Controlled release of sphingosine 1-phosphate, a potent stimulator of endothelial cell migration

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Statement of Purpose: Sphingosine 1-phosphate (S1P) is a signaling lipid that is abundantly stored in platelets and released upon platelet activation. S1P accounts for the majority of the chemokinetic activity of serum, via signaling through a family of G protein-coupled receptors that lead to activation of Akt and Rac.[1] Knockout of the main receptor for S1P is embryonic lethal, due to incomplete vascular maturation.[2] S1P has also been reported to be a complete angiogenic factor.[1] Here, we demonstrate controlled release of S1P from polyethylene glycol hydrogels crosslinked with albumin. Albumin is a transporter of S1P in the blood,[3] and high affinity binding of S1P by albumin may provide a mechanism for controlled release. These new S1P-releasing materials may be useful to promote the endothelialization of vascular grafts or as angiogenic scaffolds for tissue engineering.

Methods: PEG-octavinylsulfone (PEG-OVS) was produced from 8-arm PEG (Shearwater Polymers, Huntsville AL). NMR indicated 75% conversion of end-groups to vinylsulfone. PEG-OVS was crosslinked by reaction with fatty acid free albumin that had been previously loaded with S1P. As a control, PEG-OVS was crosslinked with PEG-diamine. [^{32}P]S1P was produced by incubating sphingosine with sphingosine kinase in the presence of [^{32}P]-ATP. The S1P was purified by extraction into chloroform/methanol. Thin layer chromatography demonstrated complete conversion of sphingosine to S1P. The chick chorioallanotoic membrane (CAM) assay was performed as follows. At embryonal day 6, the top of the egg was removed. A PEG hydrogel was placed onto the CAM. Controls (bFGF or S1P) were spotted onto Thermanox coverslips (Nunc), air dried and placed onto the CAM. The eggs were sealed with parafilm and examined after two days of incubation.

Results / Discussion: PEG/albumin/S1P materials derivatized with RGD peptide led to increased rates of endothelial cell migration on the surface of the PEG materials (Figure 1).

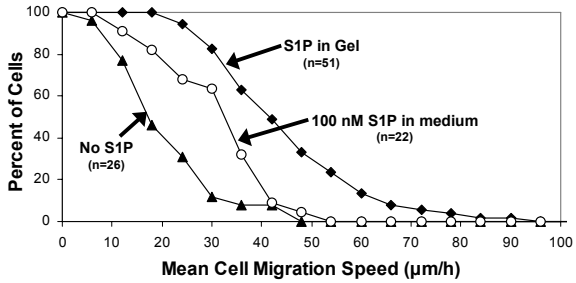

Figure 1: S1P was dissolved in PBS containing 17.5% fatty acid free-BSA (1 nmol S1P per mg of albumin). PEG hydrogels (50 μL) were formed in the wells of a 24-well plate by reacting PEG-OVS with the albumin/S1P solution, with 5 nmol S1P present in each gel. The migration speed of individual HUVEC on PEG/albumin hydrogels containing RGD peptide was measured in the presence or absence of S1P. Delivery of S1P from the hydrogel increased the mean migration speed of the HUVEC from 19.2 ± 10.1 μm/h to 43.8 ± 15.7 μm/h. ($p = 3x10^{-10}$, gel + S1P vs. gel). Error bars represent standard deviations.

In the CAM assay, controls consisting of 50 ng bFGF or 50 ng S1P spotted on coverslips led to strong angiogenic responses. PEG/albumin hydrogels alone led to no angiogenic response. PEG-OVS/PEG-diamine hydrogels incubated with S1P and then washed with buffer for 24 h led to a modest angiogenic responses. However, PEG/albumin hydrogels incubated with S1P and washed buffer for 24 h led to strong angiogenic responses. This demonstrated that albumin was necessary to load S1P within the hydrogels.

Controlled release of S1P was demonstrated by crosslinking albumin loaded with [^{32}P]S1P (Figure 2). Fickian release kinetics were observed, although the exact mechanism for controlled release is still under investigation.

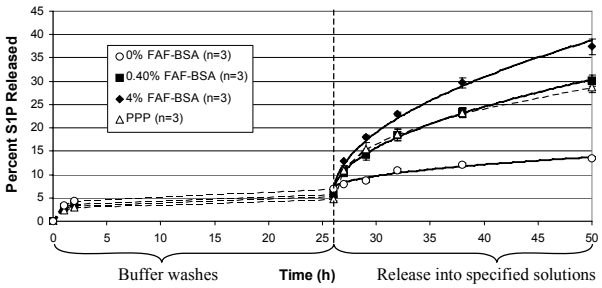

Figure 2: PEG/albumin/S1P hydrogels were formed as described in Fig. 1. The rate of S1P release was measured using [^{32}P]S1P. Approximately 5% of the S1P in the hydrogel was released into PBS washes over 26 h. The gels were then washed with solutions containing different concentrations of fatty acid free albumin, or platelet poor plasma. The amount of S1P released increased as the solution concentration of albumin increased. Dashed lines connect data points, solid lines show least-squares fit of the data to a solution of the unsteady diffusion equation. A single parameter was adjusted in the model, an effective diffusion coefficient. Error bars represent standard deviations.

Conclusions: The PEG materials described here provide for controlled release of S1P due to the inclusion of the lipid-binding protein albumin in the hydrogel. Endothelial cell migration is enhanced on S1P-releasing materials containing RGD peptides and an angiogenic response was observed in the CAM assay.

References:

1. English et al., FASEB J **14** (2000) 2255-2265.
2. Paik et al., Genes & Development 18 (2004) 1-12
3. Murata et al., Biochem. J. 353 (2000) 809-815