Advanced Ceramics Progress: Vol. 8, No. 4, (Autumn 2022) 32-41

Materials and Energy Research Center
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Advanced Ceramics Progress

Journal Homepage: www.acerp.ir

Review Article

Investigation of the Coating Methods and Types of Coatings Containing Hydroxyapatite for Applications in Tissue Engineering

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URL: https://www.acerp.ir/article_161855.html

ARTICLE INFO

Article History:

Received 11 October 2022
Received in revised form 8 November 2022
Accepted 29 November 2022

Keywords:

Hydroxyapatite
Coating
Metal Substrates
Osteoinduction
Tissue Engineering

ABSTRACT

In recent years, application of Hydroxyapatite (HA) as the coating on metal substrates for biological stabilization of implants, stimulation of bone growth around the implant, and optimization of recovery time has attracted the attention of many researchers around the world. In this regard, the current study presented a review of HA and its composite coatings for tissue engineering applications. HA is one of the bioceramics that has been an interesting subject of research in recent years owing to its in-vitro bioactivity, osteoinduction, and osteoconduction properties. According to the previous reports, coated implants were performed successfully to achieve high corrosion resistance, bone growth and regeneration, and reduction of corrosion current density. The current research presented a review of the previous research works on the coating mechanism, physico-mechanical, in-vitro bioactivity, and biocompatibility properties of HA and its composite coatings on substrates. The obtained results revealed that HA and its composites had a synergistic effect on the metal substrates in terms of improving corrosion resistance, providing biocompatibility, direct bonding to tissue, accelerating treatment, and reducing costs imposed on the health care sector.

https://doi.org/10.30501/acp.2022.365116.1108

1. INTRODUCTION

Stainless steel, Mg, Mg aloys, titanium, and titanium alloy are some of the metals used in the production of bone implants [1-5]. Due to the supply of suitable mechanical properties, implants have been used for several years to stabilize bones, teeth, and joints. Metals corrode in the body fluid that results in the release of metal ions around the tissue, hence the appearance of side effects. For this reason, surface treatment is required to improve the biocompatibility as well as bioactivity, reinforce the connection to bone tissue, and promote bone formation by proliferation of osteoblast cells. In this regard, in order to improve the surface properties of the metal implants, biocompatible and bioactive materials should be coated [6-8].

Hydroxyapatite (HA) is a bioactive calcium phosphate ceramic with the chemical formula of [$Ca_{10}(PO_4)_6(OH)_2$] [9-12]. Owing to its chemical and crystallographic characteristics similarity to the human bone, HA is currently utilized in the field of bone tissue repair and reconstruction and as bioactive coating on different metal

Please cite this article as: Doudkanlouy Milan, B., Mohammadzadeh, H., Jafari, R., Soltani, M., “Investigation of the Coating Methods and Types of Coatings Containing Hydroxyapatite for Applications in Tissue Engineering”, ***Advanced Ceramics Progress***, Vol. 8, No. 4, (2022), 32-41. https://doi.org/10.30501/acp.2022.365116.1108

substrates for orthopedic applications [6,9,13-15]. Given that HA is mainly composed of calcium and phosphate and that its chemical composition and crystal structure are similar to the mineral content of the human bone, the biocompatible and bioactive and other related products are generally not dangerous for cell viability [16]. In addition, in clinical applications, HA ensures new bone formation due to its controlled biodegradability [17].

Hence, HA is used in various medical applications such as bioactive coatings on the bone metal implants, ear implants, dental materials, and tissue engineering applications [18-20]. However, HA has some drawbacks such as its low mechanical properties and low fracture toughness [21]. Therefore, it does not conform to the mechanical properties of the human bone, which is considered an obstacle to its in-vivo applications [22]. Therefore, reinforcements such as Al_2O_3, TiO_2, Y_2O_3, Ni_3Al, and carbon nanotubes (CNTs) are composited with HA to enhance its mechanical characteristics [23-27]. However, the presence of these enhancers can sometimes cause damages to the surrounding tissues. For instance, HA decomposition occurs during the procedure manufacturing through ZrO_2, which leads to a significant degrade in the biological behavior of HA [26].

Therefore, according to the factors mentioned above, HA has a high potential for bone tissue engineering applications, drug release, and bioactive coatings. In this review study, we attemted to cover the methods for HA coating and investigate the HA reinforcing materials on the metal substrates as much as possible. Hence, it is expected that this review study will be utilized as a practical reference for researches.

2. COATING TECHNIQUES

2.1. Sol-Gel

In sol-gel method, inorganic polymers/ceramics are obtained through converting solutes-soluble precursors into sol and then into a lattice structure called a gel [28]. This method enjoys several advantages namely its choice of coating composition, coating of complicated structures, homogeneity of the coating, and simplicity of the procedure [28]. On the contrary, the limitations of this method are the slow speed of the process and presence of inherent cracks [29]. $Ca(NO_3)_24H_2O$ and P_2O_5 are usually used as precursors to prepare the HA sol. In addition, the most common solvent for dissolving the existing precursors is pure ethanol, to which a small amount of water is added to increase the hydrolysis of the prepared sol [29]. Then, the resultant solution is exposed to different temperatures at different time intervals to achieve the desired viscosity, evaporate the existing solvent, and obtain a sol-gel state [29]. Finally, the prepared sol-gel is subjected to aging, drying, and calcination processes to make it ready for use. It should be noted that to date, the sol-gel method has been extensively utilized to coat the HA and HA composites on metal substrates [30-33]. Figure 1a presents a schematic of the overall sol-gel immersion coating procedure.

2.2. Electrophoretic Deposition (EPD)

Electrophoretic Deposition (EPD) is a colloidal process in which the charged particles in suspension are coated by applying an electric field to a conductive electrode (Figure 1b) [34]. However, in the EPD tecnique, application of water as a solvent is limited owing to electrolysis and production of small bubbles near the electrode. High adaptability and inclusion of a wide range of materials are among the effective factors that have drawn considerbale attention to this method. It is worth noting that creating a stable suspension where the particles are well distributed inside the solvent is one of the important stages of EPD that should be further explored by researchers [35]. However, the disadvantages of this method can be remedied by reducing the ionic conductivity of water [35]. Moreover, the applied EPD method was utilized to coat different composites of HA on the metal substrates [36-39].

2.3.Thermal Spraying (TS)

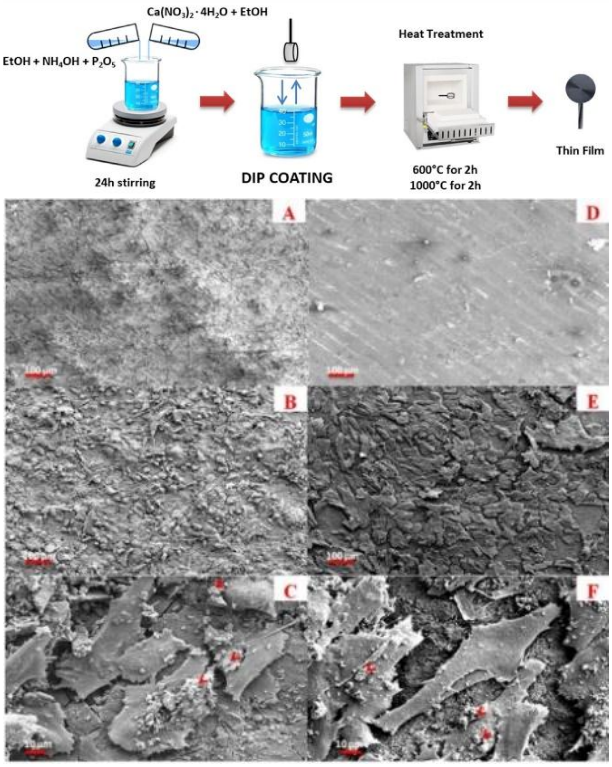
Thermal Spraying (TS) method is among the physical deposition techniques for creating HA coatings. This technique is based on processes in which coating materials are heated and sprayed on the substrate. The reasons for using thermal spray coating are to protect the surface against physical corrosion, abrasion and scratches, chemical corrosion, electrical corrosion and oxidation [6]. It should be noted that through the TS method, Ti/HA composite coating can be applied on a stainless steel substrate that yields interesting physico-chemical results [40].

2.4. Physical Vapor Deposition (PVD)

Coating from vapor phase involves a wide range of vacuum coating processes in which materials are physically separated from a source through evaporation and transferred as a film on the surface of the substrate through a partial vacuum (Figure 1c) [41]. Deposition of the thin layers of the vapor phase is accomplished using a variety of techniques used in the optical, tribology, energy storage, and medical industries [41,42]. Moreover, this technique was employed to coat HA on the Ti–35Nb–xZ substrate [43].

2.5. Biomimetic Deposition (BD)

When the coating is formed under physiological conditions, it is called biomimetic (Figure 1d). This method was developed by researchers through forming a layer consisting of calcium and phosphate on a titanium substrate in a simulated body fluid [44]. The figure below exhibits a bioactive apatite coating formed by a biomimetic method on a substrate. Of note, formation of the calcium phosphate layer is also indicative of the substrate bioactivity [45]. To date, the biomimetic deposition (BD) method has been used to coat HA on deferent metal substrates such as stainless steel, Ti, and TiO_2 [46-48].



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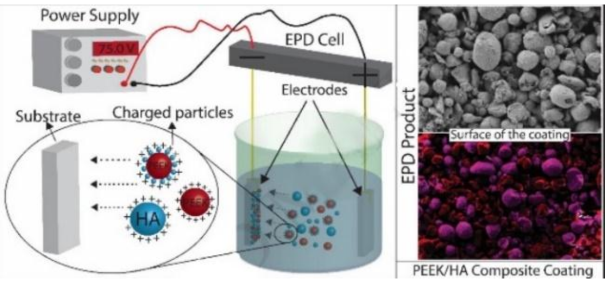

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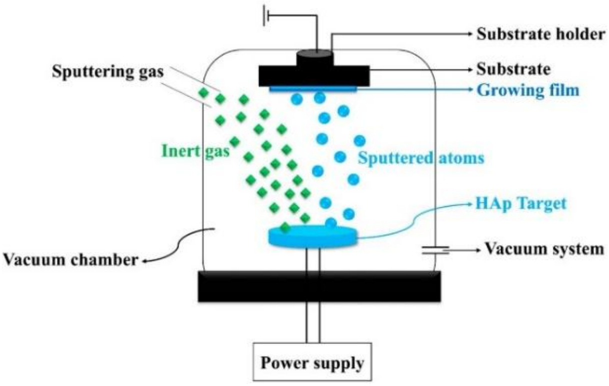

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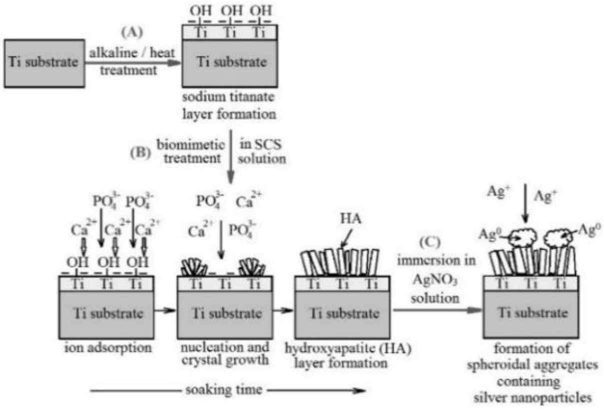


(d)

Figure 1. Schematics of HA coating through Sol-Gel, EPD, PVD, and BD, respectiveley (a-d) [34,41,49,50]

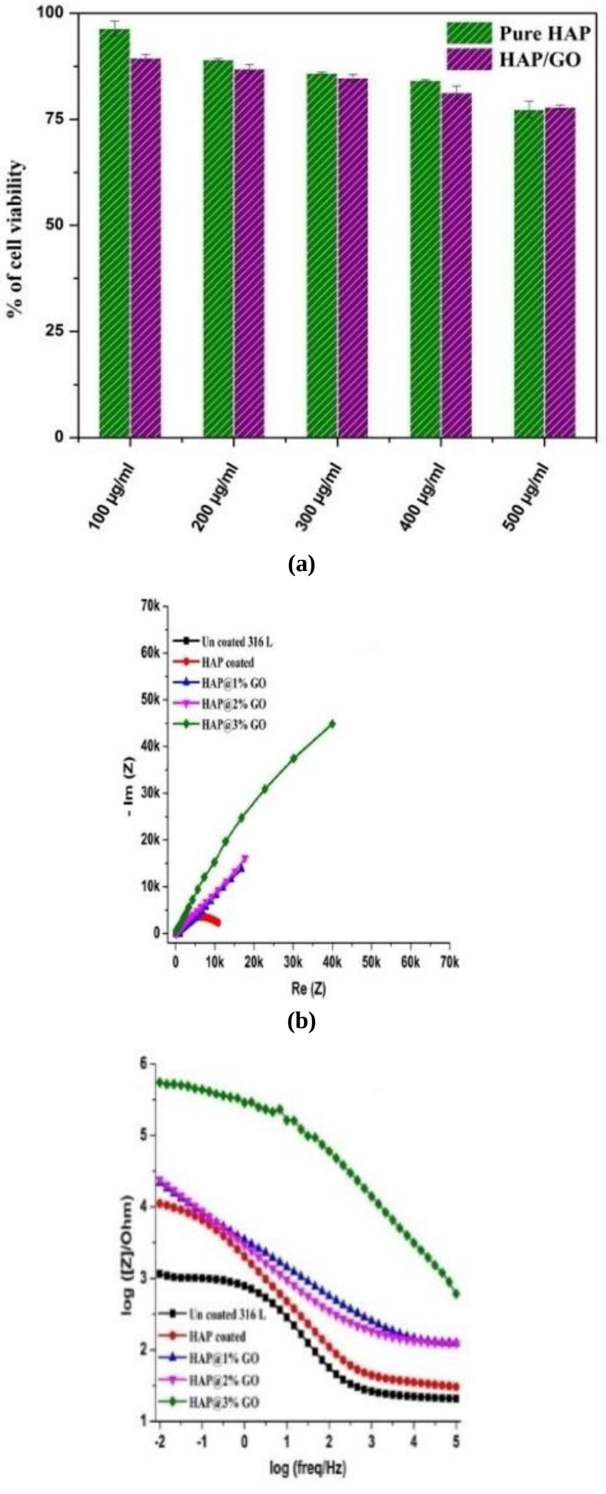
3. HA COMPOSITE COATING ON METAL SUBSTRATES

3.1. HA/Graphen Oxide

Owing to its high specific surface area, controlled drug release, good biocompatibility, and high stability Graphene oxide (GO) is widely used in various biological applications including biosensors, bio-imaging, and tissue engineering scaffolds [51,52]. In addition, GO is characterized by other acceptable mechanical properties. Among the effective factors involved in the stability of GO in solution are the oxygen groups that are placed on the edges and plates that facilitate applications of GO as an enhancer in biocomposites [53]. It should be noted that based on the previous report, upon adding 1 % by weight of GO to the composite composition, a significant increase in the biological and mechanical characteristics of the final sample was achieved. In addition, according to the literature data, in the presence of GO and HA nanoparticles, a significant increase in the bioactivity of gelatin and Polycaprolactone (PCL) was observed [54]. In a study by Sebastin et al., HA/GO composite coating was applied on the 316L stainless steel substrate. According to the results of this study, cell viability was reported to be above 95 % for HA composite coating containing 2 % by weight of GO (Figure 2a). In addition, the corrosion resistance of the HA/GO composite coating was significantly improved, compared to HA alone (Figure 2b and Figure 2c) [55].

3.2. HA/TiO_2

In recent years, TiO_2 has been highly acknowledged by researchers as a bioactive coating [56-58]. TiO_2 is characterized by good biocompatibility and good chemical stability in physiological environments [37]. Additionally, TiO_2 is currently used in biological applications such as drug delivery systems, bio-imaging, and cancer treatment [59-61]. Studies highlighted that



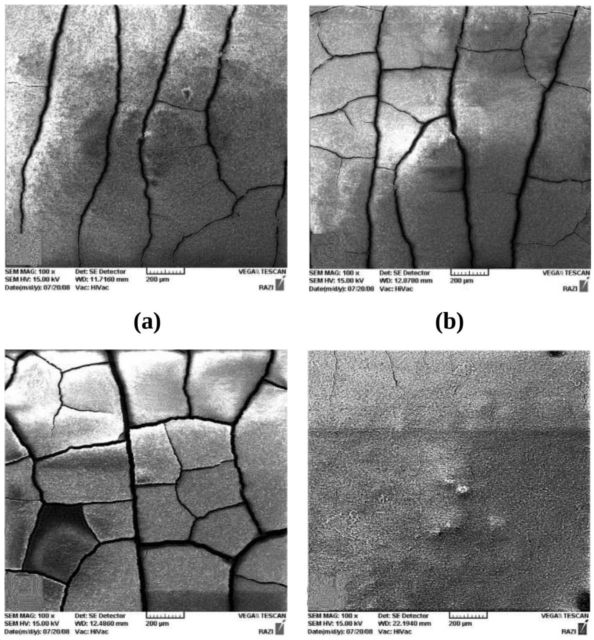
(a)

(b)

(c)

Figure 2. Viability of MG-63 cell lines (a), Nyquist (b), and Bode (c) plots [55]

HA and TiO_2 coatings could provide higher mechanical characteristics than neat HA coatings when TiO_2 was in the range of 20 to 25 by weight [62,63]. These results were further employed to design more complicated structures with the ability to improve the biological and mechanical properties of the HA-based bioactive coatings [63]. According to the morphological investigations, the prepared HA coating is characterized by a porous morphology. In this case, an increase in the amount of TiO_2 leads to the higher density of the composite coatings, which increases the adhesion strength of the coating and enhances the bond between coating and substrate (Figure 3) [37].



(a)

(b)

(c)

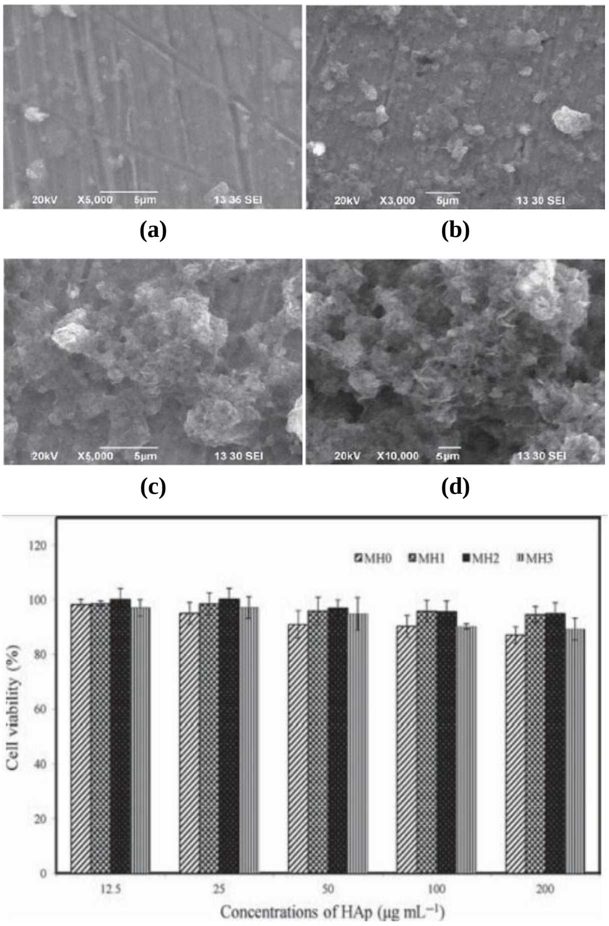
(d)

Figure 3. SEM images of HA / TiO_2 coatings deposited on 316L stainless steel [37]

3.3. HA/Chitosan

Biodegradable polymers are widely utilized in composite preparation [12,64,65]. Chitosan (CS) proved to be an excellent matrix for HA and HA composites. It is also a biocompatible, biodegradable, and available biopolymer [12,66]. Although CS has unique properties such as biocompatibility, non-toxicity, and antibacterial effect, it fails in binding to the bone [67]. According to the previous research work [68], CS coatings applied as a composite with HA on 316 L substrate revealed high in vitro bioactivity, biocompatibility, and corrosion resistance properties (Figure 4). In this study, the value range of 3.66-18.98 was the reported in GPa for Young's modulus [68].

The research studies conducted in recent years on the HA composites as a coating on mainly metallic substrates are presented in Table 1. In addition, a summary of the results already obtained regarding the addition of a new material to HA in the coating is reported.



(e)

Figure 4. SEM images of the different concentrations of Mg doped HAp/CTS coatings on the 316L (a-d) and NIH 3T3 fibroblast cell viability results (e) [66]

TABLE 1. Hydroxyapatite composite coatings that have attracted the attention of researchers in recent years

HA Composite Coatings	Results	References
Zn	Good biomineralization capacity, conversion of HA tissue to bone-like tissue, biocompatibility, bioactive, antibacterial	[69-74]
Graphene Oxide	Compact structure, increasing corrosion resistance, antibacterial, improving hardness and elastic modulus, biocompatibility	[55,75-79]
Carbon Nanotube	Increasing the shear strength between the surface of the implant and the coating, improving hardness and elastic modulus	[80-83]
Chitosan (CS)	Bioactive, biocompatibility, increasing corrosion resistance, antibacterial	[39,66,84-87]
Gelatin	Biocompatibility, bioactive osteogenesis, improving mechanichal properties	[88-90]
Collagen	Conversion of coating tissue to bone-like tissue, bioactive, improving coating bonding strength, osteogenesis	[91-94]
MgO	Increasing corrosion resistance,control of corrision of Mg substrate	[95,96]
TiO_2	Improving coating bonding strength, bioactiv, biocompatibility, decreasing prosity, increasing corrosion resistance, Improving scratch resistance	[37,63,97,98]
ZnO	Increasing corrosion resistance, antibacterial, load bearing, osteogenesis, improving coating bonding strength	[99-102]

3.4. HA/CNT

In a study conducted by Nabipour et al., HA/CNT composite coating was applied on the stainless steel substrate through EPD technique and then investigated. In this type of coating, the presence of CNT filled the gaps between the HA nanoparticles and prevented the formation of microcracks. As a result, the weight of HA-5 wt. % CNT coating became less than that of HA coating due to the lower density of CNT than that of HA particles. According to our observations, addition of CNT improved the uniformity of the coating; therefore, almost no difference was observed in the thickness of the coating [103].

According to the previous reports, one of the main applications of HA is a biocompatible and bioactive coating in all kinds of metal implants. Of note, the bioceramics based on the HA greatly reinforced the connection of bone cells to the implanted biological material and thus increased the integration of the cell with the biological material. As a result, the proliferation of the bone cells also increased. In other words, the HA coating stimulates the bone growth and consequently restores the lost bone [38,104]. In case the HA is placed in the human body, it facilitates the recovery and regeneration of the lost or damaged bone tissue mainly due to the type of protein called osteocalcin, which is a non-collagenous bone protein. Previous Studies revealed that osteocalcin protein could form the bonds with the calcium ions in HA [105,106]. It should be noted that HA, as a widely used biological material, does not cause toxicity in the body [107].

However, while using HA, researchers face a series of challenges anmely the poor mechanical properties such as brittleness and low fracture toughness as well as low hardness, low load bearing capacity, and migration from the implant site and spread in the tissue that cause some problems such as deposition in the lymph nodes, cartilage, and bone marrow.

The solution proposed in the research literature for the first limitation is the coating of HA on the metal implants, use of reinforcing materials, and formation of composites. Examples of these methods were reported in detail in Table 1. Additionally, the proposed solution for the second challenge suggests adding the organic and polymer materials along with HA. These materials improved the adhesion of HA to the implant and prevented its dispersion in the body. Hence, new HA composites can compensate for its drawbacks [108-110]. Moreover, one of the main challenges in using HA composites is that the adhesion strength of these composites in processing methods such as EPD is not enough, and other coating methods such as PVD are not cost-effective despite providing adequate adhesion. On the contrary, use of some polymeric materials is alos bound to some limitations such as toxicity and uncontrollable biodegradability [111-113]. Given what was already mentioned, more and more detailed research is still needed in this field.

In addition, in recent years, with the scientific advancements and emergence of nanotechnology, these coatings have become nanoscale. In addition to the greater adhesion to the substrate in this scale, porosity and microcracks are eliminated, hence improvements in mechanical properties and corrosion resistance. It was proved that nanomaterials interacted with cells better than their counterparts due to their dimensions and consequently yielded better results [38,104].

Among the challenges ahead in the field of coating HA composites on the metal substrates are:

1. Introducing the appropriate coating method in such a way that by changing the coating parameters, we can obtain a coating with appropriate adhesion and mechanical properties.
2. Using suitable polymers in such a way that they are biocompatible and at the same tiem, they ensure improvement of the HA adhesion to the substrate.
3. Using suitable ceramic materials of micron or nano in size to have a positive effect on the mechanical properties and biological properties of HA.
4. Using suitable metal nanoparticles to improve the biological properties of the HA coatings and bring the coating tissue closer to the bone tissue.

4. CONCLUSIONS

In the present review study, the authors attempted to investigate the physico-mechanical and biological characteristics of HA. In addition, a brief summary of the coating methods and HA composite coatings were presented. The main findings of the present research are briefly stated below:

1. The mechanical properties of HA and bioactivity of metal substrates, especially 316 L stainless steel, were enhanced by HA/metal substrate composites.
2. Sol-gel, EPD, TS, PVD, and BD were among the effective techniques for coating HA on the metal substrates.
3. GO/HA composite was the main cause of more than 95 % cell viability.
4. The combination of HA and TiO_2 as a composite played an effective role in reducing the porosity of the coatings.
5. HA/CS composite coatings applied on 316L stainless steel revealed proper corrosion resistance and biological properties.

5. ACKNOWLEDGMENT

Special thanks to the referee comitee of the 3th International Conference on Ceramics.

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