In vitro Degradation and Physical Characterization of Antimicrobial Electrospun Polyurethane Scaffolds

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Introduction: Electrospun scaffolds may be used to generate tissue engineered constructs for the regeneration of the gingival connective tissues that are destroyed during the progression of periodontal disease. However, the use of a synthetic material in the infectious oral environment has the potential to lead to a biomaterial-associated infection[1]. Antibiotic incorporated directly into electrospun scaffolds impose challenges due to drug aggregation that can affect the physical properties of the scaffold, and result in a burst release of antibiotic[2]. Polymer-based antimicrobial delivery systems have been explored as a means to deliver drug in a more sustained, controlled manner[3]. The objective of the current study was to incorporate an antimicrobial polymer containing ciprofloxacin into aligned electrospun nanofiber scaffolds and to characterize the scaffolds' properties. It is hypothesized that the antimicrobial polymer will promote a uniform distribution of drug throughout the fibers such that as the antimicrobial polymer and scaffold matrix each degrade by hydrolysis, there will be a sustained release of antibiotic.

Methods: Scaffolds were made using a degradable polyurethane (PU), synthesized with hexane diisocyanate:polycarbonate diol:butane diol in a molar ratio of 3:2:1[4]. A proprietary antimicrobial polymer (AP) was incorporated via blend electrospinning at concentrations corresponding to 7 and 15wt% equivalent ciprofloxacin (CF) with respect to the PU. Scaffolds with 15wt% free CF HCl were also fabricated. PU or PU with AP or CF materials were dissolved in hexafluoro-2-propanol and injected at a rate of 0.5 ml/h onto a cylindrical mandrel rotating at 1150 rpm (18 kV voltage difference). Matrix degradation and antimicrobial release studies were carried out in Dulbecco's PBS at 37°C for 28 days. AP and CF release were measured by high performance liquid chromatography with a photo-diode array detector. Fiber morphology was investigated using scanning electron microscopy (SEM). The distribution of CF in the fibers was imaged using confocal microscopy. Scaffold microstructure was investigated via differential scanning calorimetry. The static water contact angle (WCA) on the fiber scaffolds was measured.

Results and Discussion: The release of antimicrobial containing molecules is sensitive to the concentration loaded into the fibers. At 28 days, the scaffolds with 7wt% antimicrobial had a cumulative release of 11.21±4.02% relative to the total loaded AP, while the 15wt% scaffolds had a cumulative release of 102.97±14.05% (n= 3±SD). An increase in the wettability of the 15wt% antimicrobial scaffolds due to a higher hydrophilic character (significantly different WCA at 82.4±4.1° for 7wt% vs 67.2±8.8° for 15wt% antimicrobial scaffolds, $p < 0.05$, n=5) may have increased the penetration of water and led to a greater drug diffusion rate. Release of AP from the 7wt% antimicrobial scaffolds may in turn be dependent on degradation of the PU scaffold to allow for AP diffusion. The degree of scaffold degradation is being investigated in on-going studies. The 15wt% CF HCl scaffolds released 98.30±7.93% of the loaded antimicrobial within 1 hour, while the scaffolds with 15wt% antimicrobial in polymeric form had released 67.68±2.43% of the AP ($p<0.05$). The AP was hydrolysed to release CF slowly over the 28 day study (**Fig. 1**), while the scaffolds with CF HCl had a burst release of CF at 100x the concentration of the AP scaffolds. There was greater surface roughness visible on the fibers of scaffolds with CF HCl when compared to scaffolds with AP (SEM images not shown), and confocal microscopy revealed aggregated fluorescent CF in the 15wt% CF HCl fibers and outside the fibers in clumps (white arrows) (**Fig. 2**). Drug aggregation would increase the rapid diffusion of antibiotic from the scaffolds, and may explain the fast burst release of drug from the 15wt% CF HCl scaffolds. Glass transition temperatures were similar between scaffolds, so it is unlikely that differences in scaffold microstructure contributed to differences in antimicrobial release (data not shown).

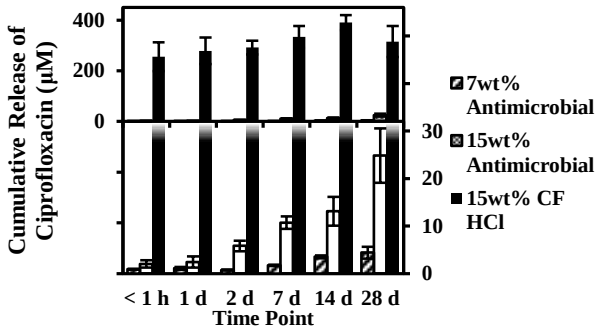

Figure 1. Cumulative release of CF in μM (n=3±SD).

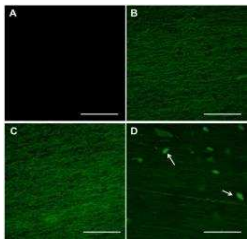

Figure 2. Ciprofloxacin distribution in the scaffold fibers. A) 0wt% antimicrobial, B) 7wt% antimicrobial (AP), C) 15wt% antimicrobial (AP) and D) 15wt% CF HCl. Scale bars = 50 μm.

Discussion and Conclusions: The results indicate the ability to use the AP to generate electrospun scaffolds with a slow and sustained (>28 days) release of CF. Future work will assess the antibacterial activity of the scaffolds against characteristic bacteria.

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