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Original Research

Surface Modification of Polycaprolactone/Gelatin Nanofibers with β-Tricalcium Phosphate

Syakirah Athirah Abdulla Hamid[1], Norhana Jusoh[1,2*], Sharifah Raihanah Kamaruddin[1], Adlisa Abdul Samad[1]

[1] *Department of Biomedical Engineering and Health Sciences, Faculty of Electrical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*
[2] *Medical Devices and Technology Centre, Institute of Human Centered Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

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ABSTRACT

Polycaprolactone (PCL) is one of synthetic biomaterials that have been used for bone scaffolds. PCL is a synthetic, linear, and semicrystalline aliphatic polymer that is widely used for bone application due to its biocompatibility and good mechanical properties. However, PCL has disadvantages due to its hydrophobicity nature that require combination with another natural biomaterial such as Gelatin. However, this composite still lacks elements to support bone mineralization environment. Besides hydroxyapatite, ß-tricalcium phosphate (B-TCP) is one of bioceramics that having similar components with natural bone minerals. ß-Tricalcium phosphate (B-TCP) is a widely used for bone scaffolds, but the study on the effect of this biomaterial coating on nanofibers remains limited. Therefore, this study focuses on the surface modification of fabricated PCL/Gelatin nanofibers with B-TCP. Prior to surface modification, PCL/Gelatin nanofibers were fabricated by using an electrospinning at two distinct PCL to gelatin volume ratio, which are 60:40 and 70:30. This approach aims to identify the optimal volume ratio for nanofiber through scanning electron microscope (SEM) result analysis. The PCL/Gelatin nanofibers with mix ratio of 70:30 was selected and subjected to surface modification using a post-treatment method, involving immersion in a B-TCP solution. Following this modification, all types of nanofibers were analyzed using SEM, Fourier transform infrared spectroscopy (FTIR), and water contact angle (WCA) measurements. The surface-modified PCL/Gelatin nanofibers treated with B-TCP (PCL/Gelatin/B-TCP) exhibited a rougher morphology, with an average diameter of 395.0799.83 nm and an average porosity of 26%. WCA analysis indicated an increase in hydrophilicity for the PCL/Gelatin/ß-TCP nanofibers, with an average contact angle of 56.17#2.87°, compared to the PCL/Gelatin nanofibers, which had an average contact angle of 89.958.43°. Based on the results obtained, the surface modified-nanofibers demonstrated characteristics that have potential in enhancing bone regeneration. Consequently, this provides valuable insights for advancing bone tissue engineering applications.

INTRODUCTION

Bone tissue engineering (BTE) has emerged as a promising alternative, overcoming issues like donor shortage and immune rejection (Gautam et al., 2021). BTE utilizes cells, growth factors, and a 3D polymeric matrix scaffold to create an environment conducive to bone tissue regeneration, offering a revolutionary approach in orthopedics with the potential for improved outcomes and patient well-being. Nanofibrous scaffolds, particularly those made of nanofibers, have gained attention for their potential in BTE due to their ability to mimic the extracellular matrix (ECM)'s physical properties (Farzamfar et al., 2020).

Nanofiber materials can be made from natural or synthetic polymers. Synthetic polymers like polycaprolactone (PCL) are

* Norhana Jusoh (norhana@utm.my)

Meditec, Institute of Human Centered Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia.

preferred due to their good mechanical properties. PCL is a synthetic, linear, and semicrystalline aliphatic polymer that is widely used as synthetic polymers in BTE applications. PCL offers various advantages, such as low cost, biocompatibility, and good mechanical properties (Lee et al., 2022). Additionally, PCL is a non-toxic polymer with fully biodegradable properties (Budi et al., 2022). However, its hydrophobicity nature limits its effectiveness in promoting cell adhesion, migration, proliferation, and differentiation (Gautam et al., 2021). In contrast, natural polymers are favored by cells for their hydrophilicity and attachment sites, but they lack mechanical strength and may pose pathogen transmission risks. Therefore, previous research suggested that combining both types of polymers can create biodegradable, and biocompatible scaffolds, potentially useful in tissue engineering (Farzamfar et al., 2020).

Gelatin is a natural polymer derived from collagen, and it possesses hydrophilic properties that can enhance cell adhesion and proliferation. Furthermore, the ability of gelatin to promote the adsorption of ECM biomolecules, facilitate cell adhesion mediators, and assist cell communication and regulation, makes it a valuable complement to PCL. Moreover, due to other properties such as low toxicity and low cost, gelatin has been applied in diverse tissue engineering such as bone, skeletal, and neural tissues (Lukin et al., 2022). Despite the benefits offered, gelatin has some limitations where gelatin short degradation time and has a low mechanical strength (Anjum et al., 2022). Therefore, the combination of gelatin and PCL is anticipated to provide a scaffold with improved mechanical properties and increased cell affinity.

Ceramic materials, specifically calcium phosphate (CaP), found in human bones and teeth, are biocompatible and widely used in dentistry and orthopaedic applications. Nathanael and Oh (2021) highlight the potential of nanohybrids by mixing CaP with polymers can offer variable mechanical properties, biocompatibility, flexibility, low toxicity, biodegradability, and mirror the organic-inorganic structure of real bone. This integration of scaffolds with ceramics is prevalent in BTE.

In addition, previous research proposed the surface modification of Gelatin-PCL nanofiber with nano hydroxyapatite (nHAp) demonstrated enhance osteoconductivity and improve scaffold surface roughness which are suitable for bone regeneration (Gautam et al.,2021). β-TCP is another form of CaP that can offer the same advantages as nHAP but β-TCP have higher solubility and reabsorb faster which promotes natural bone regeneration (Mahanty & Shikha, 2022). β-tricalcium phosphate (β-TCP) distinguishes itself among bioceramics by having similar components with natural bone minerals. It also demonstrates an optimal breakdown rate, contributing to enhanced new bone formation (Liu et al., 2021). This study focuses on fabricating PCL/Gelatin electrospun nanofibers then explore the surface modification of the composite nanofiber with β-TCP, and subjecting the modified nanofibers to characterization analyses, including scanning electron microscopy (SEM), Fourier transform infrared spectroscope (FTIR), and Water contact angle (WCA) measurement.

MATERIALS AND METHOD

Polycaprolactone (PCL) pellets (Mw=80,000), Gelatin (type B) powder and β-TCP sintered powder (≥98% β-phase basis) were purchased from Sigma-Aldrich.

Preparation of PCL/Gelatin Solution

To prepare the PCL/Gelatin solution, 10% (w/v) PCL solution and 8% (w/v) Gelatin solution was prepared. The 10% (w/v) PCL solution was prepared by dissolving the PCL pellets (10% w/v) in chloroform/methanol (3:1, v/v) using magnetic stirrer. The stirring continued until the PCL pellets were dissolved. To produce 8% (w/v) gelatin solution, gelatin powder (8% w/v) was dissolved in acetic acid (80% v/v) by using a magnetic stirrer until it fully dissolves. The PCL and gelatin solution were then mixed using a vortex at volume ratio 60:40 and 70:30. The PCL/gelatin solutions were incubated at room temperature for 48 h. Following the incubation period, gelatin was evenly dispersed within the PCL solution, resulting in an immiscible blend of gelatin and PCL (Gautam et al, 2021). This blended solution was subsequently utilized for electrospinning processes.

Fabrication of nanofiber by electrospinning method The electrospinning process started with placing the pure PCL polymer solution in a 10 mL plastic syringe. Then, the syringe was fitted with a flat needle (tip diameter: 21G). The distance between the needle and the collector was adjusted to 10 cm. Next, the electrospinning voltage was adjusted to 22 kV, and the feed flow rate was set at 0.2 mL/h. A syringe pump was used to control the feed flow rate. After that, nanofibers were collected on a 15 × 15 cm² flat aluminium plate (Gautam et al., 2023). The electrospinning process was repeated with the polymer solution shown in Table 1.

Surface Modification

For the surface modification solution, the β-TCP solution was prepared by dissolving the β-TCP sintered powder (1 wt%) in double distilled water at room temperature (25 ± 1°C) and stirred for 5 minutes (Gautam et al., 2023). The fabricated PCL/Gelatin nanofiber was then dipped in the β-TCP solution for 20 minutes and stirred using a magnetic stirrer (Gautam et al., 2023). After that, the coated PCL/Gelatin was rinsed three times using distilled water and dried overnight inside a desiccator.

Characterisation of nanofibers

The morphology of the nanofibers was observed using Scanning Electron Microscope (SEM) (Hitachi, TM3000, Japan), which measured fiber diameter and porosity. the nanofibers were cut into 0.5 × 0.5 cm2 and the samples were sputter coated with gold. After that, the samples' morphology was observed using SEM at a 15kV accelerating voltage with 5000x, 7000x and 10000x magnification. Diameter and porosity were measured using Image J software (1.35a, Rasban. W, USA). 100 measurements of the fiber diameter for each sample were taken to find the average and diameter distribution of the nanofiber. The porosity of the nanofiber was calculated using formula (1) by applying the information obtained from ImageJ software. The average porosity for 4 Region of Interest (ROI) from the SEM image were calculated.

$$Porosity\ (\%) = \left(\frac{Total\ Area\ of\ pores}{Total\ Area\ of\ nanofibre}\right) \times 100\% \qquad (1)$$

Fourier Transform Infrared (FTIR) spectroscopy (Frontier Spectrometer, PerkinElemer, UK), was used to determine the presence of functional groups in scaffolds. It was also used to investigate any chemical interactions between the components of the nanofiber scaffold and to determine the incorporation of

β-TCP on the PCL/Gelatin nanofiber. FTIR was conducted using FTIR spectroscopy throughout a wavenumber range of 4000 to 650 cm−1 (Gautam et al., 2021).

Water Contact Angle (WCA) analysis was an analysis to evaluate the surface wettability through the sessile drop method. WCA was conducted using contact angle analyzer (AST-VCAOptima, AST product inc., USA). The samples were prepared with dimensions of 1x1 cm². A drop of 2 ml distilled water was deposited on the sample. Subsequently, an image of the droplet was captured, and the contact angle was quantified using VCAOptima software. In this study 3 readings were taken for each sample and the average WCA measurement was calculated (Ehtesabi & Massah, 2021).

RESULTS AND DISCUSSION

Fabrication of PCL/Gelatin nanofibers

SEM was conducted on the fabricated PCL and PCL/Gelatin nanofibers to examine their morphology. SEM observations shown in Figure 1 revealed distinct morphological differences between PCL/gelatin (60:40) and PCL/gelatin (70:30) nanofibers. The PCL/gelatin (60:40) nanofiber displayed irregular, porous structures with discontinuous fibers, indicating pronounced phase separation and uneven polymer distribution. Conversely, PCL/gelatin (70:30) nanofiber exhibited a well-defined network of continuous fibers, densely packed and interconnected to form a more uniform nanofiber as compared to PCL/gelatin (60:40) nanofiber. Previous research suggests that a higher proportion of the more hydrophilic component leads to more pronounced phase separation due to incompatibility with the hydrophobic component, and the phase separation in the polymer blend solutions during electrospinning leads to the formation of nanofibers with uneven and porous fibers (Asano et al., 2022). This suggests that concentration ratio of 70:30 (PCL:Gelatin) resulted in a more homogeneous polymer blend.

Nanofibers with diameters ranging from 100-450 nm were said to mimic the size of collagen fibrils in natural bone ECM which therefore, provide a biomimetic structure that supports cell attachment and growth (Anjum et al., 2022). Since PCL/Gelatin (70:30) nanofiber exhibits average diameter of 312.93 ± 134.48 nm, which falls within the range of collagen fibrils in natural bone ECM, it indicates that mix concentration ratio of 70:30 is the best formulation of PCL/Gelatin nanofiber. By examining the morphologies of both PCL/Gelatin nanofibers, PCL/Gelatin (70:30) nanofiber was selected for the next phase of the experiment, which involves the surface modification with β-TCP, due to its smaller and more uniform diameters.

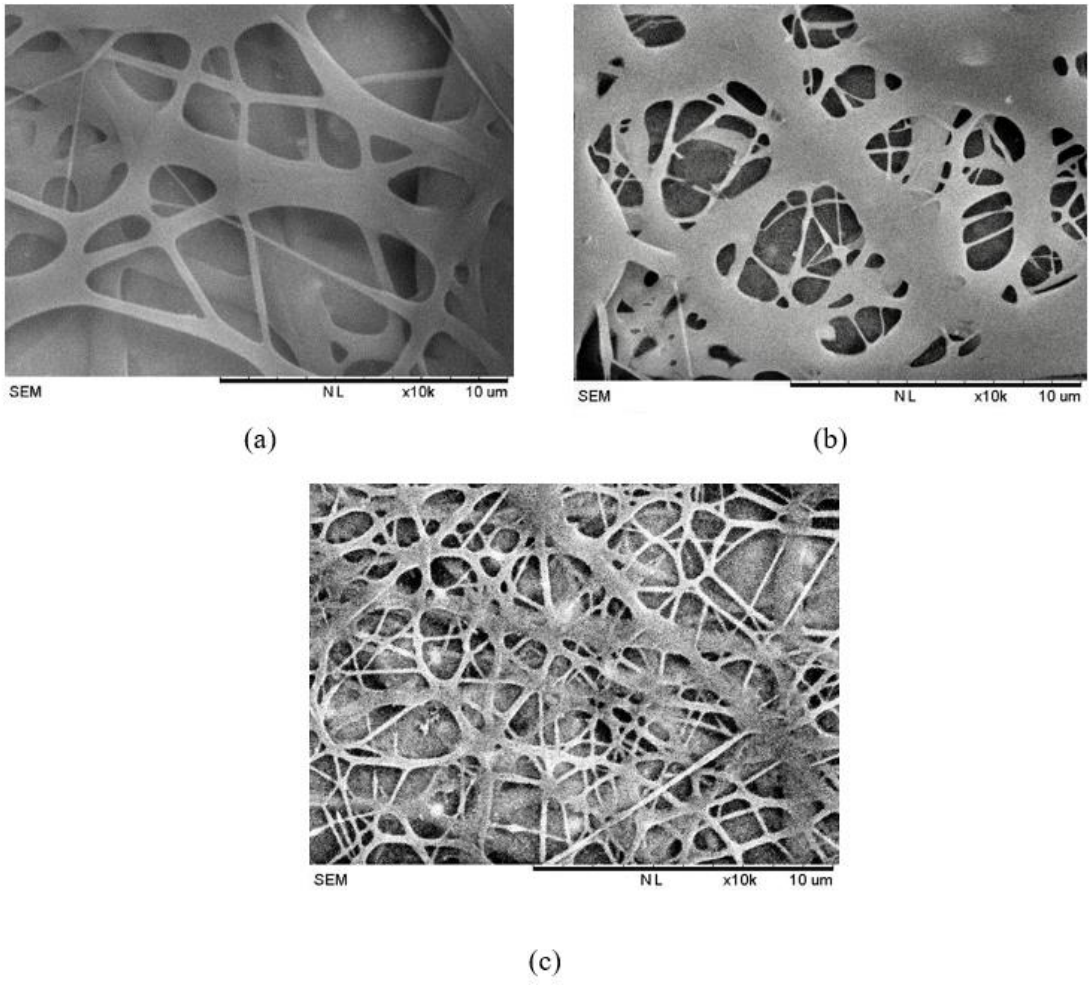

Fig 1 SEM image of nanofibers for (a) PCL, (b) PCL/Gelatin (60:40) (c) PCL/Gelatin (70:30)

Table 1 Nanofibers diameter

Nanofibers	Average diameter (nm)
PCL	1168.55 ± 451.71
PCL/Gelatin (60:40)	974.93 ± 581.23
PCL/Gelatin (70:30)	312.93 ± 134.48

Surface Modification of PCL/Gelatin nanofibers with β-TCP

Surface modification of PCL/Gelatin (70:30) nanofibers has been done by using β-TCP. As shown in **Error! Reference source not found.**2, the morphology of PCL/Gelatin/β-TCP nanofiber exhibited rough fiber morphology. This roughness was due to the β-TCP particles deposited on the coated scaffold surface, which aligned with previous studies indicating that nanofibers exhibited rough morphology after surface modification by nHAp (Gautam et al., 2021). This rough morphology can be beneficial for bone regeneration as previous study has shown that micro- and nano-rough surfaces on nanofiber scaffolds positively influence osteogenesis (Dolgin et al., 2023). This is because surface roughness increases surface area and polarity, potentially creating additional sites for cell growth and enhancing cell adhesion (Liu et al., 2021). Moreover, the rough surface mimics the natural ECM of bone, providing a scaffold that facilitates better interaction and attachment of osteogenic cells.

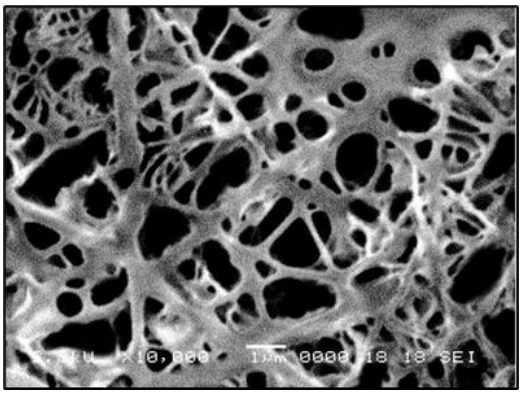

Fig 2 SEM image of PCL/Gelatin/β-TCP nanofibers at 70:30 (PCL: Gelatin) ratio

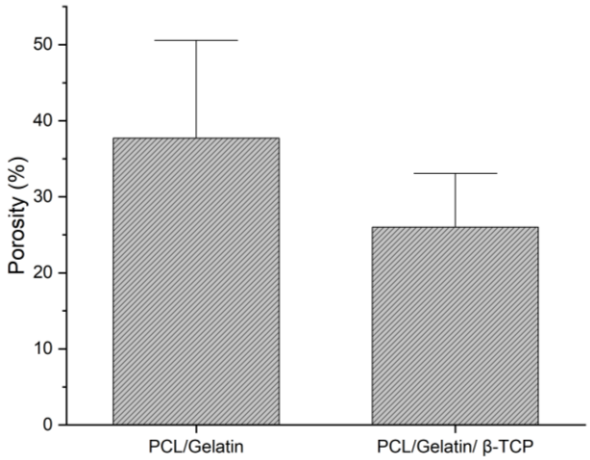

Fig 3 Porosity of PCL/Gelatin and PCL/Gelatin/β-TCP nanofiber at 70:30 (PCL: Gelatin) ratio.

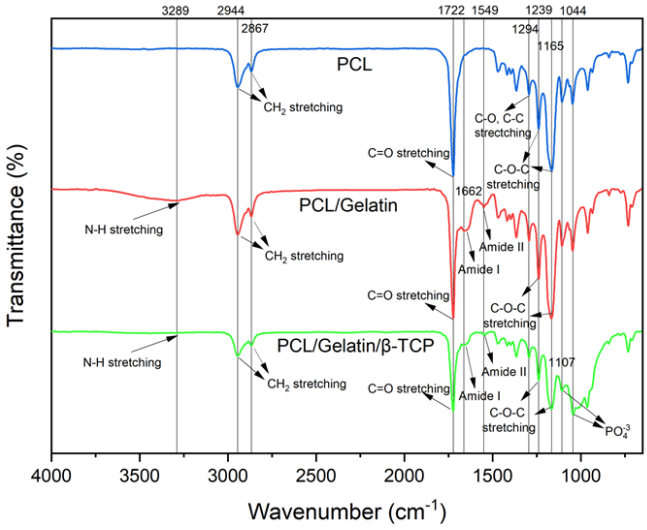

Fig 4 SEM image of PCL/Gelatin/β-TCP nanofibers at 70:30 (PCL: Gelatin) ratio

In addition, the PCL/Gelatin/β-TCP nanofiber exhibited an average diameter of 395.07±99.83 nm, which slightly larger than average diameter of PCL/Gelatin nanofiber at 312.93±134.48 nm. This increase in diameter is primarily due to the additional layer of β-TCP particles deposited on the fiber surface. Consequently, the PCL/Gelatin/β-TCP nanofiber's average porosity had decreased to 26% as shown in **Error! Reference source not found.**3.

Error! Reference source not found.4 presents the FTIR spectra of PCL, PCL/Gelatin and PCL/Gelatin/β-TCP electrospun nanofibers. The PCL spectrum exhibited characteristic bands at 2944 cm-1, 2867 cm-1, 1722 cm-1, 1294 cm-1, 1239 cm-1, and 1165 cm-1, corresponding to the asymmetric and symmetric stretching of CH2, carbonyl (C=O) stretching, C-O and C-C stretching, and asymmetric and symmetric C-O-C stretching, respectively (Jafari et al., 2020). For the PCL/Gelatin nanofibers, new absorbance peaks emerged at 3289 cm-1, 1662 cm-1, and 1549 cm-1, corresponding to OH and N-H stretching of amide bonds, C=O stretching (amide I), and N-H bending (amide II), respectively. These new peaks indicated the presence of amide groups from gelatin, confirming its successful incorporation into the PCL matrix. The FTIR spectrum of PCL/Gelatin/β-TCP displayed new characteristic bands at 1107 cm-1 and 1044 cm-1, corresponding to phosphate (PO43-) functional group of β-TCP (Gautam et.al, 2021). Besides these additional characteristic bands, the PCL/Gelatin/β-TCP nanofibers also exhibited all the characteristic bands of PCL and Gelatin at lower wavenumbers (Gautam et al., 2023).

Water contact angle (WCA) measurements were conducted to PCL, PCL/Gelatin and PCL/Gelatin/β-TCP nanofibers to assess the hydrophilicity of the nanofibers. **Error! Reference source not found.** illustrates the WCA data presented as mean ± standard deviation with n = 3. PCL nanofiber exhibited the highest average WCA measurement which is 104.87°±4.83°, reflecting its hydrophobic nature. In contrast, the average WCA measurement decreased to 89.95°±8.43° for PCL/Gelatin

nanofiber indicating a reduction in hydrophobicity due to the hydrophilic nature of gelatin incorporated into the structure.

Moreover, PCL/Gelatin/β-TCP resulted in the lowest WCA measurement among the nanofibers. According to research conducted by Lee et al. (2023), β-TCP demonstrated complete wetting confirming its hydrophilic properties. The deposition of β-TCP particles on the nanofibers reduced the average WCA measurement of PCL/Gelatin/β-TCP to 56.17°±2.87°. According to previous research, enhanced surface hydrophilicity promotes the adsorption of cell-adhesive proteins, which in turn enhances integrin-mediated cell-surface interactions and improves cell adhesion (Sutthiwanjampa et al., 2023). The improved cell adhesion can consequently enhance bone regeneration by promoting the osteoblast attachment and proliferation by promoting effective binding of cell adhesion molecules such as integrins and cadherins (Khalili & Ahmad, 2015).

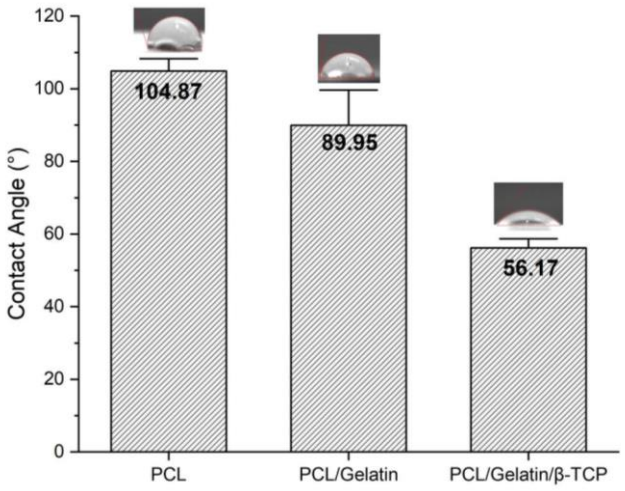

Fig 5 Contact angle measurement for PCL, PCL/Gelatin and PCL/Gelatin/β-TCP. Data shown as mean ± standard deviation with n = 3.

CONCLUSION

In conclusion, the findings from the SEM analysis of PCL/Gelatin nanofibers provide significant insights into their morphological characteristics and assist in finding the optimal mix volume ration of PCL/Gelatin nanofibers. The study shows that the best formulation mixture of PCL/Gelatin is 70:30 as it has more uniform nanofiber and its small diameter mimics the natural ECM of bone making it biocompatible and provide consistent structural support for bone regeneration. Furthermore, after surface modification with β-TCP, the PCL/Gelatin/β-TCP nanofibers exhibited a rough morphology, increased diameter, and reduced water contact angle, all of which are beneficial for bone regeneration. The rough surface, due to β-TCP particle deposition, enhances surface area and create additional sites for cell growth, improving cell adhesion. The increased hydrophilicity, as shown by the reduced WCA, further supports improved cell adhesion, which is crucial for effective bone regeneration. Overall, these findings highlight that the potential of PCL/Gelatin/β-TCP nanofibers as a promising scaffold material for BTE, offering a biomimetic structure that supports cell attachment, growth, and osteogenesis.

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