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Electrospun polyurethane nanofiber scaffolds with ciprofloxacin oligomer versus free ciprofloxacin: Effect on drug release and cell attachment

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ABSTRACT

An electrospun degradable polycarbonate urethane (PCNU) nanofiber scaffold loaded with antibiotic was investigated in terms of antibacterial efficacy and cell compatibility for potential use in gingival tissue engineering. Antimicrobial oligomer (AO), a compound which consists of two molecules of ciprofloxacin (CF) covalently bound via hydrolysable linkages to triethylene glycol (TEG), was incorporated via a one-step blend electrospinning process using a single solvent system at 7 and 15% w/w equivalent CF with respect to the PCNU. The oligomeric form of the drug was used to overcome the challenge of drug aggregation and burst release when antibiotics are incorporated as free drug. Electrospinning parameters were optimized to obtain scaffolds with similar alignment and fiber diameter to non-drug loaded fibers. AO that diffused from the fibers was hydrolysed to release CF slowly and in a linear manner over the duration of the study, whereas scaffolds with CF at the same concentration but in free form showed a burst release within 1 h with no further release throughout the study duration. Human gingival fibroblast (HGF) adhesion and spreading was dependent on the concentration and form the CF was loaded (AO vs. free CF), which was attributed in part to differences in scaffold surface chemistry. Surface segregation of AO was quantified using surface-resolved X-ray photoelectron spectroscopy (XPS). These findings are encouraging and support further investigation for the use of AO as a means of attenuating the rapid release of drug loaded into nanofibers. The study also demonstrates through quantitative measures that drug additives have the potential to surface-locate without phase separating from the fibers, leading to fast dissolution and differential fibroblast cell attachment.

1. Introduction

Periodontal disease is a chronic inflammatory disease that causes sensitivity, root caries, and tooth loss in adults. Almost half of adults over the age of 30 are affected [1]. Gingival recession occurs in adults regardless of oral hygiene, and is found in 58% of adults over 30 [2]. Autologous grafts taken from the palate are commonly used to repair gingival tissue defects due to periodontal disease or recession due to other factors. Gingival tissue engineering is a promising alternative strategy for the regeneration of soft tissue periodontium that has the potential to overcome several of the limitations associated with current regenerative therapies, including the increased pain and morbidity, as well as the potential tissue shortage associated with autologous grafts [3]. A tissue engineered gingival construct consists of a 3D scaffold seeded with fibroblasts which can be used to contribute to the reconstruction of the lamina propria and mediate epithelial cell morphogenesis [4–6].

Electrospinning has been used extensively to fabricate tissue engineering scaffolds from natural and synthetic polymers, with potential applications in periodontal tissue regeneration [7–9]. Electrospun fibers can be oriented into an aligned morphology to control bulk mechanical properties and the cellular response. Fiber alignment has been shown to promote cell alignment, and may also yield a more desirable fibroblast phenotype by promoting the production of extracellular matrix (ECM) molecules [10,11]. A degradable polycarbonate urethane (PCNU) synthesized with a hard-segment component consisting of hexane diisocyanate (HDI) and butane diol, and a soft segment polycarbonate diol (PCN) ([12,13]), has been electrospun into aligned nanofibers and coated with fibronectin to engineer connective tissues for spinal repair. The fibers formed a mechanically strong and elastic substrate for annulus fibrosis cell adhesion, phenotype maintenance and ECM

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accumulation while undergoing surface-mediated resorption [14,15]. The degradation by-products, which include CO_2 and hydroxyl containing molecules, along with hexane diamine, showed good biocompatibility with AF cells at the rate of release present in the study. Consequently, this scaffold platform showed potential as a gingival tissue engineering scaffold material.

The application of electrospun PCNU nanofiber scaffolds in the infectious oral environment potentiates the risk of a biomaterial-associated infection. As such, the feasibility of integrating anti-infective functionality within the scaffolds has been explored. Antibiotic-loaded nanofibers can be readily formed by blend electrospinning polymer and drug mixtures. However, the burst release commonly observed when antibiotics are incorporated directly into the polymer excludes their usefulness for longer-term applications [16]. Coaxial electrospinning, emulsion electrospinning and drug-loaded nanoparticle additives have all been used in an effort to slow down the release of drug [17]. Although core-shell fibers have been shown to enable more control over drug release, the strict arrangement of the electrospinning equipment in the coaxial method and the poor biocompatibility of emulsifiers in the emulsion method limit their biomedical applicability.

In the current work, an antimicrobial oligomer (AO) containing the antibiotic ciprofloxacin (CF) was provided in-kind and used to control antibiotic release from electrospun PCNU nanofibers fabricated via one-step blend electrospinning. The AO consists of two CF molecules covalently linked to triethylene glycol (TEG) via hydrolysable ester bonds (Fig. 1). The AO/PCNU blend fibers were hypothesized to control drug release as the AO must be hydrolysed for the CF to have any bioactivity. The AO was anticipated to further control drug release by promoting a more uniform distribution of drug within the PCNU scaffold matrix by increasing the strength or extent of interactions between the added antibiotic and the PCNU and minimizing interactions between the CF molecules themselves, thereby improving the compatibility of the blend system. Hence, the goal of this study was to determine if the AO can successfully prevent aggregation of CF, and to assess how the concentration of AO in the PCNU affects drug release character, antibacterial activity and gingival cell compatibility of the PCNU nanofiber scaffolds.

2. Materials and methods

2.1. Electrospun scaffold fabrication

Antimicrobial oligomer (AO) was produced and received in-kind from Interface Biologics Inc. (Toronto, Canada). AO is a oligomeric form of CF that hydrolyzes to form free CF at a controlled rate. PCNU was synthesized according to previously established methods with hexane diisocyanate (HDI, Sigma-Aldrich, Oakville, Canada) polyhexame thylene carbonate diol (PCN, Sigma-Aldrich) and butane diol (BD, Sigma-Aldrich) in a molar ratio of 3:2:1 (HDI:PCN:BD) [12]. The PCN was degassed and dissolved in anhydrous *N*,*N*-dimethylacetamide (DMAC, EMD Millipore, Etobicoke, Canada) and then reacted with HDI in the presence of dibutyltin dilaurate (DBDL, Sigma-Aldrich, ~1×10^{-3} mol catalyst/mol NCO) for 4 h at 60–70 °C to form a prepolymer. BD was then added to carry out a chain extension which proceeded overnight at 60–70 °C. The polymer was then precipitated in an ether/water solution (30% v/v) to wash the residual DBDL and unreacted prepolymer. The final polymer product was washed in water (5 × 3 h) and dried under vacuum for 72 h at 50 °C. The polystyrene equivalent weight average molecular weight and polydispersity was determined using gel permeation chromatography (GPC) with a refractive index detector (40 °C) and data acquisition software (Waters, Mississauga, Canada). The mobile phase was *N*,*N*-dimethylformamide (DMF; Sigma-Aldrich) with 0.05 M LiBr, and columns were maintained at 80 °C.

The PCNU and AO were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Aldrich) at concentrations corresponding to 0, 7 and 15% w/w equivalent CF with respect to the PCNU. A solution of PCNU and 15% w/w ciprofloxacin hydrochloride (free CF, Alfa Aesar, Ward Hill, MA, USA) was also prepared as a control. PCNU and free CF in HFIP formed a clear solution with no precipitate. The concentration of PCNU in HFIP ranged from 13.4–14.0% w/v. Concentrations were adjusted to maintain a similar solution viscosity between all samples, wherein the concentration of the solution was adjusted such that 0.1 mL of the polymer solution in a 1 mL syringe flowed through a 22-gauge needle in 64 ± 2 s under normal gravitational force. The conductivity of the electrospinning solutions was measured using a 2-cell conductivity probe (accumet AB30, Fisher Scientific, New Hampshire, USA).

The solutions were injected at a rate of 0.5 mL/h onto a cylindrical mandrel rotating at 9.25 m/s or 18.90 m/s (for PCNU alone and PCNU with AO or free CF, respectively). The needle had a positive charge of 17.5 kV (for PCNU alone) or 17 kV (for PCNU with AO or free CF). An 18 kV voltage difference between the needle and collecting surface was maintained. Scaffolds were approximately 50 μm thick. Residual HFIP was removed from scaffolds by drying under vacuum at 50 °C for 72 h. A separate scaffold was electrospun for each experiment repeat (N = 3).

2.2. Scaffold imaging

Scaffold fiber morphology was imaged using scanning electron microscopy (SEM). Scaffold sections were mounted onto steel stubs, sputter-coated to 10 nm with platinum using an SC515 SEC Coating Unit (Polaron Equipment, Uckfield, UK) and imaged using a Hitachi S2500 SEM (Hitachi, Mito City, Japan). Average fiber diameter and fiber alignment distribution were calculated using Image J image processing software (NIH, http://imagej.nih.gov/ij/). The widths of 15 fibers from each scaffold type were measured. The fiber alignment distribution was determined using the fast Fourier transform (FFT) processing technique in Image J [18]. The variance filter was applied to a representative image from each scaffold type to highlight the fiber edges. The images were then transformed to graphical representations of their frequency domains using the FFT function to extract directional information. A summation of the pixel intensities along a straight line from the centre of the FFT image for all 360° angles around the image was performed by using the Oval Profile plug-in (authored by Bill O'Connell, http://rsb.info.nih.gov/ij/plugins/oval-profile.html). Pixel intensity was then plotted from 0° to 180° to provide a graphical representation of the degree of fiber alignment.

The distribution of CF in the scaffolds and scaffold fibers was imaged using a Zeiss LSM700 confocal microscope (Advanced Optical Microscopy Facility, Toronto Medical Discovery Tower, Toronto, Canada). Dry

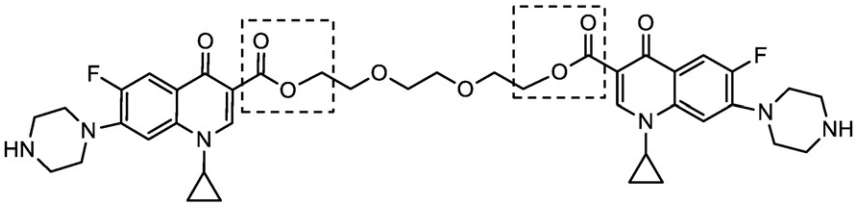

Fig. 1. The antimicrobial oligomer (AO). The AO is in the form of a trimer and consists of two CF molecules covalently linked to TEG via hydrolysable ester bonds (indicated with dashed boxes).