ORIGINAL ARTICLE

Modification of Polycaprolactone/Chitosan Nanofibers with B-tricalcium Phosphate in Improving Bone Scaffold Properties

Sharifah Raihanah Kamaruddin[1], Syakirah Athirah Abdulla Hamid[1], Adlisa Abdul Samad[1], Norhana Jusoh[1,2,3]

[1] Department of Biomedical Engineering and Health Sciences, Faculty of Electrical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
[2] Medical Device Technology Center (MEDiTEC), Institute Human Centred Engineering (iHumEn), Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
[3] Bionspired Device and Tissue Engineering Research Group, Department of Biomedical Engineering and Health Sciences, Universiti Teknologi Malysia, Faculty of Electrical Engineering 81310 UTM Johor Bahru, Johor, Malaysia

ABSTRACT

Introduction: Investigation on electrospun nanofiber for bone tissue engineering have been extensively conducted with the main focus is towards mimicking bone extracellular matrix (ECM) that have osteoconductive properties to restore injured or diseased bones. Incorporating calcium phosphates enhances the bioactivity of these nanofibers, but there has been limited exploration of β-tricalcium phosphate (β-TCP) compared to hydroxyapatite (HA). Therefore, this study investigated the surface modification of polycaprolactone (PCL)/chitosan electrospun nanofiber with β-TCP. **Materials and Methods**: Initially, PCL (10 wt.%)/Chitosan (1 wt.%) nanofibers were fabricated at different PCL-to-chitosan volume ratios (60:40, 70:30 and 80:20). Then, PCL/Chitosan nanofiber with 70:30 ratio was selected for the surface modification by immersing the nanofibers in β-TCP (1wt.%) solution, as this ratio produced the finest nanofibers, most closely resembling the bone ECM. All nanofibers underwent characterization analysis using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and water contact angle (WCA) measurements. **Results:** Based on the SEM results, PCL/Chitosan nanofibers with a 70:30 ratio exhibited a more interconnected and porous structure compared to pure PCL, with the addition of β-TCP further enhancing this porous network morphology. Wettability results demonstrated increased hydrophilicity from pure PCL (110.6°) to PCL/Chitosan (84.4°) and PCL/Chitosan/β-TCP (64.9°). The successful incorporation of chitosan and β-TCP into the PCL matrix, as evidenced by characteristic peaks of FTIR spectra for C-H, N-H, C=O, C-O-C, and PO_4^{3-} groups. **Conclusion:** These findings suggest that the PCL/Chitosan/β-TCP nanofiber scaffold holds significant potential in replicating native bone properties for tissue engineering applications.
Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP11):155-161. doi:10.47836/mjmhs.21.s11.22

Keywords: Polycaprolactone, Chitosan, β-tricalcium Phosphate, Electrospun Nanofibers, Bone Scaffold

Corresponding Author:
Norhana Jusoh, PhD
Email:norhana@utm.my
Tel: +60122535206

INTRODUCTION

Globally, bone disorders and conditions are on the rise and are expected to double by 2020, particularly in aging populations and those with low physical activity levels. Engineered bone tissue has been considered an alternative to conventional bone grafts due to their limitless supply and lack of disease transmission [1]. Although bones have the ability to heal and regenerate, bone healing cannot be achieved on its own in cases of large segmental bone defects caused by factors such as old age, traffic accidents, non-union fractures, bone tumor resections and others [2]. These problems can adversely affect the patient's health and quality of life. The goal of bone tissue engineering (BTE) is to induce new functional bone regeneration by combining biomaterials, cells, and factors in a synergistic manner [1].

Bone tissue engineering (BTE) provides a novel therapeutic strategy for the restoration of injured bone structures. It serves as an alternative to conventional tissue transplant techniques, including autografts

and allografts, BTE addresses challenges related to limited donor availability, inadequate supply, and immune rejection [3]. By integrating scaffolds, cells, and mechanical or soluble factors, BTE provides a sustainable, long-term solution for bone regeneration [2]. A key challenge in BTE is the development of bioartificial bone implants scaffolds that replicate the extracellular matrix (ECM) and possess osteoconductive properties to restore injured or diseased bones. One critical characteristic of bone scaffolds is their porosity, which facilitates the integration of osteogenic cells and tissue formation. High porosity, interconnected pore networks, and a large surface area have been shown to significantly enhance tissue in-growth. Nanofibrous scaffolds are particularly promising in BTE due to their favorable properties for bone regeneration [3]. Among fabrication techniques, electrospinning is widely employed for creating nanofiber scaffolds. This method mimics the structure of collagen fibrils and provides an increased surface area to support cell attachment [4].

A scaffold's primary function is to provide a balance between short-term mechanical support and efficient mass transport, enabling biological delivery and tissue repair. Scaffolds serve as temporary extracellular matrices, promoting cell proliferation, differentiation, and biosynthesis. Scaffolds positioned at the sites of regeneration also help prevent invasion of disrupting cells. For bone reconstruction to be achieved, scaffolds must meet a number of specific criteria [2]. The materials used for nanofibers fabrication can be natural or synthetic. Synthetic biodegradable polymers such as PCL has good mechanical properties and is widely used in BTE as the primary material for electrospun nanofibers [5,6,7]. As compared to other materials, PCL has a slow degradation rate. Additionally, natural polymers such as chitosan can be used in BTE because of their biocompatibility, biodegradability, and ability to enhance cell adhesion and proliferation. However, it has some limitations, such as low mechanical strength and potential adverse reactions [8]. Therefore, by combining PCL and Chitosan in nanofibers, the scaffold properties can be enhanced and the limitations of each material could be overcome [6,9-12], surface modifications of nanofibers can enhance their properties and effectiveness for BTE and other uses. Alongside hydrolysis and aminolysis, techniques such as plasma treatment and physical adsorption have been employed to alter the surface of nanofibers [13]. β-tricalcium phosphate (β-TCP) is a biocompatible and osteoconductive material and has the potential to be used in BTE as a surface modification material for nanofibers. As a result, it can support the growth of new bone tissue and facilitate bone regeneration [8].

Human health is at risk when bones are damaged by trauma or tumours, and therefore the development of three-dimensional (3D) scaffolding materials that promote and stimulate the regeneration of broken bone tissues has become a major research focus. In this regard, a preferential combination of materials and preparation techniques is considered crucial for the preparation of advanced BTE scaffolds to better facilitate broken bone regeneration [14]. One primary challenge lies in ensuring biocompatibility and bioactivity of engineered scaffold materials. These materials must support cell adhesion, proliferation, and differentiation while promoting bone tissue formation. Replicating native bone mechanical properties presents another significant hurdle, as the engineered scaffold needs to possess sufficient strength, stiffness, and toughness to withstand physiological loads.

In this research, PCL and chitosan are explored as polymers for the fabrication of nanofiber scaffold via electrospinning method. Nanofibrous polymer scaffolds, particularly those made from polycaprolactone (PCL) and chitosan, have shown considerable potential in the field of tissue engineering. However, their limited bioactivity and mechanical properties hinder their optimal performance in supporting cellular activities and promoting tissue regeneration. To address these challenges, the incorporation of β-TCP as a surface modification emerges as a potential solution [9,15]. Enhancing the bioactivity and osteoconductivity of scaffolds for BTE by modifying their surfaces with calcium phosphates is one of the key strategies for enhancing the bioactivity of nanofibers. While hydroxyapatite (HA) has been widely investigated for surface modification, the limited exploration of β-TCP in nanofiber surface modification creates a substantial gap in understanding its specific impact on the structural, mechanical, and biological aspects of the nanofibers. In the existing literature, HA is predominantly discussed, leaving a significant knowledge gap regarding the distinct effects of β-TCP on nanofiber scaffolds. Therefore, this research aims to address this critical gap by systematically investigating the effects of β-TCP as the exclusive coating agent for nanofiber surface modification. By elucidating the unique contributions of β-TCP in promoting bioactivity and optimizing mechanical properties, this study aims to provide valuable insights that will inform the selection and design of advanced biomimetic scaffolds, thereby advancing the field of BTE.

MATERIALS AND METHODS

Materials

PCL pellets (Mw = 80,000 kDa), Chitosan powder (Mw = 80,000 kDa). Chloroform and methanol. Glacial acetic acid at a concentration of 2% (w/w) and synthetic ≥98% β-tri-Calcium phosphate (sintered Powder) was obtained from Sigma Aldrich [16].

Preparation of PCL/Chitosan solution

A 10 wt% PCL solution was prepared by stirring in a chloroform/methanol solvent mixture (3:1, v/v) at 600 rpm for 2 hours [17]. Simultaneously, a 1 wt% chitosan

solution was prepared in 2% (v/v) acetic acid, stirred at 500 rpm for 2 hours [18]. Both solutions were prepared at room temperature (26 ± 1 °C). Subsequently, the PCL and chitosan solutions were combined in volume ratios of 100:0, 80:20, 70:30, and 60:40 (PCL/Chitosan) and incubated for 72 hours [16]. This process ensured uniform dispersion of chitosan within the PCL solution, resulting in an immiscible PCL/Chitosan polymer blend, which was later utilized for electrospinning.

Electrospinning

The PCL/Chitosan polymer blend solution was loaded into a 3 mL plastic syringe equipped with a needle tip of 0.56 mm in diameter for the electrospinning process [19]. The solution was delivered at a flow rate of 0.1 mL/h via a syringe pump. A voltage of 22 kV was applied to the tip of the needle, with a separation of 10 cm maintained between the needle and the collector. Nanofibers were deposited onto a flat aluminum plate. The process was carried out at room temperature (25 ± 1 °C) with a controlled humidity of 45%. The electrospinning process lasted for 3 to 4 hours [19]. Afterward, the sample was cut into 1 cm × 1 cm squares for further characterization, including SEM, FTIR, and water contact angle measurements.

Surface Modification of Nanofiber

The fabricated PCL/Chitosan nanofiber was then subjected to a surface modification process with β-TCP. The β-TCP solution was prepared by dissolving the β-TCP sintered powder (1wt%) in double distilled water at room temperature and stirring at 500 rpm for 5 min. Then, dip the PCL/Chitosan nanofiber inside β-TCP solution for 20 minutes and stir it moderately using a magnetic stirrer [16]. The coated nanofibers produced (PCL/CS/β-TCP) were then washed with distilled water to remove the loosely bound β-TCP and dried overnight inside a dessicator [20].

SEM Analysis

The nanofiber samples were prepared by cutting each sample into 0.5 × 0.5 cm^2 and the samples were coated with platinum using a sputter coater. Sputter coating is important step before conducting SEM to minimize the electric charging of the samples and to achieve the highest image quality possible. The sample morphology was examined using a scanning electron microscope (SEM, Hitachi TM3000, Japan) operated at an accelerating voltage of 15 kV, with magnifications set to 1000x, 3000x, and 5000x [21].

FTIR Analysis

Before FTIR, the nanofibers were cut into a small piece with a dimension of 5x5 cm^2. Next, FTIR was conducted using FTIR spectroscopy (Frontier Spectrometer, PerkinElemer, UK), throughout a wavenumber range of 4000 to 650 cm^{-1}.

Water Contact Angle

The contact angle of the nanofiber scaffold was measured using the sessile drop method with the help of contact angle analyzer (AST-VCAOptima, AST product inc., USA). The samples were prepared with dimensions of 1x1 cm^2. A small drop of water where the volume to dispense was set to 2.0 was deposited to the sample. When the water droplet fell onto the sample, a high-resolution camera captured a back-lit image of the droplet on the surface in profile. The captured image was then analyzed by VCAOptima software to measure the contact angle. In this study 3 readings were taken for each sample and the average WCA measurement was calculated.

RESULTS

The SEM images of electrospun nanofibers in Fig. 1 reveals distinct characteristics across different formulations. The SEM analysis reveals notable differences in the average fiber diameter of PCL and PCL/Chitosan nanofibers at various mixing ratios. Upon coating the PCL/Chitosan (70:30) nanofibers with β-TCP, significant changes in

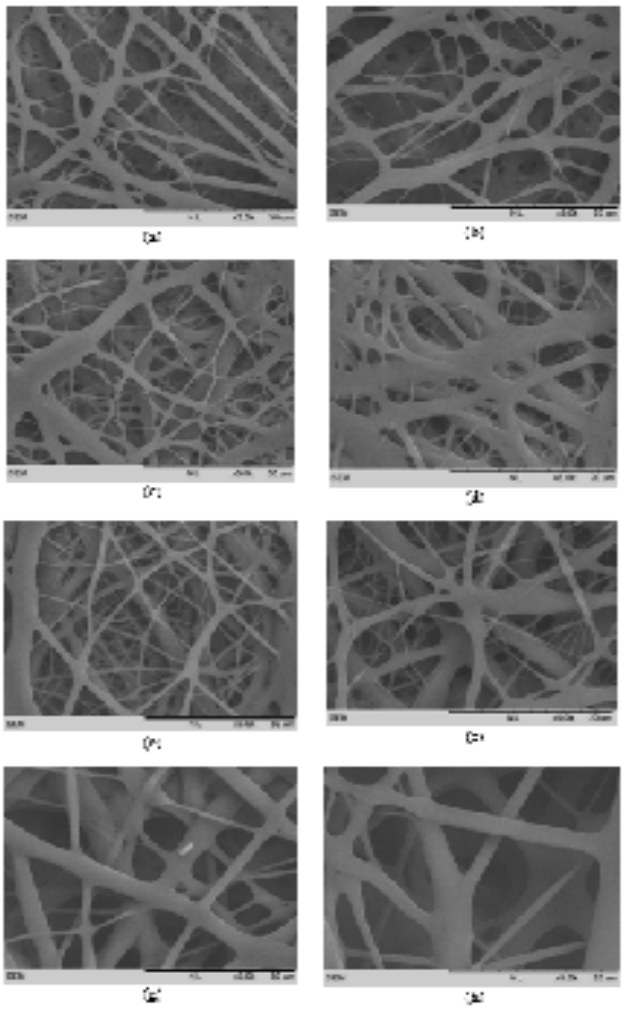
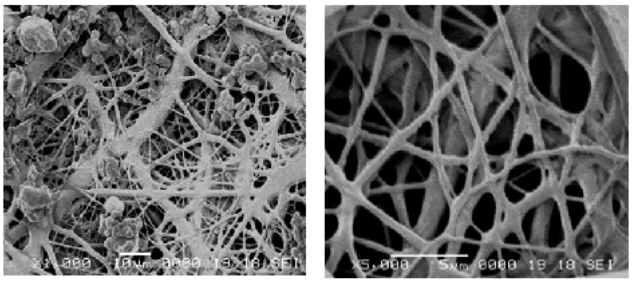


Fig. 1 SEM images of (a-b) PCL, (c-d) PCL/Chitosan (80:20), (e-f) PCL/Chitosan (70:30) and (g-h) PCL/Chitosan (60:40) electrospun nanofibers at x3k and x5k magnification respectively.

Table I: Average diameter of electrospun nanofibers.

Nanofibers	Average Diameter (nm)
PCL	1618.44 ± 614.02
PCL/Chitosan (80:20)	1061.10 ± 369.83
PCL/Chitosan (70:30)	704.47 ± 198.56
PCL/Chitosan (60:40)	1155.77 ± 411.53
PCL/Chitosan/β-TCP	803.93 ± 310.50



(a) (b)

Fig. 2 SEM images of PCL/Chitosan (70:30)/β-TCP electrospun nanofiber at (a) x1k and (b) x5k magnification.

surface morphology was observed as shown in Fig. 2. Using ImageJ software, 100 measurements of the fiber's diameter from each sample image were taken to find the average diameter of the nanofiber as shown in Table 1. Pure PCL nanofibers exhibited the largest average diameter of 1618.44 nm. Introducing chitosan into the PCL matrix resulted in a significant reduction in average diameter. The PCL/Chitosan (80:20) sample had an average diameter of 1061.1 nm, while the PCL/Chitosan (70:30) sample had the smallest diameter at 704.47 nm, indicating the formation of finer fibers. Conversely, the PCL/Chitosan (60:40) sample showed an increased average diameter of 1155.77 nm, although still smaller than the pure PCL fibers.

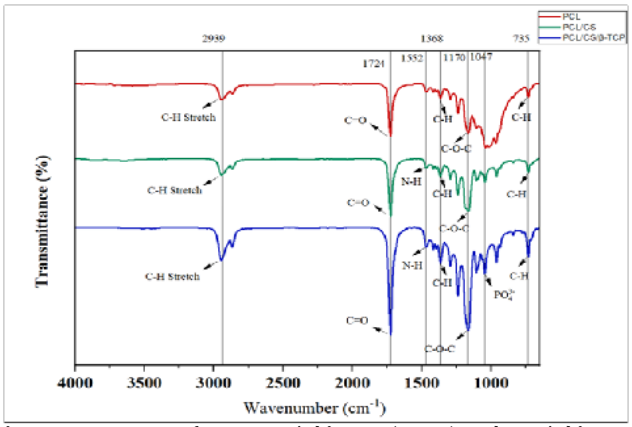


Fig. 3 FTIR spectra of PCL, PCL/Chitosan (70:30) and PCL/Chitosan (70:30)/β-TCP electrospun nanofibers.

Fig. 3 shows the FTIR spectra of PCL, PCL/Chitosan, and PCL/Chitosan/β-TCP electrospun nanofibers show distinct peaks indicating their chemical structures. Pure PCL exhibits peaks at 2939 cm^{-1} (C-H stretch), 1724 cm^{-1} (C=O stretch), 1368 cm^{-1} (C-H bend), 1170 cm^{-1} (C-O-C stretch), and 1047 $cm^{-º}$ (C-O-C stretch).

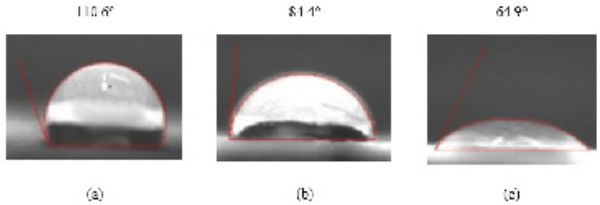


Fig. 4 Water contact angle for (a) PCL, (b) PCL/Chitosan (70:30) and (c) PCL/Chitosan (70:30)/βTCP electrospun nanofibers.

The PCL/Chitosan blend retains these peaks and adds peaks at 1552 cm^{-1} (N-H bend) and broadens some peaks due to interactions between PCL and chitosan. The WCA measurements for the PCL, PCL/Chitosan, and PCL/Chitosan/β-TCP nanofibers showed significant differences in their surface wettability. Fig. 4 below shows the average measurement of water contact angle for PCL, PCL/Chitosan and PCL/Chitosan/βTCP.

The pure PCL nanofibers exhibit a contact angle of 110.6°, which indicates a relatively hydrophobic surface. Whilst, further modification of the PCL/Chitosan nanofibers with β-TCP results in an even lower contact angle of 64.9°.

DISCUSSION

Pure PCL nanofibers exhibit a highly porous, interconnected network with smooth surfaces and some variations in fiber diameter. When chitosan is added at different ratios (80:20, 70:30, and 60:40), the nanofibers maintain their porous structure, but the addition of chitosan introduces slight variations in fiber (70:30) formulation stands out with a highly interconnected, porous network, smooth fiber surfaces, and minimal irregularities, ensuring a robust and cohesive structure, which is essential for applications requiring high porosity and structural integrity. The analysis concluded that the 70:30 PCL/Chitosan blend ratio produced the finest nanofibers, with an average diameter of 704.47 nm, which falls within the desirable nanoscale range for mimicking the extracellular matrix (ECM) of bone tissues which is typically between 10 and 1000 nm [10,22]. Previously, the optimal 70:30 PCL/Chitosan formulation have been reported as the ideal nanofibers formulation for various applications [23-25].

Following the determination of the optimal 70:30 PCL/Chitosan formulation, the nanofibers were coated with β-TCP to enhance their properties. Upon coating the PCL/Chitosan (70:30) nanofibers with β-TCP, significant changes in surface morphology were observed. The coated nanofibers display a rougher surface texture, increased fiber diameter, and a non-uniform distribution of β-TCP particles, which enhance surface roughness. Despite these modifications, the fibrous network remains interconnected and porous, with distinct junctions and intersections enhancing the overall porosity of the nanofiber mat. The coating material adheres well to the fibers, although the distribution is uneven, leading

to variations in surface texture. This enhanced surface characteristic suggests the coated nanofibers are suitable for bone scaffold applications, combining structural integrity with improved surface properties. Furthermore, the enhanced surface roughness can also lead to a greater surface area and increased polarity, which may create more sites for cell growth and improve cell adhesion [26].

The β-TCP coating process resulted in a slight increase in the average diameter to 803.93 nm, indicating the addition of a layer to the nanofibers while maintaining a relatively fine structure compared to pure PCL and other chitosan ratios. The increase in diameter was due to the deposition of β-TCP particles onto the nanofibers surface during the surface modification process. To support the finding, porosity of both coated and uncoated PCL/Chitosan nanofibers were evaluated by using ImageJ software. The average porosity for 4 of the regions of interest (ROI) from the SEM image were calculated. The analysis revealed that the uncoated PCL/Chitosan nanofibers had an average porosity of 22.63%. In contrast, the PCL/Chitosan nanofibers coated with β-TCP exhibited a significantly lower porosity of 18.39%. The incorporation of β-TCP particles into the PCL/Chitosan nanofiber during the surface modification process can result in the deposition of these particles both on the surface and within the pores of the nanofibers. This deposition can lead to the partial or complete filling of the pores, thereby reducing the overall porosity [27]. Additionally, the interaction between the PCL/Chitosan polymer matrix and the β-TCP particles may cause the nanofibers to swell. This swelling effect can further reduce the pore size, ultimately resulting in a lower porosity percentage.

FTIR spectra of PCL, PCL/Chitosan, and PCL/Chitosan/β-TCP electrospun nanofibers show distinct peaks indicating their chemical structures. The PCL/Chitosan/β-TCP composite shows all these peaks plus additional peaks at 1047 cm^{-1} and 735 cm^{-1} indicating the presence of phosphate groups from β-TCP, confirming the effective incorporation of β-TCP within the nanofiber framework. Similar phosphate peaks near 1040cm^{-1} and 560–610 cm^{-1} have been previously reported as characteristic of β-TCP in composite scaffolds [28-30].

The WCA measurements for the PCL, PCL/Chitosan, and PCL/Chitosan/β-TCP nanofibers showed significant differences in their surface wettability. The pure PCL nanofibers exhibit a contact angle of 110.6°, which indicates a relatively hydrophobic surface. This high contact angle suggests that water droplets do not spread easily on the surface, reflecting the inherent hydrophobic characteristics of PCL [31]. When chitosan was added to the PCL matrix, the contact angle decreased significantly to 84.4°. This reduction in contact angle indicates an increase in hydrophilicity, which can be attributed to the presence of chitosan. Chitosan is known for its hydrophilic properties due to the presence of amino and hydroxyl groups that enhance water interaction. Consequently, the PCL/Chitosan blend displays improved wettability compared to pure PCL [32].

Further modification of the PCL/Chitosan nanofibers with β-TCP results in an even lower contact angle of 64.9°. This substantial decrease suggests that the β-TCP coating enhances the surface hydrophilicity of the nanofibers. The presence of β-TCP, a bioceramic known for its hydrophilic nature, likely contributes to this improved wettability. The lower contact angle indicates that water droplets spread more easily on the surface of the PCL/Chitosan/β-TCP nanofiber, making them more hydrophilic than both the uncoated PCL and PCL/Chitosan nanofibers. The enhanced surface hydrophilicity of PCL/Chitosan/β-TCP nanofiber can consequently promote the adsorption of cell-adhesive proteins, improving integrin-mediated cell-surface interactions and cell adhesion, which in turn boosts bone regeneration by facilitating osteoblast attachment and proliferation [9].

These findings proved a successful integration of β-TCP into PCL/Chitosan nanofiber and due to its improved structural properties. Furthermore, previous integration of β-TCP with various type of PCL polymer scaffolds have shown promising bioactivity properties such as cytocompatibility, osteoinductivity, histocompatibility, cell differentiation and mineralization [9,15,33]. Therefore, PCL/Chitosan/β-TCP nanofibers represent a promising candidate for bone scaffold applications.

CONCLUSION

In conclusion, this study found that the best formulation of fabricating PCL/Chitosan nanofiber is 70:30 as the SEM observation shown that the PCL/Chitosan (70:30) nanofiber exhibited smallest diameter at 704.47 nm which are closely mimicked the bone ECM compared with other mix ratio. Furthermore, the surface modification of the PCL/Chitosan (70:30) with β-TCP further enhancing the morphology of the nanofiber. SEM analysis showed the modified PCL/Chitosan nanofiber with β-TCP had enhanced the nanofiber's roughness morphology which lead to a greater surface area and consequently create more sites for cell growth. In addition, the decreasing of WCA measurement of the modified PCL/Chitosan nanofiber to 64.9° indicated an improved wettability which give advantage to enhance the nanofiber's capacity to support cell growth by enhancing protein adsorption. Lastly, FTIR analysis confirm the successful incorporation of chitosan and β-TCP into the PCL matrix, indicated by characteristic peaks for C-H, N-H, C=O, C-O-C, and PO_4^{3-} groups. These findings indicated that the PCL/Chitosan nanofiber had been successfully modified by β-TCP and highlighted that the potential of surface modified PCL/Chitosan nanofiber with β-TCP as a promising scaffold material for BTE, offering a

biomimetic structure that supports cell attachment, growth, and osteogenesis.

ACKNOWLEDGEMENTS

This work was supported by RUG-UTM Matching Grant (Q.J130000.3023.04M47) from Universiti Teknologi Malaysia, Malaysia.

REFERENCE

1. Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng. 2012;40(5):363-408. doi: 10.1615/critrevbiomedeng.v40.i5.10
2. Alonzo M, Alvarez Primo F, Anil Kumar S, Mudloff, JA, Dominguez E, Fregoso G, et al. Bone tissue engineering techniques, advances, and scaffolds for treatment of bone defects. Current Opinion in Biomedical Engineering. 2021;17:100248. doi. org/10.1016/j.cobme.2020.100248
3. Udomluck N, Koh WG, Lim DJ, Park H. Recent developments in nanofiber fabrication and modification for bone tissue engineering. International Journal of Molecular Sciences. 2019;21(1); 99. doi.org/10.3390/ijms21010099
4. Li Z, Wang C. Effects of working parameters on electrospinning. SpringerBriefs in Materials, 2013;15–28. doi.org/10.1007/978-3-642-36427-3_2
5. Ashrafi F, Emami A, Navaei-Nigjeh M, Izadi E, Salehi S, Seyhoun, I. Regeneration of bone tissue using nanofibers made from electrospun polycaprolactone (PCL) and a hydrogel composed of alginate (Alg/PCL). Regenerative Engineering and Translational Medicine. 2025;1-10. doi. org/10.1007/s40883-025-00392-2
6. Cao Z, Zhuo H, Zhu W, Peng X, Li J. Rational construction of PCL-PEG/CS/AST nanofiber for bone repair and regeneration. Frontiers in Bioengineering and Biotechnology. 2025;12:515043. doi. org/10.3389/fbioe.2024.1515043
7. Gedik B, Erdem MA. Electrospun PCL membranes for localized drug delivery and bone regeneration. BMC Biotechnology. 2025; 25(1), 31. doi. org/10.1186/s12896-025-00965-7
8. Wei S, Ma JX, Xu L, Gu XS, Ma XL. Biodegradable materials for bone defect repair. Military Medical Research. 2020;7:54. doi.org/10.1186/s40779-020-00280-6
9. Ezati M, Safavipour H, Houshman B., Faghihi S. Development of a PCL/gelatin/chitosan/β-TCP electrospun composite for guided bone regeneration. Progress in Biomaterials. 2018;7(3):225–237. doi.org/10.1007/s40204-018-0098-x
10. Roozbahani F, Sultana N., Fauzi Ismail A, Nouparvar H. Effects of chitosan alkali pretreatment on the preparation of electrospun PCL/chitosan blend nanofibrous scaffolds for tissue engineering application. Journal of Nanomaterials, 2013;(1):641502. doi.org/10.1155/2013/641502
11. Xu W, Gao X, Zhang M, Jiang Z, Xu X, Huang L, et al. Electrospun polycaprolactone-chitosan nanofibers on a zinc mesh as biodegradable guided bone-regeneration membranes with enhanced mechanical, antibacterial, and osteogenic properties for alveolar bone-repair applications. Acta Biomaterialia. 2024;187:434-450. doi. org/10.1016/j.actbio.2024.08.033
12. Chen G, Xu T, Gao R, Liu W, Li W, Zeng D, et al. Poly-ε-caprolactone/chitosan/whitlockite electrospun bionic membrane conjugated with an E7 peptide for bone regeneration. Stem Cell Research & Therapy. 2025;16(1):212. doi. org/10.1186/s13287-025-04307-4
13. Yaseri R, Fadaie M, Mirzaei E, Samadian H, Ebrahiminezhad A. Surface modification of polycaprolactone nanofibers through hydrolysis and aminolysis: a comparative study on structural characteristics, mechanical properties, and cellular performance. Scientific Reports. 2023;13(1):9434. //doi.org/10.1038/s41598-023-36563-w
14. Xu C, Liu Z, Chen X, Gao Y, Wang W., Zhuang X et al. Bone tissue engineering scaffold materials: Fundamentals, advances, and challenges. Chinese Chemical Letters. 2024;35(2):109197. doi. org/10.1016/j.cclet.2023.109197
15. Zheng C, Zhang M. 3D-printed PCL/β-TCP/CS composite artificial bone and histocompatibility study. Journal of Orthopaedic Surgery and Research. 2023; 18(1):981. doi.org/10.1186/s13018-023-04489-8
16. Gautam S, Chou CF, Dinda AK, Potdar PD, Mishra NC. Fabrication and characterization of PCL/gelatin/chitosan ternary nanofibrous composite scaffold for tissue engineering applications. Journal of Materials Science. 2013; 49(3):1076–1089. doi. org/10.1007/s10853-013-7785-8
17. Bikuna-Izagirre M, Aldazabal J, Paredes J. Gelatin Blends Enhance Performance of Electrospun Polymeric Scaffolds in Comparison to Coating Protocols. Polymers. 2022;14(7):1311. doi. org/10.3390/polym14071311
18. Zhan Y, Hong Y, Wang Y. Sequential release of vancomycin and BMP-2 from chitosan/nano-hydroxyapatite thermosensitive hydrogel for the treatment of chronic osteomyelitis. Journal of Orthopaedic Surgery and Research. 2024;19(602):1-10. https://doi.org/10.1186/s13018-024-05097-w
19. Ganesh SS, Anushikaa R, Swetha Victoria VS, Lavanya K, Shanmugavadivu A, Selvamurugan N. Recent advancements in electrospun chitin and chitosan nanofibers for bone tissue engineering applications. Journal of Functional Biomaterials. 2023;14(5):288. doi.org/10.3390/jfb14050288
20. Afrash H, Nazeri N, Davoudi P, FaridiMajidi R,

Ghanbari H. Development of a bioactive scaffold based on NGF containing PCL/chitosan nanofibers for nerve regeneration. Biointerface Research in Applied Chemistry. 2021;11(5):12606-12617. doi.org/10.33263/BRIAC115.1260612617
21. SciMed. 2023. A Brief Introduction to SEM (Scanning Electron Microscopy) | SciMed. SciMed. https://www.scimed.co.uk/education/sem-scanning-electron-microscopy/
22. Zhang S, Zhang M, Bai R, Kong L, Yang H, Zhang A, et al. Electrospun coaxial nanofibers loading with perovskite and icariin to enhance the bone scaffold-mediated osteogenesis. Materials Today Chemistry. 2022;26:101246. doi.org/10.1016/j.mtchem.2022.101246
23. Saleh HM, Albukhaty S, Sulaiman GM, Abomughaid MM. Design, preparation, and characterization of polycaprolactone–chitosan nanofibers via electrospinning techniques for efficient methylene blue removal from aqueous solutions. Journal of Composites Science. 2024;8(2):68. doi.org/10.3390/jcs8020068
24. Semnani D, Naghashzargar E, Hadjianfar M, Dehghan Manshadi F, Mohammadi S, Karbasi S, et al. Evaluation of PCL/chitosan electrospun nanofibers for liver tissue engineering. International Journal of Polymeric Materials and Polymeric Biomaterials. 2017;66(3):149-157. doi.org/10.1080/00914037.2016.1190931
25. Mahoney C, Conklin D, Waterman J, Sankar J, Bhattarai N. Electrospun nanofibers of poly (ε-caprolactone)/depolymerized chitosan for respiratory tissue engineering applications. Journal of Biomaterials Science, Polymer Edition. 2016:27(7):611-625. doi.org/10.1080/09205063.2016.1144454
26. Sencadas V, Correia DM, Areias A, Botelho G, Fonseca, AM, Neves IC. Determination of the parameters affecting electrospun chitosan fiber size distribution and morphology. Carbohydrate Polymers. 2012;87(2):1295–1301. doi.org/10.1016/j.carbpol.2011.09.017
27. Kahdim QS, Abdelmoula N, Al-Karagoly H, Albukhaty S, Al-Saaidi J. Fabrication of a Polycaprolactone/Chitosan Nanofibrous Scaffold Loaded with Nigella sativa Extract for Biomedical Applications. BioTech. 2023;12(1):19. doi.org/10.3390/biotech12010019
28. Al-Qahtani AS, Tulbah HI, Binhasan M, Shabib S, Al-Aali KA, Alhamdan, MM. Influence of Concentration Levels of β-Tricalcium Phosphate on the Physical Properties of a Dental Adhesive. Nanomaterials. 2022;12(5):853. doi.org/10.3390/nano12050853
29. Choy CS, Lee WF, Lin PY, Wu YF, Huang HM, Teng NC. Surface Modified β-Tricalcium phosphate enhanced stem cell osteogenic differentiation in vitro and bone regeneration in vivo. Scientific Reports. 2021;11(1):9234. doi.org/10.1038/s41598-021-88402-5
30. Ruiz-Aguilar C, Alcбntara-Quintana LE, Aguilar-Reyes EA, Olivares-Pinto U. Fabrication, characterization, and in vitro evaluation of β-TCP/ZrO2-phosphate-based bioactive glass scaffolds for bone repair. Boletнn de La Sociedad Espacola de Cerбmica y Vidrio. 2022;61(3):191–202. doi.org/10.1016/j.bsecv.2020.09.004
31. Kao HH, Kuo CY, Tagadur Govindaraju D, Chen KS, Chen JP. Polycaprolactone/Chitosan Composite Nanofiber Membrane as a Preferred Scaffold for the Culture of Mesothelial Cells and the Repair of Damaged Mesothelium. International Journal of Molecular Sciences. 2022;23(17):9517. doi.org/10.3390/ijms23179517
32. Sutthiwanjampa C, Hong S, Kim WJ, Kang SH, Park H. Hydrophilic modification strategies to enhance the surface biocompatibility of poly(dimethylsiloxane)-based biomaterials for medical applications. Advanced Materials Interfaces. 2023;10(12): 2202333(1-18). doi.org/10.1002/admi.202202333
33. Javkhlan Z, Hsu SH, Chen RS, Chen MH. 3D-printed polycaprolactone scaffolds coated with beta tricalcium phosphate for bone regeneration. Journal of the Formosan Medical Association. 2024;123(1):71-77. doi: 10.1016/j.jfma.2023.08.009