Enhancing Stem Cell Treatment via Biosponge Encapsulation and Hypoxic Preconditioning

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INTRODUCTION: Volumetric muscle loss (VML) causes irreversible mass and function deficits and often results in permanent disability. Mesenchymal stromal cells (MSC) and their secretome can improve muscle regeneration. Placenta-derived stem cells (PDSCs), which share cell surface markers with MSCs, have better proliferation and differentiation capabilities due to their fetal origin. However, MSCs generally have a brief lifespan and do not persist in injured tissues beyond the first week following transplantation. We previously developed a biosponge composed of extracellular matrix (ECM) proteins that enhanced muscle regeneration and function after VML [1]. We hypothesized that delivery of biosponge encapsulated human (hPDSCs) to the VML defect will result in improved cell survival and engraftment. Biosponges will offer adhesion sites, biomechanical support, and shielding from inflammatory cells while allowing nutrient diffusion. Additionally, we implemented hypoxic preconditioning of PDSCs. This technique involves exposing cells to a controlled low-oxygen environment which is expected to enhance paracrine signaling, survival rate, and angiogenesis. The goal of this study was to investigate if the hypoxic preconditioned and biosponge encapsulted hPDSCs can improve muscle regeneration and function in rodent model of VML.

METHODS: hPDSCs (5 x 10^5 cells) were seeded on a 10 mm biosponge disc and cultured for a week either in normoxic (21% O_2) or hypoxic (3% O_2) conditions. A VML defect was created by removing ~20% of the tibialis anterior (TA) muscle's mass in adult male Lewis rats unilaterally. A subset of animals underwent no repair (NR). Others were treated with either an acellular biosponge (BS), normoxia-conditioned biosponge encapsulated hPDSCs (N-PDSC-BS), or hypoxia-conditioned biosponge encapsulated hPDSCs (H-PDSC-BS) (n=7-8/group). All animal procedures were approved by Saint Louis University's Institutional Animal Care and Use Committee (animal protocol number 2645). After 28 days post-injury, peak isometric torque was measured, and the muscle tissue was harvested for histological and biochemical analysis. All data were analyzed using one-way ANOVA with Fisher's LSD post-hoc tests.

RESULTS: Biosponge encapsulated hPDSCs remained viable and secreted trophic factors under both normoxic and hypoxic culture conditions *in vitro*. Hypoxic culture enhanced angiogenic factor secretion (e.g., VEGF, bFGF) compared to normoxic cultures. The TA muscle weights normalized to body weight were significantly reduced in the VML-affected limbs relative to the contralateral limbs (ANOVA $p<0.0001$). The H-PDSC-BS ($p=0.0333$) and BS ($p=0.0195$) groups had significantly higher muscle mass compared to the NR group (Figure A). Peak isometric torque was lower in all VML injured groups relative to contralateral (ANOVA $p<0.0001$). Unexpectedly, no improvements in torque were observed over the NR group. Biodistribution of the hPDSCs ($Ku80^+$) revealed that the cells did not migrate into the surrounding musculature. H-PDSC-BS group retained more cells ($Ku80^+$) within the biosponges compared to N-PDSC-BS ($p=0.0195$) (Figure B). In both groups, hPDSCs slowed biosponge remodeling compared to the BS group. Histological analysis of vascularity (von Willebrand Factor or vWF^+) showed that both acellular and cellular treatment groups had higher vessel (ANOVA $p=0.0041$) and junction density (ANOVA $p=0.0008$) than NR, indicative of higher angiogenesis and branching, respectively (Figure C).

DISCUSSION: Our findings reveal that hPDSCs delivered to a VML defect using a biosponge carrier persisted until day 28. Encapsulation of cells also slowed the degradation of the biosponges, which may be a consequence of either immunomodulation or ECM deposition by the cells. Our results show that both BS and H-PDSC-BS implantation can improve muscle mass post-VML, suggesting enhanced muscle regeneration in these groups. The implantation of biosponges with or without hPDSCs also enhanced vascularity following VML injury, further highlighting that biosponges elicit a favorable host response that supports angiogenesis.

CLINICAL RELEVANCE: There are no tissue engineered therapies available for the treatment of VML. Physical therapy is the current standard of care. Furthermore, the clinical utility of stem cell treatments is impeded by various challenges such as suboptimal in vivo engraftment, compromised bioactivity, and uncertain safety profiles. Using a biosponge carrier to deliver stem cells can address some of these challenges. Our results suggest that biosponge encapsulated and hypoxic preconditioned hPDSCs can improve muscle mass and vascularity following VML injury.

REFERENCES:

1. Gabriel Haas, et. Al. *Biomimetic sponges improve muscle structure and function following volumetric muscle loss*. JBMR, 2021. **Volume 109**(11): p. 2280-2293.

FIGURES:

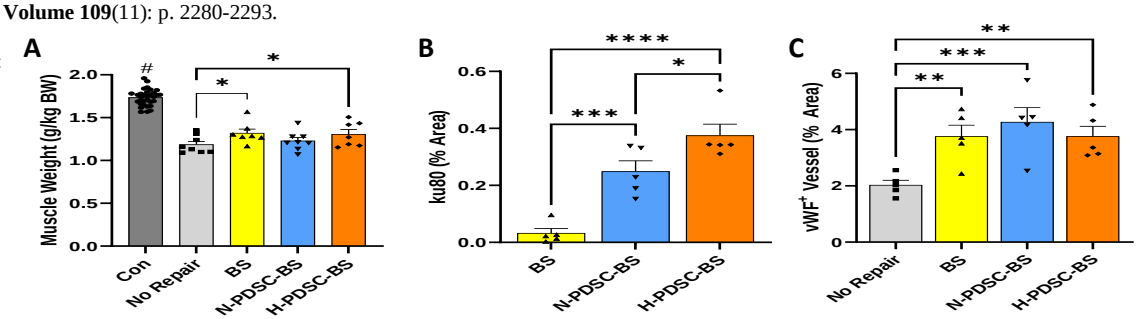

Figure 1. (A) Muscle mass was normalized to body mass of the animal at day 28 post-VML injury. (B) Immunohistological staining and analysis of human Ku80+ cells revealed biodistribution of the hPDSCs (C) Vessel density (% area) was determined through immunohistological analysis of von Willebrand factor (vWF).