**Photo-polymerized keratin-PEG sponge for growth factor delivery in bone tissue engineering**

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**Statement of Purpose:** Growth factor-mediated tissue regeneration is an appealing method of repairing damaged bodily structures because of its relative simplicity and ease of clinical usage. Fractures and segmental bone defects are now being alternatively treated using the growth factor: bone morphogenetic protein 2 (BMP-2), delivered with type I collagen. Despite some successes, failures were also encountered, primarily attributed to the carrier biomaterial itself. The collagen sponge collapses and does not hold its shape upon wetting[1] (Figure 1). It does not bind and sequester BMP-2, thereby limiting its growth factor retention ability. In this study, we employed a composite material composed of keratin and polyethylene glycol to improve the matrix material properties; keratin to bind positively-charged growth factors (such as BMP-2 and others), while polyethylene glycol (PEG) to increase the structure stability and mechanical strength.

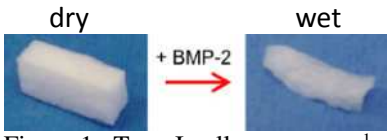

Figure 1. Type I collagen sponge[1].

**Methods:** 5 % (m/V) reduced keratin (KRT) extracts were homogenously mixed with 10 % polyethylene glycol diacrylate (PEGDA; MW = 700 g/mol) and 0.5 % Irgacure® 2959 photoinitiator (BASF). The solution was then exposed to 36-W UV (at 254 nm) for 20 min to polymerize the PEGDA monomers (PEG chain growth) and to link KRT proteins to PEG via thiol-ene reaction (Figure 2). After washing and freeze-drying to create a sponge-like scaffold (Figure 3), test specimens were cut, characterized for presence of keratin (western blot with anti-K31 antibody), and tested for their equilibrium swelling and tensile strength properties. Representative scaffolds were soaked in protein solutions with varying isoelectric points (pI): bovine serum albumin (BSA; pI ~ 5), human hemoglobin (pI ~ 7), and chicken egg white lysozyme (pI ~ 11) and transferred into a phosphate-buffered saline (PBS), pH 7.4 medium. At time points: 1, 3, 7, 14, and 28 days, protein release was assessed through enzyme-linked immunosorbent assay (ELISA). Release of proteins out of the scaffold was also quantified in acidic PBS (pH 4.5). Growth factors: vascular endothelial growth factor C (VEGF-C; pI = 8.6) and chondromodulin 1 (ChM-1; pI = 7.5) were separately absorbed into the KRT-PEG scaffolds. Human microvascular endothelial cells (HMVECs) were then seeded and cultured onto the matrix for 1 week. Live-Dead and MTT assays were utilized to determine the relative cell viability.

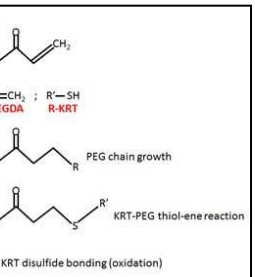

Figure 2. Chemical Reaction

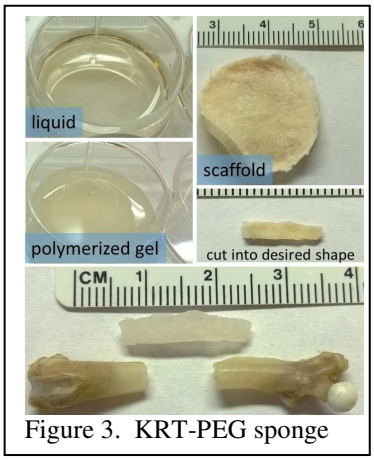

Figure 3. KRT-PEG sponge

**Results:** Keratin proteins were detected in the thoroughly-washed scaffolds at similar to initial quantities confirming that keratins covalently-link to the PEG network. The sponges swelled to 3-fold their volume and were able to hold 5-fold the mass of water while maintaining a stable 3D shape (Figure 3). The modulus of elasticity (E) of the construct was found to be higher than the E of type I collagen. Supporting our previous findings that KRT is indeed highly negatively-charged[2], the positively-charged lysozyme was tightly retained within the matrix, while the neutral hemoglobin and negative BSA were released slowly and quickly, respectively. Acidification of PBS enabled complete release of all loaded proteins due to keratin (keratin pI = 5.3) charge reversal. VEGF-C enabled growth of HMVECs into the 3D matrix at a dose-dependent manner. ChM-1 has opposite inhibitory effects to HMVEC proliferation and survival in the KRT-PEG sponge.

**Conclusions:** Electrostatic or coulombic attractive interaction enabled the sequestration of positively-charged growth factors like VEGF-C and ChM-1 into the negatively-charged KRT-based sponge. PEG provided increased strength and stability to the carrier biomaterial structure. An *in vivo* study will be an appropriate follow-up work to determine the tissue engineering capacity of the fabricated scaffold.

**References:**

1. de Guzman, R. C.; Saul, J. M.; Ellenburg, M. D.; Merrill, M. R.; Coan, H. B.; Smith, T. L.; Van Dyke, M. E., Bone regeneration with BMP-2 delivered from keratose scaffolds. *Biomaterials* **2013,** 34, (6), 1644-56.

2. de Guzman, R. C.; Tsuda, S. M.; Ton, M. N.; Zhang, X.; Esker, A. R.; Van Dyke, M. E., Binding interactions of keratin-based hair fiber extract to gold, keratin, and BMP-2. *(submitted)*.