Electrospun fabrication and direct coating of bio-degradable fibrous composite on orthopedic Titanium implant: Synthesis and Characterizations

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Abstract
For orthopedic bone regeneration, an extracellular matrix (ECM) mimicking the composite’s ability has been highly expected by natural hard tissue along with their bio-activeness, bio-compatibleness, and bio-degradable properties. An electrospinning approach is one of the high potential processes to manufacture the porous material, and the method has highly recommended for the production of porous fibrous materials in the presence of various active compounds owing to its uniqueness of producing randomly oriented fibrous matrices with appropriate porosity. In this work, we report the fibrous composite coating process on the orthopedic titanium (Ti) plate during the process of electrospinning by simply fixing the Ti metal on the aluminum foil in the drum collector. This approach gives the bio-compatible Ti implant with ECM mimicking properties. The bio-activeness of that implant will be obtained through the presence of minerals (Sr$^{2+}$ and Ce$^{3+}$) substituted hydroxyapatite (MHAP) particles. The formation of the desired composites and their phase characteristics were carefully evaluated by Fourier Transform Infra-Red (FTIR) spectroscopy and X-ray diffraction (XRD) analysis, respectively. The formation of a fibrous network and various minerals in an appropriate manner were established by a scanning electron microscope (SEM) and energy dispersive X-ray analyzer (EDX) instruments. The important notable thing is their Vickers hardness was very lower for the Poly(3-hydroxybutyrate) (PHB) polymer containing fibrous composite. These results strongly suggest the conclusion that the synthesized fibrous composite coated material will act as a better ECM mimicking implant for orthopedic applications.

1. Introduction
The biomaterials for orthopedic application should be designed to try to be like the Extracellular Matrix (ECM) structure of the bone tissues to enhance bone imperfection’s osteogenesis and healing process. The collagen (also responsible for in-vivo piezoelectricity) is the major protein present in ECM and exists in the form of nanofibers. Various synthesis techniques, including electrospinning, molecular assembly, and phase separation, were used to prepare the nano-fibrous polymeric matrix for mimicking the natural ECM [1, 2]. Among various techniques, electrospinning is the most extensively used method to produce nanofibers. The nanofiber fabrication mainly depends on the conductivity of the polymers that are utilizing in the fabrication process. The conducting polymers (CPs) are available in numerous natural and artificial biodegradable polymers. The ceramics like the HAP combined with conducting polymers will enhance the electrospun process, the osteogenic potential for tissue engineering, and overcome the reactivity barrier between the fibrous materials and bio-molecules (Proteins) and then cells [3, 4]. The resulting nanofiber from electrospinning shows the high surface area to volume ratio and porosity. It has the main advantage of a tuneable property of its diameter between several nano-meters and several micrometers [5].

Poly(3-hydroxybutyrate) (PHB) is one of the polyhydroxyalkanoates (PHAs) with the properties of biocompatible, bio-degradable, and also it is piezoelectric material with the piezoelectric constant of $d_{14} = 1–2$
pico Coulombs/N [6, 7]. In 2019, R. V. Chernozem et al prepared the novel biomaterial for bone tissue repair. They designed a novel piezoelectric fibrous material derived from two different polymers, poly[(R)-3-hydroxybutyrate] (PHB) and poly[3-hydroxybutyrate-co-3-hydroxyvalerate] (PHBV), and they make mineralization on these polymers with CaCO₃ using ultrasound (U/S) method. They analyzed the piezoelectric charge of the two mineralized fibrous polymers and they found that the piezoelectric charge and porosity were significantly higher (4.3 times and ~15%, respectively) in PHB fibrous scaffolds than PHBV fibrous scaffolds. Besides, they had also done the bio-compatibility experiment on osteoblastic MC3T3-E1 cells, and they were getting the result of the nontoxic nature of that CaCO₃ mineralized PHB brous scaffolds. Following these results, they had concluded that the bio-material based on the piezoelectric PHB fibrous polymers would act as a better bio-compatible platform for enhancing the development of bone tissue [8].

Gelatin (GEL) is a bio-compatible fibrous protein with a low immunogenicity character derived from the collapsed native collagen [9]. Besides, GEL can enhance the cell adhesion, differentiation, and proliferation processes due to its structural feature, including the arginine-glycine-aspartic acid (RGD) motif for better cell-cell communication [10]. The bone regenerating ability of GEL with other substances including chitosan-nano-bioglasses 3D scaffold [11], microbial enzyme transglutaminase (mTG) [12] and cross-linked with genipin, β-tricalcium phosphates with the incorporation of traditional Chinese herbal DangguiBuxue Tang (DBT) [13] composites has been reported by many researchers previously.

We prepared the fibrous scaffold containing PHB cross-linked with GEL by electrospinning method based on PHB and GEL properties. The fibrous scaffold was loaded with hydroxyapatite (HAP- Ca₁₀(PO₄)₆(OH)₂) ceramic which includes osteogenic potential for better bone healing, in addition with an inorganic composition which is similar to that of native bone matrix [14]. Further, the osteoblast ability of HAP ceramic will enhance by two different minerals (Sr²⁺ and Ce³⁺) with different valent (Di and Trivalent) which were substituted on the HAP lattice. For treating the disease of osteoporosis, Strontium ranelate (SrR) is one of the orally active drugs with the combination of strontium (Sr) and ranelic acid. In this skeleton, Sr is the most active component and renelic acid is just acting as a carrier [15]. Meanwhile, Sr has a dual role as it dissociates the bone remodeling by stimulating the bone regeneration since Sr acts on the calcium-sensing receptor (CaSR) articulated by bone cells. Thus Sr has sensitive to the Wnt/β-catenin signaling pathway as Sr act as Ca like entity that promotes osteogenesis and osteogenic differentiation. And another role is it inhibits osteoclast activity and reduces bone resorption [16, 17]. The Ce ion has been chosen for its antibacterial activity [18]. In 2016, Sang et al. had developed the anti-microbial Ce (III) complex derived from Tris-(5-chlorosalicylidene)amine with tripodal Schiff base ligand. They proved that their new Ce (III) complex had exhibited great antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas fluorescens pathogens [19].

The PVA-GEL/MHAP/PHB fibrous scaffold was simultaneously coated on the surface of Titanium (Ti) metal during electrospinning for in-vivo bone regeneration application. We have chosen Ti metal due to its bio-compatibility nature regarding corrosion resistance property and higher mechanical strength under load-bearing conditions [20]. From our knowledge, this is one of the smallest existing results based on the direct coating of these kinds of fibrous scaffolds on the surface of the Ti metal during electrospinning.

2. Experimental section

2.1. Materials
Calcium nitrate tetrahydrate (Ca(NO₃)₂·4H₂O), Strontium nitrate (Sr(NO₃)₂), Cerous nitrate hexahydrate (Ce (NO₃)₃·6H₂O), diammonium hydrogen phosphate ((NH₄)₂HPO₄), ammonium hydroxide (NH₄OH), anhydrous N, N-Dimethylformamide (DMF, 99.8%), chloroform (CHCl₃), Polyvinyl alcohol (PVA) with an average molecular weight of 160,000 (89% hydrolyzed), Polyhydroxybutyrate (PHB) with Mw of 2.3 × 10⁵ g mol⁻¹, gelatin and commercially pure titanium (Cp Ti) used as a substrate for a coating of the fibrous composite were purchased from Sigma-Aldrich, India. There was no essential to the additional distillation of all other reagents and solvents. During the experiment, double distilled water (DDW) was utilized for washing purposes and as a solvent.

2.2. Preparation of minerals (Sr and Ce) substituted HAP (MHAP)
MHAP was prepared by following the previously reported literature in the molar ratio of 1.68 (One mole of cationic minerals to 0.68 M of phosphate anion) [21]. Briefly, all the solutions of cationic and anionic substances were prepared separately by dissolving the corresponding cationic and anionic salts in 50 ml of DDW at the molar ratio 0.05 + 0.05 + 0.40 (Sr + Ce + Ca)/0.34 (PO₄³⁻). Then, all the cationic minerals solutions were added into the calcium solution by using a burette drop wise under constant stirring. After adding all the minerals solution, they were allowed to stir for 45 min to attain mutual interactions between the cationic ions at
room temperature (RT) followed by maintaining pH at 11.0 using the ammonia solution. The phosphate solution was then added into cationic solution over 2 h using burette under constant stirring at RT with a white color precipitate formation. The pH was maintained at 11.0 all over the reaction. The mixture solution was then sonicated using the ultrasonication method (UltraSonics model with the supplying of 150 W with the on-off time of 8 s and 5 s respectively) at 28 kHz for 10 min. The solution containing white precipitate was then filtered with Whatman filter paper after aging the solution for 8 h. The MHAP particles were then dried under a microwave oven (Morphy Richards microwave synthesizer with a maximum microwave power of 1300 W and microwave frequency of 50 Hz for 120 s). The dried MHAP particles were then sintered at 600 °C for 5 h in a muffle furnace to get pure MHAP ceramic.

2.3. Surface treatment of Ti plate
The Ti metal was first dissected with 10 × 10 × 0.3 mm³ sizes, which was employed as a substrate for further coating during the electrospinning process. Before using this Ti plate, it was subjected to some surface treatments. Initially, this Ti plate was mechanically rubbed using abrasive silicon carbide paper with a grade range between 400–500. The acid etching of the substrate was carried out by utilizing the piranha solution containing the mixture of acids included H₂SO₄/H₃PO₄ (4:1). The acid-etched Ti was sonicated with water, acetone, and ethanol individually for 30 min each of the solvents. Then, the plate was sintered for 3–4 h at 450 °C. This sintered plate was then used as a substrate for the coating process.

2.4. Preparation of electrospinning solutions for fabricating fibrous composite
Here we fabricated different fibrous composites, including PVA, PVA/MHAP, PVA-GEL/MHAP, and PVA-GEL/MHAP/PHB matrices. First, PHB solution was prepared by refluxing PHB crystals (0.5 mg ml⁻¹) at 70 °C in CHCl₃/DMF (4:1 ratio) mixture for 4 h [22]. Then PVA–GEL blend was synthesized by dissolving the PVA and GEL (10% and 5% respectively) particles in cold water. Then it was heated at 70 °C until to obtain the homogeneous solution (for 18 h) under magnetic stirring. After the homogeneous solution was obtained, the MHAP particles (1%) were added to this PVA-GEL blend solution. The solution was vigorously stirred for 2 h under a magnetic stirrer, and the solution was used as an electrospinning solution for preparing the PVA-GEL/MHAP fibrous composites. Likewise, the composites such as PVA-GEL and PVA/MHAP were also prepared without adding MHAP and GEL particles, respectively.

2.5. Electrospinning process
All the composite solutions were individually loaded into 5 ml syringes consisting of 21-gauge precision needles attached with a high voltage power supply (22 kV). The aluminum foil was used for roofing the drum collector, and the pre-treated Ti was attached to its surface. The solution’s flow rate and the drum collector’s speed were set as 0.75 ml h⁻¹ and 15 rpm, respectively. A space between the syringe needle and the drum collector was maintained as 15 cm. The PVA–GEL/MHAP/PHB fibrous composite was prepared by the double-layer method. After the completion of spraying the PVA–GEL/MHAP solution, the new 2 ml syringe was loaded with PHB solution. Then it was allowed to electrospun over the already deposited PVA-GEL/MHAP fibers on both
aluminum foil and Ti plate. Then, both aluminum foil and Ti plate coated with fibrous composites were dried at 60 °C for 10 h to evaporate the solvent entirely and get pure fibrous composites and fibrous composites coated Ti plate. Figure 1, represents the possible mechanism that responsible for the construction of PVA-GEL/MHAP/PHB fibrous composite.

2.6. Physico-chemical characterizations of prepared MHAP ceramic and fibrous composites

2.6.1. Functionality analysis

Their peaks’ appearance corresponds to their functional groups confirmed the formation of individual MHAP ceramic and the fabricated fibrous composites. This analysis of the functional groups was done using the KBr pellet method with an IRTRACER-100 Infra-Red absorption spectroscopy (Shimadzu). The spectra were recorded at 4 cm⁻¹ resolution within the wave-numbers region from 4000 cm⁻¹–400 cm⁻¹.

2.6.2. Phase analysis

The crystallinity of the prepared ceramic and fibrous composites was determined by utilizing the Diffractometer system (XPERT-PRO) with an incident of Cu Kα radiation over the diffraction angle 2θ = 10° to 80°, worked at 40 kV and 30 mA and scanning rate (2θ) of 0.02°.

2.6.3. Morphology analysis

MHAP without any polymer and the prepared fibrous composites were examined for assessing their surface morphology by using scanning electron microscopy (SEM) collective with energy-dispersive X-ray spectroscopy (EDX) (JEOL JSM-6400, Japan). Individual ceramic morphology was analyzed by dispersing the samples in cold water and then coating them on the glass plate. In the case of fibrous composites, their morphology was examined after coated on the Ti plate.

2.6.4. Vickers microhardness analysis

The Ti plates with surface modification by different coating composites were subjected to a Vickers microhardness test. The hardness tester of the Akashi AAV-500 series with Vickers pyramid indenter was used for this experiment. The dwell time and load were set as 20 s and 490 mN, respectively. Three replicated tests were done to obtain the average value of Hv.

2.6.5. Porosity and surface area measurements

The surface area and the distribution of pore size were detected by the methods of Barrett–Joyner–Halenda (BJH) and Brunauer–Emmett–Teller (BET). It was performed on the sample to be tested using a Quantachrome Nova Winversion 11.05 under non-stop adsorption conditions at 77 K. Before nitrogen adsorption and desorption analysis, all samples are degassed at 100 °C–120 °C for 5 h.

Figure 2. Functional group analysis of (a) MHAP, (b) PVA-MHAP fibrous composite, (c) PVA-GEL/MHAP fibrous composite, and (d) PVA-GEL/MHAP/PHB fibrous composite.
2.6.6. The bio-degradation studies in Tris-HCL solution in-vitro
The ability to degrade the fibrous composite coated Ti plate at particular time duration was evaluated by simply immersing the sample in a 0.05 M of tris-HCL solution at 37 °C. The tris-HCL solution was prepared according to already published literature [23, 24]. Initially, Tris(hydroxymethyl)aminomethane was dissolved in DD water and then adjusted the pH of the solution to 7.4 by using HCL solution. The solution was refreshed at every 24 h of the time interval. The immersed samples were then characterized by SEM analysis, found their weight loss at 1, 3, and 7 days to access their bio-degradation ability. Before analysis, the samples were dried entirely under an oven at the temperature of 75 °C for 8 h.

3. Results and discussions

3.1. Functional group analysis
The formation of all the prepared materials had been confirmed by evaluating their functional group analysis through the FT-IR technique. An influence of the substitution of Sr and Ce (divalent and trivalent ions respectively) on the HAP lattice was first analyzed, and the results were given in figure 2A(a). The ionic minerals (Sr, Ce) substituted on HAP lattice, which are replacing Ca2+ ions, affect the intensity and position of PO4³⁻ absorption peak. The obtained peak for MHAP is mainly arising due to the presence of PO4³⁻ group in the MHAP ceramic. The fundamental vibrational modes appeared at 1151.1 cm⁻¹ and 996.84 cm⁻¹ (ν3), 487.1 cm⁻¹ (ν2), 623.8 cm⁻¹ (ν4) and 940.0 cm⁻¹ (ν1) are corresponds to the functional PO4³⁻ group. Those vibrational peaks are greatly varied from their original position of the pure HAP [25]. This FT-IR spectrum had undoubtedly shown that both intensity and position of the absorption peaks of pure HAP had distorted. The vibrational absorption peak at 3568.9 cm⁻¹ corresponds to the –OH group was vanished due to the distorting the electrical charge balance in the HAP lattice made by the replacement of Ca2⁺ ion by different valent ions Sr2⁺ and Ce3⁺ [26]. The functional groups of the fibrous materials such as PVA-MHAP, PVA-GEL/MHAP, and PVA-GEL/MHAP/PHB composites were analyzed successively, and the results were plotted in figures 2A(b)–(d). Figure 2A(b) corresponds to the vibrational modes of PVA-MHAP fibrous composite. The broadband

![Figure 3. Phase analysis of (a) MHAP, (b) PVA-MHAP fibrous composite, (c) PVA-GEL/MHAP fibrous composite, and (d) PVA-GEL/MHAP/PHB fibrous composite coated on Ti plate.](image)
centered at 3123.5 cm\(^{-1}\) in figure 2A(b) corresponds to the –OH absorption stretching band of PVA polymer. An interaction between C-H in the alkyl groups of PVA gives the strong band at 2738.9 cm\(^{-1}\). The smaller and less intense bands at the 1448.5 & 1349.4 cm\(^{-1}\) correspond to the bending and wagging of CH\(_2\) vibration. The vibrational bands at 1721.9 and 1516.8 cm\(^{-1}\) confirmed the carbonyl group (C=O) in PVA polymer. H-O-H bending mode of vibration has appeared at 1647.6 cm\(^{-1}\). These vibrational bands of PVA were appeared along with the vibrational peaks of MHAP’s PO\(_4^{3-}\) (1045.9 cm\(^{-1}\) and 940.0 cm\(^{-1}\) (\(\nu_3\)), 469.9 cm\(^{-1}\) (\(\nu_2\)), 648.6 cm\(^{-1}\) (\(\nu_4\))) group confirms the presence of MHAP-PVA fibrous matrix [27]. The interactions between PVA-GEL and MHAP ceramic were given in figure 2A(c). The broadband that occurred at 3200–3500 cm\(^{-1}\) corresponds to the stretching vibrations of –OH in PVA polymer and N–H and O–H group in gelatin molecule. This increase in the –OH group’s wave-number may occur due to the intermolecular hydrogen bonding interaction between PVA and gelatin. The newly formed peak at 1665.6 cm\(^{-1}\) attributed to the carbonyl (C=O) group presents an amide functional group of gelatin. Moreover, the strong peak at 1740.7 cm\(^{-1}\) corresponds to the formation of an ester bond between the alcohol (–OH) group of PVA and carboxyl (–COOH) group of gelatin molecules [28]. In addition to these peaks, the already existed PVA and MHAP peaks have also appeared with slight deviation, which confirms PVA-GEL/MHAP fibrous composite formation. The spectrum in figure 2A(d) is attributed to the PVA-GEL/MHAP/PHB fibrous composite. In this spectrum, the PVA-GEL/MHAP composite’s original peak position had been changed after adding the PHB polymer as a second layer on the previous fibrous matrix. Also, the new peak corresponds to PHB was appeared. The peak at 1802.3 and 3005.5 cm\(^{-1}\) has attributed to the carbonyl (C=O) stretching and –CH stretching. Moreover, the C-O stretching band also appeared at 1110.5 cm\(^{-1}\) [29]. The extra sharp band at 3500–3600 cm\(^{-1}\) may arise due to the functional -OH group in PHB polymer. From these results, we can understand that HAP ceramic functionality was not affected even after the formation of composite with other organic compounds. This similar result was obtained for Mukherjee et al. They have also made the composite of HAP with carbon nanotubes without changing HAP’s functionality [30]. We could finally confirm all fibrous composites’ formation as the expected structure from these observations of FT-IR analysis.
3.2. X-Ray Diffraction (XRD) analysis

XRD analysis was performed in this study to identify the phase characteristics of the all prepared composite fibers. The XRD patterns of the sintered (600 °C) MHAP was demonstrated in a crystalline phase with decreased in their crystallinity nature. The peaks were obtained at 2θ = 17.25°, 22.1, 25.8°, 28.5, 31.2°, 34.7°, 48.1°, and 53.2°. This pattern slightly deviates in their peak position to lower 2 theta values from the pure HAP matrix [31]. The diffraction patterns are overlapped due to their lower crystallinity, as shown in figure 3(a). The intensity of the peak and position decreased due to the formation of MHAP with a calcium-deficient matrix [32]. From this MHAP pattern, we can confirm the incorporation of Sr2+ and Ce3+ ions within the HAP matrix by replacing Ca2+ ions. After forming the MHAP/PVA fibrous composite, the crystalline phase of MHAP was completely degraded into amorphous nature. The prominent peak of PVA shows at 2θ = 19.2° corresponds to the doublet reflection of (101) and (10–1) planes of semi-crystalline PVA, and a second peak corresponds to the plane of 220 planes of PVA [33]. Meanwhile, the primary crystalline peak of HAP in MHAP pattern at 2θ = 31.5° has also appeared in MHAP/PVA fibrous matrix shows the formation of MHAP/PVA fibrous composite with amorphous nature as demonstrated in figure 3(b) [33]. Figure 3(c) indicates the phase characteristics of PVA-GEL/MHAP fibrous composite. The broadening of PVA peak at 2θ = 19.2° may be due to the overlapping the peaks of PVA and GEL (2θ = 20°) [34]. At the same time, the MHAP peaks also appear with a slight change in their peak position. Figure 3(d) stands for the diffraction pattern of PVA/MHAP/PHB fibrous composite coated on Ti metal. The intensity of all peaks is greatly reduced due to the main Ti phase. The peaks with low intensity at 2θ = 13.5° (020), 16.4° (110) correspond to the PHB matrix [35], and other peaks for PVA, gelatin, and MHAP have also appeared with slight variation. Besides, the main peaks of Ti correspond to the planes of (002), (102), and (103) were appeared for the Ti phase [36]. We could conclude that all prepared fibrous composites' phase characteristics are included as a coating on Ti plate from these diffraction patterns.

3.3. Morphology analysis

The surface morphology of all the prepared fibers, namely PVA, PVA-MHAP, PVA-GEL/MHAP, and PVA-GEL/MHAP/PHB composites, along with pure MHAP particles, were observed through SEM analysis and are given in figures 4(A)–(G). It is clear that from the images, all the prepared fibers by electrospinning method (at the voltage of 22 kV and flow rate of 0.75 ml h^-1) are well arranged randomly oriented structure and exhibits an interconnected porous 3D network. Any type of composites with these porous 3D networks can mimic the ECM of the natural bone. Hence, they will facilitate the cell attachment and proliferation to enhance the significant bone regeneration in that favorable microenvironment. The pure electrospun PVA’s average fiber diameter is ~350 nm without any beds formation (figure 4(B)). After adding MHAP particles into the PVA solution by in situ preparation, the obtained PVA-MHAP fiber consisted of some agglomerated particles of MHAP on the fibrous network and had an increased mean diameter from ~350 to ~460 nm (figure 4(C)) as showed in previous PVA/PVP/HAP fibers prepared by B. Chaudhuri et al in 2016 [37]. This increase in the mean diameter of the fiber after the addition of the MHAP particles is well correlated with the previous result reported by Uma Maheshwari et al. They had also got the increased mean diameter of the PVA-Polyvinylpyrrolidone (PVP) fiber from 189 nm to 261 nm after the addition of HAP particles [38]. The same effect of the increased mean diameter of the fiber was obtained in 2008 by Gyeong-Man Kim et al. They found that the mean diameter of PVA was 160 nm while PVA/HAP was 170 nm [33]. Figure 4(D) corresponds to the fibrous composite made up of PVA-GEL/ MHAP matrices. The mean diameter of this composite is surprisingly decreased from ~460 nm to ~307 nm. This decrease in the mean diameter may be due to the variation of the electrospinning solution’s properties upon
the gelatin’s addition into the PVA solution. The morphology of the final composite (PVA-GEL/MHAP/PHB) was shown in figure 4(E) which exhibits relatively dense fibers due to the second time spinning of PHB solution on the already existed PVA-GEL/MHAP fibers with the increasing mean diameter of 370 nm. Figures 4 (A) and (F) corresponds to the SEM and TEM images of MHAP particles, respectively. The MHAP ceramic was formed with uniform agglomerated particles with some pores, and the average diameter is ~60 nm. The cross-sectional view of the final fibrous composite coated Ti plate was showed in figure 5(A). The elemental composition of MHAP ceramic alone and the final composite are shown in figure 4(G) and figure 5(B), respectively. The average fiber diameter distribution histograms were showed separately in figure 6.
3.4. Vickers microhardness (Hv) analysis

During the fabrication of an implant material for hard tissue regeneration in the load-bearing application, the determination of its mechanical strength is a vital thing. Vickers microhardness analysis is the surface phenomena that predict the coating’s ability to resist plastic deformation. Two different composites such as PVA-GEL/MHAP and PVA-GEL/MHAP/PHB fibers coated Ti plates, were taken into account for Vickers hardness analysis. Interestingly, PVA-GEL/MHAP fibrous composite coated Ti plate exhibits very low hardness value of 4.9 ± 0.44 Hv than the pure uncoated or unmodified Ti plate which exhibits 829.7 ± 14.95 Hv [21]. This considerable variation may be arising due to significantly softer spongy like fibrous composites on the Ti plate’s surface. In the case of PVA-GEL/MHAP/PHB fibrous composite coated Ti plate, the Hv value was increased to 90.37 ± 1.12 Hv. It may occur due to the addition of PHB polymer as a second layer, which will enhance the resisting character of that particular fibrous composite to plastic deformation. These results indicate that the PVA-GEL/MHAP/PHB fibrous composite coated Ti implant would be a better choice for applying in-vivo implantation. The maximum, minimum, and mean values of the microhardness test for the Ti plates coated with PVA-GEL/MHAP and PVA-GEL/MHAP/PHB fibrous composite was given in table 1.

| Table 1. Vickers Microhardness value of PVA-GEL/MHAP and PVA-GEL/MHAP/PHB fibrous composite coated Ti plate. |
|---------------------------------------------------------------|
| Test No | Hardness (HV) | Test No | Hardness (HV) |
|---------|---------------|---------|---------------|
| 1       | 4.81          | 1       | 91.63         |
| 2       | 4.51          | 2       | 90.00         |
| 3       | 5.34          | 3       | 89.47         |
| Max     | 5.34          | Max     | 91.63         |
| Min     | 4.51          | Min     | 89.47         |
| Mean    | 4.90          | Mean    | 90.37         |

| Table 2. In-vitro degradation of PVA-GEL/MHAP/PHB fibrous composite at different periods. |
|---------------------------------------------------------------|
| Days of immersion in a tris-HCL solution | Initial weight (W1) (mg) | Final weight (W2) (mg) | Weight loss (%) |
|------------------------------------------|---------------------------|------------------------|-----------------|
| 1                                        | 55                        | 54                     | 1.82            |
| 3                                        | 54.8                      | 52.5                   | 4.20            |
| 7                                        | 59                        | 54                     | 8.47            |

3.5. Porosity and surface area measurements

The curves of adsorption and desorption isotherms and the pore size distribution of PVA-GEL/MHAP/PHB fibrous composite were given in figures 7(A) and (B), respectively. The methods of Barrett–Joyner–Halenda (BJH) and Brunauer–Emmett–Teller (BET) were adopted for determining the surface area, pore-volume, and pore diameter of the tested samples. According to IUPAC, the hysteresis loop is visible; indicating the formation of type IV isotherm. The BJH studies obtained for the PVA-GEL/MHAP/PHB fibrous composite exhibited a surface area in the range of 7.182 m²g⁻¹. Following this surface area, the fibrous composite’s average pore volume and pore diameters were indicated at 0.014 c.c./g and 2.2 nm, respectively. The pore size distribution graph shown in figure 7(B) shows that the most intense pore peaks appeared at the mesoporous range (2–50 nm). These point out that, in this PVA-GEL/MHAP/PHB fibrous composite, the mesopores are more dominant than micropores. These pores will form new bone tissues on this ECM, mimicking fibrous composite coated Ti plate.

3.6. In-vitro degradability of the fibrous composite

The PVA-GEL/MHAP/PHB fibrous composite’s degradability was determined in terms of weight loss percentage (%) in the tris-HCL solution for 1, 3, and 7 days and the results were tabulated in table 2. After one day of the immersing time, the degradation of PVA-GEL/MHAP/PHB fibrous composite had reached 1.82%. Then, it was increased to 4.19% after 3 days of immersion period. A higher percentage of degradation was obtained for 7 days of immersion period. It shows up to 8.47% of the composite had degraded. We could
understand that the PVA-GEL/MHAP/PHB fibrous composite has bio-degradable nature and that degradability depends on time. The SEM morphology of the composite after degradation at every particular time period was also given in figure 8.

In the application for bone fixation, reasonable degradation in a physiological atmosphere is one of the biomaterial attributes. This study shows that the final fibrous composite exhibits the degradation rate of 8.47% at 7 days of immersion, which point out that this fibrous composite is bio-degradable in time-dependent manner. Figure 8 stands for the PVA-GEL/MHAP/PHB fibrous composite morphology after immersing in tris-HCL solution for 1, 3 and 7 days. The degradation of the composite was well evidenced from these images. The fibrous morphology was almost degraded quickly on 1st day of immersion. We could see the small fibrous nature (indicated by yellow arrows) of the composite on 1st day of immersion in figure 8(A). But after 3 and 7 days of immersion, as shown in figures 8(B) and (C), the fibrous morphology was collapsed with the retention of small pores. These pores are well defined in 7 days of immersion than 3 days. From these morphological results of the fibrous composite after degradation, we can understand that, with the help of these pores, the newly formed bone will be viable to adjust and curing the bone defects.

The superior coating on the surface of the bio-implant material is still need for improving the osteogenesis during the bone reformation. The implant material made from this research work through direct coating of fibrous composite on Ti plate during an electrospinning is also able to act as better bio-implant material like various implant materials prepared by coating of various bio-materials through electrophoretic deposition, electrochemical deposition and pulsed electrochemical deposition methods [25, 39–41].

4. Conclusions

This present research work produced the novel orthopedic biomaterial surface coated with bio-degradable electrospun fibers. Attaching the Ti plate on the drum collector was adopted for efficient coating of the fibrous material on the Ti plate. The porosity present in this fiber will enhance the formation of bone tissues on their surface. Moreover, the surface morphology reveals that this new composite made up of PVA-GEL/MHAP/PHB has an ECM mimicking property due to their fibrous network evidencing from SEM analysis. The main notable
thing is that, the very lower microhardness for the final composite was attained due to its more spongy nature. But, the degradation ability of the fibrous composite was increased with an increase in the period. It exhibited that about 8.47% of the composite was degraded after 7 days of immersion. After confirming its bio-active and bio-compatible nature by in-vitro and in-vivo studies, this implant material will move towards clinical application and commercial production for orthopedic bone repair.

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Data availability statement

The data generated and/or analysed during the current study are not publicly available for legal/ethical reasons but are available from the corresponding author on reasonable request.

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