Peptide-Delivering Fibro-porous Mats to Accelerate Skin Regeneration

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Statement of Purpose: Although burn wound care has advanced over the years, there is still a critical need to develop treatments that prevent burn injury progression and promote robust burn wound healing [1]. To achieve this goal, the development of a topical, biocompatible therapy that can prevent cell death, rapidly recruit host tissue cells, and stimulate tissue regeneration is required [2]. A novel peptide has recently been elucidated by the Clark laboratory at Stony Brook University and shows significant promise in the treatment of burns. This peptide provided the necessary protection of human dermal fibroblasts against oxidative and cytokine stress and reduced burn injury progression in a rat hot comb burn model (unpublished). Thus, we hypothesize that the incorporation of this peptide into a biodegradable polymer matrix will produce a controlled delivery of the peptide over a desired period of time. To this end, tyrosine-derived polymers were chosen due to their biocompatibility, tailorable resorption kinetics, and processability [3]. In the current study, two polymers with different compositions were evaluated for their ability to (a) produce electrospun fibro-porous mats containing peptide; (b) provide optimal release profiles of the peptide; and (c) accelerate granulation tissue formation and minimize scarring in a porcine full-thickness excisional wound model.

Methods: The fibro-porous mats were fabricated from the poly(DTR-co-y% DT-co-z% PEG_x carbonate) family (Figure 1). Polymer solution was prepared in glacial acetic acid and electrospun to produce either unloaded or peptide-loaded fibro-porous mats. The morphology (size and surface) of mats was assessed using a SEM. Fibro-porous mats were incubated in 1 mL of buffer (pH = 7.4) at 37 °C. At each time point, the resorption of the fibro-porous mats was evaluated using GPC and peptide release was measured using HPLC.

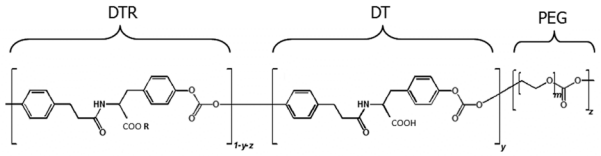

Figure 1. General structure of tyrosine-derived polycarbonates: DTR - desaminotyrosyl-tyrosine alkyl ester, DT - desaminotyrosyl-tyrosine, PEG - poly(ethylene glycol).

Results: Experimental conditions such as polymer composition, peptide input and compatibility with polymer solvent, and various electrospinning parameters were explored to produce optimal peptide-containing fibro-porous mats. Hexafluoroisopropanol induced a conformational change in the peptide (determined by CD) that prevented its incorporation into the fibro-porous mats resulting in 5% binding compared to the 90% binding obtained with acetic acid. The mats showed significantly different resorption profiles dependent on the polymer compostion: 50-mol% DT (DTR-50) resorbed within 8 hours while 25-mol% DT (DTR-25) resorbed in 4 days. The DTR-25 polymer produced slightly larger fibers (1.2 $\pm$ 0.4 μm) than DTR-50 (1.0 $\pm$ 0.4 μm) at the same 0.06% w/w peptide loading (Figure 2-A v. 2-B). However, further increase in pepide loading to 0.6% w/w produced smaller fibers in both polymers (DTR-25: 1.1 $\pm$ 0.2 μm, Figure 2-C). Similar peptide binding efficiency was measured regardless of the polymer composition: 90 $\pm$ 3 and 82 $\pm$ 6% in DTR-50 and DTR-25 mats, respectively.

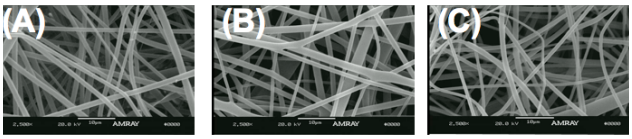

Figure 2. SEM of peptide-loaded fibro-porous mats (w/w): DTR-50 with 0.06% of peptide (A); DTR-25 with 0.06% (B) and 0.6% (C) of peptide. Scale bar: 10 μm.

The polymer composition showed a profound effect on the performance of the fibro-porous mats. The DTR-50 mats released 36% of the peptide within the first 0.5 hours and a total of 70% within the first 2.5 hours of incubation (Figure 3). The DTR-25 mats showed a burst release of peptide (63%) within the 24 hours, while the remaining peptide was continuously released over the 4 days (Figure 3). In addition, the release profiles parallel the resorption rates of the fibro-porous mats (not shown).

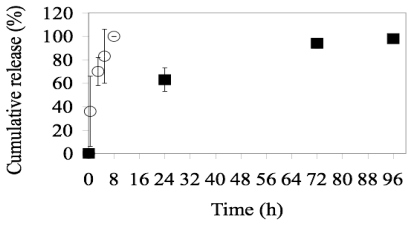

Figure 3. Peptide release profiles from electrospun (○) DTR-50 and (■) DTR-25 fibro-porous mats.

Conclusions: Peptide-loaded fibro-porous mats were fabricated with desired fiber morphology and high peptide loading. Control of the peptide dose delivered to the injured site can be achieved using different polymer compositions of fibro-porous mats. High DT-containing mats resorb significantly faster and thus, most of the peptide is rapidly released to the injured site. Reducing the DT content slows both the polymer resorption and kinetics of peptide release. In the latter case, a more sustained therapeutic treatment is applied. Fibro-porous mats containing varying doses of the peptide are currently being evaluated for their effects on granulation tissue formation and scarring in a porcine full-thickness excisional wound model.

References: *[1]* Singer, A et al. Am J of Emer Med. 2007;25:666-671; *[2]* Macri, L et al. Skin Pharmacol Physiol. 2009;22:83-93; *[3]* Bourke, SL et al. Adv Drug Deliv Rev. 2003;55:447-466.

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