Wnt7a-Releasing Hydrogel for Enhancing Local Skeletal Muscle Regeneration

Woojin Han, Shannon Anderson, Mahir Mohiuddin, Andrés Garcia*, Young Jang*
*Georgia Institute of Technology, Atlanta, GA | *co-corresponding authors*

Statement of Purpose: Skeletal muscles efficiently regenerate upon minor injury, but their regenerative capacity is debilitated with traumatic injuries (lacerations, volumetric muscle loss) and diseases (Duchenne muscular dystrophy). Recent evidence suggests that Wnt7a induces muscle fiber hypertrophy[1] and symmetric expansion of muscle stem cells[2], stimulating muscle regeneration. When muscle stem cells are pre-treated with Wnt7a *ex vivo*, they exhibit significantly increased migration and engraftment upon transplantation.[3] Although direct intramuscular injection is the simplest method to deliver Wnt7a, direct injection is not viable in many muscle trauma cases where the structural integrity is severely compromised.[4] Furthermore, in Duchenne muscular dystrophy, a delivery vehicle is required to deliver Wnt7a locally to the diaphragm, a muscle critical for respiration, where direct injection is not feasible due to its thin dimensions. The objective of this work is to engineer a synthetic matrix to facilitate the delivery of Wnt7a to skeletal muscles affected by severe trauma or muscular dystrophies.

Methods: 20 kDa 4-arm poly(ethylene glycol)-maleimide macromer (PEG-4MAL) was conjugated to RGD adhesive peptide, mixed with human recombinant Wnt7a, and crosslinked using protease-degradable peptide (GCRDVPMSMRGGDRCG) to form hydrogels. Wnt7a Release Assay: Release kinetics of Alexa 488-labeled Wnt7a from 4% (w/v) and 8% 20 kDa PEG-4MAL hydrogels were fluorescently quantified over time in PBS at 37C. To quantify Wnt7a release kinetics in a degrading condition, Wnt7a-loaded 4% PEG-4MAL hydrogel was incubated in collagenase I (3.9U/mL) containing PBS. In Vitro Bioactivity Assay: Wnt7a (75-ng)-loaded PEG-4MAL hydrogels (4%, 6%, 8%), free Wnt7a (75-ng), and PBS control were introduced to differentiated C2C12 cells. After 5 days, myotube diameters were measured to assess the degree of hypertrophy. In Vivo Bioactivity Assay: Wnt7a (2.5 μg)- and PBS-loaded PEG-4MAL hydrogels were delivered to the supramuscular surface of freeze-injured tibialis anterior (TA) muscles of mice. On day 14, the animals were euthanized, and TA mass, muscle fiber area, and number of $Pax7^+$ muscle stem cells were quantified. Effects of Wnt7a on Muscle Stem Cells: Primary muscle stem cells were isolated and encapsulated in Wnt7a (50-ng/ml)- and PBS-loaded 4% PEG-4MAL hydrogels. On day 4 of culture, myogenic colony size and proliferation were assessed. To evaluate the effect of Wnt7a on muscle stem cell migration, primary cells were seeded atop Wnt7a (50-ng) and PBS-loaded 4% PEG-4MAL hydrogels. On day 3, cell migration distance into the hydrogels was quantified.

Results: Wnt7a released at a faster rate from 4% PEG-4MAL hydrogels than from 8% PEG-4MAL hydrogels ($p<0.0001$ at 74 hr; Fig 1a). The release rate of encapsulation of Wnt7a was further accelerated in the presence of collagenase ($p<0.0001$ at 74 hr; Fig 1a), suggesting that the rate of release can be enhanced by targeting the protease-sensitive cross-linking peptides to induce hydrogel degradation. Treating C2C12 myotubes with free Wnt7a induced hypertrophy ($p<0.05$ vs. control). Wnt7a released from 4% PEG-4MAL hydrogels also induced myotube hypertrophy ($p<0.05$ vs. control) comparable to myotubes treated with free Wnt7a, suggesting that the hydrogel-released Wnt7a retains its biological activity. However, Wnt7a released from 6% and 8% PEG-4MAL hydrogels induced a significantly lesser degree of myotube hypertrophy compared to gel-free and 4% PEG-MAL conditions ($p<0.05$), likely due to slower release kinetics attributed to higher polymer density. In vivo, treating cryo-injured TA muscles with Wnt7a-loaded 4% PEG-4MAL hydrogels resulted in an accelerated regenerative response, measured by increased muscle fiber cross-sectional area ($p<0.05$; Fig 1b) and bulk TA mass ($p<0.01$), compared to the TA muscles treated with saline-loaded 4% PEG-4MAL hydrogels. Wnt7a-hydrogel treatment also increased muscle stem cell number at the site of injury ($p<0.05$; Fig 1c, d). Culturing muscle stem cells within RGD-presenting 4% PEG-4MAL hydrogels in the presence of Wnt7a had no effect on myogenic colony formation; however, Wnt7a-treated muscle stem cells exhibited significantly increased migration through the RGD-presenting 4% PEG-4MAL hydrogels ($p<0.0001$).

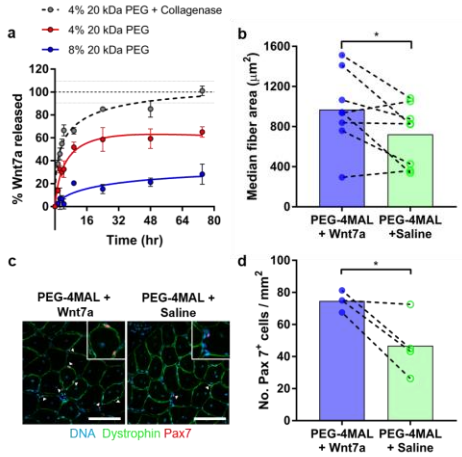

Figure 1. (a) % Wnt7 released over time. **(b)** Median fiber area of TA muscles. **(c)** Representative micrographs of TA muscles. Scale bar: 100 μm. **(d)** Number of muscle stem cells present per mm^2. Day 14. *$p<0.05$ paired 2-tailed t-test.

Conclusions: A synthetic hydrogel that enables local delivery of Wnt7a for treating skeletal muscle injuries and diseases, where direct injection is not applicable, has been engineered. Wnt7a released from the engineered hydrogel retains biological activity. This system is currently being evaluated for co-delivering Wnt7a and muscle stem cells to treat volumetric muscle loss.

References: 1. Von Maltzmahn et al. 2012 Nat Cell Biol. 2. Le Grand et al. 2009 Cell Stem Cell. 3. Bentzinger et al. 2014 JCB. 4. Oliva et al. 2013 MLTJ.

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