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Hybrid electrospun chitosan-phospholipids nanofibers for transdermal drug delivery

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ABSTRACT

Chitosan (Ch) polysaccharide was mixed with phospholipids (P) to generate electrospun hybrid nanofibers intended to be used as platforms for transdermal drug delivery. Ch/P nanofibers exibithed average diameters ranging from 248 ± 94 nm to 600 ± 201 nm, depending on the amount of phospholipids used. Fourier Transformed Infra-Red (FTIR) spectroscopy and Dynamic Light Scattering (DLS) data suggested the occurrence of electrostatic interactions between amine groups of chitosan with the phospholipid counterparts. The nanofibers were shown to be stable for at least 7 days in Phosphate Buffer Saline (PBS) solution. Cytotoxicity studies (WST-1 and LDH assays) demonstrated that the hybrid nanofibers have suitable biocompatibility. Fluorescence microscopy, also suggested that L929 cells seeded on top of the CH/P hybrid have similar metabolic activity comparatively to the cells seeded on tissue culture plate (control). The release of curcumin, diclofenac and vitamin B12, as model drugs, from Ch/P hybrid nanofibers was investigated, demonstrating their potential utilization as a transdermal drug delivery system.

1. Introduction

Electrospinning (ES) is a simple and effective fabrication process for producing non-woven fibrilar structures made of (bio)polymers and long-chain molecules whose diameters can be controlled from the nano to the micro scale (Frenot and Chronakis, 2003). Due to their high surface area, tunable diameter and surface functionality, the use of electrospun fibers have also been investigated in transdermal drug delivery applications (Kataria et al., 2014; Madhaiyan et al., 2013; Rasekh et al., 2014; Taepaiboon et al., 2007; Zamani et al., 2013). Electrospun nanofibers made of poly(vinyl alcohol) and sodium alginate were loaded with the antibiotic ciprofloxacin and investigated as transdermal patches in wound healing applications (Kataria et al., 2014). It was observed that patches loaded with ciprofloxacin decreased the time of wound healing comparatively to the patches without drug. Poly (vinylpyrolidone) electrospun nanofibers loaded with indomethacin were also used for layering wound dressings and adhesive patches, without altering their mechanical functionality and allow the release of this anti-inflammatory drug to further enhance wound healing process (Rasekh et al., 2014). In another study, nanofibers of electrospun cellulose acetate (CA) were developed for transdermal delivery of vitamin A and E (Taepaiboon et al., 2007). A gradual release of the vitamins was observed from CA nanofibers, in comparison with a burst release observed from CA casted films. Madhaiyan et al. (2013) loaded hydrophilic vitamin B12 within Polycaprolactone (PCL) electrospun nanofibers and observed a sustained release suitable for transdermal delivery of vitamin B12.

Chitosan(s), a chitin-derived polysaccharides made of glucosamine and N-acetyl glucosamine, have received particular attention due to their remarkable properties, including non-toxicity, biocompatibility, biodegradability, hemostatic activity, antibacterial and antimycotic (Balan and Verestiuc, 2014; Jayakumar et al., 2010; Luo and Wang, 2014; Menchicchi et al., 2015). The efficacy of chitosan(s) to be used as transdermal penetration enhancers (Sarmento and das Neves, 2012), and as transdermal drug delivery carriers (in the form of hydrogels, nanocapsules and membranes), has been investigated in previous studies (Anitha et al., 2014; Bhattarai et al., 2010; Thein-Han and Stevens, 2004). Moreover, phospholipid nanostructures have been also fabricated in the form of vesicles, liposomes, and films and used in

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transdermal drug delivery applications (Ghanbarzadeh and Arami, 2013; Kumar and Katare, 2005; Neubert, 2011; Taghizadeh, 2011). Encapsulation of bioactives within phospholipid formulations often offer desired delivery, enhanced stability, protection, biocompatibility and permeability, depending on lipid composition and properties(Mouritsen, 2011). Subsequently, the combination of lecithin with chitosan to engineer carriers such as nanocapsules for transdermal drug delivery of clobetasol-17-propionate (Şenyiğit et al., 2010) and melatonin (Hafner et al., 2011) has also been investigated.

Vitamin B12, also known as cyanocobalamin, is a water-soluble vitamin and is considered to function as an essential factor in DNA synthesis (for chromosomal replication and division) and a mediator of hematopoietic and nervous systems (Goto et al., 2015). Microemulsions (Salimi et al., 2013) and recently electrospun nanofibers (Madhaiyan et al., 2013) were developed to delivery vitamin B12 *via* transdermal and overcome the issues related with poor bioavailability found in oral delivery. Diclofenac belongs to the most frequently administered class of Non-Steroidal Anti-inflammatory Drugs (NSAIDs) and is known for its potent anti-inflammatory, analgesic and antipyretic properties (Shen et al., 2011). Due to the gastrointestinal side effects, poor bioavailability and short biological half-life of Diclofenac, transdermal administration route is preferred comparatively to oral route (Thakkar et al., 2014). Microemulsions (Thakkar et al., 2014) and liposomal systems (Taghizadeh, 2011) have been investigated to serve as carriers to transdermally release Diclofenac. Curcumin, a polyphenol derived from the rhizomes of Curcuma longa, has been recognized by its pharmaceutical properties as an antioxidant, an anti-inflammatory agent and an inhibitor of tumorigenesis and metastasis (Sun et al., 2013). Due to the poor bioavailability of Curcumin new delivery carriers to release this drug have been developed. However the transdermal pathway has been recently explored through the encapsulation of Curcumin within dried polymeric film-type matrix (Patel et al., 2009) and using microemuslsions as release vehicles (Sintov, 2015).

The production of stable and functional chitosan electrospun nanofibers has never been assessed regarding transdermal drug delivery applications. Overall the lack of stability of the individual electrospun chitosan nanofibers in aqueous media limits its application in different fields including transdermal drug delivery. To address this drawback, neutralization and crosslinking methods are required. However the aforementioned methods follow a two-step protocol or use components with latent cytotoxicity, which are not desirable for drug delivery applications. The present study aimed to develop stable, biocompatible chitosan electrospun nanofibers through the inclusion of phospholipids within chitosan solutions, in one single step, to produce hybrid electrospun chitosan/phospholipid nanofibers for transdermal drug delivery. The morphology, water uptake, stability, and cytotoxicity of the nanofibers were evaluated using different contents of phospholipids and the encapsulation and *in vitro* release of curcumin, diclofenac and vitamin B12, as model drugs, were performed.

2. Materials and methods

2.1. Materials

Asolectin from soybean (containing approximately 25–33% of lecithin, cephalin and phosphatidylinositol, 24% saturated fatty acids, 14% mono-unsaturated and 62% poly-unsaturated fatty acids), chemicals and drugs (Curcumin, Diclofenac and Vitamin B12) were obtained from Sigma-Aldrich. Chitosan, Mw 211 kDa, DA 13% was obtained from GILLET CHITOSAN (product 112). All of the consumables were used as received.

2.2. Chitosan/phospholipid interactions in solution-DLS measurements

ζ-Potential and size measurements were made using a Zetasizer NanoZS Instrument (ZEN 3600, Malvern Instruments, Worcestershire, UK). Prior to the analyses, chitosan, phospholipid and chitosan/phospholipid solutions were prepared at a concentration of 0.1 wt% in TFA (1% v/v). The solutions were filtered using a pyrogen free, 0.45-mm disposable membrane filter (Schleicher and Schuell Bioscience, Germany).

2.3. Fabrication and characterization of electrospun hybrid chitosan/phospholipid nanofibers

2.3.1. Electrospinning process

Chitosan was dissolved in a mixture of TFA/DCM (70:30) at a concentration of 2%.wt/v. Asolectin was then added to chitosan solution at different ratios, 1:1; 1:3 (wt:wt) and the samples were designated as Ch/P1 and Ch/P3 respectively. The mixture was allowed to react under continuous stirring for 2 h. For the *in vitro* drug release studies, Vitamin B12, Curcumin and Diclofenac were loaded in chitosan/phospholipid solutions at a concentration of 1% (wt/wt). The electrospinning process was conducted at room temperature by applying a voltage of 25 kV (Gamma High Voltage Research, USA) to the polymer solution at a feed rate of 0.02 mL/min (syringe pump from New Era Pump Systems, USA) using a 24G needle (Proto Advantage, Canada). The electrospun samples were collected on a plate made of stainless steel covered with aluminum foil placed 10 cm from the needle tip.

2.3.2. Fiber morphology

The morphology of the fabricated fibers was evaluated by scanning electron microscopy (SEM). The specimens were mounted on aluminum stubs and sputter-coated with gold prior to visualization in a scanning electron microscope (FEI Inspect S, USA). The diameters of the electrospun fibers were measured using image visualization software Image-J (National Institutes of Health, USA). The average fiber diameters and diameter distributions were determined by measuring 100 fibers from 3 distinct samples.

2.3.3. FT-IR spectroscopy

The samples were dried at room temperature and placed in a desiccator before the analysis. The spectra were scanned from 0 cm^{-1} to 4000 cm^{-1} in a PerkinElmer Spectrum One model 2000 spectroscope.

2.3.4. Water uptake

The amount of water uptake (swelling) was determined by immersion of the samples in PBS and incubation at 37 °C over a period of 1 and 7 days in dynamic conditions. After each time point, samples were taken out of the solution, rinsed with distilled water, blotted on filter paper to remove surface water and immediately weighed (*mt*, mass of wet sample). Samples were then dried until they achieved constant weight (*mf*), and the percentage of water uptake was determined following equation 1:

$$\boldsymbol{WU}(\%) = \left(\frac{\boldsymbol{mt} - \boldsymbol{mf}}{\boldsymbol{mf}}\right) \times 100 \quad (1)$$

Three replicates were performed for each sample [N = 3].

2.3.5. Weight loss

The percentage of weight loss (WL) was determined to make inferences about the stability of the chitosan/phospholipid nanofibers. Samples were weighted, immersed in PBS and incubated at