Regulation of *In Vivo* Angiogenesis by Heparin-controlled Dual Growth Factor Release from Hyaluronan Hydrogels

R. A. Peattie[1], P.W. Fuegy[1], C.M. Riley[1], S. Cai[2], B. Yu[2], R.J. Fisher[3], M.A. Firpo[4], X.Z. Shu[2], G.D. Prestwich[2].
1. Dept. Of Chemical Engineering, Oregon State University, Corvallis, OR 97331.
2. Center for Therapeutic Biomaterials, Dept. Of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84108.
3. Dept. of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139.
4. Dept. Of Surgery, University of Utah School of Medicine, Salt Lake City, UT 84132.

Statement of Purpose: Synthetic biopolymer matrices can serve as cytocompatible scaffolds for cell and tissue growth provided that functional capillary networks can be developed within the matrices. In previous studies[1,2], we have shown that a strong, localized angiogenic response can be elicited *in vivo* by implantation of hydrogel films fabricated from modified hyaluronic acid (HA) preloaded with a single cytokine growth factor. We now hypothesize that the maturity of the resulting microvessel beds could be significantly improved if sequential release of multiple GFs from the gels could be sustained. In that way, delivery of one growth factor selected to initiate new microvessel development (*e.g.* Vascular Endothelial Growth Factor, VEGF) along with another chosen to stimulate later vessel maturation (*e.g.* Angiopoietin-1, Ang1) could be coordinated. Accordingly, here we show that inclusion of small amounts of modified heparin (Hp) in the gels effectively regulates release of preloaded growth factors and retains the bioactivity of the GFs.

Methods: To test this hypothesis, hydrogels were formed by crosslinking chemically modified hyaluronan (HA-DTPH), gelatin (Gtn-DTPH), and heparin (Hp-DTPH) with poly(ethylene glycol) diacrylate (PEGDA).

In vitro: One of six cytokine growth factors (VEGF, Ang1, basic Fibroblast Growth Factor, bFGF, Keratinocyte Growth Factor, KFG, Transforming Growth Factor β1, TGF-β, or Platelet-derived Growth Factor AA, PDGF) was preloaded in the gel prior to crosslinking. Gel samples were immersed in a DPBS solution, and GF release measured by sandwich ELISA over 42 days. All measurements were performed in triplicate ($n = 3$). The released GF mass, $m(t)$, was fit to a first-order kinetics model: $m(t) = A + Be^{-t/\tau}$.

In vivo: Gels made with 0.3%Hp w/w were dried to a film, and 4mm disks containing 100ng each of VEGF and Ang1 implanted in a mouse ear pinna. The surgical and contralateral ears were retrieved at 7 and 14 days post-implant, sectioned and stained with H&E. Microvessels were counted under a microscope. A nondimensional neovascularization index (*NI*) was defined as:

$$NI = \frac{\overline{(treatment - CL)} - \overline{(sham - CL)}}{mean\ \ CL} \qquad (1)$$

Six animals received implants for each treatment case ($n = 6$). Statistical significance was determined with two-way ANOVA and Fisher's post-hoc PLSD analysis, with significance taken at $p < 0.05$.

Results/Discussion: For all 6 growth factors, in both the absence and presence of gelatin, release profiles increased monotonically with time *in vitro* and were well fit by first order exponential kinetics ($R^2 > 0.9$ for all cases, figure 1a). Remarkably, the total mass released over the duration of the experiment varied from as little as 1.8% for PDGF to as much as 90.2% for bFGF, a variation of nearly two orders of magnitude. Even with as little as 0.3% Hp in the gels, total released mass at 14 days varied from 1.5% to 55%, in the order bFGF > TGF-β > KGF > VEGF > Ang-1 > PDGF. These data strongly suggest that incorporation of small amounts of Hp in the gels can lead to a coordinated sequence of GF releases.

Histologic examination showed that the vessel networks produced *in vivo* by co-delivery of both GFs were more mature than control cases delivering neither or only one GF, with intact endothelial borders. Quantitatively, the most effective treatment group was also found to be delivery of both GFs (*NI* = 4.67 at day 14, figure 1b), for which the mean value of *NI* was 29% greater than either case delivering a single GF ($p < 0.04$).

Conclusions: (1) HA-based hydrogels can serve as effective vehicles for sustained, localized delivery of bioactive biochemicals. (2) Specificity was dramatic; the total mass released varied from 90.2% for bFGF to 1.8% for PDGF. (3) Growth factors delivered in this way can generate continuous development of new microvessels over at least a 14 day period.

References:

1. Peattie RA. Biomaterials 2004;25:2789-2798.
2. Peattie RA. Biomaterials 2006;27:1868-1875.

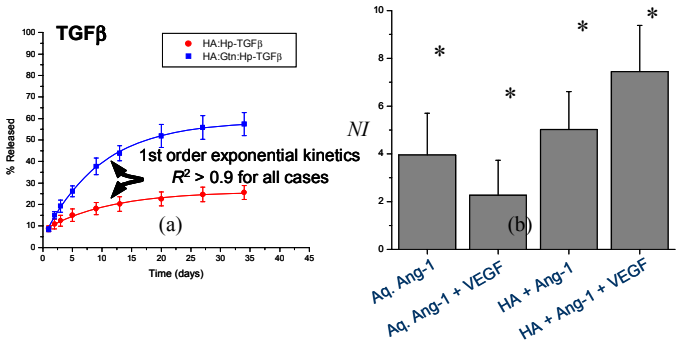

Figure 1. (a) Representative time course of *in vitro* GF release, data shown is for release of TGFβ. All GFs were released from the gels with first order kinetics. (b) *In vivo* neovascularization response, day 14 post-implantation. The greatest response was to co-delivery of both GFs. * indicates statistical significance.