Sequential Delivery of VEGF and S1P in an Angiogenesis Model

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Introduction: Angiogenesis is an organized series of events, beginning with vessel destabilization, followed by endothelial cell proliferation and migration, ending with vessel maturation. Vascular endothelial growth factor (VEGF) is thought to be important in vascular permeability and endothelial cell proliferation, and migration (early stage angiogenesis)(1), while sphingosine 1-phosphate (S1P) is known to stimulate vascular stability (late stage angiogenesis)(2). For this reason, we hypothesized that inducing angiogenesis by sequentially delivering angiogenic growth factors, controlling their presence and absence, would better mimic the temporal role of each factor during the progression of native angiogenesis in situ. To this end, we utilized a delivery system based on porous cellulose hollow fibers that, for the first time, permits sequential delivery of an early stage factor followed by a late stage growth factor *in vivo*, where previous attempts have only results in different rates of delivery. Our delivery system addresses the idea that factors involved in one stage of angiogenesis may inhibit other stages of angiogensis, causing absence of one factor to be just as important as the presence of another factor. Using a modified murine Matrigel plug model, it is apparent that delivery strategies where VEGF alone is delivered before S1P alone not only lead to greater recruitment of endothelial cells, but also higher maturation index of associated vessels. Sequential delivery was also optimized by examining varying delivery schedules. Sequential delivery strategies such as this one have potential to improve wound healing strategies involving angiogenesis as well as other types of tissue formation that occur in a series of organized stages.

Materials and Methods:

Hollow Fiber Fabrication. Cellulose was used as a model, dissolvable material. Polymer solution and antisolvent are injected separately into a coaxial nozzle for extrusion. Beginning in the air gap, and continuing into an antisolvent bath, solvent is extracted from the polymer solution, precipitating a porous, hollow fiber.

Sequential Delivery In Vivo. Growth factor reduced Matrigel was injected into the subcutaneous dorsal space in C57BL/6 mice (left and right flank). A 12G needle was used to thread cellulose hollow fibers through the skin and Matrigel plugs. On the day of implantation and every day for the next 6 days, hollow fibers on the left side were injected with saline (internal negative control), and hollow fibers on the right side were injected with 10µL of an angiogenesis promoting factor: 100µg/mL VEGF and/or 1800µM S1P. In the sequential delivery groups, factor switching occurred on the third day after implantation, following five rinses with saline. Cross-sectionsof the Matrigel plugs were analyzed for cellular infiltration and cell phenotype.

Results:

FIGURE 1. *Delivery of VEGF followed by S1P results in a greater recruitment of CD31+ cells than other delivery schedules.* (a-f). Immunoflourscent staining of CD31 (green) and nuclei (blue) in Matrigel plug crossections, scale bar=100µm. (a) Saline. (b) VEGF (100µg/mL). (c) VEGF (100µg/mL), followed by S1P (1800µM). (d) S1P (1800µM). (e) S1P (1800µM), followed by VEGF (100µg/mL). (f) VEGF (100µg/mL) and S1P (1800µM).

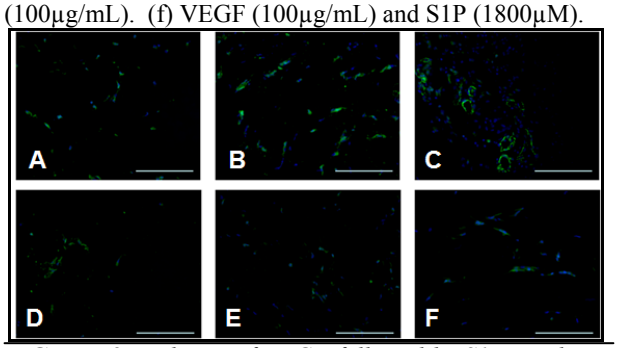
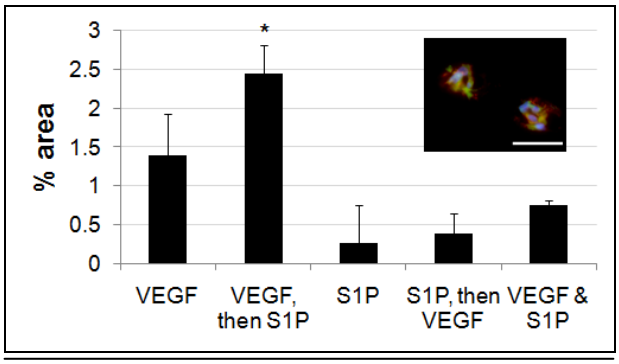


FIGURE 2. *Delivery of VEGF followed by S1P results in greater colocalization of CD31 and □SMA than other delivery schedules.* Maturation index calculated by the percent of CD31+ blood vessel that are colocalized with αSMA staining in areas where CD31+ blood vessels were observed. Inset: colocalization of CD31+ and αSMA+ in plugs where VEGF delivery is followed by S1P delivery. *p<0.035 when compared to all other groups.



Conclusion: We have created a system capable of exploring true sequential delivery of growth factors, where only one factor is presented at a time. When using this system to explore sequential delivery of VEGF and S1P for the purpose of promoting angiogenesis, we demonstrated that delivery of VEGF followed by delivery of S1P resulted in recruitment of more endothelial cells and a higher maturation index than the reverse sequential delivery schedule, single factor delivery or dual delivery. We also optimized the delivery schedule according to the day at which we switch factors. This system can be used to explore any number of delivery schedules, allowing for a facile way to explore different delivery schedules of growth factors in vivo for therapeutic responses as well as for studying the basic biological signals that accompany stage-wise regeneration of tissues.

References:

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