described already that because of the presence of the second phase particles, the surface roughness of HA–A and HA–A16C coatings is more than 5 times higher than that of HA. After three days, HA, HA–A and HA–A16C coatings show further proliferation of 23%, 70% and 71% respectively. Hence the lower attachment and higher proliferation rate for HA–A and HA–A16C coatings are most likely due to the rough surfaces of the composite material which drives the osteoblasts to secrete factors that enhance cell differentiation [40]. HA–A16C displays higher proliferation rate than HA–A which is due to apatite crystallization on the CNT surface which acts as a bioactivity booster promoting cell proliferation.

Fig. 6. Globular osteoblast cells are observed to be extending tentacles in (a) HA coating (1-day culture), and (b) HA coating showing flattened cells (3-day culture) (c) HA–A coating eliciting globular osteoblasts (3-day culture) and, (d) HA–A16C coating evincing additional filopodia like osteoblast growth (3-day culture). Insets depict an overall morphology of cell-coverage.

Fig. 7 shows apatite precipitation on CNT surface in HA–A16C coating after 1 day of culture. HA precipitation on MWCNT surface renders them bioactive potentially aiding the differentiation of the fOB. Precipitation of HA on the outer wall of MWCNT when exposed to simulated body fluids has been documented by other researchers as well [14,41–45].

After seven days of cell culture, all coatings achieved a similar cell

Table 5
Quantitative data of cell proliferation on HA, HA–A and HA–A16C.

<table>
<thead>
<tr>
<th>Coating</th>
<th>1 Day (cells/cm$^2$)</th>
<th>3 Days (cells/cm$^2$)</th>
<th>7 Days (cells/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>23,750 ± 3862</td>
<td>29,250 ± 2986</td>
<td>52,500 ± 11,561</td>
</tr>
<tr>
<td>HA–A</td>
<td>14,250 ± 1707</td>
<td>24,250 ± 1707</td>
<td>47,000 ± 2943</td>
</tr>
<tr>
<td>HA–A16C</td>
<td>15,500 ± 1290</td>
<td>26,500 ± 3109</td>
<td>54,250 ± 11,295</td>
</tr>
</tbody>
</table>

Fig. 7. Apatite precipitation on CNT present in HA–A16C coating after 1 day.
Bioceramic composite coatings of HA reinforced with bioinert Al2O3 and carbon nanotubes (CNTs) were successfully synthesized using plasma spray technique. HA–A coating, consisting of 20 wt.% Al2O3 displayed an improvement of fracture toughness by 158% over HA coating. HA–A16C coating, achieved via addition of 18.4 wt.% Al2O3 and 1.6 wt.% CNTs to HA, showed ~300% improvement in the fracture toughness, as compared to HA coating. This improvement in the toughness is attributed to dual toughening mechanisms: i) introduction of second phase material (Al2O3) with superior mechanical properties and ii) rebar mechanism achieved through CNTs. Addition of the reinforcements has no negative effect on the biocompatibility of the coating. CNTs behave as a bioactive booster, precipitating apatite on its surface. Al2O3 particles retained their bio-inertness without affecting the biocompatibility of HA. Biocompatibility is correlated with the fraction of bioactive phase (FBC) in the coatings. It is concluded that the optimization between FBC and the fracture toughness gives the most suitable coating material for orthopedic implants. HA–A16C coating, with improved fracture toughness and comparable biocompatibility, is the best choice for this purpose.

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References


Fig. 8. Correlation of Fraction Bioactive Content with cell density (shown as the lines) and fracture toughness (depicted as the bars).