Growth Factor Delivery via a Keratin Biomaterial for Volumetric Muscle Loss Therapy

Tomblyn S[1], Baker HB[2], Passipieri JA[2], Siriwardane M[2], Stewart CE[3], Okoukoni C[2], Ellenburg M[1], Christ GJ[2], Burnett L[1].
[1]KeraNetics, LLC, Winston-Salem, NC
[2]Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC
[3]University of Virginia, Charlottesville, VA

Statement of Purpose: Therapeutic strategies to replace or regenerate damaged tissues have involved using autografts, allografts, cells cultured in porous biomaterial scaffolds, and delivery of cells or biological factors that augment or stimulate endogenous repair mechanisms. However, despite the technological advances of these strategies, each has commercialization and regulatory challenges to overcome in order to become a widely used solution to regenerating functional muscle tissue. One potential solution is the use of keratin biomaterials. Keratins are naturally-derived proteins whose formulation flexibility can be utilized for cell and drug delivery that offers an inexpensive but effective regenerative therapy for these injuries. Keratins can be extracted by either reductive methods (kerateine, KTN) or oxidative methods (keratose, KOS). The different methods produce biomaterials that can be polymerized into hydrogels by endogenous covalent cross-linking or entanglement, respectively. Their utility in delivering therapuetic agents (Ciprofloxin and rhBMP-2) has also been demonstrated (Saul 2011; de Guzman, 2013). Here we describe a keratin formulation that provides sustained delivery of growth factors releated to muscle repair and regeneration, basic Fibroblast Growth factor (bFGF) and Insulin-like Growth Factor 1 (IGF-1), and demonstrate the ability to improve functional recovery in an extremity model of volumetric muscle loss.

Methods: Human hair keratin was purified using a patented process by KeraNetics. Growth factors (IGF-1 and bFGF) are dissolved in sterile water and added to lyophilized keratin powders to generate a 7% or 10% weight to volume hydrogel and incubated at 37 °C overnight. A KOS hydrogel is formulated to 15% weight to volume in DPBS. The KOS and KTN hydrogels are mixed at ratios or 50:50 and 70:30 by passing between coupled syringes (final growth factor concentration was 100 μg/mL of each). Growth factor release was quantified by exchanging 100 μL DPBS placed on100 μL aliquots of the keratin hydrogels in microcentrifuge tubes at regular intervals.

A rat model of volumetric muscle loss was utilized to demonstrate applicability for muscle regeneration. The model entailed a 20% resection of the rat tibialis anterior (TA) with ablation of the extensor digitorum longus and the extensor hallucis longus. Defects were filled with a 70:30 mixture of KOS/7%KTN with the growth factor loaded in the KTN. The impact of cells was assessed using muscle progenitors cells (MPCs) mixed into the gel using serum-free growth media in lieu of DPBS. Control treatments included no treatment and bladder acellular matrix (BAM). Muscle function was analyzed prior to surgery and at 4 and 8 weeks post surgery as contraction force resulting from peroneal nerve stimulation and measured with a footplate force transducer. The results were normalized to pre-injury measurements and weight. The TA muscle was harvested at 8 weeks post defect and assessed histologically.

Results: The rates of release and the maximal amount of material released from the gels are expressed as a percentage of the initial mass of the growth factor loaded into the gels. While all growth factors' release showed high correlation to the keratin degradation, the release of the growth factors varied slightly. The bFGF release from the KTN/KOS mixture gels was bracketed by the release of bFGF from either KTN or KOS alone. The release of IGF-1 showed a release profile from mixtures that more closely matched the release from the KTN alone. Functional data collected at 8 weeks indicated that keratin+IGF1 (n=7) recovered to approximately 70% of baseline contractile force (Figure 1). This was significantly greater recovery than no repair (n=7)*, keratin+IGF-1+bFGF*, and BAM (n=8)** (*p<0.05, **p<0.01). Keratin+FGF treated animals performed significantly better (p<0.01) than BAM. Histological findings from tissue explants at 8 weeks illustrate a range of regenerative responses among the groups including neo-muscle tissue formation as well as adipose and scar formation.

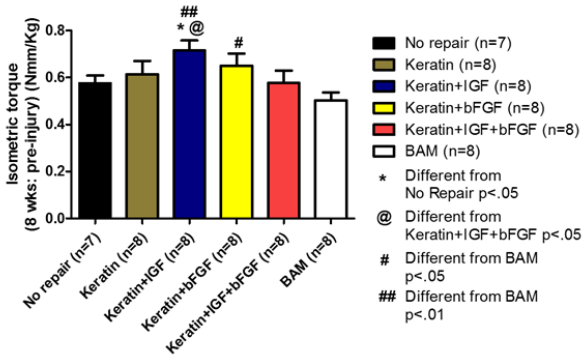

Figure 1. Functional recovery measured at 8 weeks post injury.

Conclusions: Through modulation of the disulfide cross-linking of the keratin hydrogel, we have demonstrated control of the carrier degradation rate as well as the release of growth factor from the system. Using this material in a volumetric muscle defect, significant functional recovery was observed in the keratin hydrogels containing IGF-1.

References:

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