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Investigation of biomimetic HAp formation on graphite

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Abstract. According to reports, coating implants with hydroxyapatite (HAp) promotes bone repair and combines the biological effectiveness of the material with the mechanical properties of standard metallic implants. In this contribution, bioactive nanographite particles were synthesized using a novel technique to stimulate HAp deposition using a biomimetic method. The rapid breakdown ionization (RBA) technique was used to synthesize graphite nanoparticles. The electrophoretic deposition (EPD) technique was utilized to deposit the nanoparticle on titanium substrates. Scanning electron microscope (SEM) images showed the creation of nanoparticles with a size of around 65nm. An X-ray diffraction (XRD) test confirmed the polycrystalline structure of graphite with a dominant peak (002). UV-VIS absorption and FTIR spectra confirmed the production of graphite powder. To test the bioactivity of the graphite layer, it was immersed in simulated body fluid (SBF) for 30 days. The formation of a HAp layer on graphite is depicted by an XRD pattern, and SEM images illustrate nanoclusters of this layer.

Keywords: bioactivity, electrophoretic deposition, graphite, hydroxyapatite, ionization

INTRODUCTION

One of the important inorganic components of human teeth and bone is HAp. It has exhibited a wide variety of applications in the domains of biomaterials research, tissue engineering, and biomedicine due to its straightforward manufacture, low cost, and great biocompatibility (Haider *et al.,* 2017). However, because of its relatively low toughness and tensile strength, pure HAp is difficult to utilize for bone tissue engineering and biomedical implants. To address this issue, the mechanical qualities of HAp-based materials have been improved by combining them with other types of bioactive tough materials, such as carbon materials (carbon nanotubes and graphene) (Gang *et al.,* 2019). Numerous beneficial characteristics of graphite include its low weight (in comparison to metal), wear resistance, antithrombogenicity, and biocompatibility. The major features of graphite are that it is chemically inactive (bioinert), and the issue with the development of better biocompatibility still has to be solved (Yuichiro *et al.,* 2013). Biomaterials having antibacterial and anti-demineralization properties, such as bioactive glass and fluorinated graphite, have been shown by Hyung *et al.* to be useful and simple to utilize in clinical settings (Hyung *et al.,* 2019). In 2009, Luis *et al.* showed how graphite could supply HAp with the reinforcement it requires without compromising the system's biocompatibility (Luis *et al.*, 2009). Graphite is a crystalline form of carbon. It is made up of graphene layers layered on top of each other. The carbon atoms in each layer are organized in a honeycomb lattice with 0.142 nm bond lengths and 0.335 nm between planes. Because of their weak van der Waals bonds, the graphene like layers may easily separate and glide past one another. (Chung *et al.,* 2002). Many methods have been used to form bioactive materials (Muhammad *et al.,* 2018; Francesco *et al.* 2020; Weidong *et al.* 2021; Hiroaki *et al.* 2001), but rapid breakdown anodization has many advantages, including the simplicity of the equipment, the cheapness of the materials used,

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and the possibility of operating it at normal atmospheric pressure and room temperature. This method was used by the authors to produce various bioactive materials such as TiO_2, ZnO (Mustafa *et al.,* 2020), and MgO (Murtadha *et al.,* 2022). The soaking time required for coating using a biomimetic approach must be long enough to obtain the necessary calcium phosphate deposition thickness on the sample's surface. This technique has several benefits, including low cost, simplicity, and the capacity to cover porous surfaces or intricate geometries (Elkoca *et al.,* 2012). After immersing the substrate in SBF, an HAp layer forms, grows, and crystallizes on the surfaces automatically. SBF's created apatite crystals mimic human bone on implant surfaces. Numerous literatures mention this method. This bioactive process can help with the healing process and bone apposition (Dalal, 2018). It is important that the bioactive material has the property of killing bacteria that may be present with medical implants in the mouth. In addition to the ability of graphite to stimulate the precipitation of hydroxyapatite, it also has the ability to kill different types of bacteria (Liu *et al.,* 2011). Esquivel confirmed the antimicrobial activity of graphite oxide doped with silver against Bacillus subtilis, Candida albicans, Escherichia coli, and Staphylococcus aureus (Esquivel *et al.,* 2021). In this paper, an attempt is made to produce bioactive particles from graphite using a simple method and uncomplicated equipment and to test their bioactivity.

MATERIALS AND METHODS

Production of graphite

To produce graphite nanoparticles, two pieces of graphite were submerged in an electrolyte of (0.1 M) HCO_4. The cathode electrode was in one piece, while the anode electrode was in the other. The electrodes were spaced apart by 0.5 cm, and twenty volts were put between them. One hour was spent on the RBA procedure. During this period, the graphite sample changed into a black powder. A glass container was used to deposit the graphite layer by EPD. A magnetic stirrer was used to mix 0.5 grams of graphite powder with 50 ml of methanol for 10 minutes. The cathode and anode were made of two graphite pieces. Both electrodes were linked to a 30 volt power source. There was a 5 minute deposition period. Images of graphite that was deposited using the EPD method are shown in (Figure 1).

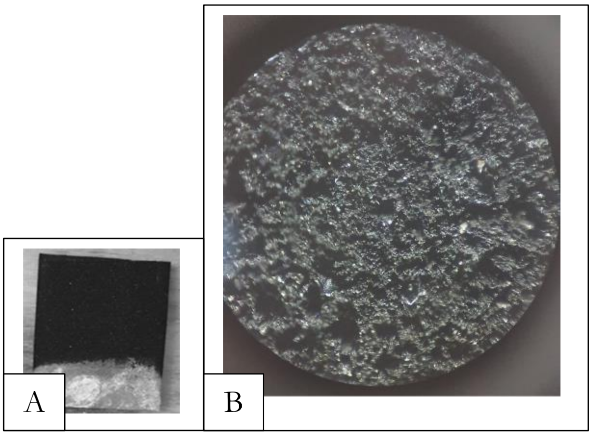

Figure 1. Images of deposited powder by EPD: A-Deposited Graphite on Ti base .B- Magnified image X10 of Graphite.

Biomimetic procedure of HAp coatings

Coated Ti substrate with graphite was immersed in simulated body fluid (SBF) concentrated five times (SBF×5) for one month to investigate the creation of HAp. The composition of SBF is listed in Table 1 (Dalal, 2018).

Characterization of graphite

To verify the materials' crystalline nature, a powder X-ray diffractometer (Shimadzu, Japan) was utilized. XRD Powder Diffraction Files (PDF) for Ti (05-0682), Graphite (0000-49), TiO2 (21-1276), and HAp were used to index the data (09-0432). The topographies of the surfaces and the sizes of the generated particles were determined using the SEM method. The system being used has a Carl Zeiss model (Germany). Each compound's component elements were identified using the dispersive X-ray spectroscopy (EDX) approach and model (Carl Zeiss, Germany).

RESULTS AND DISCUSSION

The XRD pattern of the deposited graphite on Ti base is shown in (Figure 2). There are two phases that can be noticed inside this pattern: the Ti base and the graphite layer. Miller indices of graphite pattern peaks are (002), (020), and (111). The rest of the peaks inside the pattern belong to the Ti base; this might be attributed to the low thickness of deposited graphite on Ti. The SEM image in (Figure 3) confirms the nano dimensionality of the prepared graphite particles and that they have irregular shapes. By checking the image, it can be noticed that the graphite layers are arranged on top of each other in a random manner. The EDX spectrum (Figure 3) shows the formation of carbon as well as oxygen as two dominant elements. The existence of oxygen is a natural result of the creation of nanoparticles by the RBA technique (Reem *et al.,* 2019).

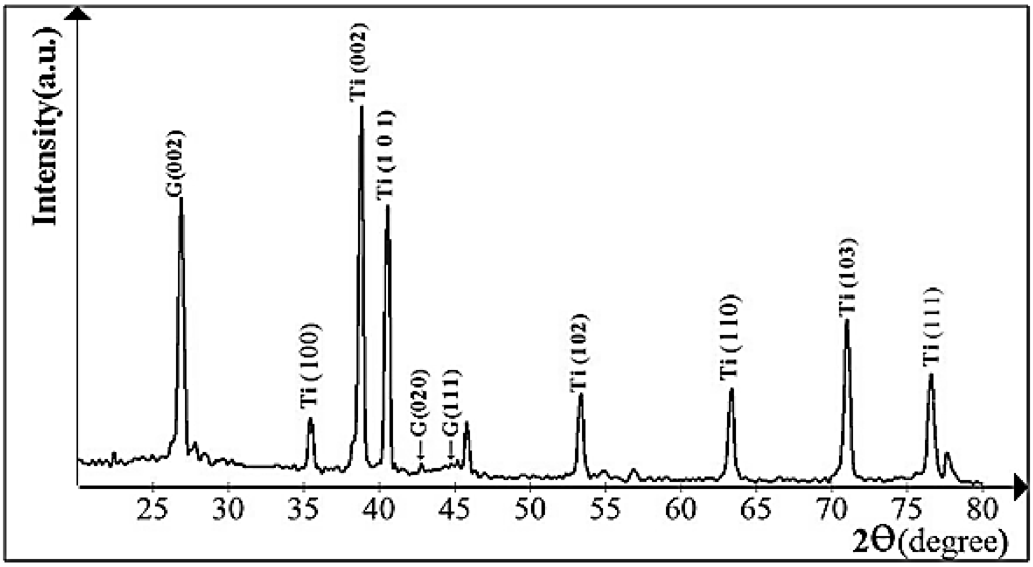

Figure 2. XRD patterns of the deposited Graphite on Ti

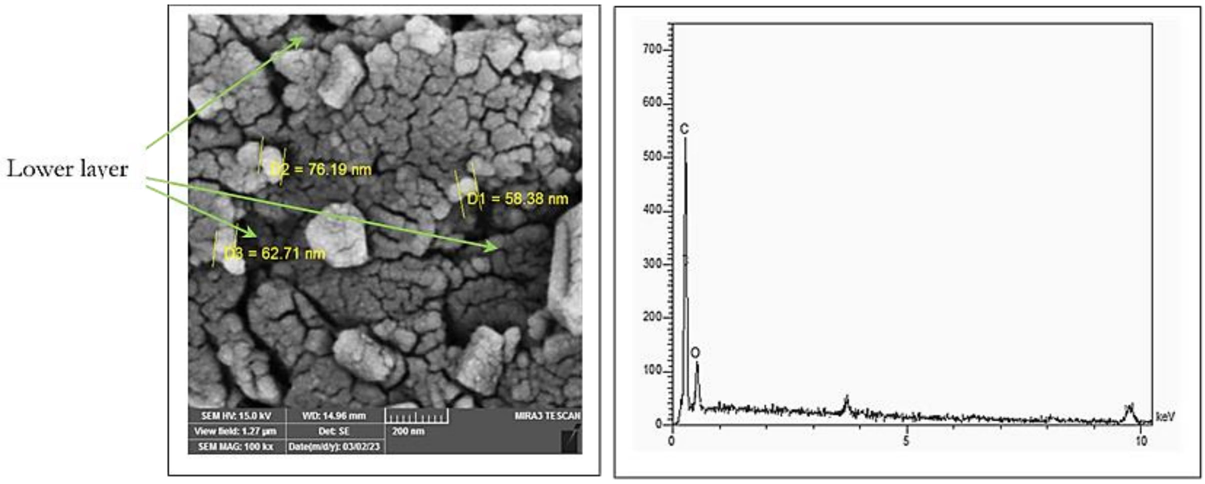

Figure 3. As prepared graphite powder SEM image and EDX spectrum.

The absorption spectrum of the graphite collides, shown in Figure 4, exhibits a very strong peak centered at 206 nm. It is attributed to the π- π* transition of C=C (Suzuki *et al.,* 2017). This result is in agreement with (Akansha *et al.,* 2019).

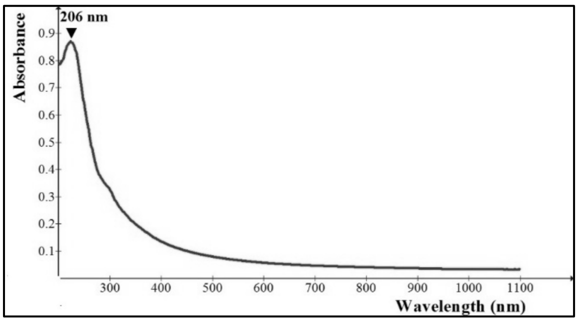

Figure 4. UV-VIS absorption spectra of Graphite.

In Figure 5, the FTIR spectra of graphite powder have peaks at 3322, 3215, 2919, and 2852, cm−1. It can be attributed to vibrations OH stretching. Peaks at 1602 and 1066 can be ascribed to C=C and C-H bending (Chen *et al.,* 2016). While the OH, C=O, and C-H bending peaks indicate that the graphite had OH, C=O, and C-H surface moieties attached to it, the presence of the C=C peak shows that the powder was made of a graphitic structure (Guanxiong *et al.*, 2016). After soaking in SBF and then the heat treatment at 600^0C for 2 hours, three HAp peaks with low intensity were observed in the XRD pattern of this sample, see Figure 6. The appearance of TiO_2 peaks is attributed to the annealing process in the ambient atmosphere. The scenario of building HAp on graphite oxide is described by Zebin *et al.* (Zebin *et al.* 2021). According to their report, Ca^{2+} ions were driven to the Graphite oxide by the negatively charged oxygen functional groups or by the carboxyl groups' ability to exchange ions with H^+. The positively charged Graphite oxide started drawing in the negatively charged $PO4^{-3}$ions and OH^- ions after a large quantity of Ca^{2+} ions had accumulated on the Graphite oxide particles. Hence, the oxygen functional groups attached to these Ca^{2+} ions served as the HAp's nucleation sites. With constantly added $PO4^{3-}$ and OH^-ions, the generated HAp transformed into nano shaped during the course of the reaction.

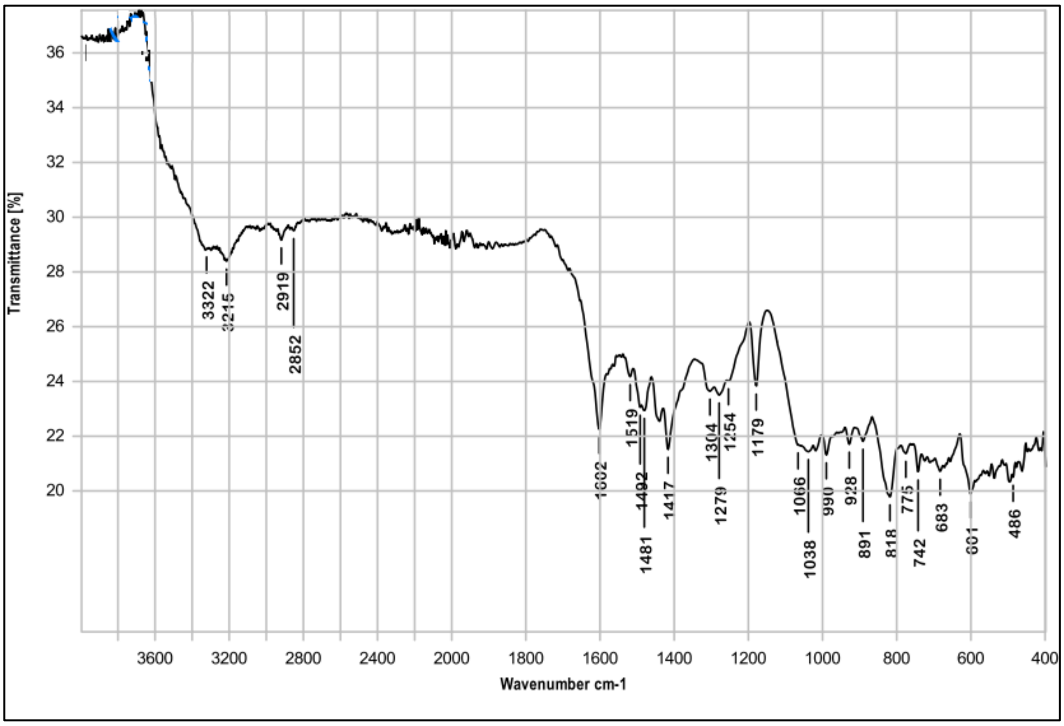

Figure 5. FTIR of graphite powder.

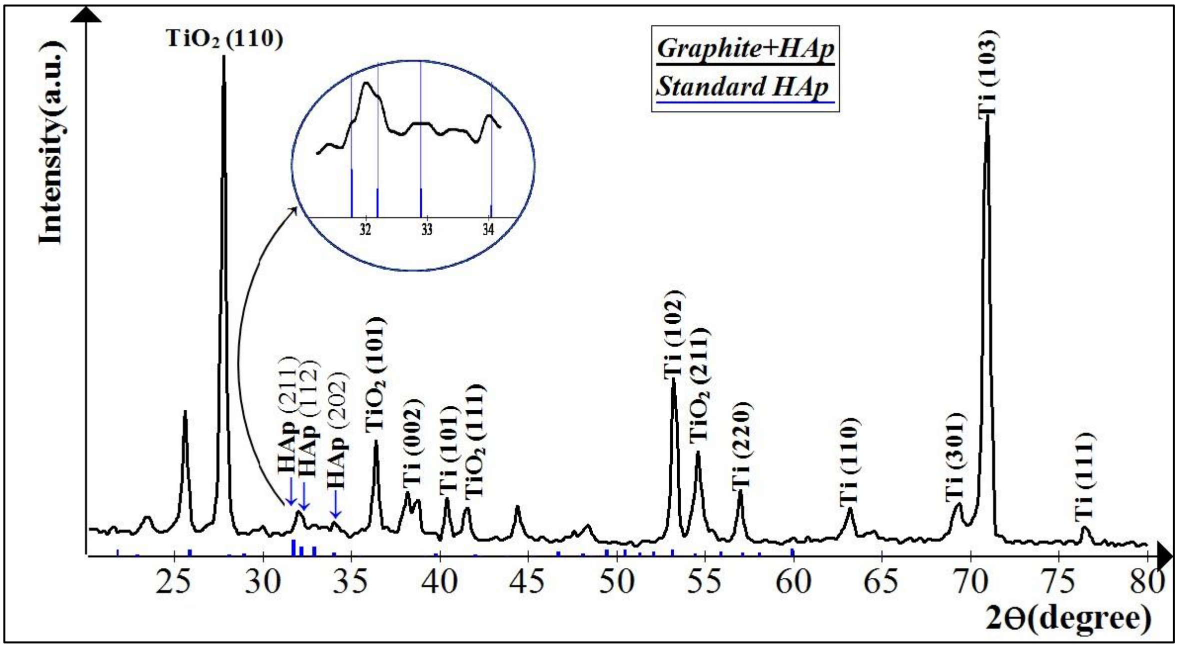

Figure 6. XRD patterns of Graphite after soaking in SBF for one month.

EDS spectra of the sample after soaking inside SBF are illustrated in (Figure 7). Beside Ti peaks, there are peaks belonging to Ca and P, indicating the formation of the HAp layer. Figure 8 depicts the shape of the generated HAp particles on the surface of graphite. SEM scans revealed that HAp was in the form of nanoballs joined to each other to form "clustered nano balls". After the biomimetic process, there are no cracks formed in the HAp layer, despite the mismatching of the thermal expansion coefficient between this layer and the graphite. Similar to biological apatite, nano HAp releases calcium ions into the surrounding environment. Higher surface area and surface roughness of nano HAp lead to enhanced cell adhesion and interactions with the matrix. Ceramic biomaterials based on nano-sized HAp were shown by Dong *et al.* to promote bioactivity and resorbability (Dong *et al.* 2009).

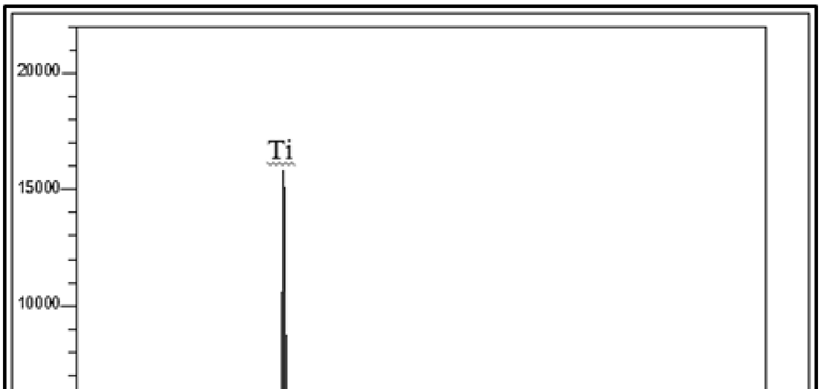

Figure 7. The elements of the layer that was deposited on Ti during a biomimetic process.

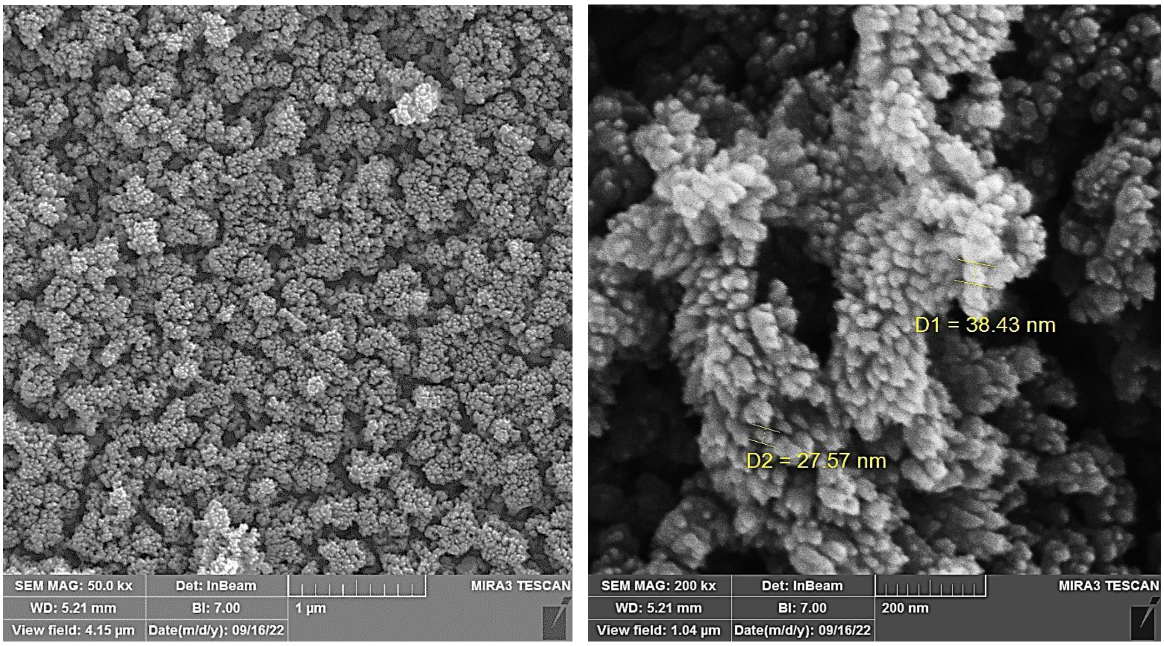

Figure 8. SEM images of HAp layer.

CONCLUSION

1. The prepared graphite by RBA is a bioactive material and can be considered a candidate material for coating implants to enhance bone growth and osseointegration.
2. EPD is an active and simple technique to deposit graphite nanoparticles on Ti bases.
3. The biomimetic technique is a successful procedure to stimulate HAp deposition on graphite.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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