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Electrospun composite matrices of poly(ε-caprolactone)-montmorillonite made using tenside free Pickering emulsions

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ABSTRACT

The production of composite electrospun matrices of poly(ε-caprolactone) (PCL) using an emulsifier-free emulsion, made with minimal organic solvent, as precursor is reported. Pickering emulsions of PCL were prepared using modified montmorillonite (MMT) clay as the stabilizer. Hydrophobic tallow group of the modified MMT clay resulted in analogous interaction of clay with oil and aqueous phase and its adsorption at the interface to provide stability to the resultant emulsion. Composite fibrous matrices of PCL and MMT were produced using electrospinning under controlled conditions. The fiber fineness was found to alter with PCL concentration and volume fraction of the aqueous and oil phases. A higher tensile strength and modulus was obtained with inclusion of MMT in PCL electrospun matrix in comparison to a matrix made using neat PCL. The presence of clay in the fibrous matrix did not change the cell proliferation efficiency in comparison to neat PCL matrix. Composite fibrous matrices of PCL/MMT bearing enhanced tensile properties may find applications in areas other than tissue engineering for example food packaging and filtration.

1. Introduction

Poly(ε-caprolactone) (PCL) is one of the most researched polymers for application as tissue engineering scaffolds due to its biocompatibility and biodegradability [1–3]. There are various conventional and advanced methods, including particulate leaching, phase separation, gas foaming, solid freeform and rapid prototyping used extensively for fabrication of PCL scaffolds [1,4–6]. Amongst them, electrospinning has gained significant attention due to its ease of operation and advantages of a fibrous scaffold obtained using electrospinning [7–9]. Additionally, the choice of using various precursors and ease of introduction of functionality has added to the desirability of PCL electrospun fibrous scaffolds [10–13]. A variety of fiber morphologies have been developed by varying the process parameters of electrospinning like voltage, polymer concentration, distance, flow rate, etc. [14–17]. The use of excessive organic solvents that cannot be retrieved and low production rate, however, are issues still need to be resolved with electrospinning.

Melt electrospinning was developed as a solvent free process; however, it was not feasible for many polymers sensitive toward high temperature processing (e.g. PCL) and otherwise was a complex intricate process requiring additional setup and cost [18,19]. Incorporation of a drug or biofactor in the scaffold was also difficult during high temperature melt electrospinning. The use of emulsions as precursor for electrospinning technique has recently been reported due to advantages originating from a water-based system with minimal use of organic solvents [20–22]. The addition of an emulsifier or a template polymer was a strict requirement to maintain emulsion's stability prior to and during jet formation in electrospinning. The presence of such extra chemicals affects scaffold's biocompatibility, cell proliferation properties, degradation profile and thermo-mechanical properties. Additional steps were therefore required to remove such materials in order to make the scaffold suitable for the desired application. If the emulsifier/template polymer is biocompatible, its removal from the scaffold material is not essential, but washing out of these materials in aqueous media may significantly reduce the mechanical properties of the scaffold.

A possible solution to overcome the issues originating from presence of emulsifiers or template polymers in electrospun scaffold could be use of PCL-based Pickering emulsions as the precursor. Unlike conventional emulsions, Pickering emulsion are stabilized using very less quantity of particulate matter that form a strong layer at the oil and water interface [23–26]. These solid particles can also act as reinforcing agent which can potentially enhance the strength. Various types of particles, including silica, titania, talc, starch, carbon nanotubes, graphene oxides, and others, have been reported in literature for formation of Pickering emulsions [27–33]. Amongst the stabilizers used in Pickering emulsions, clays were found to have special characteristic due to their high aspect ratio compared to spherical particulates. Laponite clay has been used

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to stabilize styrene water system [34] and 2,2,3,3,3-pentafluoropropyl acrylate methyl methacrylate and *n*-butyl acrylate miniemulsion systems [35]. Polymerization of heptafluoro butyl acrylate in miniemulsion stabilized by sodium montmorillonite clay using 2-cyanopropyl dodecyl trithiocarbonate was also reported [36]. In another study, Closite 20A was used to stabilize water and canola oil dispersion [37], and montmorillonite clay was used to stabilize oil in seawater emulsion [38]. Moreover, the addition of clay was found to enhance the mechanical properties in many composites [39–44]. The polymerization of Pickering emulsions was used for formation of various types of nanocomposite particles, e.g. polystyrene [45] and polystyrene-silica-based core-shell nanocomposite particles [46]. Pickering emulsions are also used for encapsulation of drugs [47], food hydrocolloid industry [48] and for preparation of hollow microspheres [49].

Electrospinning of Pickering emulsion to produce tissue engineering scaffolds as a sustainable approach has not been explored yet. The present study aims to develop a fibrous scaffold suitable for tissue engineering by electrospinning of a PCL-based Pickering emulsion. An emulsifier-free formulation was therefore developed and the emulsion was made with minimally required organic solvent. Montmorillonite clay was used as the stabilizer for Pickering emulsion and the thermo-mechanical properties of the fibrous scaffold thus obtained were evaluated in addition to its performance as support for cell proliferation.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (PCL) having number average molecular weight (M_n) = 80,000 g/mol (Sigma Aldrich, Bengaluru, India) was used for preparation of Pickering emulsions and their electrospinning to obtain fibrous matrices. Shelsite 30B, modified montmorillonite clay of purity >99% and average particle size <80 nm (as reported by supplier) and unmodified montmorillonite clay were purchased from Nanoshel LLC, Wilmington, DE, USA, and used without any purification. DI water (Millipore, Bengaluru, India), toluene, dichloromethane (DCM) and dimethylformamide (DMF) (Merck, Bengaluru, India) were used as received. Human breast cancer cell line (MCF7, a kind gift from Indianapolis University, source ATCC) was maintained in RPMI 1640 (GIBCO) medium supplemented with 10% FBS (Fetal Bovine Serum) and 1% penstrep.

2.2. Preparation of Pickering emulsions

The clay particles with various concentrations were dispersed in toluene with sonication of 15 h. The organic phase was made of PCL solution in toluene containing the dispersed clay particles. PCL content was varied from 10, 15, 20, 25 and 30 wt% with respect to toluene. Aqueous phase was added drop wise to the oil phase in pre-determined volume fractions (oil: aqueous phase at 0.74: 0.26) under a high speed homogenizer (IKA Ultra Turrax T25) operating at 20,000 rpm. The emulsions thus formed were stirred for five minutes and stored at room temperature for electrospinning. Different emulsions with varying PCL and clay content were prepared following the procedure described above. Sample identification for different emulsions is presented in Table 1 under results and discussion section.

2.3. Electrospinning of Pickering emulsion

For preparation of nanofibrous matrices, electrospinning was conducted on an electrospinning instrument (Physics Equipment Co. India) using a plate collector at 25 ± 3 °C and 65 ± 5% RH. HIPE was transferred to a 2 ml disposable syringe attached with blunt tip metallic needle and a computer controlled pump was used to pump the emulsion at a constant flow rate of 0.5 ml/h. The needle was subjected to a constant high voltage power supply of and continuous stream of fibers was collected on a plate collector placed at a distance of 20 cm from needle tip. For electrospinning of neat PCL samples, the desired concentration of PCL was dissolved in DCM/DMF in the ratio of 70/30 and was electrospun under same conditions.

Table 1
Sample composition for stable Pickering emulsion.

Sample ID	Clay (wt%)	PCL (wt%)	Φ_{aq}[a]	Stability[b]	Droplet size (μm)[c]	Fiber diameter (nm)[d]e*
PE-1	1.0	10	0.26	Stable	174 ± 12	170 ± 7
PE-2	1.0	15	0.26	Stable	150 ± 9	206 ± 6
PE-2 m*	1.0	15	0.30	Stable	205 ± 13	230 ± 7
PE-3	1.0	20	0.26	Stable	116 ± 11	604 ± 17
PE-3 m*	1.0	20	0.35	Stable	175 ± 12	595 ± 11
PE-4	1.0	25	0.26	Stable	104 ± 10	950 ± 9
PE-4 m*	1.0	25	0.40	Stable	140 ± 11	978 ± 10

[a] Volume fraction of aqueous phase.
[b] No visual phase separation for 24 h at room temperature.
[c] Determined from optical microscope images using ImageJ software.
[d] Calculated from SEM images using ImageJ software.
[e] m denotes emulsion containing maximum aqueous phase; emulsion destabilized beyond this level of water.

2.4. Characterization techniques

2.4.1. Scanning electron microscopy (SEM)

The surface morphology of the fibers in matrices (before and after washing with water) was studied from SEM images acquired using a Zeiss Evo 50 Scanning Electron Microscope. The samples were pre-coated with gold before analysis. For diameter measurement, an average of 100 readings was taken using ImageJ software.

2.4.2. Transmission electron microscopy (TEM)

Fiber morphology was studied using transmission electron microscopy (TEM, FEI, Tecnai G^2) at a voltage of 200 KV by directly placing the sample onto copper TEM grid. TEM analysis of clay particles was done by dispersing clay in toluene and drop casting it onto the TEM grid.

2.4.3. Wide angle X-ray diffraction (WAXD)

Crystal structure analysis was carried out using PANalytical XPERT-PRO WAXD with Cu K-Alpha1 [Å]:1.54060. A mesh of electrospun web was analyzed with 2 theta values from 3° to 40° at a constant temperature of 25 ± 1 °C.

2.4.4. Tensile testing

The mechanical properties of PCL and Clay reinforced PCL electrospun matrices were measured using Instron micro-tensile tester (Model-5848, Singapore). For measurements, the matrices were deposited on a 5 × 5 cm template for 30 min. It was then removed and preconditioned at 25 ± 1 °C and 65% RH. The samples were then fixed on a window template axially with the help of a scotch tape. 10 N load cell was used with gauge length of 2.5 cm and cross-head speed of 10 mm/min. An average of 5 measurements was reported.

2.4.5. Differential scanning calorimetry (DSC)

Thermal analysis of the samples was performed on TA Q2000 Differential Scanning Calorimetry (DSC) instrument. All the samples were first heated at 10 °C/min to 80 °C and kept under isothermal condition for 5 min. The samples were then cooled to −75 °C at a cooling rate of 5 °C/min and reheated at 10 °C/min to 80 °C. Melting (T_m) and crystallization (T_c) temperature, as well as change in heats of fusion (ΔH_f) and crystallization (ΔH_c) were determined from the second heating and cooling thermographs, respectively. Relative crystallinity (w_c) of PCL in different samples was calculated according to Eq. (1), where