**Modulating MMP-2 Expression in HUVECs using PEG-conjugated Biomolecules**

Joshua A. Hammer, Ryan M. Schweller, and Jennifer L. West.
Department of Biomedical Engineering, Duke University, Durham, North Carolina 27708

**Statement of Purpose:** Matrix metalloproteinases (MMPs) are cell-secreted enzymes responsible for breaking down elements of the extracellular matrix (ECM). MMP activity is an integral part of cellular processes that require ECM remodeling such as angiogenesis, while aberrant MMP activity is associated with many diverse pathologies such as tumor metastasis and scar tissue formation after cardiac infarct. Hydrogels are a promising modular biomaterial platform for tissue engineering approaches to treating a variety of disease states. The ability to regulate MMP activity within synthetic biomimetic hydrogels could broadly impact future tissue engineering strategies. For instance, enhancing the production of MMPs within and surrounding an MMP-degradable hydrogel-based tissue construct may lead to quicker remodeling of the hydrogel material and more rapid vascularization of the implanted tissue engineered construct, thereby reducing the chance of necrosis due to oxygen and nutrient starvation. Conversely, by applying MMP-inhibiting hydrogel to a tumor site, it is possible that metastasis could be delayed through local disruption of angiogenesis. To study the effects of hydrogel-mediated MMP regulation on capillary network formation, we must first investigate optimal dosing concentrations of factors which affect MMP expression in vascular cells, and validate their ability to be incorporated into a gel matrix without loss of bioactivity. To this end, human umbilical vein endothelial cells (HUVECs) were incubated with PEG-conjugated or native forms of various MMP-modulating bioactive factors. MMP-2 was selected as a model MMP due to its commonality among many cell types and its highly generalized ability to degrade ECM. This work lays the foundation for incorporating MMP-2 regulation into a 3D PEG hydrogel-based model of vasculogenesis, with the hopes of achieving tight control over capillary network formation through material-mediated MMP-2 regulation.

**Methods:** TIMP metallopeptidase inhibitor 2 (TIMP-2), and the κ-elastin-derived peptide Val-Gly-Val-Ala-Pro-Gly (kE) were conjugated to 3400 MW acrylate-PEG-NHS ester by reaction of the NHS ester to primary amines. Successful PEGylation of kE and TIMP-2 was confirmed via gel permeation chromatography and SDS-PAGE, respectively. HUVECs (passage 3) were added to 96 well polystyrene plates (10,000 cells/well) and incubated overnight at 37ºC, 5% $CO_2$ in endothelial growth media (EGM-2, Lonza) to achieve cell attachment. The following morning the media was replaced with fresh EGM-2 containing various concentrations of TIMP-2 or kE, either with or without a PEG linker. Conditioned media was collected at 6 hours and 12 hours, with new factor-containing EGM-2 added after the 6 hour time point. MMP-2 activity in sampled cell media was measured using gelatin zymography.

**Results:** Both kE and TIMP-2 have been previously shown to affect active MMP-2 expression in HUVECs. We observed both time-dependent and concentration-depended effects on MMP-2 expression in HUVEC cultures incubated with kE or TIMP-2. At the 6 hour time point, several conditions inhibited MMP-2 activity, with only 0.1 nM PEG-TIMP-2 showing no statistical difference from control cells cultured in media alone. By 12 hours, previously inhibitory conditions showed either no effect or an enhancement of MMP-2 activity compared to media alone. These transient responses indicate the potential to achieve multiple outcomes from a single factor. There was no statistical difference in bioactivity between PEG conjugated and native forms of the MMP-modulating factors tested, indicating they can be crosslinked into PEG hydrogels without loss of function.

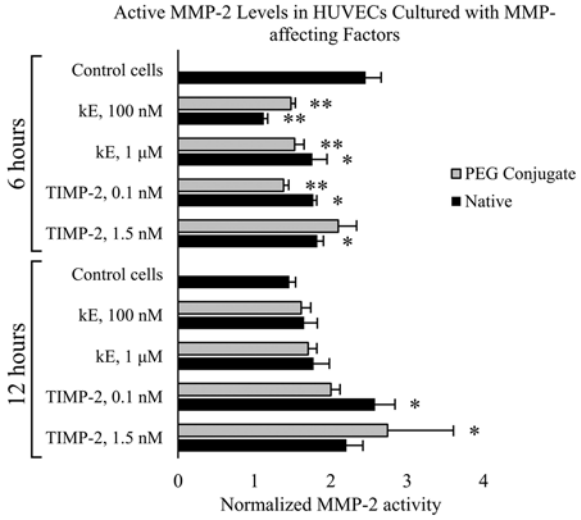


Figure 1 – Zymography analysis of active MMP-2 expression in HUVEC cultures normalized to serum-containing EGM-2. Statistical significance of MMP-2 activity was compared to HUVECs cultured in media alone (n = 3-4, *: $p < 0.05$, **: $p < 0.001$).

**Conclusions:** TIMP-2 and kE have both been confirmed to affect MMP-2 expression in HUVEC monocultures in a time and concentration dependent manner. This result illustrates the feasibility of temporal control over MMP-2 expression in HUVEC cultures through the considered integration of specific MMP-regulating factors into cell culture conditions. These MMP-modulating factors can also be incorporated into 3D PEG hydrogels as highly localized MMP-2-regulating pendant moieties without reducing their bioactivity due to PEG conjugation. In future work, PEG hydrogels functionalized in this way will be used to perturb an *in vitro* model of vasculogenesis, with the ultimate goal of controlling basal vasculature formation *in vivo* through biomaterial-mediated MMP regulation.

**References:** Haas TL. TCM. 1999;9;70-77, Kupai K. J Pharmacol Toxicol. 2010;61;205-209, Lu KV. Lab Invest. 2004;84;8-20, Rauch BH. Circ Res. 2002;90;1122-1127, Robinet A. J Cell Sci. 2004;118;343-356