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Emulsion electrospun composite matrices of poly(ε-caprolactone)-hydroxyapatite: Strategy for hydroxyapatite confinement and retention on fiber surface

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ABSTRACT

Fabrication of polymeric scaffolds for various applications usually involve organic solvents or high temperature, causing the process to be unsuitable for sensitive polymers or ingredients. Here we present a method to make stable oil-in-water emulsions of poly(ε-caprolactone) (PCL) with hydroxyapatite (HA) and produce porous composite matrices from resultant emulsions via electrospinning. The emulsions are made using minimal quantity of an organic solvent and the matrices are produced via emulsion electrospinning thereby retaining the HA activity and yield in the final product. HA is confined within the PCL solution which is then dispersed in aqueous phase containing poly(vinyl alcohol) (PVA) used as a template polymer. Subsequent treatment of matrices is done to enhance HA's presence on fiber surface of the matrix and the effect of HA loading on emulsion properties, fiber morphology and proliferation profile of osteoblasts on resultant matrices is studied. Enhanced osteoblast attachment and proliferation is observed on the matrices developed via emulsion electrospinning due to uneven surface of the fibers and presence of HA on the surface. The method adopted to develop PCL/HA based porous matrices via emulsion electrospinning is substantiated to be an advantageous way to generate scaffolds favorable for applications including biomedical besides others.

1. Introduction

Fabrication of polymeric porous scaffolds is a research area that has not only gathered attention from academia but also from industry due to their high surface area to volume ratio and vast applications including tissue engineering in which they can be used [1–3]. The choice of scaffold material and its microstructure are fundamental requirements for a successful culture of artificial tissues as the cell behavior is highly influenced by surface texture, pore size and interconnectivity [4]. The scaffold has to support 3D growth in order to produce functional tissues and it needs to satisfy a number of requirements viz. biocompatibility, biodegradability and/or bioresorbability, suitable mechanical properties, adequate physicochemical properties to direct cell-material interaction matching the tissue to be replaced and ease in regaining the original shape of the damaged tissue [1,5]. There are many advanced techniques that have been adopted for producing 3D porous scaffolds today. Solid freeform fabrication (SFF) and rapid prototyping are the most common names given to a host of related technologies that are used to fabricate physical objects directly from computer aided data sources [3,5–7]. These methods are unique in that they add and bond material in layers to form objects. Such systems are also known by the names additive fabrication, three dimensional printing and layered manufacturing. Many of these advanced fabrication methods e.g. selective laser sintering, fused deposition modeling, 3D printing and plotting, stereo lithography and electrospinning have been applied for the production of scaffolds for tissue engineering [2,8–11]. In addition to these techniques, there are some basic methods e.g. particulate leaching, phase separation, fiber bonding and gas foaming that have been used for long to introduce porosity in materials [12].

Interest in electrospinning is emerging not only due to the flexibility and ease of operation but most advantageously because of production of a highly porous nano-sized (fiber's cross-section) mesh that mimics the extracellular matrix of cells thereby

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Table 1
Electrospinning of oil-in-water emulsions of HA/PCL-PVA – effect of emulsion composition on particle size and fiber diameter.

Sample ID	PCL wt%[a]	HA wt%[b]	Particle diameter (nm)[c]	Inter-particle distance h_m (nm)[d]	Average number of particles ($n \times 10^{12}$) formed by oil phase[e]	Average total surface area ($S_t \times 10^4$ cm^2) of particles formed by oil phase [f]	Fiber diameter before washing (nm)[g]	Fiber diameter after washing (nm)[h]
PCL-1	15.0	0.0	649 ± 11	201 ± 36	6.98	9.24	450 ± 40	300 ± 70
PCLHA-1	15.0	10.0	666 ± 97	206 ± 30	6.47	9.01	430 ± 90	340 ± 60
PCLHA-2	15.0	15.0	554 ± 70	171 ± 21	11.3	10.8	400 ± 70	310 ± 50
PCLHA-3	15.0	20.0	660 ± 68	204 ± 21	6.65	9.09	390 ± 190	330 ± 180
PCLHA-4	15.0	30.0	571 ± 90	176 ± 28	10.3	10.5	390 ± 150	340 ± 100
PCLHA-5	15.0	40.0	745 ± 18	230 ± 54	4.62	8.06	420 ± 200	330 ± 120

[a] wt% of PCL taken with respect to toluene.
[b] wt% of HA taken with respect to PCL.
[c] Average particle size determined from DLS with dilution from 5 to 17 ml of water.
[d] Inter-particle distance calculated using equation $h_m = d_m[(\phi_{max}/\phi)^{1/3} - 1]$, where d_m is particle diameter, ϕ_{max} is maximum volume fraction = 0.74 for hexagonal packed particles in the lattice and ϕ is the volume fraction of particles = 0.33 in continuous phase [47].
[e] Average number of particles (n) in 10 ml of oil phase calculated using equation $n = 6/\pi d_m^3$, where d_m is the average particle diameter [47].
[f] Average total surface area (S_t) of n particles having diameter d_m, calculated using equation $S_t = 6V_t/d_m$ for a given volume V_t (10 ml) of oil phase [47].
[g] Average fiber diameter of unwashed mesh calculated from imageJ analysis of SEM pictures.
[h] Average fiber diameter of washed mesh with water calculated from imageJ analysis of SEM pictures.

providing receptive environment to direct cell growth. Fibers with micron or nanometer sized diameters can be produced depending on the selected materials and other process parameters e.g. solution concentration, flow rate and voltage. Fibers of these dimensions have an extremely high surface area to volume ratio, a desirable feature in optimizing cellular interaction with the scaffold [9,13,14]. The disadvantageous features of all basic or SFF techniques including electrospinning is that a molten polymer or a solution of polymer in organic solvents is used generally. If the process is carried out in melt, aliphatic polyester like poly(ε-caprolactone) (PCL) tend to show degradation and it becomes difficult to incorporate bioactive agents which are sensitive to high temperatures. On the other hand, use of organic solvents, if not thoroughly removed from the scaffold, reduces the ability of cells to form new tissues in-vivo. It otherwise requires long processing time to completely remove these solvents from the scaffolds. The combination of toxic chemicals and extreme temperature presents difficulties if cells or growth factors are to be included in the scaffold during processing.

One possible solution for this issue is to carry out electrospinning in an aqueous solution of the polymer thereby avoiding use of any organic solvent. Since most of the polymers intended to be used for electrospinning, including aliphatic polyesters, are water insoluble, it is therefore desirable to look for alternatives with minimal involvement of organic solvents in the process. Electrospinning of a water based emulsion or suspension of the polymer is one option where use of water as the continuous medium decreases the amount of organic solvent and in addition its high dielectric constant results in rapid formation of fine fibers [15–20]. Emulsion electrospinning, thus referred to as "Green Electrospinning" has already been adopted for hydrophobic polymers such as polystyrene [21,22] or its copolymers [22,23], polyacrylates [18] and block-copolymer of PCL [24]. Interest in PCL has been increasing over years due to the beneficial properties of this polymer [25] and electrospinning of PCL to generate porous scaffolds has also been studied in detail [26,27]. However electrospinning of PCL is mostly conducted in solution form using various organic solvents [28] and in presence of comparatively hydrophilic polymers such as chitosan [29], collagen [30], polyvinyl alcohol [31–33], silk [34] or gelatin [35–37]. A tenside free emulsion electrospinning of PCL-polyethylene glycol block copolymer has been reported recently [24]. Electrospun matrices of PCL and hydroxyapatite (HA) are also made using organic solvents [35,38–43] however the drawbacks of the process, as discussed above, still persisted. Recently, we have presented a strategy to develop uneven surfaced mesh of PCL via emulsion electrospinning of an oil-in-water emulsion of PCL [44]. Electrospinning in particular is also restricted for commercialization due to its lower production rate. Newer methods e.g. pressurized gyration and others have been reported recently to enhance rate of production in electrospinning [45,46].

The objective of present study is to develop a porous matrix based on PCL and HA for proliferation of osteoblasts. A stable oil-in-water emulsion of PCL and HA is formed in presence of a template polymer poly(vinyl alcohol) (PVA) with minimal use of organic solvent and porous matrix is produced via electrospinning of the resultant emulsion. The matrix is further treated to maximize the presence of HA on the fiber surface and the effect of HA loading on the properties of emulsion, fiber morphology and growth profile of osteoblasts on developed matrices is studied in detail.

2. Experimental

2.1. Materials

Poly (ε-caprolactone) (PCL) having peak molecular weight (M_p) = 80,000 g/mol (as reported by the supplier, Perstrop UK) was used for emulsion electrospinning and construction of matrices. Polyvinyl alcohol (reported weight average molecular weight M_w = 125,000 g/mol, CDH India), Brij58 (used as non-ionic emulsifier, having linear formula $HO(CH_2CH_2O)_{20}C_{16}H_{33}$ and reported number average molecular weight M_n = 1124 g/mol) (Sigma Aldrich India), DI water (Millipore India), toluene (Merck India), Hydroxyapatite (HA) (Sigma Aldrich, India) and 3-(4,5-dimethylthiazolyl-2-yl)-2-5 diphenyl tetrazolium bromide (MTT, Sigma Aldrich USA) were used as received. Osteosarcoma cell line MG-63 was obtained from NCCS, Pune cell repository, India. The cells were maintained in DMEM (GIBCO) supplemented with 10% fetal bovine serum with 1% Penstrep at 37 °C and 5% CO_2 in an incubator (Shell labs).

2.2. Preparation of emulsion

A solution of 15 wt% PCL in toluene is prepared and predetermined amount of HA is dispersed in the solution under constant stirring in a nitrogen atmosphere. In a separate vial, a solution of 7 wt% PVA and 5 wt% Brij 58 was made in DI water. 10 ml of oil phase (PCL-HA in toluene) was added drop wise to 20 ml of aqueous phase (PVA-Brij58 in DI water) under constant stirring at room temperature. The resultant emulsion was sonicated for next 20 min and stirred additionally for 5 h. Stable