Porous EH and EH-PEG Scaffolds as Gene Delivery Vehicles to Skeletal Muscle

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Statement of purpose: Cyclic acetal polymers show promise in tissue engineering applications as biodegradable biomaterials that reduce the acidic byproducts associated with other materials[1-3]. We created cyclic acetal porous single component and bi-component scaffolds from 5-ethyl-5-(hydroxymethyl)-β,β-dimethyl-1,3- dioxane-2-ethanol diacrylate (EHD) and polyethylene glycol (PEG). These EH and EH-PEG scaffolds, respectively, were used as a delivery device for a therapeutic plasmid that produced an insulin-like growth factor-1 fused to green fluorescent protein (IGF-1 GFP).We hypothesized that the modifying the scaffolds' surface and bulk architectures would provide a controlled system for the non-viral delivery of a therapeutic IGF-1 GFP plasmid. Our objectives were to evaluate the impact of scaffold architecture on plasmid release and transfection of human skeletal muscle myoblast cells (hSkMMs).

Materials, Methods and Analytical Procedures Used: Single (EH) and multi-component (EH-PEG) scaffolds were fabricated with 5-ethyl-5-(hydroxymethyl)-β,β-dimethyl-1,3-dioxane-2-ethanol diacrylate (EHD) and PEG diacrylate (PEGDA). FuGENE HD transfection reagent was used to transfect hSkMMs with IGF-1/GFP plasmid. Transfection efficacy was evaluated by hSkMMs expression of IGF-1 and GFP using an IGF-1 ELISA and FACS, respectively. To determine plasmid integrity after scaffold release a 1% agarose gel electrophoresis (AGE) was used. Scaffold adsorption mapping and plasmid release were evaluated with picogreen.

Summary of Results: Plasmid released from the scaffolds was determined to be intact and contained the IGF-1 gene (Fig. 1). The physical loading characteristics of each scaffold showed that the EH scaffold had 0.285%, 1.032% and 1.139% of the total DNA per mm^2; compared to the EH-PEG scaffold which had 0.43%, 0.7%, and 1.05% of the total DNA per mm^2 in the outer ring, middle ring and inner disc. (Fig. 2). This showed that the plasmid was distributed throughout the scaffold with increasing amounts of DNA toward the center of the scaffold and the formation of the PEG layer on the EH scaffold allowed for more of the DNA to migrate through the porous network. Successful hSkMMs transfection was observed by increased levels of in GFP and IGF-1 expression in transfected hSkMMs compared to the control hSkMMs (Fig. 3). Compared to control hSkMM at 1.2% GFP expression the transfected hSkMMs had 11.1% GFP expression (Fig. 3) as well as a statistically significant IGF-1 expression compared to the control hSkMMs.

Conclusions: We have shown that intact IGF-1 GFP plasmids are released by EH and EH-PEG scaffolds and by modulating the surface architecture of the scaffolds and by using differing porosities and scaffold bi-layer architectures, we improved plasmid distribution throughout the scaffold. This result combined with the successful transfection of hSkMMs lead us to believe that manipulation of the EH scaffold architecture could be used to tailor release rates for the delivery of IGF-1.

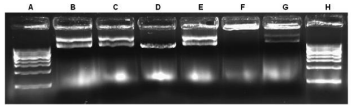

Figure 1: 1% AGE (A) 1 kb DNA ladder, (B) plasmid stock, (C) plasmid in Opti-MEM and loading dye, (D) *EcoNI* digested plasmid (E) plasmid released from scaffold, (F) FuGENE complex, (G) FuGENE complex released from scaffold, (H) 1kb DNA ladder

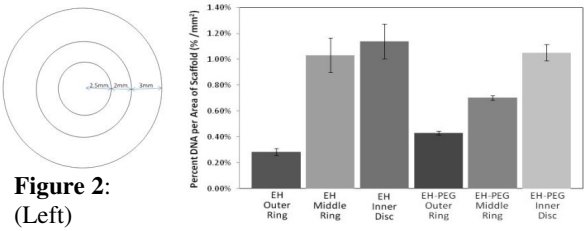

Figure 2: (Left) Concentric ring structure of EH and EH-PEG scaffolds. (Right) Distribution of DNA within scaffold geometry.

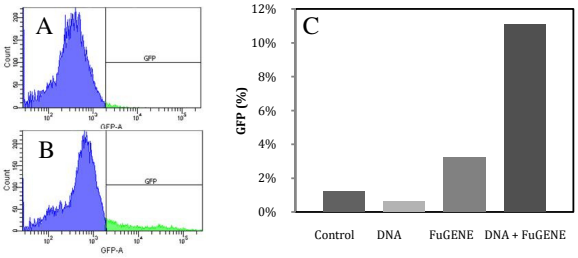

Figure 3: (A) FACS histogram of control hSkMM sample expressing GFP. (B) FACS histogram of the transfected hSkMM sample expressing GFP. (C) GFP expression of control, DNA only, FuGENE only and transfected hSkMM cells (DNA+FuGENE).

References:

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