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Research Paper

"Nano in micro" architecture composite membranes for controlled drug delivery

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ABSTRACT

A novel "nano in micro" architecture composite electrospun membrane has been fabricated with Poly (ε-caprolactone) (PCL). Nano sheets of montmorillonite (Mt) can act as drug cargoes in PCL electrospun membranes. The fiber morphology and diameter of PCL/Mt. electrospun membranes were tuned by optimization of various electrospun parameters. Higher concentration of Mt. loaded electrospun membranes exhibited improved crystallinity and hydrophilicity. The *in vitro* degradation studies of PCL/Mt. electrospun membranes explored a new scenario of degradation mechanism. PCL/40Mt membranes showed enhancement in human dermal fibroblast (HDFs) adhesion and proliferation. The vitamin B12 loaded Mt. nano-sheets incorporated in electrospun membranes exhibited extended release of the drugs over 15 days. Moreover, the pH responsive nature of PCL/40Mt electrospun membranes was also investigated. Gentamicin is the antibacterial drug loaded on PCL/40Mt membrane and showed bioactivity against *E. coli* for 15 days. The newly fabricated nano in micro architecture membranes can be used as potential material for tissue engineering and sustained drug delivery.

1. Introduction

Over the past decade, natural clay incorporated nano and micro structures acquired a great deal of attention in the field of catalysis (Soni et al., 2017), filtration (Suja et al., 2017; Ménesi et al., 2008), tissue engineering (Li et al., 2012) and controlled drug delivery (Wang et al., 2012). Nanoclay incorporated drug delivery vehicles were widely attracted due to its high selectivity, controlled and stimuli responsive release behaviour. Tissue engineering membranes based on nanoclay loaded with therapeutic drugs can be a suitable material for controlled delivery of antibiotics in specific sites and reduces the risk of systemic toxicity in human body.

Engineering of "nano in micro" architecture materials is a promising approach for the fabrication of drug delivery vehicles (Kankala et al., 2017a, 2017b). Electrospinning is one of the most efficient, simple and cost effective method for the fabrication of "nano in micro" structured material by incorporating nanoparticles into the polymer matrix (Kuthati et al., 2015; Kankala et al., 2017a, 2017b). Many other strategies were also introduced for fabricarion of 3D scaffolds (Chen et al., 2017; Kankala et al., 2018; Kankala et al., 2017a, 2017b). High electric field and elongation of polymeric dope during electrospinning process enhance the homogeneous dispersion of nanoparticles. Thus, nanoclay incorparated electrospun fiber is an easy and effective strategy for the fabrication of polymer/clay/drug hybrid. The micro-nano scale fibrous structures and high porosity of electrospun membranes resemble with the natural extracellular matrix (Xue et al., 2015). During electrospinning process the manipulation of scaffold morphology, porosity and control of drug release kinetics can be easily achieved by varying polymer composition and processing parameters (C. R et al., 2017b; Chong et al., 2007; Khalf and Madihally, 2017; Liu et al., 2015).

Naturally occurring and organically modified clays such as montmorillonite (Fejér et al., 2001; Deák et al., 2015; Janovák et al., 2009), halloysite nanotubes (Tu et al., 2013), LAPONITE® (Wang et al., 2014), and aminoclay (Yang et al., 2014) were usually act as functional nano containers for encapsulation of drug in various polymer composites. Among these, montmorillonite (Mt) has been selected for this study due to its nano sheet like structure with excellent encapsulation efficiency against biologically active molecules. Mt. is inexpensive natural clay belongs to smectite group exhibited anisotropic properties, hydrophilicity and high aspect-ratio morphology, thus a possibility of high surface interactions between polymers and nanoparticles have been occurred (Gao and Guo, 2017). Mt. has muco adhesive as well as highly efficient detoxifier properties essential for drug delivery across the gastrointestinal barrier, wound healing, hem-orrhoids, stomach ulcers and intestinal problems (Othman et al., 2016). Recently researcher developed many polymer/Mt. nano drug carrier systems for various

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biomedical applications. Monalisha et al. investigated the release kinetics of drug from carboxymethyl cellulose-g-poly (acrylic acid)/Organically modified Mt. (OMt) nanocomposite hydrogels (Boruah et al., 2014). Poly(D,L-lactide) anti-solvent nano precipitation scaffolds loaded with Mt. nanoparticles were also investigated as drug delivery systems (Othman et al., 2016). Many researchers were investigated the improvement in physicochemical properties of polymer/Mt. electrospun membranes (Marras et al., 2008; Nouri et al., 2013). Despite interesting improvements in physic-chemical properties, only a few researches have been done in focus of Mt. based electrospun membranes for drug encapsulation and controlled drug delivery applications (Oliveira et al., 2017) (Rapacz-Kmita et al., 2017).

To fabricate electrospun nanoclay loaded membranes, polycaprolactone (PCL) has been selected as the host polymer. PCL exhibited excellent biocompatibility, mechanical properties, electrospinnability (van der Heijden et al., 2016). Moreover, various PCL composite electrospun scaffolds were widely used in tissue engineering, wound dressing and drug delivery materials (Diba et al., 2012). But versatile applications of PCL scaffolds have been restricted due to its hydrophobicity, slow degradation, lack of bioactivity, and limitations in controlled drug release (Cipitria et al., 2011). Thus, loading of Mt. nanoparticles into PCL electrospun scaffold can dramatically improve the biomaterial properties of scaffold.

Gentamicin and vitamin B_{12} were chosen as a model drug in PCL/Mt. electrospun membrane due to its significant inhibition against wide variety of bacteria and therapeutic importance. Gentamicin is a widely used antibiotic with low cost, good stability and acts against several infection caused by Gram-positive and Gram-negative bacteria (Rouabhia et al., 2014). Controlled local delivery of antibacterial drugs to the infection site with sufficient inhibitory concentration has been desired to avoid systemic toxicity compared to conventional oral and intravenous administrations. More over it enhances the therapeutic efficacy of the drug. Vitamin B_{12} is a water-soluble vitamin with an important role life sustainable biological functions such as the formation of blood vessels and red blood cells, functioning of brain and nervous system (Carlan et al., 2017).

In this study, we developed a novel drug-loaded PCL/Mt. electrospun nano in micro architecture fibrous membrane with sustained drug release kinetics. The current study aimed to (i) optimise the electrospun parameters of PCL/Mt. matrix (ii) to investigate the effect of Mt. is physico-chemical and degradation of properties PCL electrospun membranes (iii) to evaluate the blood compatibility and cell viability on PCL/Mt. membranes and (iv) to evaluate *in vitro* drug release and permeation kinetics PCL/Mt. electrospun membranes. The PCL/Mt. electrospun membranes were expected to be highly efficient for sustained drug release with desired duration and dosage in target tissues.

2. Experimental

2.1. Materials

Polycaprolactone (PCL, Mn-80,000) and montmorillonite (Mt) nano clay were purchased from Sigma-Aldrich USA. Nano Mt. was prepared by following the procedure of Hosseinpour et al., (Hosseinpour et al., 2014). The solvents such as dichloromethane (DCM) and dimethylformamide (DMF) were bought from Avera Chemical, India and Merck, India respectively. The drug gentamicin sulfate and vitamin B12 were purchased from Sigma-Aldrich, China and Himedia India respectively. Albumin bovine (BSA) purchased from SRL India. Micro BCA™ protein assay kit (Thermo Scientific, USA) and Mueller-Hinton agar (Himedia, India) were purchased. Human dermal fibroblast was purchased from Himedia India. Cell culture media and supplements and the other chemicals were obtained from Sigma-Aldrich, USA. Phosphate buffered saline (PBS) buffer was prepared according to the literature (Dong et al., 2009).

Table 1
Sample codes and composition of PCL/Mt. electrospun membranes.

Sample code	PCL (wt%)	Mt (wt%)
PCL	100	0
PCL/5Mt	95	5
PCL/10Mt	90	10
PCL/20Mt	80	20
PCL/30Mt	70	30
PCL/40Mt	60	40
PCL/50Mt	50	50

2.2. Fabrication of PCL/Mt. electrospun membranes

For fabrication of pristine PCL electrospun membrane, 10 wt% polymer is dissolved in DCM:DMF 7:3 *v*/v solvent mixture at room temperature. For fabrication of PCL/Mt. nanocomposite electrospun membranes, various wt% of Mt. such as 0 wt%, 5 wt%, 10 wt%, 20 wt%, 30 wt%, 40 wt% and 50 wt% with respect to PCL concentration has been taken by maintain total concentration of PCL/Mt. as 10 wt%. Sample codes and composition of PCL/Mt. electrospun membranes were briefed in Table 1. For fabrication of composite membrane, an appropriate amount of Mt. nanoparticles was sonicated with DCM:DMF mixture for 10 min. PCL has been added to the uniformly dispersed Mt. solution and stirred for 3 h. Prior to electrospinning, the PCL/Mt. solution was subjected to 30 min probe sonication to confirm the uniform dispersion of Mt. The schematic representation of composite preparation is showed in Fig. 1. The PCL/Mt. solution has been taken in a 10 mL syringe with blunt ended 21 gauge needle mounted on a syringe pump with programmed flow rate. A high voltage DC power supply (Holmark HO-NFES-041) was used to generate potential of 18 kV. One electrode of high voltage supply was connected to tip of the needle and other electrode connected to the collector rotating mandrel. Mandrel rotates in a speed of 500 rpm with a working distance of 12 cm. During fabrication of drug loaded PCL/Mt. composite electrospun membranes the appropriate concentration of drug has been added to the uniform mixture of Mt. in DCM:DMF (7:3 v/v). The drug Mt. uniform mixture has been sonicated for 2 h for the formation of drug Mt. inclusion complex, after that appropriate amount of PCL has been added and stirred for 3 h. 10 wt% of antibacterial drug gentamicin and vitamin B12 were loaded in PCL/Mt. membranes for *in vitro* drug release studies.

2.3. Characterization

The surface morphology of electrospun fibers has been analyzed with scanning electron microscope (Hitachi 6600) operating at an accelerating voltage of 10 kV. The SEM images were examined by image J software and average fiber diameter has been calculated from the average of at least 50 measurements. The thermal properties of the sample also analyzed using thermogravimetric analyzer (TGA, TA Instruments, Q50) with a temperature scan of 10 °C/min in a range of 0 °C to 700 °C under nitrogen flow of 60 mL/min. X-ray diffraction (XRD) analysis on the membranes was performed with Rigaku Miniflex 600 diffractometer (Japan) The 2Θ range from 3°-90°at a scanning rate of 10° to 15° min^{-1}. The mechanical properties of electrospun scaffolds of dimension 0.5 × 5 cm were investigated by using a universal testing machine (Schimadzu Autograph, AG-Xplus series) with an applied load of 10 N. For each sample, 5 specimens were tested. Water contact angle was measured by goniometer processed by a software program (DIGIDROP). Water drop of 1 μL was placed on 1×3 cm^2 dimension membranes and images of water drop on the sample surface were recorded using a digital camera. Each sample was measured 15 different locations and readings were averaged. The porosity (ε) of the electrospun scaffold was determined using the following equation.