Spatial Control of Composite Musculoskeletal Tissue Regeneration Using Magnetic Microgels

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INTRODUCTION: Humans have a limited ability to restore musculoskeletal tissues of the limb after amputation [1]. Research using mouse models of digit tip amputation suggests that this regenerative failure is not due to irreversible loss of potential, but rather the absence of essential morphogenetic cues [2–4]. Indeed, recent studies reveal that bone morphogenetic protein-2 (BMP-2) and BMP-9 can promote bone elongation and synovial joint formation, respectively, when delivered at precise times and locations to the amputated digit [4]. However, a minimally invasive method for spatiotemporal delivery of multiple growth factors is necessary for clinical translation of this therapy. To address this need, we previously developed magnetic, micron-sized hydrogels ("microgels") to spatially direct protein release after injection [5]. In this study, we tested whether magnetic microgels could be physically translocated within a 3D tissue-like environment using an external magnetic field.

METHODS: Synthesis: 8-arm 40 kDa Poly(ethylene glycol)-norbornene (PEG-NB, 5% w/v), PEG-dithiol (3.8 mM), and photoinitiator (Lithium phenyl-2,4,6-trimethylbenzoylphosphinate, 2.5 mM) were dissolved in PBS to create the polymer precursor, which was mixed with either 0 (Control) or 21.6 mM (Magnetic) of iron oxide (Fe_3O_4) particles (Sigma-Aldrich) [5]. The precursor was added to a synthetic oil, agitated using a vortex mixer to generate microgel droplets, and photopolymerized (λ: 365 nm) [5] (**Fig. 1A**). To optimize microgel morphology, various mixing speeds (1990, 2550, 3200 RPM at 3 seconds) and durations (3, 5, 7 seconds at 2550 RPM) were evaluated. Analysis: Brightfield images were analyzed in ImageJ to determine microgel diameter and circularity ($4\pi \cdot \text{area}/\text{perimeter}^2$) (n=278–300 microgels/group). To assess microgel translocation in response to an external magnetic field, Control or Magnetic microgels were embedded in type I collagen hydrogels (0.5, 0.75, or 1 mg/mL) formed within silicone wells. A Weak (38.7 lb pull force) or Strong (47.8 lb pull force) neodymium magnet (K&J Magnetics) was applied ~3 cm from the collagen, and microgel displacement was determined after 5 minutes (n=14–17 microgels/group) (**Fig. 2A**). Microgels without magnetic exposure (No Magnet) acted as a negative control. Simulation: A finite element model was developed in FEBio [6] to simulate microgel (diameter: 50 μm) translocation through a channel (diameter: 40 μm) within the collagen (**Fig. 3A**). Both domains were modeled as biphasic viscoelastic materials with porous neo-Hookean elasticity. Microgel stiffness (E_M) was fixed at 2 kPa, while collagen stiffness (E_C) was varied to assess the E_C/E_M ratio. A prescribed displacement was applied to the microgel along the channel, and the peak von Mises stress and Lagrangian strain were measured at the contact interface (n=3 measurements/group). Significance was assessed by the Scheirer-Ray-Hare test with Dunn-Šidák post-hoc corrections and 2- or 3-way ANOVA with Tukey's post-hoc test ($p<0.05$).

RESULTS: Using a water-in-oil emulsion, we generated PEG-NB microgels with a median diameter of ~60 μm and a median circularity of ~0.9 (**Fig. 1C**). Morphology depended on vortex mixing speed and time, with Magnetic microgels showing greater sensitivity than Controls. Magnetic microgels fabricated at 3200 RPM exhibited larger diameters compared to those fabricated at lower speeds, whereas Control diameters remained stable (**Fig. 1D**, $p<0.05$). Similarly, Magnetic microgels mixed for 7 seconds were larger compared to those mixed for shorter durations (**Fig. 1E**, $p<0.05$). Magnetic microgel circularity increased with mixing speed and time but was lower compared to Control microgels (**Figs. 1F & 1G**, $p<0.05$). With an external magnetic field, Magnetic microgels moved through collagen towards the magnet, with displacement increasing with higher magnet strength and lower collagen concentration, respectively (**Fig. 2C**, $p<0.05$). As expected, Control microgels displayed minimal displacement in response to magnetic exposure, and microgels from all groups remained stationary in the absence of a magnet. Our computational model showed that the microgel experienced higher peak von Mises stress and Lagrangian strain as the stiffness of the collagen increased relative to the microgel (**Figs. 3B & 3C**, $p<0.05$). In contrast, the collagen exhibited increased peak stress and decreased strain at higher stiffness ratios.

DISCUSSION: Spatiotemporal delivery of multiple growth factors has the potential to promote limb/digit regeneration after traumatic injury [4]. To this end, we developed a novel biomaterial-mediated platform utilizing magnetically responsive microgels, which can be spatially separated into discrete populations after injection based on their magnetic responsivity to an external magnet [5]. Previously, we found that using synthetic oil in the water-in-oil emulsion significantly reduced microgel size [5], facilitating administration by needle injection. Here, we showed that microgel morphology can also be modulated by vortex mixing speed and time, with microgels formed at 2550 RPM and 5 seconds exhibiting optimal size and shape. Both experimental and simulation data show that microgel translocation is sensitive to the mechanical properties of its microenvironment, suggesting that microgel stiffness must be tuned to avoid excessive deformation. Future work will explore how tuning crosslinking density and incorporating degradable linkers can affect protein release [7] and movement through tissue. Ultimately, this platform may offer a translatable strategy for composite musculoskeletal regeneration.

SIGNIFICANCE: As of 2017, over 57 million people were living with limb loss, many of whom experience complications related to prosthetics that lack biological integration [8]. The successful development of a drug delivery system that promotes limb/digit regeneration may improve clinical outcomes for amputees.

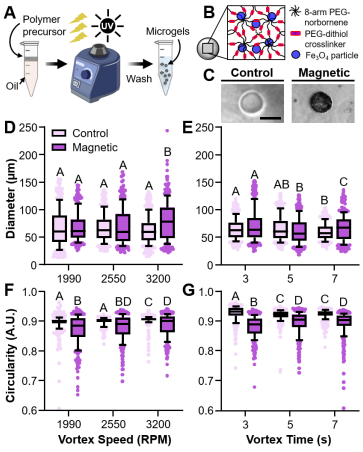

Fig. 1. Schematic of (**A**) microgel synthesis and (**B**) polymer network. (**C**) Brightfield images of Control and Magnetic microgels. Scale: 100 μm. Microgel (**D, E**) diameter and (**F, G**) circularity for various vortex speeds and times (n=278–300/group, median±10-90 percentile). Different letters indicate significant differences ($p<0.05$).

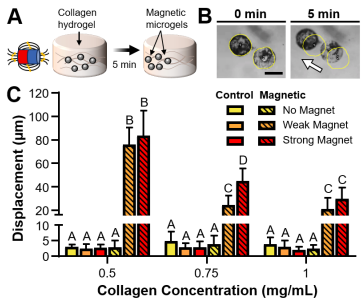

Fig. 2. (**A**) Schematic of microgel translocation through collagen. (**B**) Brightfield images of Magnetic microgels before and after magnetic exposure. Arrow shows displacement direction. Scale: 50 μm. (**C**) Control and Magnetic microgel displacement after exposure to various magnets (n=14–17/group, mean±SD). Different letters indicate significant differences ($p<0.05$).

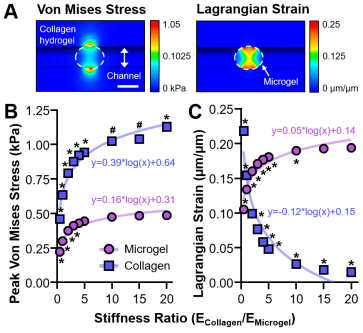

Fig. 3. (**A**) Finite element analysis of microgel moving through collagen channel. (**B**) Peak von Mises stress and (**C**) Lagrangian strain during microgel-collagen contact at various stiffness ratios (n=3/group, mean±SD). #: $p<0.05$ vs. Microgel. *: $p<0.05$ vs. all other groups.

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