Designer growth factor gradients produced by microsphere-assembled scaffolds

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Statement of Purpose: The formation of complex gradients in growth factors, drugs, cell adhesion peptides, degradability, mechanical properties and even cells may be useful for a variety of tissue engineering applications. We have previously developed novel methods to form micron-scale poly(ethylene glycol) (PEG) microspheres with different functionalities (cell adhesion, degradability, drug delivery).[1] The microspheres are able to form scaffolds following centrifugation. Successive centrifugation of microspheres loaded with different amounts of growth factor, heparin and crosslink density potentially allow the release kinetics to be tuned in space and time with exquisite precision. We demonstrate sustained release of the growth factor GDNF from multi-tiered scaffolds for at least 12 days, with excellent agreement between theoretical predictions and the measured GDNF spatial distribution/release kinetics.

Methods: Recombinant human GDNF (Peprotech, Rocky Hill, NJ) was dissolved in 8 mM sodium phosphate buffer (pH 7.4). Dylight-488 was added to the solution for a final GDNF concentration of 10 µg/mL and a final Dylight-488 concentration of 50 ng/mL and incubated overnight at 10˚C. The solution was then dialyzed using Slide-A-Lyzer MINI Dialysis Units in 8 mM sodium phosphate buffer (pH 7.4) to remove unbound Dylight-488. To form microspheres, PEG-octaamine (PEG_8-amine, MW 10K) was combined with PEG-octavinylsulfone (PEG_8-VS, MW 10K) at a 1:2 mol ratio and diluted to 20 mg/mL PEG with PBS and PBS + 1.5 M sodium sulfate to a final sodium sulfate concentration of 0.6 M.[1] The PEG_8-VS/PEG_8-amine solutions were then incubated above the cloud point at 70˚C for various times. Microspheres were subsequently buffer exchanged into PBS 2x to remove the sodium sulfate. Heparin (50 mg/mL) was activated via a 30 minute, room temperature incubation in a 500 mM EDC, 12.5 mM NHS solution. Activated heparin was immediately added to 200 mg/mL PEG_8-amine at a 1:20 or 1:160 mass ratio. Heparin-PEG_8-amine solution was used with PEG_8-VS to form microspheres as described above. Vinyl sulfone groups were quenched by incubation with cysteine solutions (1 mg/mL) overnight. The PEG-heparin microspheres were either immediately used to form scaffolds or first incubated with Dylight 488-labeled GDNF (250 ng/mL) overnight. To aid in fluorescence microscopy, scaffolds were formed in glass Pasteur pipettes. To prevent adhesion of the microspheres to the glass, Pasteur pipettes were first filled with 2% PLL-g-PEG solution, incubated for 30 seconds, and washed with DI water. The tips of the glass pipettes were then sealed with silicone aquarium sealant. To form scaffolds, microspheres were resuspended in buffer and added to the pipette. The pipette was placed in a 15 mL conical vial and spun at 1000 *g* for 10 min for each tier and then incubated at 37˚C for different times before imaging by confocal microscopy.

Results: GDNF release from a two-tiered scaffold made from PEG/heparin microspheres is shown in Figure 1.

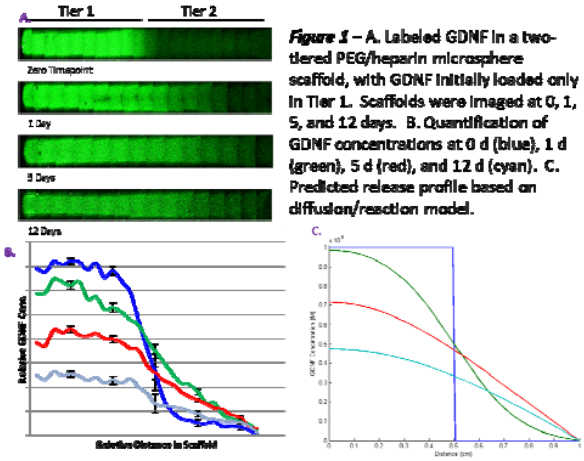

Figure 1 **– A. Labeled GDNF in a two-tiered PEG/heparin microsphere scaffold, with GDNF initially loaded only in Tier 1. Scaffolds were imaged at 0, 1, 5, and 12 days. B. Quantification of GDNF concentrations at 0 d (blue), 1 d (green), 5 d (red), and 12 d (cyan). C. Predicted release profile based on diffusion/reaction model.**

GDNF was loaded only in the lower tier (green) and the upper tier initially appears dark. The growth factor is released out of the right side of the scaffold. The GDNF concentration profile is shown at 0, 1, 5 and 12 days. The measured release kinetics are shown, and deviate greatly from release profiles expected for a homogenous scaffold.

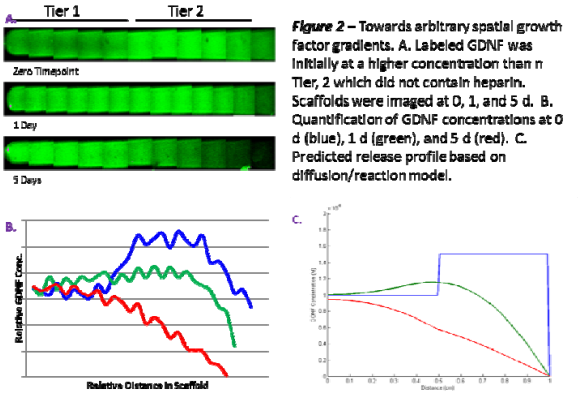

Figure 2 **– Towards arbitrary spatial growth factor gradients. A. Labeled GDNF was initially at a higher concentration than n Tier, 2 which did not contain heparin. Scaffolds were imaged at 0, 1, and 5 d. B. Quantification of GDNF concentrations at 0 d (blue), 1 d (green), and 5 d (red). C. Predicted release profile based on diffusion/reaction model.**

To demonstrate more arbitrary growth factor concentration profiles, we used microspheres without heparin in Tier 2, but loaded more GDNF within this tier. A concentration profile with a maximum in the middle was generated. We have also produced up to four tiered scaffolds, as shown in Figure 3.

Figure 3 **- Four tier scaffold with GDNF gradient at day 0 and day 1**

Conclusions: We have demonstrated that scaffolds assembled from hydrogel microspheres may be used to generate complex spatial profiles of a diffusible growth factor with designable release kinetics.

References: (1) Roam, J.L., *Biomaterials* 31 (2010) 8642-8650