BIOACTIVE PROPERTIES AND MORPHOLOGICAL FEATURES OF HYDROXYAPATITE/ZINC OXIDE HYBRID REINFORCED BIO-BASED EPOXY RESIN COMPOSITES FOR DENTISTRY

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ABSTRACT

In this work, hybrid reinforced epoxy biopolymer composites were developed using natural hydroxyapatite and zinc oxide (nHAp/ZnO). The epoxy was reinforced with varying addition of nHAp and ZnO in the steps of 3, 5 and 7 wt. % respectively using stir casting molding process. The developed composites were immersed in simulated body fluid (SBF) at 37°C to determine their bioactivity. Microstructure evaluation using scanning electron microscopy was also used to assess the morphology of the developed composites before and after in vitro test, respectively. The microstructure evaluation in vitro showed good deposition of white-like layers on the surface indicating good bioactivity reaction with the SBF and affirm that the developed biopolymers will react well with living tissue when used in vivo. However, hybrid sample BP_5HB showed better apatite-like layer deposition.

Keywords: bioactive, hydroxyapatite, biocomposites, hybrid, simulated body fluid.

INTRODUCTION

Epoxy is more widely used than other thermosets in a variety of applications because of its easy processing procedures, superior strength, and best performance at high temperatures. Despite its higher cost than other polymer matrices, epoxy accounts for more than two-thirds of the polymers used in aerospace applications [1]. However, recent advances in polymer resin reinforced composites have improved their properties and broaden their applications in restorative dentistry [2].

Dentistry as a profession is thought to have begun around 3000 BC in Egypt. The first denture is thought to have been created around 2500 BC in Egypt [3]. The dentures were hand carved and secured in place with silk threads, and they must be taken out before eating [4]. Dentures have been made of metal and its alloys, polymers, and ceramics for many years. However, not all of these implants degrade naturally, resulting in higher health-care expenses, infection risks, and difficulties. Furthermore, dental biomaterials are subjected to a harsh environment in which pH, salivary flow, and mechanical loading alter often. To meet these obstacles, extensive research and development is required to provide goods to clinical practitioners.

Implantable devices are designed to mimic one portion of the human body and they are utilized for replacement of a damaged organ or structure to maintain normal body functionality [5]. The most used medical implants include the bones, heart, breast, eyes, ears, thighs, cardio-vascular implants and knees. Synthetic polymers, Ceramics and Metals have been used in the past, but there are significant disadvantages, such as immunological rejection by the body. Furthermore, synthetic polymers may also provide an issue in terms of biodegradation of products in the body, which could

trigger an immune response. As a result, biopolymers are becoming increasingly essential in medical implant materials.

Biopolymers are renewable, environmentally benign, and biodegradable materials that can be used to replace petroleum-based synthetic polymers [6]. Renewable materials such as modified plant oils, trepans, sugars, and polyphenols are used to make bio-based resins [7], with some resins containing up to 55 % bio-content [8]. As a result, bio-based polymers have emerged as viable alternatives to synthetic polymers. They have a more complex and well-defined structure, as well as the ability to decay and regenerate, which synthetic polymers do not have. Bio-epoxy is a bio-based thermosetting polymer [9].

Biopolymers have been used to build medical materials, cosmetics, packaging and food additives, water processing chemicals, apparel textiles, industrial plastics, data storage elements, absorbent, and even biosensor. However, their shock resistance, tensile strength, permeability, and thermal stability are all low. As a result, including the reinforcing material into the micro or nano regime is the greatest way to improve the characteristics and economic value of biopolymers [6]. Environmentally friendly polymer composites or biopolymer composites are the resultant products. Zinc oxide and hydroxyapatite have been demonstrated to be key biomedical engineering materials over the years, with their bioactive and biodegradable qualities allowing them to be employed as scaffolds for bone grafts and filler material for teeth. They are well-known biomedical engineering materials with desirable characteristics such as osteoconductivity, biocompatibility, thermodynamic stability, corrosion resistance, and high strength comparable to bone strength.

Several attempts have been made to incorporate bioactive fillers in polymer matrix for application in dentistry. These bioactive fillers include bioactive glasses [10, 11], niobium and zirconia [12], nanostructured hydroxyapatite [13] and natural hydroxyapatite [14] with much improvement in properties investigated reported. Alhashimi et al. investigated the bioactivity and the cytocompatibility of polyethylene reinforced with bioactive glass (BAG) composite for root-canal filling and reported their suitability for a root canal system due to improve bioactivity, biocompatibility and easy removal [10]. Collares et al. also evaluated methacrylate-based root canal sealers incorporated with nano-hydroxyapatite (nHAP) fillers and reported that the inclusion of nHAP up to 40 % did not affect the film thickness or radiopacity [13]. Heid et al. investigated epoxy resin incorporated with nanometric and micrometric BAG particles as root canal sealer and reported that the incorporation of the BAG resulted in quicker calcium phosphate precipitation [11]. However, little or nothing has been done on incorporation of two or more bioactive fillers as hybrid inclusion in epoxy resin for dentistry purpose.

In this regard, the present work aimed to develop epoxy resin hybrid biocomposites reinforced with natural hydroxyapatite (nHAp) and zinc oxide for application in dentistry. The in vitro bioactivity and morphological features were investigated to assess the bioactivity of the developed biocomposites.

EXPERIMENTAL

Materials

Materials used in this research include the following: simulated body fluid (BZ_{173}), silica gel, lubricant oil, zinc oxide, hydroxyapatite, epoxy resin and hardener. The simulated body fluid was purchased from BIOCHEMAZONE located in Canada while epoxy resin and its hardener were purchased from Malachy Enterprise Ltd., Lagos.

Preparation of hydroxyapatite from cow bone

Waste cow bones were gotten from abattoir and washed with tap water before being broken into smaller fragments. The broken bones were boiled with hot water for 3 hours to burn off the fluids in the bone marrow and as well remove any remaining soft tissue with knife. To denaturalize the protein, the bone pieces were dehydrated in an oven at 80°C for 72 hours (3 days). Laboratory jaw crusher was used to reduce dry bone fragments to small particles. The crushed bone was calcined in carbolite furnace at 950°C for 3 hours with a heating and cooling rate of 5°C min^{-1}. Calcination of the cow bone produced a pure hydroxyapatite (nHAp) as an inorganic phase of the bone that is free of fat and protein. The degradation of the organic phase resulted in the formation of nHAp. The nHAp was ball milled to and sieved to obtain particle seizes of hydroxyapatite.

Composite development

The composites were developed by mixing the materials in predetermined proportions within 3 and 7 wt. % as shown in Table 1. A total weight of 240 g was used to determine the quantity of the matrix and reinforcement in the production of the composites. The hybrid reinforced hydroxyapatite-zinc oxide-based epoxy biocomposites (nHAp/ZnO BPCs) samples as shown in Table 1 were developed by stir cast molding. The epoxy composites were produced by randomly dispersing predetermined weight of zinc oxide and hydroxyapatite particles (in ratio 50:50) in epoxy resin (in a ratio 2:1 with its hardener). The mixture was poured into a wooden mould of different cavities containing predetermined dimension for various test analyses and were allowed to cure at room temperature. This process was for the development of samples according to the sample dimensions required for ASTM standards. While producing the samples, lubricant oil was applied at the edge of the mould to prevent the composite mix from flowing into the open pores of the wood.

Preparation of simulated body fluid (SBF)/in vitro bioactivity test

Simulated body fluid (SBF) solution was prepared by dissolving chemical grade reagent in accordance to Kokubo and Takadama [8]. The ion concentrations of SBF in comparison to human blood plasma (HBP) are given in the Table 2. Each selected bio-polymer samples (Control, BP_1HB, BP_3HB, BP_5HB and BP_7HB) were then immersed in the SBF solution placed inside a plastic beaker, respectively. The plastic beaker was then placed inside water bath whilst the temperature is maintained at 37°C for soaking time of 10 hours. After the period, respective sample was brought out and drop inside acetone for 5 seconds to stop the surface mineralization followed by drying in desiccator. The samples were afterward analyzed by scanning electron microscopy and X-ray diffraction to assess its bioactivity by assessment of carbonate-hydroxyapatite (CHA/HAp) phase deposited.

RESULTS AND DISCUSSION

Microstructure evaluation before immersion in SBF

Figs. 1 - 4 revealed the SEM images of the samples with 0, 3, 5 and 7 wt. % reinforcements. It can be observed from Fig. 1 which indicates the morphology of the unreinforced epoxy resin matrix that the microstructure showed an irregular arrangement of the molecules somewhat bonded firmly together to form a rigid appearance. This can be attributed to good interaction between the epoxy resin and the hardener. However, the dull and white colors observed on the surface of the morphology might be attributed to the epoxy molecules. Similar result has been observed by Bello et al. [1]. However, for samples BP_3HB, BP_5HB and BP_7HB showed in Fig. 1 - 4, it was observed that somewhat white particles layers can be seen on their morphology embedded in a dark matrix. The dark matrix indicates the epoxy phase while the dispersed white color represents the natural hydroxyapatite particles used as filler alongside zinc oxide. The white portion is more obvious because zinc oxide is a dull particulate material. It was observed from the morphology that the sample tested contains well dispersed reinforcing phase to a large extent within the epoxy matrix.

Table 1. Composition of the developed biocomposites.

Sample	Composition (wt. %)	Epoxy Resin (g)	Hardener (g)	nHAp (g)	ZnO (g)
BP_3	3	155.20	77.60	3.60	3.60
BP_5	5	152.00	76.00	6.00	6.00
BP_7	7	148.80	74.40	8.40	8.40

Table 2. Ion concentration (mM/ Liter) of SBF and HBP in accordance with [8].

Ion	Na^+	K^+	Mg^+	Ca^+	HCO_3^-	HPO_4^{2-}	SO_4^{2-}	Cl^-
Simulated body fluid	142.0	5.0	1.5	2.5	4.2	1.0	0.5	147.8
Human blood plasma	140.0	5.0	1.5	2.5	27.0	1.0	0.5	103.0

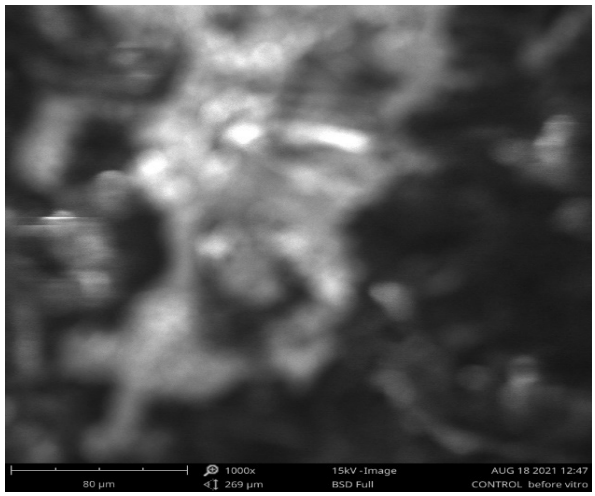

Fig. 1. SEM micrograph of control.

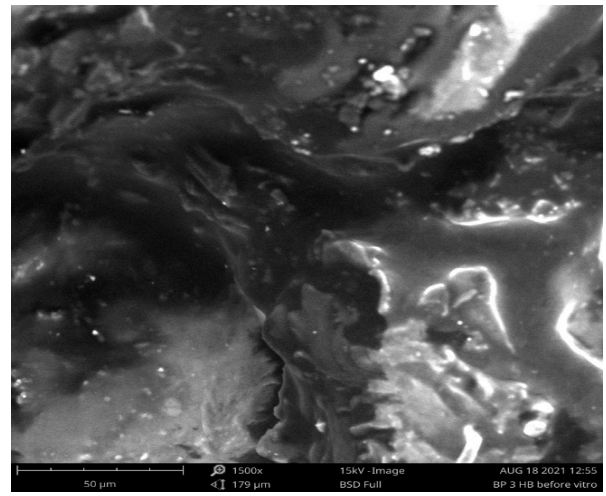

Fig. 2. SEM micrograph of BP_3HB.

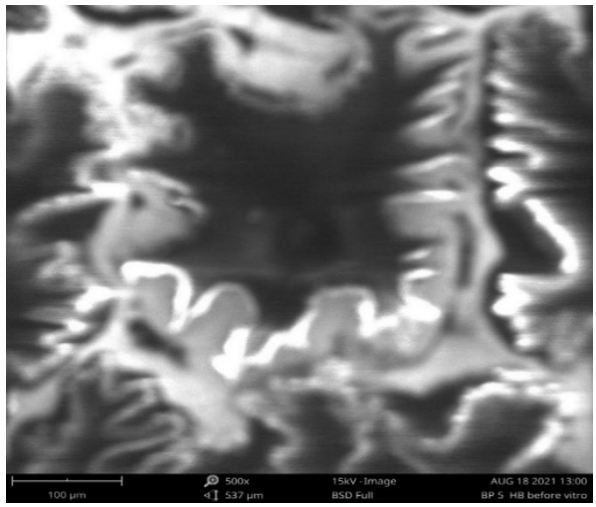

Fig. 3. SEM micrograph of BP_5HB.

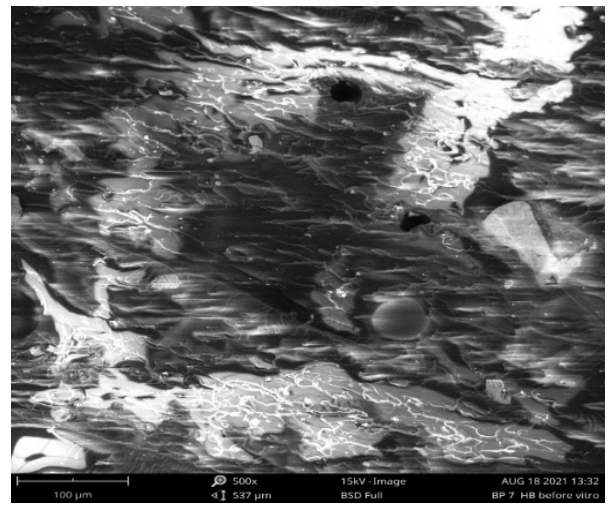

Fig. 4. SEM micrograph of BP_7HB.

Microstructural evaluation after in vitro

Fig. 5 - 8 showed the representative morphology for the selected samples after immersion in the SBF. It can be observed clearly from the micrograph that the microstructure consists of a regular and dense structure after immersion in SBF solution during the in vitro test. It is clearly seen that the surfaces of the tested sample where covered with somewhat finely dispersed whitish particles which can be attributed to the depositions of carbonate-hydroxyapatite layers (CHA/HAp). It was observed that there was an increase in hydroxyapatite formation after immersion in SBF which is as a result of good combination of natural hydroxyapatite used alongside zinc oxide as fillers in the hybrid biopolymers. The CHA/HAp layers observed after immersion in SBF as in vitro were an indication of good bioactivity reactions of the biopolymers [8]. However, it was observed that the CHA/HAp layers were more pronounced in sample

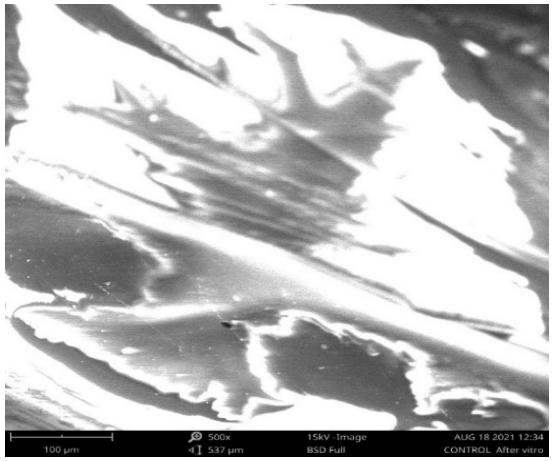

Fig. 5. SEM micrograph of control.

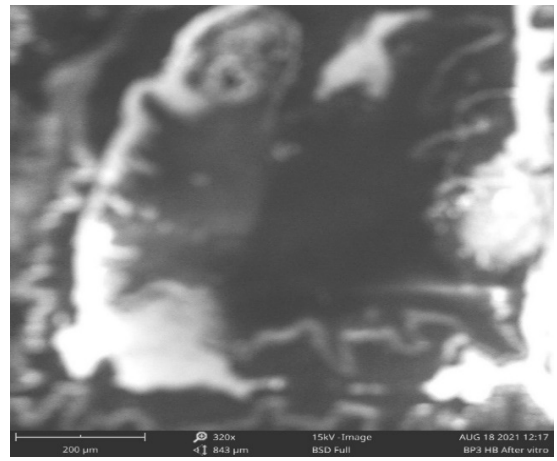

Fig. 6. SEM micrograph of BP_3HB.

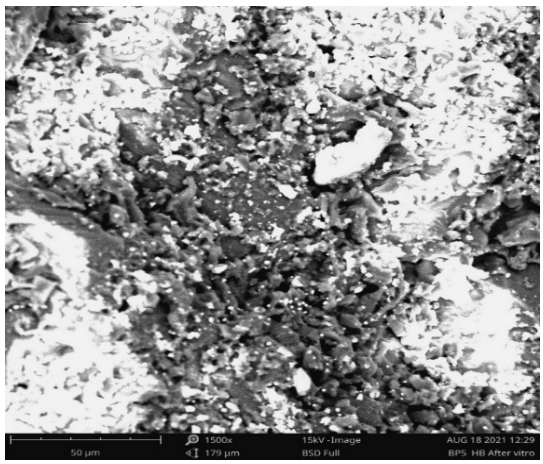

Fig. 7. SEM micrograph of BP_5HB.

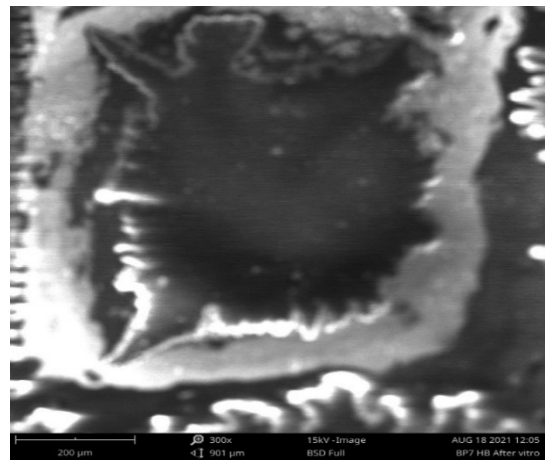

Fig. 8. SEM micrograph of BP_7HB.

BP_5HB which was an indication that the sample showed better bioactivity performance than the rest which can be as a result of good blend of nHAp and ZnO.

Phase evaluation after in vitro SBF

Figs. 9 - 11 showed the result of the phase evaluation on the selected samples using X-ray diffraction analysis after immersion in SBF solution for 10 hours. The phase evaluation is necessary to complement the result of the microstructure in vitro and to justify the CHA/HAP forming ability of the developed biopolymer composites. It is observed from the XRD spectra that there is a presence of two major apatite compounds in form of hydroxyapatite and carbonate hydroxyapatite alongside the polymeric mineral. The presence of calcite can also be observed during mineralization period in the SBF solution. The phase evaluation also showed characteristic amorphous diffractogram typical of cured epoxy resin.

General information

Analysis date	2021-08-13 16:56:43	Measurement start time	2021-08-13 12:40:22
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Multiple Profile

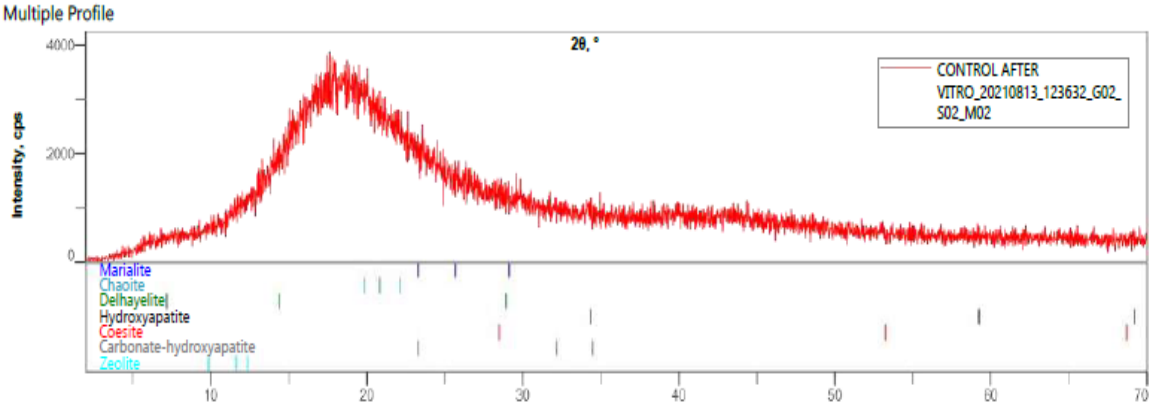

Fig. 9. X ray diffraction of control sample (*in vitro*).

Gener

Analysis date	2021-08-13 15:56:20	Measurement start time	2021-08-13 12:36:32
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Sample name	BP3 HYBRID AFTER VITRO	Comment	
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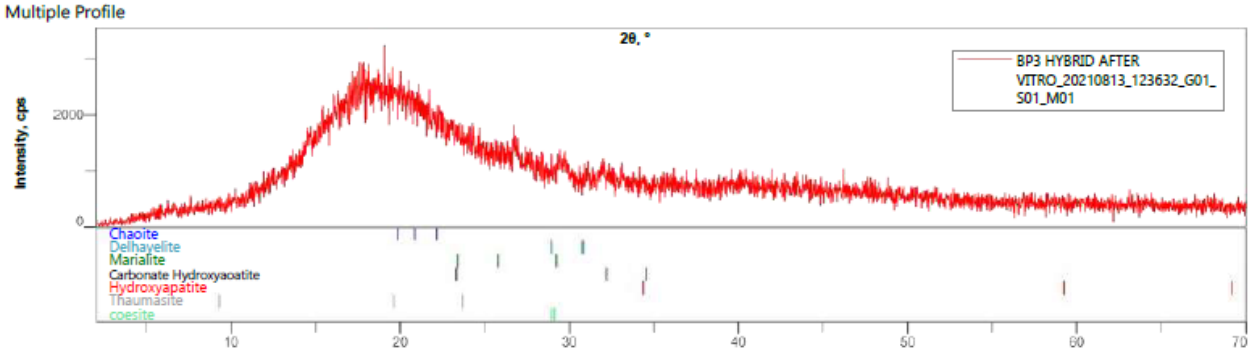

Fig. 10. X-ray diffraction of BP_3HB sample (*in vitro*).

General information

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Sample name	BP5 HYBRIDE AFTER VITRO	Comment	
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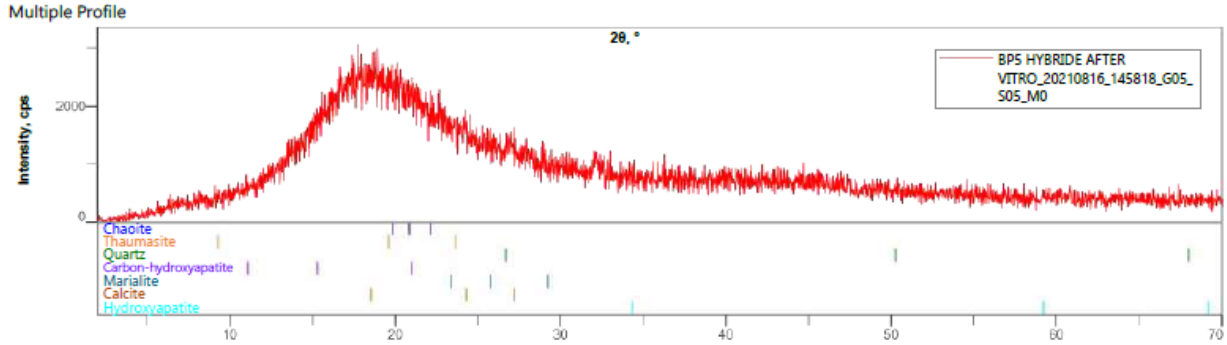

Fig. 11. X-ray diffraction of BP_5HB sample (*in vitro*).

CONCLUSIONS

Within the limits of this work, the hybrid reinforced epoxy resin samples showed good bioactive performance in SBF solution. However, Bp5HB displayed the overall better bioactivity performance, this bioactivity performance in SBF solution is an indication that the developed biopolymer composite is suitable and can bond well with living tissues.

In terms of the phase evaluation, the presence carbonate hydroxyapatite indicates the apatite forming ability of the developed biopolymer composites.

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