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Fabrication and characterisation of drug-loaded electrospun polymeric nanofibers for controlled release in hernia repair

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ABSTRACT

The chemical distribution and mechanical effects of drug compounds in loaded electrospun scaffolds, a potential material for hernia repair mesh, were characterised and the efficacy of the material was evaluated. Polycaprolactone electrospun fibres were loaded with either the antibacterial agent, irgasan, or the broad-spectrum antibiotic, levofloxacin. The samples were subsequently characterised by rheological studies, scanning electron microscopy (SEM), atomic force microscopy (AFM), contact angle goniometry (CAG), *in vitro* drug release studies, antibacterial studies and time-of-flight secondary ion mass spectrometry (ToF-SIMS). Increased linear viscoelastic regions observed in the rheometry studies suggest that both irgasan and levofloxacin alter the internal structure of the native polymeric matrix. *In vitro* drug release studies from the loaded polymeric matrix showed significant differences in release rates for the two drug compounds under investigation. Irgasan showed sustained release, most likely driven by molecular diffusion through the scaffold. Conversely, levofloxacin exhibited a burst release profile indicative of phase separation at the edge of the fibres. Two scaffold types successfully inhibited bacterial growth when tested with strains of *E. coli* and *S. aureus*. Electrospinning drug-loaded polyester fibres is an alternative, feasible and effective method for fabricating non-woven fibrous meshes for controlled release in hernia repair.

1. Introduction

Hernia repair, one of the most common general surgeries performed, is complicated by bacterial infections and implant rejection (Earle, 2010). Commercially available mesh devices currently employed in hernia repairs contain brained or knitted fibres. The mechanical properties of the mesh and the biocompatibility of the material are critical to the healing process. Tissue incorporation, a key factor in the success of the graft device is dependent on the material type, density, compliance and electrical properties of the mesh (Procter et al., 2009). Graft failure motivates research into new fabrication methods for incorporating biomaterials and drug encapsulation in novel mesh matrices, such as hot-melt extrusion (Li et al., 2013), electrospinning (Toncheva et al., 2011), 3D printing (Holländer et al., 2016) and high-speed rotary spinning (Sebe et al., 2013).

Electrospinning is the most popular and preferred technique for nanofiber fabrication due to its simplicity, cost-effectiveness, flexibility, and ability to spin a broad range of polymers (Zamani et al., 2013). The method allows for the simple and direct functionalization of fibres with drug compounds and is compatible with solvents such as chloroform and dimethyl sulfoxide. In addition, the process of electrospinning with the use of solvents such as chloroform, dimethyl sulfoxide etc., allows functionalisation of the scaffolds through the inclusion of drugs in the polymer-solvent solution without the need for a complicated preparation

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process (He et al., 2015). Electrospinning has previously been applied to the fabrication of triclosan/cyclodextrin inclusion complexes (Celebioglu et al., 2014), the construction of scaffolds with perlecan domain IV peptides (Hartman et al., 2011), manufacture of biocatalytic protein membranes (Kabay et al., 2016), and encapsulation of levofloxacin in mesoporous silica nanoparticles (Jalvandi et al., 2015). Given the broad applications of electrospinning, there has been previous research specifically focused on the development of electrospun polymeric materials for hernia repair mesh devices. Electrospinning produces scaffolds containing micro-fibres and this is an advantageous feature not observed in braided mesh commercial devices – these microfibers also introduce mechanical anisotropy and provide topographic features to guide cell alignment (Goldstein and Thayer, 2016). However, electrospun fibres typically incorporate the use of organic solvents and for applications such as hernia repair or tissue engineering, the toxicity of organic solvents used could be highly critical – avoiding organic solvents is of outmost importance for applications in medicine and pharmacy (Agarwal and Greiner, 2011; Bubel et al., 2014).

The purpose of this study is to examine the physicochemical properties, bacteria response, and drug loading of electrospun scaffolds. The polymer chosen for this study is *polycaprolactone* (PCL); a biodegradable polyester commonly used in biomedical applications for controlled release and targeted drug delivery (Bhavsar and Amiji, 2008). PCL, a biodegradable aliphatic polyester (Azimi et al., 2016), is an obvious candidate for drug delivery systems due to its high biocompatibility and ease of degradation in the human body (Bikiaris et al., 2007). Drug loading of structures that mechanically resemble interfacial tissue and which allows short or long-term release of suitable bioactives may be utilisable in hernia-repair meshes. PCL was chosen in this research as it has a high permeability to a variety of drug molecules (e.g. gentamycin, chitosan) and low toxicity (Murthy, 1997). The matrix was loaded and electrospun with two drugs, *irgasan* (an antibacterial agent used commonly in soaps, detergents and surgical cleaning agents) or *levofloxacin* (a broad-spectrum antibiotic used commonly to treat gastrointestinal infections). The mechanical characteristics, morphology, surface hydrophobicity, drug efficacy and chemical distribution were characterised with an array of analytical techniques. The results from this study should help to build platform to aid future work with various fabrication methods, such as extrusion and shaping using 3D printing.

2. Materials & methods

2.1. Materials

Polycaprolactone (PCL) with a mean molecular weight of 80 kD, Irgasan (variation of Triclosan, >97%), Levofloxacin (>98%), and all the solvents used for the electrospinning were obtained from Sigma Aldrich. The solvents consisting of chloroform (anhydrous, containing amylenes as stabilizers, >99%) and *N,N*-dimethylformamide (DMF, anhydrous 99.8%).

2.2. Preparation of PCL solutions

Different solutions with a polymer concentration of 12% (w/w) were prepared to be used within the electrospinning method – this particular concentration was used due to its possessed suture retention and tensile strengths appropriate for hernia repair, as specified for similar electrospun scaffolds described by Ebersole et al. (2012). Various PCL formulations were constructed of a total weight of 25 g per solution, which allowed for PCL (12% w/w) and a 9:1 (w/w) ratio of chloroform (CLF) to *N,N*-dimethylformamide (DMF). For the unloaded polymer solution, 3 g of PCL was dissolved in 22 g of CLF:DMF (9:1) which was initially mixed through 30 min in a centrifuge, a further 30 min in a sonicator (Elma S30 Elmasonic) and a final 1 h with a magnetic stirrer. This process was vital to ensure that the solution was fully homogeneous. The solution was left overnight, and a further 30 min of sonication applied the following morning in order to confirm the homogeneity of the solution. For the irgasan-loaded solutions, the same method was applied, except the solution contained 1% (w/w) irgasan. The concentration of the levofloxacin-loaded solutions was 0.5% (w/w), providing sufficient sensitivity in the release cell for accurate UV analysis. All the preparations turned to clear solutions. These observations were interpreted to determine that the solutions had successfully homogenised. The solutions were then subsequently used in the electrospinning process and for rheological analysis.

2.3. Electrospinning of PCL solutions

The PCL test specimens were fabricated for each polymeric solution, using a custom in-house electrospinning apparatus, which consisted of a syringe pump (Harvard Apparatus PHD 2000 infusion, US) and two 30 kV high-voltage power supplies (Alpha III series, Brandenburg, UK). The polymer solution was loaded into glass syringe and fed through tubing with a metal needle tip attached at the end. The needle was clamped into place, to allow a high-voltage supply to run through it, which allowed an electric field to be created between the needle and the target plate. The syringe was clamped to a pump, which determined the specific injection flow rate of the polymeric solutions. For each of the three solutions (e.g. unloaded, irgasan-loaded, and levofloxacin-loaded), 3 varying flow rates of 0.5, 1 and 1.5 ml h^{-1} were applied across varying voltages of 2 kV–5 kV (needle) and 10 kV–18 kV (target plate). The variation in flow rate and applied voltages was to correct any problems that occurred during fabrication, i.e. 'spitting' of solution at the target plate, or any potential beading (which was examined through SEM). The fabrication of this solution was electrospun onto the target that was covered with aluminium foil, in order for the final material to be removed and used for further characterisation. The final yield of electrospun PCL resulted in thin, flexible sheets of material.

2.4. Rheological studies

A Thermo Scientific HAAKE MARS II rheometer with a P35 TiL cone and plate was used to measure the rheological and mechanical behaviour of the different unloaded and loaded polymeric solutions. The objective of this experiment was to examine the viscoelastic properties of the PCL solution, specifically to determine whether the irgasan or levofloxacin is having an effect on the mechanical properties of the polymer. The method used was taken and modified from the rheological study undertaken by Bubel et al. (2014). In briefly, an oscillating amplitude sweep between 0.1 Pa–1000 Pa at a frequency of 1 Hz was used to determine the linear viscoelastic region (LVER) of the samples. Once the LVER is determined from the amplitude sweep, a downwards oscillating frequency sweep from 10 Hz–0.1 Hz with a shear stress (Pa) within the LVER was then used in order to help understand the nature of the solutions concerning strength and stability. The experiments were repeated 4 times per solution, and for each experiment, each data point (20 data points per method) was optimised to repeat each measurement 5 times.

2.5. Scanning electron microscopy (SEM)

The morphology and diameter of individual fibres spun from PCL solution were determined from scanning electron micrographs