Microporous Annealed Particle Hydrogel-based Muscle Repair in Rat Model of Volumetric Muscle Loss

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Statement of Purpose: Volumetric muscle loss (VML) occurs when the severity of an injury to skeletal muscle surpasses the natural regenerative capacity of muscle, resulting in both loss of tissue and loss of function. There currently is no effective treatment available. Within tissue engineering, a commonly used approach to muscle repair in VML is the implantation of a scaffold – which may be acellular or cellular – to regenerate muscle *in vivo*. However, this method's success is often limited by insufficient vascularization, poor cellular integration, and inadequate structural support. To address these limitations, our approach uses Microporous Annealed Particle (MAP) gel – an injectable, space-filling hydrogel with a porous structure – to form an acellular scaffold *in situ*. MAP is a bioinert, polyethylene glycol (PEG)-based hydrogel made up of a slurry of spheres that may be covalently bound together, or *annealed*, on demand via radical chain polymerization. We have shown that MAP gel has a high rate of cellular infiltration, promotes formation of vascular networks, and can be tuned to match the mechanical properties of surrounding tissue.[1] Passive muscle stiffness is approximated by 2 wt% PEG MAP gel. To increase bioactivity, we immobilized high molecular weight (HMW) heparin and included soluble HMW hyaluronic acid (HA) to increase vascularization and muscle progenitor cell recruitment, respectively.[2,3]

Methods: Following a modified protocol[1], we prepared two types of non-degradable MAP hydrogel: 2 wt% PEG (unmodified MAP), and 2 wt% PEG with 5 mg/ml heparin (hep-MAP). We used an established rat model of VML in the tibialis anterior (TA), in which two synergist muscles (extensor digitorum longus and extensor hallucis longus) and 20% of the TA are removed from one leg. The defect was filled with MAP, which was annealed *in situ* with 505 nm light exposure. Rats were divided into four study groups according to treatment method. Group 1 received unmodified MAP; group 2 received a mixture of unmodified MAP and hep-MAP (9:1); group 3 received a mixture of hyaluronic acid (HA) gel (4 wt% HMW HA in PBS), unmodified MAP, and hep-MAP (3:6:1); and group 4 did not receive treatment. We performed a functional test on each rat before defect creation (baseline) and at several time points post-surgery (4 weeks, 8 weeks, 12 weeks). This test measures the force of dorsiflexion upon stimulation of the TA with needle electrodes at several frequencies ranging from 0 Hz to 200 Hz. Forces measured during the functional test were normalized to the rat's body weight to account for growth during the study period. We also conducted a small pilot study in which we measured perfusion in the defect with gadolinium-enhanced MRI at 16 weeks. The injured TAs and contralateral control muscles were harvested and preserved at 17 weeks to be analyzed via IHC.

Results: MAP groups showed rapid improvement in function compared to the no repair group; the unmodified MAP group (group 1) recovered more function in 4 weeks than the no repair group did in 12 weeks (Fig 1A). Group 1 also achieved nearly 80% of its baseline function at 12 weeks, close to what is considered the maximum recovery possible in this model due to the removal of synergist muscles. Using MRI analysis, we found a 72% increase in blood flow in the defect for this group compared to the no repair group (0.38 ml/g/min and 0.22 ml/g/min, respectively). IHC shows a substantial infiltration of cells into the MAP implant, as well as branched vascular networks of $CD31^+$ cells in groups 2 and 3 (Fig 1B).

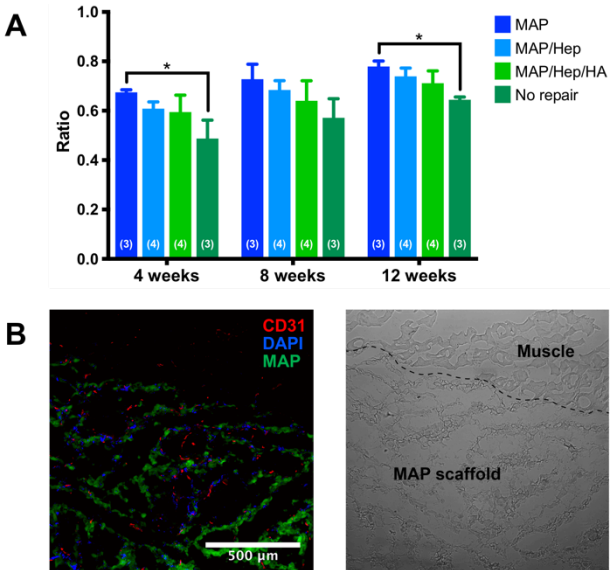

Figure 1. *(A) Ratio of normalized force generated at each time point to normalized force generated at baseline (B) MAP/Hep/HA in defect at 17 weeks. Brightfield image and fluorescent image are taken from the same location.*

Conclusions: These findings support the use of MAP gel for muscle repair in VML. Due to the significant improvements in function demonstrated by unmodified MAP, we conclude that this porous material provides a structure conducive to vascular network formation, cell infiltration, and force generation. Based on the results of this study, we have a new, on-going study with an enzymatically degradable formulation of MAP gel. We hypothesize that this formulation will support the formation of a vascular network before degradation and allow for the reorganization and alignment of infiltrating myocytes after degradation, resulting in complete scaffold replacement with functional muscle.

References:

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