TUNABLE SPATIOTEMPORAL CONTROL OF ANGIOGENIC AND OSTEOGENIC GROWTH FACTOR DELIVERY VIA HYDROGEL ENCAPSULATION AND COVALENT CONJUGATION

Matthew J. Meagher and Ryan K. Roeder
Department of Aerospace and Mechanical Engineering, Bioengineering Graduate Program, University of Notre Dame

Statement of Purpose: Growth factor delivery is a promising strategy to repair critical size bone defects [1]. Bone morphogenetic protein-2 (BMP-2) is a potent regulator of osteogenesis [1], but current clinical delivery methods are limited by burst release which necessitates superphysiological doses resulting in adverse outcomes and high treatment costs [2]. Vascular endothelial growth factor (VEGF) promotes angiogenesis [3], which is critical for bone regeneration [4], but clinical translation is hindered by the lack of a suitable delivery strategy. Therefore, the objective of this study was to develop a new growth factor delivery platform to enable tunable, independent control over the dosing, spatial presentation, and release kinetics of VEGF and BMP-2 from collage-hydroxyapatite (col-HA) scaffolds (Fig. 1).

Methods: Col-HA scaffolds, 3 mm in diameter and height, were prepared with 85% porosity and 40 vol% HA whisker reinforcements using established methods [5]. Prior to scaffold preparation, BMP-2 and 1% of collagen fibrils were biotinylated in equimolar EZ-link sulfo-NHS-PEG12-biotin (50 mM $NaHCO_3$, pH 8.0) for 8 h at 4°C and 2 h at 25°C, respectively. Scaffolds were incubated with streptavidin to allow binding to biotinylated collagen fibrils. Biotinylated BMP-2 was then added dropwise to bind to streptavidin at a total loaded dose of 1 µg/scaffold. A collagen solution was prepared from 6 mg/mL collagen fibrils with 10 µg/mL VEGF, infiltrated into scaffold pore spaces, and gelled at 37°C to achieve a total loaded dose of 2.5 µg/scaffold VEGF. VEGF and BMP-2 release kinetics were measured by incubating scaffolds in 1 mL PBS (pH 7.52, 0.1% BSA) at 37°C and sampling aliquots at longitudinal timepoints for 28 days followed by scaffold digestion with collagenase to measure all residual growth factor. Eluted VEGF and BMP-2 concentrations were measured at each timepoint via ELISA.

Results: VEGF and BMP-2 were loaded in col-HA scaffolds by hydrogel encapsulation and covalent conjugation, respectively (Fig. 1). This new growth factor delivery platform enabled independent control over the *in vitro* release kinetics of VEGF and BMP-2 (Fig. 2). VEGF was completely and gradually released over 3 weeks, with a peak release rate at days 1-5. In contrast, the entire dose of BMP-2 was immobilized within col-HA scaffolds for 31 days *in vitro* and BMP-2 release was only observed following digestion of the scaffold with collagenase (Fig. 2). Thus, BMP-2 will be immobilized *in vivo* until the collagen matrix is metabolized by infiltrating cells. In contrast, delivery of BMP-2 from an absorbable collagen sponge occurs in <14 d either *in vitro* or *in vivo* [6].

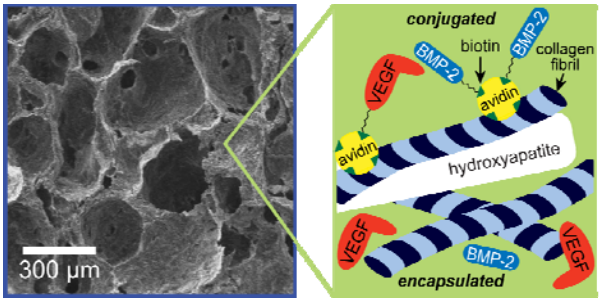

Figure 1. VEGF and BMP-2 were delivered from col-HA scaffolds using a novel platform for tunable spatiotemporal control: short term release (1-3 weeks) was controlled by growth factor encapsulation in a collagen hydrogel filling scaffold pore spaces while sustained release (>3 weeks) was controlled by streptavidin-biotin mediated conjugation to the scaffold matrix.

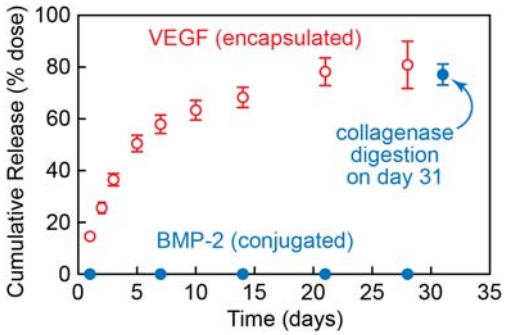

Figure 2. Cumulative *in vitro* release (% dose) of a 2.5 µg dose of VEGF encapsulated within a collagen hydrogel filling scaffold pore spaces and a 1.0 µg dose of biotinylated BMP-2 covalently conjugated to collagen fibrils in the scaffold. Error bars show one standard deviation of the mean.

Conclusions: A new growth factor delivery platform comprising two independent delivery mechanisms, hydrogel encapsulation and covalent conjugation, was used to tune the delivery of an angiogenic (VEGF) and osteogenic (BMP-2) growth factor to correspond with physiological timelines. Tunable release kinetics is achieved in a straightforward manner by tailoring the proportion of each growth factor loaded by each mechanism. Moreover, this platform can be applied to a variety of scaffold biomaterials and other growth factors.

References:

1. De Biase P. Injury. 2005;36 Suppl 3:S43–6.
2. Cahill KS. JAMA. 2014;302:58-66.
3. Street J. Proc Natl Acad Sci. 2002;99:9656–61.
4. Kanczler JM. Biomaterials. 2010;31:1242–50.
5. Kane RJ. Biomaterials. 2014, (submitted).
6. Geiger M. Adv Drug Deliv Rev. 2003;55:1613–29.