

## Original Article

# Characterisation and *In Vitro* Biocompatibility of Polycaprolactone: *Cissus quadrangularis* Electrospun Scaffold for Bone Regeneration

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The aim of the present study is to develop an osteogenic bone graft material with a herbal component. *Cissus quadrangularis*, a perennial climber widely used in traditional Indian is reported to possess bone fracture healing activity. Polycaprolactone (PCL) an FDA approved biocompatible polymer and *Cissus Quadrangularis* (CQ) were blend and electrospun to obtain a nanofibrous scaffold. The fiber morphology was characterised by scanning electron microscopy, FT-IR, XRD and TGA. MG63 osteoblast-like cells were used to evaluate the biocompatibility using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The *in vitro* experiment revealed that the PCL-CQ composite electrospun scaffold showed better MG63 cell adhesion and growth compared to the control PCL scaffold and thus could be a potential scaffold material for bone defect applications.

## Introduction

Bone defects are usually treated by autografts, allografts, xenografts and by synthetic biocompatible materials. Autografts are being the gold standard which needs additional surgery of the donor site and associated complications. Allografts and xenografts have the risk of immunogenicity. Synthetic bone substitute materials would overcome the need for secondary surgery and immune related problems. Polymeric bone substitute materials should have biocompatibility, sterilisability, adequate mechanical and physical properties. PCL is a FDA approved hydrophobic, semi crystalline synthetic polymer; its crystallinity tends to decrease with increasing molecular weight. The good solubility of PCL, its low melting point (59–64°C) and exceptional blend-compatibility has stimulated extensive research into its potential application for various biomedical applications in the biomedical field [1].

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world [2]. *Cissus quadrangularis* (CQ), a perennial climber widely

used in traditional medicinal systems of India has been reported to possess bone fracture healing, antibacterial, antifungal, antioxidant, anthelmintic, antihemorrhoidal and analgesic activities. It is commonly known as Veldt Grape or Devil's Backbone. The plant commonly known as "bone setter" and is referred to as *Asthisandhani* in Sanskrit and *Hodjod* in Hindi because of its ability to join bones. CQ belongs to the taxonomic group Magnoliopsida and family Vitaceae.

Phytochemical screening of CQ revealed numerous bioactive compounds such as alkaloids, resveratrol, piceatannol, pallidol, parthenocissin, quadrangularins, ascorbic acid, carotene, phytosterol substances, calcium, flavinoids, vitamins, enzymes, nicotinic acid, tyrosin, and triterpenoids [3]. It also contains a variety of terpenoid components, such as the balsam ketone, amyrin, taraxerol, acetyl taraxerol and friedelin ketone. The main chemical constituents are tetracyclic triterpenoids, onocer-7-ene-3 $\alpha$ , 21 beta-diol and onocer-7-ene-3 $\beta$ , 21 alpha-diol and two steroidal principles I and II, alpha-sitosterol and deltaamyrin. The presence of  $\beta$ -sitosterol,  $\delta$ -amyrin,  $\delta$ -amyrone, and flavanoids (quercetin) having different potential metabolic and physi-

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ological effects has also been reported [4]. The stem has revealed unique stilbene derivatives, which are termed *quadrangularins* A, B and C10. Other lipids and several phytosterols like heptadecyl octadecanoate, icosanyl icosanoate, 4-Hydroxy-2-methyltricos-2-en-22-one, 9-methyl-octadec-9-ene,  $\alpha$ -amyrin,  $\alpha$ -amyrone taraxeryl acetate, friedelan-3-one, taraxerol, P-sitosterol and isopentacosanoic acid are identified in this plant. *Cissus quadrangularis* is rich in vitamin C and beta-carotene in addition to calcium oxalate [5].

The petroleum ether extract of *Cissus quadrangularis* can stimulate the differentiation of mesenchymal stem cells into osteoblasts in a dose-dependent manner even in the absence of osteogenic conditioning media. This activity of the plant extract is increased further in the presence of osteogenic media. The plant extract also facilitated extracellular matrix mineralization, which was more pronounced in the presence of osteogenic media. Wnt signaling pathway has been shown to play a significant role in the control of osteoblastogenesis and bone formation [6-8].

Electrospinning technique play an important role in preparing a medical tissue scaffold, since the resulting fibre diameters are in the size range (submicron to nanometer) of the extracellular matrix (ECM) microstructures, particularly the higher-ordered collagen microfibrils [9]. The flexibility of the electrospun fibres, due to the very high aspect ratio (length/diameter), is also beneficial in allowing seeded cells to remodel their surroundings [10]. Thus, in the current study a composite nanofiber electrospun scaffold incorporating PCL and CQ was spun, characterized and evaluated for biocompatibility focusing for bone defect application.

## Materials and Methods

Polycaprolactone (PCL) medical grade was provided by Dr. Jean Claude Matasa, Ortho cycle, California, USA in beads form having a molecular weight of 80,000 g/mol. The various chemicals used are Chloroform (Himedia, India), Dimethylformamide DMF (Rankem, India), Dimethylsulfoxide DMSO (Sigma-Aldrich), Calcium nitrate tetrahydrate and diammonium hydrogen phosphate (Merck, India), Dulbecco's Modified Eagles Medium, fetal bovine serum and Trypsin/EDTA (Invitrogen, USA). CQ powder was purchased from an government approved siddha medicine pharmacy (Chennai, India)

## Fabrication of the PCL and PCL-CQ Scaffold

PCL-CQ scaffold was prepared by Electrospinning process (E-spin Nano, India). A typical procedure is as follows: neat PCL 12 wt% was dissolved in a solvent mixture of chloroform and DMF (9:1). In another similar sample, CQ 5wt% was added the mixture was kept in an ultrasonic bath for 30min to achieve a homogenous dispersion of CQ particles. Samples were loaded in a 2ml syringe with a needle inner diameter of 0.8mm and 3.5cm length for electrospinning. The parameters for the electrospinning were optimized as 13kV, needle tip-collector distance of 9cm, syringe pump at 1.5ml/hr, spinning chamber temperature of 26°C with 50% humidity. The collecting rotary drum was covered on clean aluminum foil operated at 1100 rpm and the nanofibres were collected randomly but uniformly. The collected mat were dried in vacuum for 12 hours to ensure removal of any residual solvent.

## Characterization of the nano-scaffolds

PCL and PCL-CQ scaffolds were characterized by SEM, FT-IR, XRD and TGA. The morphology of the scaffolds were analyzed by FEI Quanta FEG 200 - high resolution scanning electron microscope. The size distribution of the fibers was quantified using Image J software. FTIR spectroscopy was performed to identify the functional groups of the electrospun samples by ATR-FTIR using Perkin Elmer Spectrum RX1. The spectra were recorded from 4000 to 400  $\text{cm}^{-1}$  in transmission mode. The nanofibers were collected on glass slides during electrospinning which were then mounted onto Philips X'pert Pro X-ray diffractometer. Incident and transmitted beam angles were varied so as to scan the  $2\theta$  angles from  $0^\circ$  to  $90^\circ$  with scan speed  $2^\circ/\text{min}$  and step rate 0.02 per second in order to determine the crystal structure. The resulting diffraction pattern was recorded and analysed using X'pert High score plus software. Thermo-gravimetric analysis (TGA) was carried out to evaluate the degradation temperature of PCL. TGA samples were subjected to a temperature ramp from room temperature until  $700^\circ\text{C}$ , at  $20^\circ\text{C}/\text{min}$  under nitrogen flow (50mL/min).

Human osteoblast-like MG63 cells was obtained from National center for cell science (NCCS) -Pune, India and cultured in  $25\text{ cm}^2$  cell culture flask at  $37^\circ\text{C}$  in Dulbecco's Modified Eagles Medium supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin in a 5%  $\text{CO}_2$

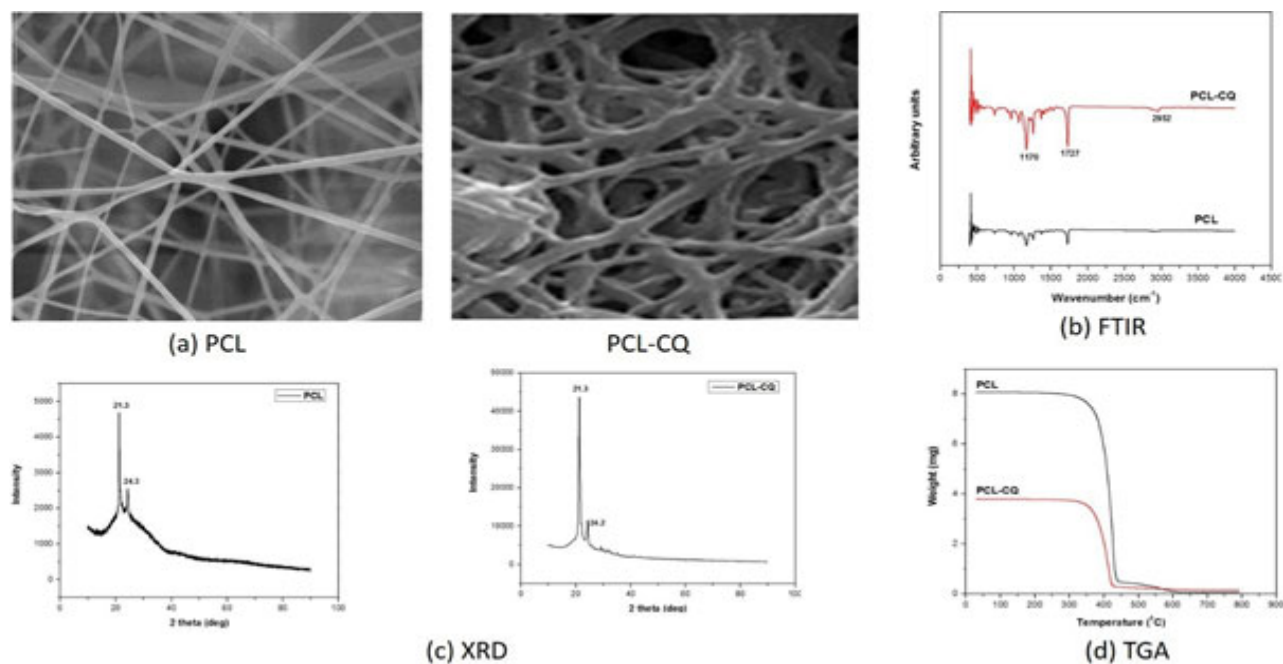


Figure 1: (a) SEM, (b) FTIR, (c) XRD and (d) TGA analysis of PCL and PCL-CQ electrospun scaffolds

incubator. When the cells attained confluency, they were detached using 0.25% Trypsin/EDTA and seeded into 48 well plates (Axygen, California) at a cell density of  $5 \times 10^3$  cells per well. The seeded cells were incubated overnight to allow cell adherence at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$  atmosphere and was further used for cell proliferation study with the electrospun scaffolds. The sterile scaffolds were cut into squares of  $5 \times 5$  mm. Triplicates of each scaffold type were placed in 24-well culture plates.

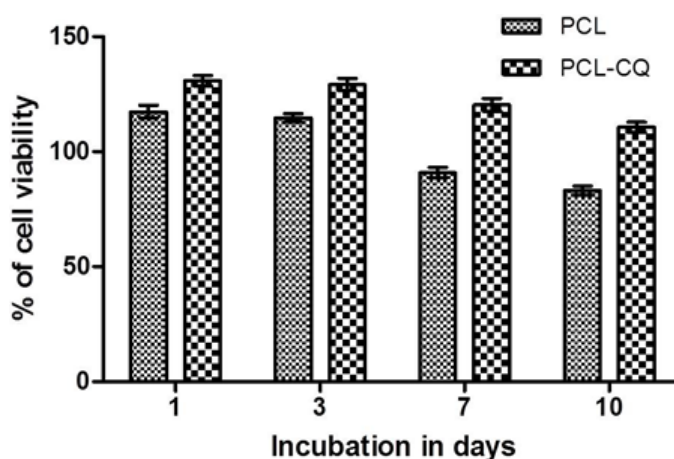
Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. At the end of the cell culture time periods of 1, 3, 7 and 10 days, 50  $\mu\text{L}$  of MTT reagent (5  $\mu\text{g}/\text{mL}$ ) was added to each well and incubated for 24 hr at  $37^\circ\text{C}$ . The water-insoluble formazan product was dissolved by adding 500  $\mu\text{L}$  of DMSO and absorbance was measured at 570 nm in an Elisa (Thermo Scientific) reader [11].

## Results and Discussion

PCL is a biodegradable polymer that has been intensively investigated as a biomedical material [14] and CQ is considered as a traditional bone healer and thus the composite was fabricated by electrospinning in the development of the scaffolds for bone regeneration. Scanning electron micrographs revealed the nanofibrous morphology of PCL and PCL-CQ scaffolds, randomly aligned, homogeneously distributed as shown in figure 1(a). The fibrous network was seen in multiple layers. It is observed that the fiber diameter distribution of PCL and PCL-CQ scaffolds were found to be on an average of  $122 \pm 26$  nm and  $218 \pm 56$  nm respectively. There is an increase in fiber thickness in the PCL-CQ scaffold which is due to the incorporation of CQ particles and also more of fibrous junctions were seen.

The FT-IR spectra of electrospun neat PCL and PCL-CQ nanofibers were shown in figure 1(b). PCL shows characteristic carbonyl ( $\text{C}=\text{O}$ ) peak at  $1720\text{ cm}^{-1}$ ,  $\text{CH}_2$  stretching at  $2950\text{ cm}^{-1}$  (asymmetric) and  $2865\text{ cm}^{-1}$  (symmetric),  $\text{C}-\text{O}$  stretching at  $1050\text{ cm}^{-1}$ , and  $\text{C}-\text{O}-\text{C}$  stretching at  $1240\text{ cm}^{-1}$  (asymmetric) [12,13]. The XRD spectrum shows crystalline peaks of PCL at about  $23.8$  and  $21.38$ , indicating that PCL is highly ordered chain folding characteristics and is seen in both scaffolds as in figure 1(c). The thermal properties of PCL was studied by TGA. Thermal analyses showed the melting point of both samples at  $60^\circ\text{C}$ , decomposition starting at  $350^\circ\text{C}$  and ending by  $450^\circ\text{C}$ . The PCL scaffold left no residues while PCL-CQ scaffold showed minimal residues as in figure 1(d). Thus, the thermal analysis revealed the stability of the PCL-CQ electrospun scaffold.

MTT assay was done for MG63 cells which were cultured for 1, 3, 7 and 10 days with PCL and PCL-CQ scaffolds. The assay showed more



**Figure 2:** MTT assay of MG-63 osteoblastic cells on PCL and PCL-CQ electrospun scaffolds

cells present in PCL-CQ scaffolds on all days and comparatively lesser cells in PCL mat. The amount of cell proliferation was maximum on day one and gradually reduced in other observed days. In all the observed days PCL-CQ scaffolds showed more than 100% viability of cells as seen in figure 2. The hydrophobicity of PCL, demonstrated by their limitedly wettable surfaces, allows for protein adsorption and cell adhesion [15]. The mixture of CQ particles with PCL also would have increased the wettability of the scaffold's surface as shown by more viability of MG63 cells compared to only PCL scaffolds.

In the emerging field of tissue engineering, biodegradable polymers are used for making polymer scaffolds to assist tissue and cell growth during the formation of artificial organs [16]. In such applications, biodegradable polymers allows for successful cell attachment, proliferation and functioning. The nano fibrous architecture created by electrospinning of the tested scaffolds will allow neo-vascularization and flow of nutrients for cell proliferation. In this study, PCL-CQ scaffolds supports well MG63 cells adhesion and growth, which shows that the developed scaffold can be beneficial for bone tissue engineering and for bone defect applications as a bone graft material.

## Conclusion

In our study, a PCL-CQ composite was electrospun with dispersion of CQ particles. In-Vitro biocompatibility tests with MG63 osteoblast-like cells showed the polymeric-herbal composite electrospun scaffold is nano-structured, highly osteogenic in nature compared to plain PCL scaffold. Therefore, we conclude that the novel PCL-CQ composite nano-scaffold is biocompatible, osteoconductive and osteoinductive, and could be a potential material for bone regeneration by grafting bone defects to expedite the healing process.

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