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Fabrication of polycaprolactone/β-tricalciumphosphate based nano scaffolds using electrospinning method for biomedical applications

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In this study, we have focused on preparation, fabrication and characterization of polycaprolactone and beta tricalcium phosphate (PCL/β-TCP) composite scaffolds that are used for bone tissue engineering applications. The electrospun composites have been characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and field emission scanning electron microscopy (FESEM). The developed scaffolds are effectively simulated the morphology, mechanical property and bioactivity for load-bearing tissue engineering applications. The bioactivity of the scaffolds has been evaluated with *in vitro* cell adhesion and growth studies. The results confirm the nontoxic behaviour of the composite biomaterials and developed scaffolds with MG-63 osteoblast-like cell line. The synthesized scaffolds have shown promising bioactivity with the growth as well as proliferation of new bone cells with considerable osteoconductive properties. The nanoscaffolds possess better physical properties and support high cell adhesion suggesting their application in bone tissue engineering field.

Keywords: Bone tissue engineering, Electrospinning, PCL/β-TCP hybrid nanoscaffolds, Nanocomposites, Osteoconductive

Nano/micro dimensional scaffolds are star trove for biomedical applications as they significantly influence cellular actions in tissue regeneration. Scaffolds must have specific features including extraordinary porosity, relevant pore size roughly (300-400 μm) for the osteons or bone spicules to grow into the scaffold and shape, sponginess or 3D dimensional pore interconnectivity related to be relevant in bone tissue regeneration. Besides, the viable mechanical properties for biomedical applications they should be biodegradable in the course of bone tissue growth and biocompatible material in point of cell colonization. Based on the studies related to scaffolds, the hydrophilic property enclosed by the 3D scaffold is an essential parameter to succeed homogeneous cell uniformity and fluid diffusion. In the material composition, polycaprolactone (PCL) and beta tricalcium phosphate (β-TCP) are two widely used and medically graded materials utilized for orthopedic applications individually[1-3]. The biocompatibility, durable biodegradability, FDA approval brands the synthetic polymer, PCL castoff in biodegradable implants[4,5]. PCL is a commonly used base material for preparing scaffold used in bone restructuring[6-8] and for proper administration of drugs and treatment of burn injuries[9-12]. In recent years, biodegradable bioceramics has received an increasing attention for bone tissue engineering applications. β-TCP is a calcium phosphate derivative with biodegradable properties that involves 60 and 70% of natural bone, thus presenting an inherent biomimetic potential in encouraging new bone growth, thus reducing patient recovery time due to the generation of natural bone tissue[13]. Bio ceramics including β-TCP is one of the promising biomedical constituents for bone tissue regeneration, for the reason that it exhibits good bioactivity, in comparison with synthetic biopolymers. It possesses bone healing properties and shows osteo-inductive properties in 3D pore structures. Conversely bio ceramics possess unique properties, such as low mechanical fracture and brittleness.

The grouping of these two materials bids the unique properties and boons great interest for regenerative medicine, and many studies have been accompanied on this particular composite for medical

devices, implants and constructs for orthopedic applications[14,15]. Electrospinning helps widely in preparation of blended scaffolds using polymer-bioceramic mixtures. The alteration of spinning parameters help us to vary the porosity, nanodimensions and length of the fibers. The use of different solvents, variation of applied voltage and electrode distance also affects the properties including the growth of new cells on the surface of the scaffolds[16-20]. A few reports are available on the use of combination of PCL/β-TCP for design and fabrication of multimaterial biomimetic implant for potential disc replacement[21]. The bilayer polycaprolactone membrane for guided bone regeneration was fabricated by combining the electrospinning and emulsion templating methods[22].

The present work is focused on fabricating hybrid PCL/β-TCP based nanofibrous scaffolds for tissue engineering applications. Firstly, we synthesized electrospun materials by varying β-TCP concentrations in the PCL matrix. The preparation of PCL nanofibrous scaffolds was carried out using PCL (10%) with dual solvents chloroform and dimethylformamide. The prepared hybrid nanoscaffolds were subjected to various characterization and biocompatibility assessments.

Materials and Methods

Materials

PCL with the average molecular weight of 80,000 (80 kDa) was obtained from Sigma Aldrich. Analytical grade (AR) chloroform and dimethyl formamide (DMF) from Merck was used as solvent for the fabrication of PCL. Synthesis of nano β-TCP powder was carried out by wet chemical precipitation methods. The chemicals used for nano β-TCP synthesis are calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$, Alfa Aesar), Ammonium dihydrogen phosphate ($NH_4.H_2PO_4$, Alfa Aesar) and cetyl trimethyl ammonium bromide (CTAB, Alfa Aesar).

Nano- fibrous scaffold fabrication by electrospinning

The electrospinning process was carried out using ESPIN Nano (Physics equipment, Chennai, India), equipment at room temperature was dissolved into equal parts of chloroform and DMF at a concentration of 10% (wt./vol). For composite scaffolds, the PCL solution was mixed with 0.1 wt. % and 0.5 wt. % of β-TCP. The precursor solutions were loaded in a syringe (2.0 mL) fitted with a metal needle (diameter 0.2 mm). The needle was connected to the power supply which generated 20kV and the obtained fibers were collected on aluminium foil kept at a distance of 10 cm. An infrared radiation (IR) heater was used to assist the Nano fiber drying process. The flow rate was fixed at 0.5 mL per hour with a syringe pump. The collected fibers were placed in a vacuum dryer to remove a trace amount of residual moisture and organic solvents. The same procedure was followed for other combination.

Nanoscaffold characterization

The characterization of pure and hybrid nanoscaffolds was performed by measuring the diameter and observing the morphology of the scaffolds using a field emission scanning electron microscope (Model Carl Zeiss Supra 55). FTIR spectra were obtained using Attenuated total reflectance-Fourier transform infrared (ATR-FTIR, Perkin Elmer, Spectrum II) spectrophotometer. Tensile test for each scaffold strip (0.69, 0.65, 0.60mm) was carried out on an Instron 3342 using a 10 N loading cell.

Cell viability and Proliferation

MG63 (P10) cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) supplemented with antibiotics. They were seeded at a density of 10^5 cells per scaffold with 100 µL media per well to immerse the scaffolds in a 96 well-plate. For 0, 24 and 48 h, the MTT measurements were recorded. Cells without scaffold served as the positive control and the media alone served as the blank control. Each time the experiment was executed using three scaffolds. The electrospun nanofibrous scaffolds of PCL/β-TCP exhibit outstanding physicochemical properties such as high surface area, high porosity and resistance against microorganism. The increase in the formation of nanofibers increases the surface area, which leads to better cell proliferation in the scaffolds.

Osteogenic differentiation

MG-63 (P10) cells were cultured on scaffolds in growth media and after two days of seeding growth media was replaced with osteogenic differentiation media, which was supplemented with 10 nM dexamethasone, 20 mM β-glycerophosphate and 50 µM L-ascorbic acid. Every alternate day the media was changed and the induction was carried out for 21 days, then the scaffolds were fixed with absolute ethanol and stained with Alizarin red for 1 h. Then the quantification of uptake was observed by leaching the stain and the absorbance

was measured at 450 nm in a spectrophotometer which was zeroed to plain leaching solution.

Results and Discussion

FTIR

The IR spectra of the PCL/β-TCP nano composite are shown in Fig. 1. In PCL, the main absorbance band at 1723 cm^{-1} corresponds to carbonyl stretching while the bands at 1294, 1240 and 1162 cm^{-1} corresponds to the stretching vibrations of the C-O-C groups[23]. In all spectra, except for the PCL and PCL/β-TCP, the bands at 557 and 615 cm^{-1} correspond to the absorption bands related to PO_4^{3-} which is an indication of incorporation of the β-TCP in the polymeric fibers. β-TCP was well-embedded in the PCL nano scaffold which confirmed the presence of β-TCP loading into the PCL matrix.

X-ray diffraction (XRD)

The nano composites were characterized with XRD and are presented in Fig. 2. It can be seen from the diffractogram that PCL/β-TCP showed two strong peaks located at 2θ value of 21.5° and 23.9°, which were respectively associated with the (110) and (200) reflections of PCL[24]. The characteristic peaks of the β-TCP particles in the PCL scaffold were observed at 2θ =25.7°, 27.7°, 31.14°, 34.26° and these correspond to the (1010), (214), (0210), (220) reflection intensities of β-TCP in the composite and were shown to increase qualitatively with an increase in the content of the weight percent of β-TCP. The observed diffraction patterns were matched with standard JCPDS (File no. 09-1069) file. These results indicate the formation of PCL bioceramic composite.

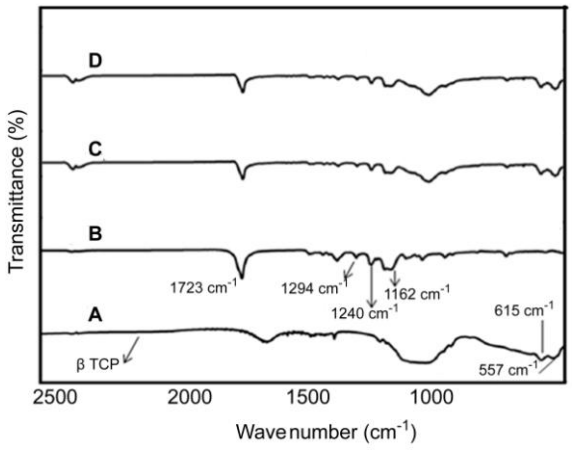

Fig. 1 — FTIR spectra obtained for (A) β-TCP, (B) PCL, (C) PCL/β-TCP (0.1 wt.%) and (D) β-TCP (0.5 wt. %) scaffolds

The electron microscope images of PCL/β-TCP are shown in Fig. (3-5). The surface morphology and stoichiometry of the β-TCP/PCL nano fibrous scaffold indicates that the fabricated scaffolds exhibited a well interconnected structure and uniformly distributed fibrous structure. It can be understood from the Fig. 3A that the fibrous scaffold fabricated was in the nano scale which is free from agglomeration in the matrix. It also shows that the

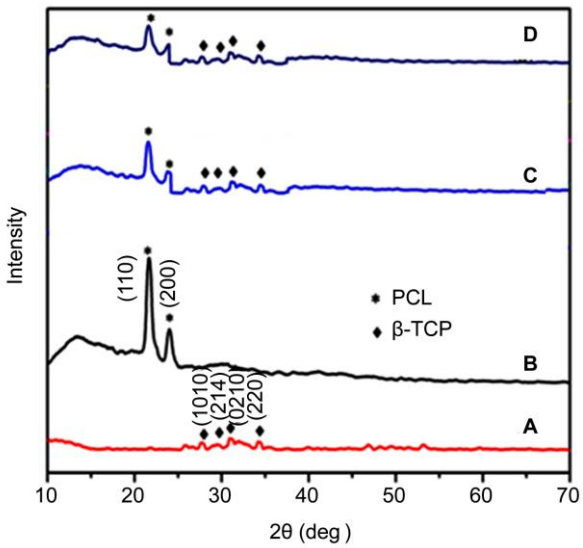

Fig. 2 — XRD patterns of (A) β-TCP (B) PCL, (C) PCL/β-TCP (0.1 wt.%), and (D) PCL/β-TCP (0.5 wt.%)

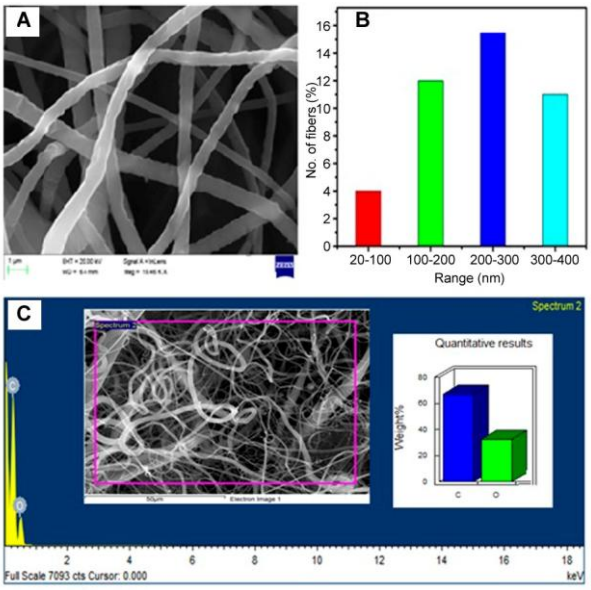

Fig. 3 — (A) FESEM images of PCL scaffold, (B) Histogram of corresponding fiber distribution and (C) EDAX spectra of scaffolds

electrospun PCL is available with beadless structure. Fig. 3B shows the size distribution histogram of the nano fibers from 100-500 nm with maximum in the range of 300-400 nm. Fig. 3C shows the elemental analysis of PCL with 67 wt.% of carbon and 33 wt.% of oxygen, which confirms the purity of PCL.

Fig. 4A represents the incorporation of β-TCP at the minimum percentage of (0.1 wt.%) as mentioned in the preparation. The size distribution histogram as shown in Fig. 4B and the stoichiometric EDAX spectrum shown in Fig. 4C elucidated the compositional change in the PCL incorporated with β-TCP.

Furthermore, the increase in β-TCP concentration to 0.5 wt. % and above in the PCL matrix, increases the fiber diameter with more bead formation. This could be due to addition of β-TCP at 0.5 wt.% in PCL which lowers the viscosity of the polymer and entanglements between polymer chains which drastically increased the net charge density[25] and become unfit for the stable jet flow which is shown in the Fig. 5 A-C.

The EDAX spectrum of PCL/β-TCP with varied concentrations of β-TCP confirms the gradual increase in atomic percentage for each proportion of 0.1 to 0.5 wt.% β-TCP. From these investigations it is finally concluded that the β-TCP in to the PCL scaffold matrix did not affect the actual properties of the scaffold. It can be concluded that β-TCP concentration at 0.1 wt.% by weight showed uniform smooth formation of fiber with desired properties and smaller size distribution.

Mechanical properties of electrospun β-TCP Scaffolds

Analysis of the mechanical performance of composite fibrous scaffolds is required to understand their stability and strength. The polymer PCL having lower tensile strength when compared with the β-TCP composites. In the electrospun of PCL/β-TCP composites existing here, it has been found that mechanical properties were drastically pretentious by incorporation of β-TCP. The tensile modulus of composites containing 0.1 wt.% of β-TCP and 10% PCL is high when compared to the other scaffold PCL/β-TCP (0.5 wt.%) this behaviour is due to the development of an interfacial adhesion between the polymer and the ceramic particles. The Young's modulus value of 10% PCL/β-TCP (0.5 wt.%) scaffolds showed a similar trend resulting in a decreased value with increase in the filler content, the behaviour being notable for electrospun composites containing high filler amount. It is also known that

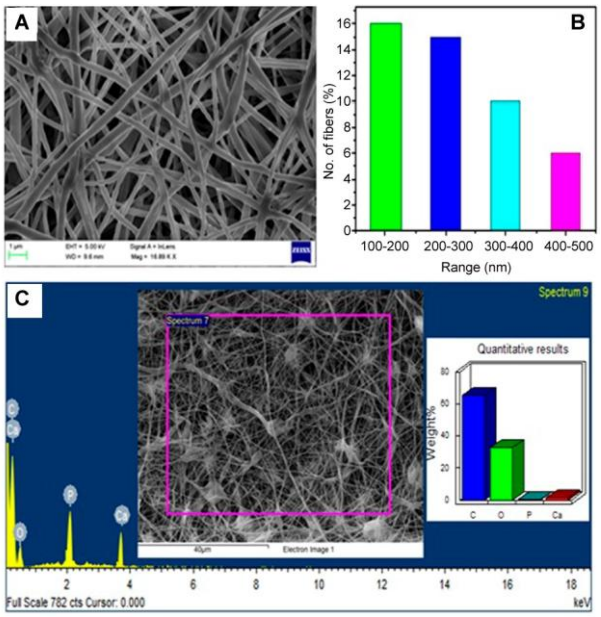

Fig. 4 — (A) FESEM images of PCL/β-TCP (0.1 wt.%) scaffold, (B) Histogram of corresponding fiber distribution, and (C) EDAX spectra of scaffolds

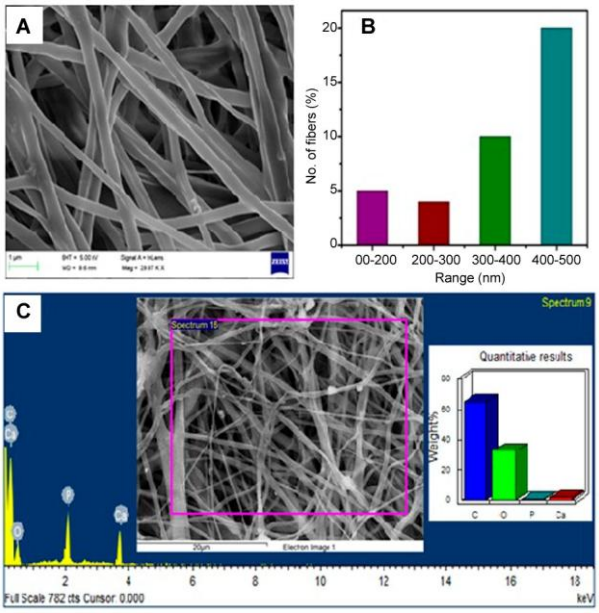

Fig. 5 — (A) FESEM images of PCL/β-TCP (0.5 wt.%) scaffold, (B) Histogram of corresponding fiber distribution and (C) EDAX spectra of scaffolds

mechanical properties of hybrid samples depend on the nature of the polymer/ceramic interphase as well as on the efficiency of the polymeric matrix to transfer the stress to the reinforcing phase in the presence of increasing amounts of micrometric β-TCP agglomerates. The bar diagram showing the tensile strength of PCL and PCL/β-TCP (0.1 and 0.5 wt.%) is represented in Fig. 6.

MTT assay

The presence of β-TCP (0.1 and 0.5 wt.%) with the PCL reveals that the PCL/ β-TCP (0.1 wt.%) shows (Fig. 7) the higher proliferation adhesion and have more biocompatible nature than the PCL/ β-TCP (0.5 wt. %). This indicates the loaded β-TCP in PCL at (0.1 wt.%) shows high cell proliferation and it is highly suitable for use in tissue engineering applications[26].

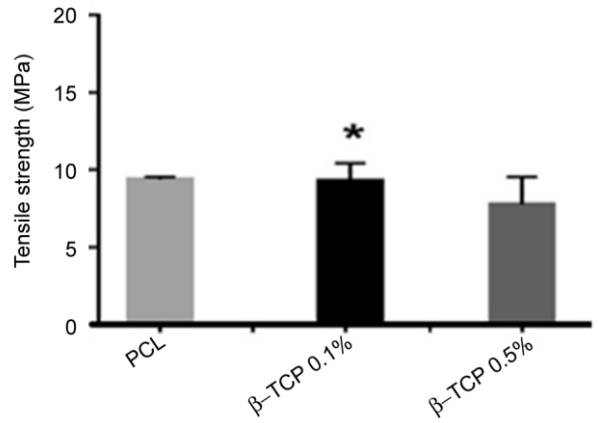

Fig. 6 — Tensile strength of PCL/β-TCP (0.1 wt.%) and PCL/β-TCP (0.5 wt.%) scaffolds

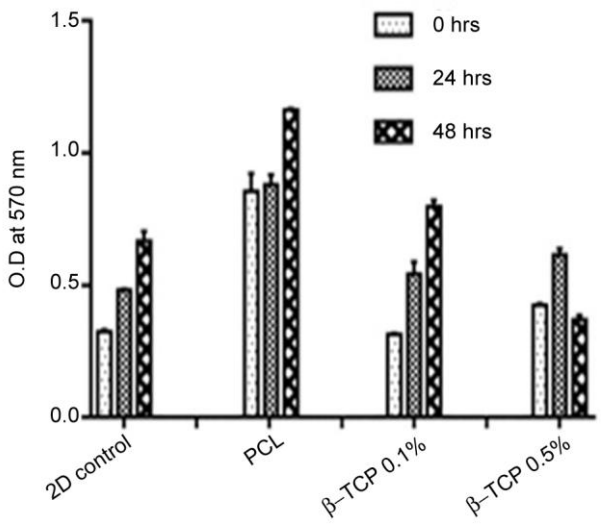

Fig. 7 — MTT assay obtained for pure PCL nano scaffolds and PCL/β-TCP based nano scaffolds

Osteogenic differentiation

The alizarin red stain binds to the mineralization and calcification deposits secreted by the cells and confirms the osteogenic nature of the cells induced. The intensity of stain correlates to the level of osteogenic differentiation of the cells that has happened on the scaffolds. In these PCL and PCL/β-TCP (0.1 and 0.5 wt.%) scaffolds, PCL/β-TCP (0.1 wt.%) showed maximum transformation in differentiation of MG-63(P10) cells into osteocytes and it was confirmed by high levels of alizarin red stain uptake whereas the PCL and PCL/ β-TCP (0.5 wt.%) showed the least stain uptake as it is represented in the Fig. 8 (A-C) and the Osteogenic induction bar diagram is represented in the Fig. 9.

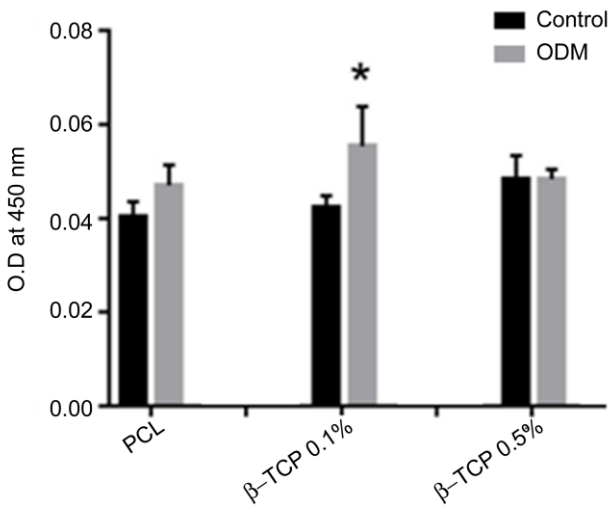

Fig. 9 — Osteogenic induction of PCL, PCL/β-TCP (0.1 wt.% and 0.5 wt.%) scaffolds

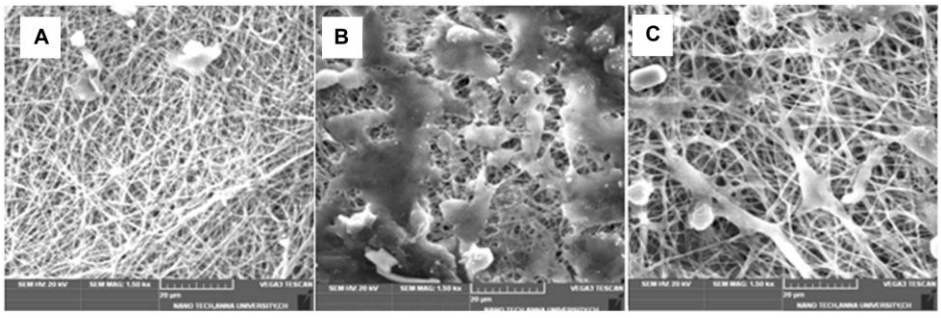

Fig. 8 — SEM micrograph of (A) PCL, (B) PCL/β-TCP (0.1 wt.%) and (C) PCL/β-TCP (0.5 wt.%) scaffolds cultured with MG-63 (P10) cell line

Conclusions

Here, we fabricated PCL nano fibers with different concentrations of β-TCP loaded PCL by electrospinning method. The β-TCP was distributed homogeneously inside PCL nano fibers. The results indicate the optimized conditions for electrospinning as PCL/β-TCP (0.1 wt.%) concentration. Further, increasing β-TCP concentration (0.5 wt.%) on PCL, increased fiber diameter with bead formation. The tensile strength was higher for PCL/ β-TCP (0.1 wt.%) scaffolds. MTT assay revealed the cell proliferation of hybrid nanoscaffolds. These results suggest that the electrospun nanofiber PCL/ β-TCP (0.1 wt.%) fiber is potential candidate for drug delivery applications.

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Conflict of interest

The authors declare no conflict of interests in this study.

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