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Biomimetic hybrid nanofibrous substrates for mesenchymal stem cells differentiation into osteogenic cells

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ABSTRACT

Mimicking native extracellular matrix with electrospun porous bio-composite nanofibrous scaffolds has huge potential in bone tissue regeneration. The aim of this study is to fabricate porous poly(L-lactic acid)-co-poly-(ε-caprolactone)/silk fibroin/ascorbic acid/tetracycline hydrochloride (PLACL/SF/AA/TC) and nanohydroxyapatite (n-HA) was deposited by calcium-phosphate dipping method for bone tissue engineering (BTE). Fabricated nanofibrous scaffolds were characterized for fiber morphology, hydrophilicity, porosity, mechanical test and chemical properties by FT-IR and EDX analysis. The results showed that the fiber diameter and pore size of scaffolds observed around 228 ± 62–320 ± 22 nm and 1.5–6.9 μm respectively. Resulting nanofibrous scaffolds are highly porous (87–94%) with ultimate tensile strength observed in the range of 1.51–4.86 MPa and also showed better hydrophilic properties after addition of AA, TC and n-HA. Human mesenchymal stem cells (MSCs) cultured on these bio-composite nanofibrous scaffolds and stimulated to osteogenic differentiation in the presence of AA/TC/n-HA for BTE. The cell proliferation and biomaterial interactions were studied using MTS assay, SEM and CMFDA dye exclusion methods. Osteogenic differentiation of MSCs was proven by using alkaline phosphatase activity, mineralization and double immunofluorescence staining of both CD90 and osteocalcin. The observed results suggested that the fabricated PLACL/SF/AA/TC/n-HA biocomposite hybrid nanofibrous scaffolds have good potential for the differentiation of MSCs into osteogenesis for bone tissue engineering.

1. Introduction

Biomimetic nanofibrous scaffolds mimicking the important features of native extracellular matrix (ECM) offer a positive approach to repair functions or complete favorable reactions for tissue regeneration. The prevalence of many bone deficiencies particularly massive bone defects due to trauma, infections, cancer or genetic malformations, presents a major task for clinicians [1,2]. It has been revealed that implantation of large bone grafts without suitable vascularity normally results in apoptosis and cartilage development. Hence, angiogenesis and osteogenesis are integral for bone regeneration in massive bone defects. The tissue-engineered graft consisting of novel bioactive scaffolds with mesenchymal stem cells (MSCs) could be a potential bone graft for huge bone defects, due to its ability to improve angiogenesis and osteogenesis [3,4]. Biomaterial scaffolds play a critical role in BTE. Polymeric scaffolds garner attention due to their characteristic properties such as high surface-to-volume ratio, high porosity, biodegradation and tensile properties. Most of these material necessities are fulfilled by PLACL, which is a synthetic, biocompatible, biodegradable copolymer of PCL and PLLA [5]. It has been used as a substrate for culturing smooth muscle cells and endothelial cells [6,7]. Silk fibroin (SF) is a FDA approved naturally obtained protein polymer that has been used for centuries in the formation of clinical sutures [8,9]. SF biomaterials support cell proliferation, differentiation of primary cells and cell lines [10–12]. The stimulating cytocompatibility and flexibility of SF materials make silk a standard biomaterial for tissue engineering scaffolds used in the healing of several tissues including skin and bone [13–17]. However, PLACL alone is not sufficient for bone tissue regeneration as cell binding sites are absent and growth factor absorbing capacities have a limited range. In addition to SF, ascorbic acid (AA) specifically regulates cell differentiation of multilineage mesenchymal cells (adipogenesis, chondrogenesis and myogenesis). Takamizawa et al. reported increased cell proliferation and differentiation into osteoblasts and chondrocytes when using low calcium cell culture medium with N-acetyl-L-cysteine (NAC), and L-ascorbate-2 phosphate [18]. AA is a main supplement for the differentiation of osteoblast — like cells by encouraging Type I collagen formation and alkaline phosphatase activity [19]. Tetracycline (TC) is a broad-spectrum antibiotic with low toxicity. It shows anti-collagenase activity, inhibition of bone damage and the ability to stimulate cell attachment and connective tissue to the root surface [20]. The bone is an organic–inorganic mixture of collagen and hydroxyapatite.

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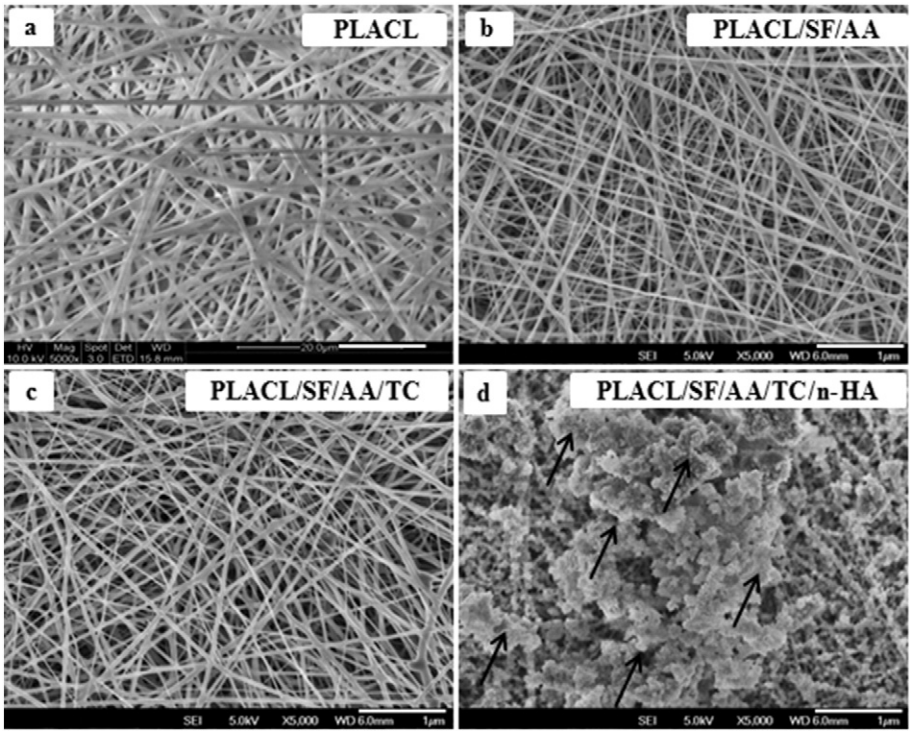

Fig. 1. FESEM images of the electrospun nanofibers. a) PLACL b) PLACL/SF/AA, c) PLACL/SF/AA/TC and d) PLACL/SF/AA/TC/n-HA nanofibrous scaffolds.

Hence, biodegradable polymers, bio-ceramics and other biomaterials can be combined to mimic the natural ECM for engineering artificial organs. Composite materials usually strike an excellent balance between strength and toughness with generally enhanced characteristic properties compared to their separate constituents. Hydroxyapatite (HA) is a major inorganic mineral constituent of bone and is generally used as a bio-ceramic plaster in polymer-based bone substitutes because of its great bioactivity and biocompatibility [21]. The present study proved in vitro response of MSCs cultured on the surface mineralized PLACL/SF/AA/TC nanofibrous scaffolds in terms of cell proliferation, osteogenic differentiation and mineralization for bone tissue regeneration.

2. Materials and methods

2.1. Materials

Poly(L-lactic acid)-co-poly-(ε-caprolactone) (70:30, Mw 150 kDa) obtained from Boehringer Ingelheim Pharma, GmbH & Co., Germany. 1,1,1,3,3,3-Hexafluoro-isopropanol (HFIP), Alizarin Red-S and cetylpyridinium chloride, AA, TC and methanol were purchased from Sigma-Aldrich, Singapore. SF was purchased from Zhang Peng International Trading, Singapore. Crystalline hydroxyapatite was obtained from Department of Metallurgical and Materials Engineering, Indian Institute of Technology, Chennai, India. Dulbecco's modified Eagle's medium (DMEM), Nutrient Mixture F-12 (HAM), fetal bovine serum (FBS), antibiotics and trypsin-EDTA were purchased from GIBCO Invitrogen USA. CellTiter 96® Aqueous one solution was obtained from Promega, Madison, USA.

2.2. Fabrication of nanofibrous scaffolds

Poly(L-lactic acid)-co-poly-(ε-caprolactone) (PLACL) 10% (w/v), PLACL/SF 80:20 (w/w), PLACL/SF/AA 70:25:5 (w/w) and PLACL/SF/AA/TC 70:20:5:5 (w/w) solutions were prepared at the concentration of 10% in HFIP. All the solutions were magnetically stirred at room temperature overnight for better distribution and homogenization. The biocomposite polymer solution was loaded into a 5 mL syringe which was then attached into a 21G-grounded needle using syringe pump (KD 100 Scientific Inc., Holliston, MA., US) at a flow rate of 1.2 mL/h with a high voltage electric field of 16 kV (DC high voltage power supply from Gamma high voltage research, Florida, USA). The nanofibers were collected on an aluminum foil wrapped plate kept at a distance of 15.5 cm between the tip of the spinneret and the plate. Nanofibers were collected on 15 mm coverslips for cell culture experiments. Fabricated nanofibrous scaffolds were subsequently dried overnight under vacuum oven to eliminate remaining solvents and used for further studies. Bio-mineralization process was then carried on the PLACL/SF/AA/TC nanofibrous scaffolds to precipitate n-HA by calcium phosphate dipping method [22].

2.3. Characterization of nanofibrous scaffolds

The fiber morphology was observed under field emission scanning electron microscope (FESEM, FEI-QUANTA 200F, Netherland) at an

Table 1
Characterization of biocomposite nanofibrous scaffolds.

Nanofiber constructs	Fiber diameter (nm)	Water contact angle (°)	Pore size (μm)	Porosity (%)	Tensile break (%)	Tensile strength (MPa)
PLACL	320 ± 22	122.70 ± 2.6	3.2–7.4	92 ± 2.6	41.62	1.51
PLACL/SF/AA	254 ± 36	70.80 ± 2.28	2.4–6.8	91 ± 1.8	40.34	2.76
PLACL/SF/AA/TC	228 ± 78	53.90 ± 1.83	1.5–5.6	94 ± 3.2	28.88	4.86
PLACL/SF/TC/n-HA	228 ± 62	29.70 ± 3.42	2.0–6.9	87 ± 2.3	31.83	4.32