Characterization of DNA Release from Composites of Oligo(poly(ethylene glycol) fumarate) and Cationized Gelatin Microspheres In Vitro

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Introduction: A primary advantage presented by cationized gelatin in DNA release applications is the formation of electrostatic complexes between the cationized gelatin and plasmid DNA.[1-2] Complexation of released DNA with degradation fragments of cationized gelatin may reduce degradation of the DNA by nucleases and may facilitate cellular entry through interaction of the positively charged complexes with cell membranes.[2] However, the duration of plasmid DNA release from cationized gelatin and cationized gelatin microspheres (CGMS) is limited by the enzymatic degradation of the matrix.[1-2] A potential method to retain the benefits provided by cationized gelatin, while extending the duration of the release of DNA and the persistence of the scaffold may be found in the formation of composites of CGMS with oligo(poly(ethylene glycol) fumarate) (OPF). The objectives of the present study were: (1) to characterize the release of plasmid DNA in vitro from composites of OPF and CGMS and from control hydrogels of OPF alone, and (2) to characterize the swelling and degradation of these materials in vitro.

Methods: OPF hydrogels and composite hydrogels of OPF and CGMS were prepared with plasmid DNA loaded into either the OPF or the CGMS component following established procedures.[3-4] Respective control samples containing no DNA were prepared similarly. Hydrogel samples (8 mm in diameter and 2 mm in thickness), were housed individually in 3 ml of phosphate buffered saline (pH 7.4) with 373 ng/ml of bacterial collagenase 1A and were agitated on a shaker at approximately 70 rpm at 37°C for the duration of the study. The release solution was completely removed individually from the samples and replaced with fresh solution at periodic time points. The amount of double-stranded DNA (dsDNA) and total DNA in the release solution aliquots was quantified fluorescently with PicoGreen dsDNA and OliGreen ssDNA Quantitation Reagents (Molecular Probes, Eugene, OR), respectively. The cumulative fraction DNA release for each specimen was normalized with respect to the volumetrically calculated initial DNA content. The swelling ratio of the hydrogels at each time point was calculated as,

$$\text{Swelling Ratio} = (W_w - W_i)/(W_i),$$

where W_w is the wet weight of the sample and W_i is the initial weight of the sample immediately after fabrication. Complete degradation of a hydrogel sample was noted visually when the presence of the disk or fragments of the disk were no longer apparent.

Results / Discussion: As show in Fig. 1, it was found that plasmid DNA can be released in a sustained fashion over the course of 49 to 140 days in vitro, with the release kinetics depending upon the material composition and the method of DNA loading. The swelling ratio reflects the change in the mass of a gel at each time point with respect to the initial mass of the gel. As a hydrogel degrades, the wet weight (W_w) of the gel approaches zero and the swelling ratio approaches a value of -1. Indeed, the swelling ratio of all the gels in the present study reached final values of approximately -1 (Fig. 2). Comparison of the swelling ratio of the gels within a group (Fig. 2) with the corresponding profile of DNA release (Fig. 1) demonstrates a strong relationship between the release of DNA and the swelling ratio of the gels.

Figure 1: Plasmid DNA Release (n=6)

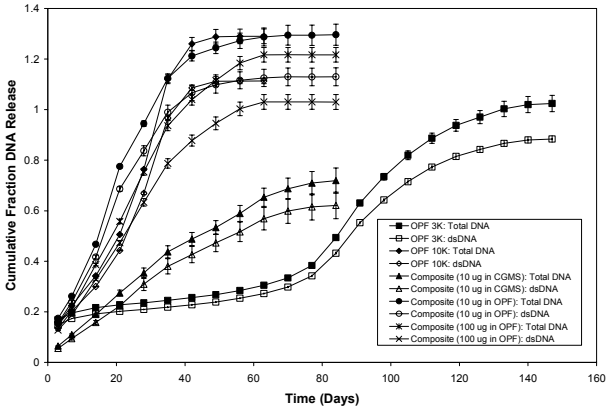
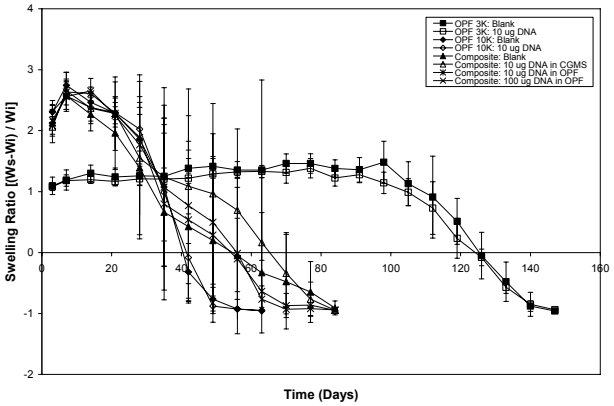


Figure 2: Hydrogel Swelling Ratio (n = 6)



Conclusions: Plasmid DNA can be released from hydrogel composites of OPF and CGMS in a controlled, sustained manner, with the release kinetics depending upon the material composition and the method of DNA loading. The swelling ratios reflect the degradation of the hydrogels and correspond directly with the observed release within each group. The results suggest that the DNA release from hydrogel composites of OPF and CGMS is dominated by the degradation of the OPF component of the gels

References:

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