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Investigation of crystallinity, mechanical properties, fracture toughness and cell proliferation in plasma sprayed graphene nano platelets reinforced hydroxyapatite coating

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Abstract
Graphene nanoplatelets (GNPs) (0, 1 wt% and 2 wt%) reinforced hydroxyapatite (HA), denoted by HA, HA-1G and HA-2G respectively, coatings were fabricated on titanium substrate (Ti-6Al-4V) through atmospheric plasma spraying. The major parameters such as porosity, crystallinity, mechanical properties, toughness and cell proliferation were manipulated by varying plasma power from 15 kW to 35 kW and content of GNPs. For the coating synthesized at all plasma power, GNPs were found to be retained by Raman spectroscopy. GNPs reinforcement has led to an improvement in the crystallinity of the composite coatings as compared to HA coatings. On the contrary to it, increase in plasma power from 15 kW to 35 kW resulted in decrease in crystallinity for all three individual coating. Further, Increment in plasma power from 15 kW to 35 kW delivered a significant enhancement in hardness, elastic modulus and fracture toughness up to 81%, 149% and 282% respectively for HA-1 wt% GNPs coating, while it improved to 20%, 50% and 173% respectively on the addition of 2 wt% GNPs in HA coating fabricated at 35 kW. Enhancement in hardness, elastic modulus and fracture toughness was due to three simultaneous reasons: (1) Reduction in porosity (2) Uniform dispersion of GNPs and (3) Toughening mechanism offered by GNPs. Further, the addition of GNPs showed a remarkable improvement in the rate of cell proliferation in the HA coating. A detailed discussion over the reasons behind every results have been made profoundly.

1. Introduction

Atmospheric plasma spraying (APS) is a versatile, FDA-approved technique for fabricating hydroxyapatite [HA;Ca₁₀(PO₄)₅(OH)₂] coatings on metallic substrates such as titanium or stainless steel. These HA coatings can be potentially applied in the biomedical sector such as in orthopedics and for dental implants and maxillofacial surgery [1, 2]. However, the porosity and phase transformations of plasma-sprayed HA coatings limit their application in the industrial sectors [3–5]. Porosity can exponentially degrade the hardness, elastic modulus, and toughness by generating voids and cracks in the coating. Furthermore, the transformation of the HA coating from a crystalline to an amorphous phase during plasma spraying results in the fast dissolution of the coating, thus causing the premature failure of HA-coated implants [5–7]. Researchers have devoted considerable efforts for solving these two issues by manipulating the process parameters and reinforcing the second phase in the HA matrix individually [1, 4, 6–8]. However, no study has focused on solving both problems together in plasma-sprayed HA coatings simultaneously.
According to several research studies, the plasma power (one of the plasma process parameters) can be effectively used to optimize the mechanical properties and crystallinity of the coating. Researchers have reported significant improvements in the hardness, elastic modulus, and toughness in plasma-sprayed HA coatings with increased plasma power [7–10]. However, they have not investigated the crystallinity of plasma-sprayed HA coatings. Hence, the coating quality cannot be gauged to improve its suitability for implants [9].

Other researchers have investigated the crystallinity of plasma-sprayed HA coatings. Unlike the mechanical properties, the crystallinity percentage decreases with increasing plasma power in plasma-sprayed HA coatings. The degradation of the crystallinity was attributed to the formation of higher amounts of amorphous phases such as oxy-hydroxyapatite, tetra-calcium phosphate (TTCP), tri-calcium phosphate (TCP), and calcium oxide (CaO) phases at high temperatures (≥1000 °C) in the HA coating during the APS process [10, 11]. An increase in amorphous phases in the coating could be detrimental to the durability and performance of HA-coated be concluded that an increasing plasma power has opposing effects on the mechanical properties and crystallinity. This could be one of the major reasons for the unavailability of study reports covering both properties simultaneously.

Tercero et al aimed to establish a good correlation between the toughness and crystallinity by adding a second-phase material instead of varying the plasma power [1]. They used carbon nanotubes (CNTs) as the second-phase material in plasma-sprayed HA coatings and fabricated the coatings at 22–23 kW plasma power. Plasma-sprayed HA, HA–20 wt% Al2O3, and HA–18.4 wt% Al2O3–1.6 wt% CNT coatings were deposited on Ti–6Al–4 V substrates. According to their results, the 1.6 wt% CNTs reinforcement increased the crystallinity by 12% and the fracture toughness by 61% compared with those of HA. The improved crystallinity was attributed to the higher thermal conductivity of CNTs (3000 W m⁻¹ K⁻¹) than that of HA (0.7 W m⁻¹ K⁻¹), which helped to lower the cooling rate of the neighboring HA splats. The resulting onset delay in the cooling rate might have accelerated the nucleation process and resulted in an increased crystallinity [1]. Moreover, enhanced fracture toughness was reported, which was due to the rebar toughening mechanism and homogenous distribution of CNTs in the matrix. Based on the results of 1D materials as reinforcements, 2D materials were more promising as second-phase materials because they facilitate higher thermal conductivities (approximately 3000–5000 W m⁻¹ K⁻¹) and stronger interfacial contact with the HA matrix.

In the present study, plasma-sprayed HA coatings were synthesized on Ti–6Al–4 V substrates at five different plasma powers (15 kW, 20 kW, 25 kW, 30 kW, and 35 kW). The mechanical properties and crystallinity percentages of the coatings synthesized at each plasma power and the respective optimized properties were investigated. In addition to the plasma power optimization, second-phase reinforcement (graphene nanoplatelets (GNPs)) was applied to the HA powder. Furthermore, three different compositions (HA, HA–1 wt% GNP and HA–2 wt% GNP) were synthesized at five different plasma powers. The combined effect of the plasma power and GNP's on the mechanical properties and crystallinity percentage was extensively studied with a bare HA coating as reference. Cell proliferation tests were also conducted for all three sets of coatings fabricated at 15 kW and 35 kW. Finally, the optimized properties of the bare HA coating and GNP-reinforced HA coatings were analyzed.

2. Experimental procedures

2.1. Powder preparation

Commercially available HA powder with purity ≥ 99% (average particle size of 200 nm) was obtained from Clarion Pharmaceutical Co., India. The GNP's with 20–30 graphene sheets were obtained from XG Sciences Inc. (Lansing, MI, USA). The nanosize powders cannot be directly plasma sprayed due to its tendency of clogging the plasma gun nozzle. High inter-particle friction between the fine powder particles leads to inconsistent flow resulting non-uniform coatings. Hence, powder processing i.e. Spray drying method was chosen to prepare the spherical agglomerates of the powder using these ultrafine nano powders. During spray drying method, nano-sized HA powder particles were mixed with polyvinyl alcohol to produce slurry. This slurry used to be passed through an atomizing orifice and resulting the spray dried agglomerate. Spray dried agglomerates reduces the inter-particle friction and leads to much better flowability during the plasma spraying resulting the uniform coating. Three dissimilar compositions of powder agglomerates (HA, HA–1 wt% GNP, and HA–2 wt% GNP) were prepared with spray drying. Their sample names are HA, HA–1G, and HA–2G respectively.

2.2. Deposition of plasma-sprayed coating

The spray-dried powders (HA, HA–1G, and HA–2G) were sprayed with a 9MB atmospheric plasma gun (Oerlikon Metco, Switzerland) on a Ti–6Al–4 V substrate (100 mm × 20 mm × 3 mm). The temperature and velocity of the in-flight powder particles were continuously measured with an AccuraSpray™ in-flight diagnostic sensor (Tecnar Automation Ltée, QC, Canada). All three composite powders were sprayed at different plasma...
process parameters (listed in Table 1). Table 1 shows the five different plasma powers (15 kW, 20 kW, 25 kW, 30 kW, and 35 kW). With all other plasma parameters (i.e., the stand-off distance, powder feed rate, and primary and secondary gas flow rates) kept constant. For each composition, five coatings were produced at different plasma powers.

2.3. Microstructure and phase identification of plasma-sprayed coatings
In order to examine the cross-sectional image of plasma sprayed coatings were cut by low speed diamond saw (IsoMet™, Buehler, USA). Now, to hold the coating piece and to perform the grinding and polishing of the coating, this needs to be mounted in an epoxy resin. Mixture of epoxy resin with hardener (in a ratio of 4:1) was prepared and gently poured in the sample cup containing the coating piece. This was allowed to dry for 24 h. After drying, the mounted samples were grinded via different grades of grit papers, such as 120, 240, 600, 800 and 1200 grit size and then polishing was performed using diamond suspension. This prepared polished cross-sectional morphology was seen in FE-SEM having substrate, which shows three surfaces namely epoxy at one end, coating in the middle portion and the substrate at another end. The morphology of the spray-dried powder and the cross-section and fracture surface of the coating were examined by field-emission scanning electron microscopy (FE-SEM; Zeiss, Sigma HD, UK) at working voltages of 10–15 kV. In addition, the radial crack propagation was observed with FE-SEM after the indentation test. The porosity of the plasma-sprayed coatings was calculated with the empirical equation (1) [12]. The theoretical densities of HA and GNPs were considered to be 3.16 g cm$^{-3}$ and 2.25 g cm$^{-3}$, respectively [13]. According to the rule of mixture, the calculated theoretical densities of HA-1G and HA-2G were 3.14 g cm$^{-3}$ and 3.13 g cm$^{-3}$, respectively. The real densities of all plasma-sprayed coatings were measured with the Archimedes principle.

\[
\text{Porosity} = 1 - \frac{\text{Real Density}}{\text{Theoretical Density}} \times 100
\]

The phase identification was conducted through x-ray diffraction (XRD; TTRAX III, Rigaku, Japan) at a Cu K$_\alpha$ wavelength of 1.54 Å. The 2θ range and scanning rate were 20°–60° and 2°/min, respectively. Furthermore, the crystallinity percentage was measured based on the area under the sharp crystalline peaks of HA ($A_{\text{HA}}$) and the area under all peaks ($A_{\text{all peaks}}$), as shown in equation (2) [1].

\[
\text{Crystallinity (\%)} = \frac{\sum A_{\text{HA}}}{A_{\text{all peaks}}} \times 100
\]

2.4. Retention of GNPs in plasma-sprayed coatings
Raman spectroscopy (Renishaw, UK) measurements of the spray-dried powder and the plasma-sprayed coatings synthesized at the lowest and highest plasma powers (15 kW and 35 kW, respectively) were conducted to validate the presence of GNPs. The Raman spectra were obtained with an excitation laser wavelength of 514 nm, spectral resolution of 1 cm$^{-1}$ and 2 cm$^{-1}$, and acquisition time of 10 s. Furthermore, the presence of GNPs and degree of damage in the composite coatings were verified by high-resolution transmission electron microscopy (HR-TEM; FEI Tecnai, USA) operating at 200 kV. The high-resolution lattice fringes of the coatings were investigated to confirm the presence of GNPs and other phases in the HA-2G coating.

2.5. Mechanical properties and fracture toughness of plasma-sprayed coatings
The micro-hardness and elastic moduli of all plasma-sprayed coatings were examined through instrumented micro-indentation (Microtest, Model MTR 3, Spain) at a load of 1 N and dwell time of 15 s. During the indentation, a load-versus-depth curve was recorded, and the elastic modulus of the coating was measured based on the unloading curve. The elastic moduli of all plasma-sprayed coatings were calculated with equation (3).
The optical density of the resulting purple solution was measured at 570 nm with an ELISA plate reader.

Mechanical properties

The probable cytotoxic effects of all plasma-sprayed coatings were determined with human MG63 osteosarcoma cells through a viability assay. The cells were cultured in Dulbecco’s Modified Eagle Medium containing 1.5 g/l glucose (DMEM–Low Glucose, Hi Media) and supplemented with 10% fetal bovine serum (heat inactivated) (Invitrogen, Carlsbad, CA, USA) and 1% antibiotic (100 U/ml of penicillin and 100 μg/ml streptomycin) (HiMedia Laboratories, India) in an incubator at 37 °C with 5% CO2 and 95% relative humidity (Forma Series, Thermo Fisher Scientific, India). All HA and HA–GNPs coated samples were UV-irradiated overnight and then inserted into the growth medium. Subsequently, the cells were seeded on the implants in a 96-well plate, and the viability was checked after 24 h, 48 h, and 72 h. The 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay was conducted by adding 10 μl of 10 mg ml⁻¹ MTT reagent to each well and incubating the samples for 4 h at 37 °C. The formed formazan crystals were dissolved in 200 μl dimethyl sulfoxide, and the optical density of the resulting purple solution was measured at 570 nm with an ELISA plate reader (Fluostar Optima, BMG Labtech, Germany). The results were used to calculate the cell survival percentage with equation (5) [16].

\[
\text{% Cell Survival} = \frac{\text{Optical density from cells grown on implant}}{\text{Optical density from cells grown on regular tissue culture well}} \times 100
\]

3. Results and discussion

3.1. Powder assessment and fabrication of plasma-sprayed coatings

Figures 1(a)–(c) present the low-magnification FE-SEM images of the spray-dried HA, HA–1G, and HA–2G powders, respectively. All three powders exhibit spherical particles with an average particle size of 39 ± 4 μm. The spherical shape of the powder particles reduces the inter-particle friction, thereby resulting in an improved flowability of the powder and a uniform coating thickness during APS [15]. Figures 1(d), (e) present the magnified FE-SEM images of the HA–1G and HA–2G powders, respectively; the arrows indicate the well-distributed GNPs on the HA matrix.

Because the GNPs have a lower surface energy (0.45 J m⁻²) than that of HA (1.69 J m⁻²), the overall surface energy of the powder might be reduced, which helps the GNPs to disperse on the HA matrix [15]. All three powder compositions were used to fabricate the coating at five different plasma powers (see table 1) on a Ti–6Al–4 V substrate.

During the coating fabrication, the in-flight particle temperature and velocity were continuously monitored. The results are provided in figures S1(a), (b) is available online at stacks.iop.org/MRX/7/015415/mediain the supplementary file. According to the results, the particle velocity increases with increasing plasma power, whereas the particle temperature decreases with increasing plasma power. This might be due to the shorter residence time of the particles in the plasma owing to the higher velocity.

For the sake of brevity, the cross-section images of the HA, HA–1G, and HA–2G coatings fabricated at the lowest plasma power (15 kW) and that fabricated at highest plasma power (35 kW) are shown in figures 2(a)–(f), respectively.

Coatings synthesized at 15 kW have relatively higher thickness than that of the coatings fabricated at 35 kW. Furthermore, a reduction in the coating thickness was observed owing to the addition of GNPs on the HA matrix. Both results are directly related to the densification of the coatings, which is the result of a higher in-
flight particle velocity and the filling of pores with GNPs. Because the GNPs experienced the aggressive plasma environment, the retention of the GNPs should be investigated in a Raman analysis.

### 3.2. Retention and dispersion of GNPs in plasma-sprayed coatings

Figure 3(A) illustrates the Raman spectra of the HA, HA-1G and HA-2G powders and plasma-sprayed HA-1G and HA-2G coatings fabricated at 15 kW and 35 kW. The HA powder does not have any Raman active between 1200–1800 cm⁻¹, there is no peak observed in the Raman spectra of HA powder in the range of 1200–1800 cm⁻¹ as shown in figure 3(A). All HA-1G and HA-2G powders and coatings exhibit a weak D band at 1348 cm⁻¹. Figure 3(B) presents a magnified view of the D band, which was clearly visible in all spray-dried powders and coatings. The weak D band in the powders and coatings is the signature of fewer incorporated defects [17]. In addition, a prominent G band was observed at 1577 cm⁻¹ for all powders and coatings. The presence of a G band and weak D band confirms the presence of GNPs in the coatings fabricated at 15 kW and 35 kW.

Figures 4(a)–(c) show the FE-SEM images of the fractured surface of the HA, HA-1G and HA-2G coating at 15 kW respectively. Similarly, figures 4(d)–(f) present the fracture surface of the HA, HA-1G and HA-2G coating at 35 kW, respectively. It could be evident from figure 4 that increases in plasma power as well as reinforcement of GNPs makes coating denser. According to these images, the GNPs are well-distributed on the HA matrix. It is believed that a dispersion of GNPs on the entire matrix can toughen the HA matrix additionally. Because the major aim of this study is to achieve a balance between the crystallinity and toughness of the coatings by varying the plasma power and GNPs reinforcement, the investigation of each coating is presented separately. Finally, a comparison of their characteristics is presented.

### 3.3. Porosity evaluation in plasma sprayed coatings

Since porosity is the integral factor in any plasma sprayed coating, an attempt has been taken to tailor the amount of porosity in the coating by varying the plasma power and the reinforcement of GNPs. According to table 2, increasing plasma power from 15 kW to 35 kW leads to a significant reduction in the porosity of up to 53% for HA coatings. Similarly, for HA-1G coatings, porosity is reduced up to 54%, while reduction of 57% in porosity was noticed for HA-2G coatings on varying plasma power from 15 kW to 35 kW. This could be due to
the higher velocity of the in-flight particles at 35 kW. The molten particles strike the substrate, which improves the packing of the layer and reduces the inter-splat region. Similar results have been reported in literature. For instance, Mohammadi et al. also reported a reduction in the porosity of 37% for an increasing plasma power from 28 kW to 36 kW [8]. However, they mentioned that the reduced porosity was due to the formation of a large amorphous phase, which could fill out the pores between the splats [8].

The reinforcement of the HA coating with 1 wt% GNPs results in a slightly reduced porosity up to 13% at 35 kW compared with that of the HA coating. Similarly, 2 wt% GNPs reinforcement in the HA coating leads to a remarkable reduction in porosity of up to 36% compared with that of the HA coating fabricated at 35 kW. The
Porosity reduction could be due to two parallel phenomena. First, the GNPs can easily access and fill the intersplat region, which is the major source of porosity. Second, the HA splats melt more easily as a result of the well-distributed GNPs and the higher thermal conductivity of the GNPs (approximately 5000 W m$^{-1}$K$^{-1}$) compared with that of the HA coating (approximately 0.7 W m$^{-1}$K$^{-1}$). The higher thermal conductivity of the GNPs contributes to an instant heat transfer from the plasma to the HA matrix [1, 15]. Hence, a slight decrease in the porosity of the coating could have a high impact on various properties such as the crystallinity, mechanical properties, and toughness of the coating.

3.4. Phase identification of plasma sprayed coatings
It is well known that HA transforms to mainly three different phases such as TCP, TTCP, and CaO at high temperatures. During an APS process, these phases can occur in the HA coating and thereby lower the crystallinity of the coating. Hence, an XRD phase evaluation of the fabricated HA coatings was conducted.
3.4.1. Phase identification of plasma sprayed HA coatings

Figure 5 shows the XRD patterns of the HA powder and plasma-sprayed HA coatings fabricated at five different plasma powers. A major intensity peak of HA appears in the powder and all coatings at 31.86°. In addition, the diffraction pattern clearly indicates the formation of additional phases such as Ca$_4$(PO$_4$)$_2$O (TTCP) and Ca$_3$(PO$_4$)$_2$ (TCP) at the peak positions of 29.83° and 31.26° in all HA coatings. The intensities of the TTCP and TCP phases slightly increased with increasing plasma power, which could be due to the subsequent reduction in the in-flight particle temperature. Furthermore, CaO phase could be observed only in the coating fabricated at 20 kW, which experienced a lower in-flight particle temperature. Cizek et al reported that the CaO content decreases with increasing in-flight particle temperature [18]. In the present study, the in-flight particle temperatures are higher for the coatings fabricated at 15 kW and 20 kW, which could be the reason for the absence of a CaO phase.

As reported in literature, the presence of TTCP and TCP phases has a detrimental impact on the crystallinity of the coating. The crystallinity percentages of the plasma-sprayed HA coatings were calculated with equation (1), and the results are listed in table 3. The crystallinity percentages were 94% at 15 kW, 92% at 20 kW, 91% at 25 kW, 87% at 30 kW, and 74% at 35 kW. Thus, increasing the plasma power from 15 kW to 35 kW led to a 21% reduction in the crystallinity, which could be due to the increasing TTCP, TCP, and CaO phases in the HA coatings. As previously mentioned, a reduction in the crystallinity accelerates the fast dissolution of the HA coating. Hence, a higher plasma power degrades the crystallinity. However, a higher plasma power decreases the porosity, which could increase the mechanical properties of the coating.

### Table 3. Crystallinity percentage of plasma sprayed HA coatings.

| S. No. | Nomenclature | 15 kW  | 20 kW  | 25 kW  | 30 kW  | 35 kW  |
|--------|--------------|-------|-------|-------|-------|-------|
| 1. HA  |              | 93.77 | 92.00 | 90.80 | 87.39 | 74.00 |

3.4.2. Phase identification of plasma sprayed GNP (1–2 wt%) reinforced HA coatings

Figure 6(A) shows the XRD patterns of the HA-1G spray-dried powder and plasma-sprayed coatings synthesized at all plasma powers. The TCP phases occurred in all GNP-reinforced coatings. However, the TTCP and CaO phases only occur in the coatings fabricated at 30 kW and 35 kW. The absence of TTCP and CaO phases at plasma powers of 15–25 kW could be due to the combined effect of a higher in-flight temperature and higher thermal conductivity of the GNPs (approximately 5000 W m$^{-1}$·K$^{-1}$) than that of HA (approximately 0.7 W m$^{-1}$·K$^{-1}$). Owing to the higher particle temperature and thermal conductivity of the GNPs, more heat is transferred to the HA matrix. Hence, the cooling rate of the HA matrix decreases and delays its thermal decomposition process during the APS process. Figure 6(B) shows the XRD patterns of the HA-2G spray-dried powder and plasma-sprayed coatings synthesized at all plasma powers. All phases in the diffraction pattern of the HA-2G coating are similar to the phases observed in the HA-1G coating. These phases will have a significant impact on the crystallinity.
According to Table 4, the crystallinity percentage of the HA-1G and HA-2G coating decreased with increasing plasma power. Increasing plasma power from 15 kW to 35 kW led to decrease the crystallinity up to 20% for HA-1G coating and 15% for HA-2G coating. The addition of 1 wt% GNPs in the HA matrix leads to a slight improvement in the crystallinity up to 3% compared with HA coatings at 35 kW. Further, addition of 2 wt% GNPs to the HA coating remarkably increases the crystallinity by up to 12% compared with that of the HA coating at 35 kW. This could be due to the lower amount of secondary phases (e.g., TTCP, TCP, and CaO phases). Tercero et al reinforced HA coatings with 20 wt% Al₂O₃, which led to a 7% improvement in crystallinity. The reinforcements with 18.4 wt% Al₂O₃ and 1.6 wt% CNTs led to crystallinity improvements of 12% with respect to that of HA. They reported that the higher thermal conductivities of Al₂O₃ and CNTs than that of HA led to the increased crystallinity [1].

3.5. Mechanical properties of the plasma sprayed coatings
The Micro-hardness and elastic moduli of the HA coatings fabricated at different plasma powers were measured with the instrumented Vickers indentation technique. With increasing plasma power, the hardness and elastic moduli increased continuously for all HA, HA-1G and HA-2G coatings. The results of the indentation experiments are listed in Table 5. An increasing plasma power from 15 kW to 35 kW improved the hardness and elastic moduli by 73% and 185% for HA coatings respectively. Similarly, remarkable improvement in micro-hardness and elastic moduli of HA-1G coating is 62% and 128%, while drastic improvement was noticed for HA-2G coating up to 81% and 149% by varying plasma power from 15 kW to 35 kW. These results are mainly attributed to the decreased porosity with increasing plasma power (see section 3.3). Furthermore, the generation

![Figure 6. XRD pattern of spray dried powders and plasma sprayed coatings of (A) HA-1G and (B) HA-2G.](image)
of secondary phases (e.g., TCP, TTCP, and CaO) with increasing plasma power promotes the pore filling in the coatings and ultimately improved the hardness and elastic modulus. According to table 5, the 1 wt% GNPs reinforcement improved the hardness by 3% and the elastic modulus by 14% compared with those of the HA coatings fabricated at 35 kW. Further, addition of 2 wt% GNPs to the coating improves the hardness and elastic modulus by 20% and 51% compared with those of the HA coatings fabricated at 35 kW, respectively. The enhanced hardness and elastic moduli of the GNP-reinforced coatings are due to the slight reduction in porosity. In addition, the higher elastic modulus of the reinforcing GNPs (approximately 0.5–1 TPa) and their uniform dispersion could be another reason for the drastically improved elastic modulus.

3.6. Fracture toughness of the plasma sprayed coatings
Fracture toughness of HA, HA-1G and HA-2G coatings fabricated at all different parameter were calculated by an Anstis equation, which relies on the radial crack formation during the Vickers indentation. Values for the fracture toughness of all the coatings are tabulated in table 6. Moreover, an increasing plasma power from 15 kW to 35 kW enhanced the fracture toughness of the HA coating by approximately 430% (from 0.33 MPa-m$^{1/2}$ to 1.75 MPa-m$^{1/2}$; as shown in table 6), while it improved from 0.71 to 2.48 MPa-m$^{1/2}$ (i.e. 249% increase) for HA-1G coating. Further, for HA-2G coating, increasing plasma power from 15 kW to 35 kW led to an improvement in fracture toughness from 1.25 to 4.78 MPa-m$^{1/2}$, i.e., increase of 282%. One of the main reason of an increase in fracture toughness with increasing plasma power is due to the lower porosity at higher plasma power, which is again due to higher in-flight particle velocity and generation of secondary phases (TCP, TTCP and CaO).

The fracture toughness of the HA-1G coating increased from 1.75 MPa-m$^{1/2}$ to 3.02 MPa-m$^{1/2}$ (i.e., increase of 73%), while further enhancement for HA-2G coatings from 1.75 MPa-m$^{1/2}$ to 4.78 MPa-m$^{1/2}$ (increase of 173%) was observed. This enhancement in fracture toughness can be ascribed to three reasons: (1) a reduced porosity, (2) different toughening mechanism caused by the GNPs, (3) strong interface between HA and GNPs. The toughening mechanism and strong interface is discussed in detail in the following section.

3.6.1. Toughening mechanism provided by GNPs
Figure 7(a) presents an FE-SEM image of the fracture surface of the HA-2G coating, which shows the presence of GNP pull-out on the HA matrix. Because the GNPs have a higher interfacial area, pull-out requires the dissipation of more energy on the application of an external force. Hence, the fracture process can be delayed, which ultimately leads to an increased toughness. Figure 7(b) depicts the FE-SEM image of the fracture surface of the HA-2G coating at 35 kW. According to the figure 7(b), the GNP are bent in the HA matrix, which could be due to the heavy strike rate of GNPs on the substrate owing to the higher in-flight particle velocity. The bending of GNPs is considered as an energy dissipation mechanism because more energy is required to create a crack in the substrate.

Figure 7(c) shows the FE-SEM images of the fracture surface of the HA-2G coating synthesized at 35 kW. The GNPs are embedded into and glued between the two HA splats. This could be due to the entrapment of GNPs between the splats during the layer-by-layer deposition of the plasma-sprayed HA coating. The splat gluing should lead to a higher bonding force between the HA splats and GNPs, which eventually enhances the load bearing capacity of the coating and increases the fracture toughness. Mukherjee et al also observed the splat gluing of GNPs in plasma-sprayed, GNP-reinforced Al$_2$O$_3$ coatings [15]. Figure 7(d) presents GNP bridges between the HA splats, which prevents a further widening of the crack and improve the fracture toughness. In addition, GNP bridging restricts the crack widening owing to the larger surface area of the GNPs. Hence, it can improve the fracture toughness.

Furthermore, the strong HA–GNP interface was considered an additional toughening factor. The interface between the GNPs and matrix plays an essential part in the stress transfer through the matrix to the GNPs, which eventually affects the mechanical properties. To investigate the interface between the HA coating and GNPs, the plasma-sprayed HA-2G coating synthesized at 35 kW was studied with HR-TEM. According to the HR-TEM results in figure 8(a), the sample contains randomly oriented planes. To investigate these planes, inverse fast Fourier transform-processed images were recorded (see figures 8(b)–(f)). Figure 8(b) illustrates a lattice spacing of 0.35 nm, which is associated with the (002) plane of the GNPs. Figure 8(c) confirms a lattice spacing of

| Table 6. Fracture toughness of plasma sprayed HA, HA-1G and HA-2G coatings. |
| --- | --- | --- | --- | --- | --- |
| S. No. | Properties | 15 kW | 20 kW | 25 kW | 30 kW | 35 kW |
| 1. | HA (MPa m$^{1/2}$) | 0.33 | 0.49 | 0.99 | 1.18 | 1.75 |
| 2. | HA-1G (MPa m$^{1/2}$) | 0.71 | 0.99 | 1.33 | 2.76 | 3.02 |
| 3. | HA-2G (MPa m$^{1/2}$) | 1.25 | 1.96 | 2.41 | 3.28 | 4.78 |
Figure 7. High magnification FE-SEM micrographs display (a) GNP pull-out (b) GNP Bending (c) splat gluing by GNP (d) crack bridging by GNP in HA-2G coating fabricated at 35 kW.

Figure 8. (a) Shows the HR-TEM images of plasma sprayed HA-2G coating at 35 kW and shows IFFT (Inverse Fast Fourier Transformation) image assured the lattice spacing of, GNP, HA, TCP, TTCP and CaO (b)–(f).
0.27 nm between the planes, which is associated with the (300) plane of HA. The lattice spacing of 0.28 nm corresponds to the (0210) plane of TCP in figure 8(d). Moreover, TTCP (figure 8(e)) and CaO (figure 8(f)) phases occur in the HA-2G coating treated at 35 kW. All planes were matched with the JCPDS card numbers (HA: 9–0432, TCP: 9–348, TTCP: 25–1137, and CaO: 4–0777). The results are in agreement with the XRD results in section 3.4.2.

Figure 8(a) shows the randomly oriented GNPs (marked by arrows), which create a strong interfacial bonding with HA matrix leading to mechanical interlocking between the GNPs and HA coating. Owing to these interlocks, the crack propagation requires more energy dissipation, which increases the toughness of the coating. Liu et al. discovered similar randomly oriented GNPs, which were anchored in the interface of reinforced HA pellets and provided mechanical interlocking [19]. In addition, the interface between the HA coating and GNPs seems clear with no interfacial products. The clean interface between the HA coating and GNPs might provide additional load transfer capacity to the GNPs in the HA matrix, which finally leads to the enhanced fracture toughness of the coating.

Like the HA and HA-1G coatings, the HA-2G coating synthesized at 25 kW exhibits a balance between the crystallinity, mechanical properties, and toughness. The 2 wt% GNPs reinforcement in the HA coating fabricated at 25 kW leads to an improved crystallinity from 91% to 96%, increased hardness from 2.94 GPa to 3.79 GPa, increased elastic modulus from 51.3 GPa to 81.24 GPa, and improved toughness from 0.99 MPa·m$^{1/2}$ to 2.41 MPa·m$^{1/2}$. Figure 9 provides a comparison of the crystallinity, porosity, hardness, elastic modulus, and fracture toughness of the plasma-sprayed HA, HA-1G and HA-2G coatings fabricated at 25 kW. Hence, a 2 wt% GNPs reinforcement for HA coatings could be the better substitute for implants used in the biological sector.

Table 7 lists studies conducted on the enhancement of the crystallinity and mechanical properties of plasma-sprayed HA coatings. According to table 7, the increase in the plasma power and the reinforcement of secondary phases in HA lead to significantly enhanced mechanical properties (e.g., the hardness, elastic modulus, and fracture toughness) of the plasma-sprayed HA coatings. Nevertheless, the results of our study show a more significant improvement in the crystallinity and mechanical properties.

### 3.7. Cytotoxicity test of plasma-sprayed coatings

To investigate the biocompatibility of the coatings, MG63 cells were grown on the plasma-sprayed HA, HA-1G, and HA-2G coatings synthesized at 15 kW and 35 kW for 24 h, 48 h, and 72 h. Figure 10(a) shows the calculated MG63 cell viability of cells on the plasma-sprayed HA, HA-1G and HA-2G coatings fabricated at 15 kW. Figure 10(b) shows the viability results of cells cultured on the three coatings fabricated at 35 kW in DMEM for 24 h, 48 h, and 72 h. Compared with that of the control sample, the survival percentage of the MG63 cells grown on the HA coating fabricated at 15 kW increases by 3% after 24 h, 5% after 48 h, and 20% after 72 h. On the HA coating prepared at 35 kW, the cell survival percentage increases by 8% in 24 h, 11% in 48 h, and 31% in 72 h compared with that of the control sample.

Similarly, for a 1 wt% GNPs reinforcement of the HA coating prepared at 15 kW, the cell survival percentage increases by 5% in 24 h, 13% in 48 h, and 24% in 72 h compared to that of the control sample. Regarding the
Table 7. Summarizes the list of notable works on plasma sprayed HA coatings.

| S. No | Material system | Crystallinity (%) | Hardness (GPa) | Elastic modulus (GPa) | Fracture toughness (MPa m$^{1/2}$) | Porosity (%) |
|-------|-----------------|-------------------|----------------|----------------------|-----------------------------------|--------------|
| 1.    | HA [7]          | 88                | —              | —                    | —                                 | —            |
| 2.    | HA-Strontium [11] | 73                | —              | —                    | —                                 | —            |
| 3.    | HA [8]          | 62                | 40%            | 16%                  | 24%                               | 37%          |
| 4.    | HA-CNT [20]     | 27                | —              | —                    | —                                 | —            |
| 5.    | HA [21]         | 68                | —              | —                    | —                                 | —            |
| 6.    | HA [10]         | 90                | 71%            | —                    | —                                 | —            |
| 7.    | HA-BNNPs [22]   | —                 | 8%             | 13%                  | 40%                               | —            |
| 8.    | Present Study   | 97                | 81%            | 185%                 | 430%                              | 58%          |

Figure 10. Percentage cell survival of plasma sprayed HA, HA-1G and HA-2G at (a) 15 kW and (b) 35 kW coatings.

HA-1G coating synthesized at 35 kW, the cell survival percentage increases by 11% in 24 h, 18% in 48 h, and 43% in 72 h. Furthermore, a 2 wt% GNPs reinforcement of the HA coating prepared at 15 kW improves the cell survival percentage by 7% in 24 h, 15% in 48 h, and 26% in 72 h compared with that of the control sample. Finally, the cell survival percentage of the HA-2G coating fabricated at 35 kW increases to 15% in 24 h, 22% in 48 h, and 50% in 72 h.

In summary, the increase in the plasma power from 15 kW to 35 kW leads to an increase in the cell survival percentage on all three composition coatings (HA, HA-1G, and HA-2G), as shown in figure 10. This could be attributed to the increasing CaO content with increasing plasma power, which improves the bone reconstruction. Demnati et al reported that the enhancement of the biological activity of the coating is mostly associated with the presence of amorphous phases that support the bone reconstruction [23]. In addition, the incorporation of GNPs increases the cell survival percentage too. This could be due to the natural affinity for protein attachments on their surfaces, which improves the cell proliferation.

4. Conclusion

Three different compositions of GNPs reinforced plasma-sprayed HA coatings were successfully fabricated on Ti–6Al–4 V substrates by increasing the plasma power from 15 kW to 35 kW. Owing to the increased plasma power, the porosity and crystallinity of the HA-2G coating decreased by 57% and 15%, respectively. However, the addition of 2 wt% GNPs to the HA coating remarkably decreased the porosity by up to 36% and increased the crystallinity by up to 12%. The increasing plasma power and addition of GNPs significantly improved the hardness and elastic modulus of the HA-2G coating, which was attributed to the reduced porosity. For an increasing plasma power from 15 kW to 35 kW, the fracture toughness of the HA-2G coating increased up to 282%. In addition, it increased by 173% for an addition of 2 wt% GNPs. The improved fracture toughness was attributed to the reduced porosity, strong HA–GNP interface, and different toughening mechanism provided by the GNPs. Finally, the presence of GNPs improved the proliferation rate of MG63 cells, which was attributed to the increase in the natural affinity for protein attachments on coating surfaces upon reinforcement.
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