Injectable Biomimetic Liquid Crystalline Scaffolds enhance Muscle Stem Cell Transplantation

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Statement of Purpose: Muscle stem cells (also known as satellite cells) are essential for endogenous skeletal muscle regeneration but their utility in cell transplantation therapy is currently limited by their inefficient survival, self-renewal, and differentiation after injection into muscle tissue. To improve the contributions of MuSCs post-transplant, we developed biomimetic scaffolds based on synthetic peptide amphiphiles (PAs) that gel to encapsulate cells and growth factors within a muscle-like unidirectionally-ordered environment of long and aligned nanofibers directly as they are injected into damaged muscle tissue. In comparison to other cell-injection materials, this liquid-crystalline scaffold gels *in vivo* due to divalent ion bridging not covalent or photo-initiated crosslinking, and have tunable mechanical properties.

Methods: We generated PAs consisting of an aliphatic (C_{16}) palmitoyl tail and hydrophilic nine amino acid cap (e.g. $V_3A_3E_3$, in various rearrangements) using a custom peptide synthesizer (*1*). We annealed PAs into randomly-ordered, long-axis nanofibers, which externally expose the amino acid cap and internally aggregate the hydrophobic tail, by entropic mixing. We extruded annealed PAs (aPAs) into physiological calcium concentrations in culture medium to fabricate a stable, noodle-like aPA scaffolds with liquid crystalline properties due to their highly-ordered nanofibers in parallel to the direction of extrusion. We measured the storage modulus of aPA scaffolds using shear rheometry. We evaluated myogenic progenitor cell viability, proliferation and differentiation after encapsulation in assembled aPA scaffolds *in vitro*. We transplanted FACS-isolated α7-integrin$^+$ CD34$^+$ muscle stem cells from *GFP*/*Luciferase* transgenic mice (*2*) mixed with a 1wt%/vol aPA solution by rate-controlled injection into hindlimb muscles of irradiated immunodeficient *NOD/scid* mice, and measured muscle repair by bioluminescence imaging and anti-GFP immunohistochemistry up to one-month post-transplant. aPA scaffold degradation *in vivo* was assayed using a Gd(III)-doped PA molecules (*3*) and MRI imaging every five days.

Results: We generated aPA gels with diverse amino acid compositions and observed shear storage moduli (G') spanning three orders of magnitude, from G' = 3-20 kPa, encompassing a range of physiological muscle stiffnesses. We observed that the aPA scaffold stiffness determined the macroscopic degree of muscle progenitor cell alignment with the ordered nanofiber template. The aPA scaffolds supported primary myoblast survival and proliferation *in vitro*, and, when tuning their stiffness to G' = 9 kPa, optimal myoblast differentiation into mature myosin heavy chain$^+$ myofibers. Upon injection into hindlimbs, we observed co-alignment of scaffold nanofibers with recipient muscle myofibers to achieve desired templating. These scaffolds displayed characteristic degradation rates *in vivo* matching the time course of tissue regeneration ($t_{1/2}$ = ~14 days). When transplanted within aPA scaffolds, we observed profound improvements in the frequency of MuSC engraftment and greatly enhanced donor cell-derived myofibers, compared to MuSCs in control solutions.

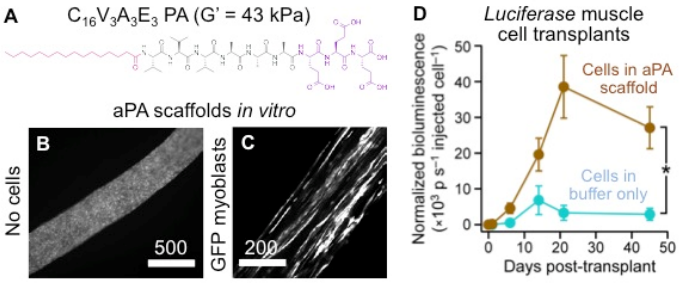

Fig 1. Annealed PA scaffolds have tunable rigidity based on their terminal amino acid sequence (A) to enhance muscle cell alignment *in vitro* (B-C) and muscle stem cell engraftment *in vivo* (D).

Conclusions: This work establishes a new stem cell encapsulation technology to aid muscle cell therapies. Importantly, this scaffold system reversibly gels *in vivo* without the need for polymerization or cross-linking and has emergent nanofiber alignment to template muscle cell maturation. Simple rearrangement of PA amino acid sequence controls scaffold stiffness and can be varied to optimize muscle stem cell function *in vitro* and *in vivo*.

References: (*1*) Zhang S. Nat Materials. 2010;9(7):594-601. (*2*) Sacco A. Nature. 2008;456(7221):502-6. (*3*) Preslar AT. ACS Nano. 2014;8(7):7325-7332.

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