Introduction

Electrospun scaffolds may be used to generate tissue engineered constructs for the regeneration of 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]. Different methods have been explored as a means to slow the burst release of loaded drug including coaxial electrospinning [3], emulsion electrospinning [4], and electrospinning using nanoparticles for drug encapsulation [5]; however, these methods significantly complicate
the electrospinning fabrication process. Antimicrobial polymers have previously been explored as a means to deliver drug in a more sustained, controlled manner [6]. The objective of the current study was to incorporate an antimicrobial polymer containing ciprofloxacin into aligned electrospun polyurethane nanofibre scaffolds via a one-step blend electrospinning protocol and to characterize the scaffolds’ properties. It is hypothesized that the antimicrobial polymer will promote a uniform distribution of drug throughout the polyurethane fibres such that as the antimicrobial polymer and scaffold matrix each degrade by hydrolysis, there will be a sustained release of antibiotic.

Materials and 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 [7]. 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 PBS at 37°C for 28 days. AP and CF release were measured by high performance liquid chromatography (HPLC) with a photo-diode array detector. The amount of surface CF on the fibres was investigated by measuring the atomic concentration of fluorine on the surface of the scaffolds using angle-resolved x-ray photoelectron spectroscopy (XPS). Take-off angles of 20°, 40° and 60° corresponding to 3.4 nm, 6.4 nm and 8.7 nm from the surface, respectively, were used for the study.

Results

Fig. 1 shows the cumulative concentration of AP released from the 7 and 15wt% antimicrobial scaffolds in the system throughout the 28 day study. It is evident that the rate of release for the AP is sensitive to the concentration loaded into the
fibres. Since fibre alignment and diameter were maintained the same for all scaffold types, the disparity in release rate between the 7 and 15wt% antimicrobial scaffolds cannot be attributed to differences in fibre surface area causing diffusion-mediated release differences. It has been reported that drug may localize on the surface of electrospun fibres due to charge accumulation of an ionic drug molecule [8], and so it was hypothesized that surface segregation of CF may be playing a role in the drug release rate differences observed between the low and high concentration antimicrobial scaffolds. Atomic fluorine concentrations measured at varying degrees of surface sensitivity revealed a greater than theoretical bulk concentration of fluorine on the surface of the 7 and 15wt% antimicrobial fibres, as well as for the 15wt% CF HCl fibres (Fig. 2). A surface sensitive gradient was evident for the 15wt% antimicrobial scaffold fibres, suggesting a more exaggerated segregation of drug at the high concentration of AP. The polar drug on the surface of the fibres was found to increase the hydrophilicity of the antimicrobial scaffolds. An increase in the wettability for 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 also be contributing to an increased drug diffusion rate due to a greater penetration of water. 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.) As the study progressed, the released AP was hydrolysed to slowly release CF, while the scaffolds with CF HCl had a burst release of CF at 100x the concentration of the AP scaffolds (Fig. 3). Using scanning electron microscopy (SEM) and confocal microscopy, drug aggregation was previously shown to be evident on the surface of the 15wt% CF HCl fibres and outside the fibres in clumps. Such aggregation would have increased the rapid diffusion of antibiotic from the scaffolds, and may explain the fast burst release of drug from the 15wt% CF HCl scaffolds.

Discussion and Conclusion

The results indicate the ability to use the AP to generate electrospun scaffolds with a slow and
sustained (>28 days) release of CF via a simple one-step blend electrospinning process. Future work will assess the antibacterial activity of the scaffolds against characteristic bacteria and the cell compatibility of the scaffolds using human gingival fibroblasts.

Figure 1. Total concentration of cumulatively released antimicrobial polymer (µg/mL) at each time point for the antimicrobial polymer polymer scaffolds (n=6±SD).
Figure 2. Surface fluorine concentration (at%) of the scaffold fibres at varying take-off angles (where 20° is the most surface sensitive) compared to the theoretical concentration as calculated using statistical methods ($n=3 \pm SD$, * represents statistically significant difference, ANOVA, $p<0.05$)
Figure 3. Cumulative release of ciprofloxacin in μM (n=6±SD).

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References