Effect of a Degradable Methacrylic Acid-Based Hydrogel on Macrophage Phenotype in Skeletal Muscle

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Statement of Purpose: Skeletal muscle has a remarkable ability to repair itself; however, large muscle injuries cannot heal on their own[1]. The current treatment for large muscle trauma, autologous tissue grafts, is of limited supply and results in scar tissue formation[2]. Regenerative medicine approaches using biomaterials and/or cells are promising, but the resulting muscle function remains well below that of native tissue[3]. Reasons for this impairment include a persistent "pro-inflammatory" macrophage response[4]. A novel method of enhancing muscle repair is through regenerative biomaterials, such as those containing methacrylic-acid (MAA). In the skin, MAA-based materials bias macrophages towards a "pro-regenerative" phenotype[5]. This project investigates the ability of a MAA-poly(ethylene glycol)(PEG) hydrogel to polarize macrophages in skeletal muscle with the goal of enhancing regeneration.

Methods: Degradable hydrogels were synthesized by blending acrylate-terminated PEG with the sodium salt of poly-MAA and dithiothreitol. A 100 mol% PEG hydrogel (PEG gel), a 20 mol% MAA/80 mol% PEG hydrogel (MAA gel), or PBS was injected into the tibialis anterior (TA) muscle of CD1 mice. For *in vivo* degradation rate studies, Fluor^{TM} 647 C_2 Maleimide was grafted onto the hydrogels and the fluorescence was monitored using a Kodak *in vivo* multispectral imaging system. To examine the effect on macrophages, the TA muscle was explanted, after 3, 5 or 7 days, and analyzed using flow cytometry.

Results: The hydrogel injected into the T.A. muscle degraded in 7 days (Fig 1). At day 3, more than half of the leukocytes (CD45+ cells) in the muscles were macrophages (CD45+CD11b+Ly6G-F4/80+) (Fig 2A). In the PBS-injected muscles, most macrophages disappeared by day 5. Macrophages were still present at day 7 in all hydrogel injected muscles with MAA gel-injected muscles having the most macrophages. Finally, the phenotype of the macrophages was examined (Fig 2B). Macrophages were classified based on their expression of MHCII as MHCII+ macrophages have been shown to contribute to type 1 inflammation and hinder muscle repair[6]. At day 7, the PEG gel-injected muscles had a significantly higher percentage of MHCII+ macrophages than the MAA gel-injected muscles. Moreover, the percent of MHCII+ macrophages increased over time for the PEG gels but not the MAA gels. This analysis was not performed on the PBS injected muscle due to the low number of macrophages.

Conclusions: We demonstrated that degradable MAA gels affect the macrophage response in skeletal muscle. MAA gels prolonged the presence of total macrophages while reducing the presence of pro-inflammatory MHCII+ macrophages. We expect that the reduction of these pro-inflammatory macrophages will be beneficial in the context of large injuries. Further studies are underway to determine the therapeutic effect of these gels in regenerating muscle.

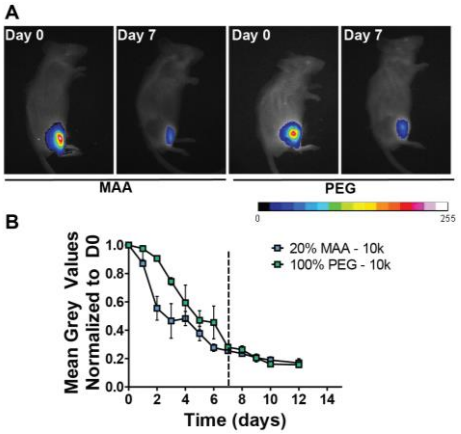

Figure 1: A) Image of MAA- and PEG gel injected mice with overlaid mean grey values. B) Mean grey values normalized to day 0. Error bars are S.E.M; n = 2

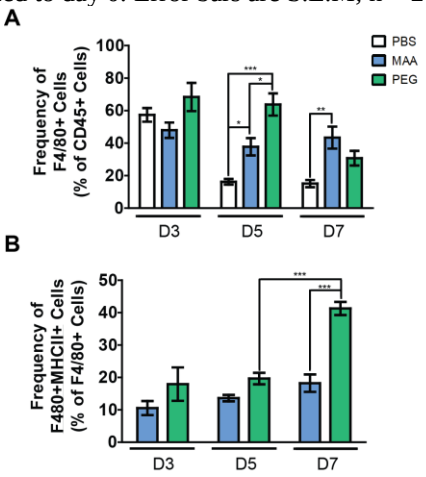

Figure 2: Flow cytometry analysis of all macrophages (A) and MHCII+ macrophages (B) in muscles injected with PBS, MAA or PEG gels. Error bars are S.E.M; n = 3; *p<0.05, **p<0.01, ***p<0.001

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