Intramuscular Injection of Stem Cells in an *In Situ*-Gelling Hydrogel Scaffold: Cell Retention, Angiogenesis, and Inflammation

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Statement of Purpose: A promising strategy for treating peripheral arterial disease involves the injection of mesenchymal stem cells into the ischemic limb with the goals of mediating inflammation and promoting angiogenesis and functional recovery.[1] This strategy has been limited by poor rates of cell retention and survival following injection.[2] Injectable cell scaffolds have the potential to overcome these limitations; however, few injectable scaffold materials can withstand the dynamic mechanical loading conditions of the intramuscular (IM) environment.

In this work, a mechanically robust, *in situ*-gelling composite hydrogel was used to deliver allogeneic adipose-derived stem cells (rASCs) to the adductor muscle of rats. An immune-competent outbred animal model was chosen to investigate the *in vivo* angiogenic and inflammatory responses induced by the combined cell therapy approach in comparison to controls of the injection of rASCs in saline or the hydrogel alone. The treatments were examined via histology and immunohistochemistry over four weeks to compare the retention of rASCs at the site of delivery; the angiogenic response; and the macrophage response in terms of M1-M2 polarization and distribution using multiple markers.

Methods: The composite hydrogel was composed of two polymers: 4% w/v poly(trimethylene carbonate)-*b*-poly(ethylene glycol)-*b*-poly(trimethylene carbonate) diacrylate PEG-$(PTMC\text{-}A)_2$ and 1% w/v methacrylated glycol chitosan functionalized with an RGD peptide (MGC-RGD). Both polymers were dissolved in PBS with 5 mM of redox initiators ammonium persulfate and tetramethylethylenediamine. rASCs were isolated from the epididymal fat pad of Wistar rats, expanded *in vitro* to passage 2, and labeled with PKH26 membrane dye 24 h prior to delivery. Wistar rats received 50 µL IM injection to the adductor muscle of one of the following:

A) Composite hydrogel with $20x10^6$ rASC/mL,
B) Composite hydrogel alone,
C) $20x10^6$ rASCs/mL in saline.

At 1, 2, and 4 weeks, the muscle tissue at the injection site was cryo-sectioned for immunohistochemical analysis at a minimum of three positions across the tissue or scaffold. The number of $PKH26^+$ cells at each position was quantified and normalized to cross sectional area. The number of $CD31^+$ blood vessels was quantified at the scaffold border or at the site of PKH^+ cells. To characterize the immune response at the hydrogel border, the density of $CD68^+$ (as a pan-macrophage marker) and $CD163^+$ (M2) cells was quantified. The density of $CD68^+$ cells expressing inducible nitric oxide synthase ($iNOS^+$; M1) or arginase-1 ($Arg\text{-}1^+$; M2) was quantified. The association of cytokines TNF-α and IL-10 with $CD68^+$ cells was assessed. All animal protocols were approved by the animal care committee at Queen's University.

Results: When delivered in the hydrogel, rASC density in the adductor muscle was significantly higher at all time points by an average factor of 4, compared to delivery in saline (Figure 1a). At 2 and 4 weeks, $CD31^+$ vessel density was significantly higher around rASC-containing hydrogels, in comparison to unseeded hydrogels and rASCs alone (Figure 1b). A similar density of $CD68^+$ cells was observed at the border of both rASC-seeded and unseeded gels (Figure 1c); however, a significantly higher ratio of $CD163^+$ to $CD68^+$ was observed surrounding rASC-seeded hydrogels (Figure 1d).

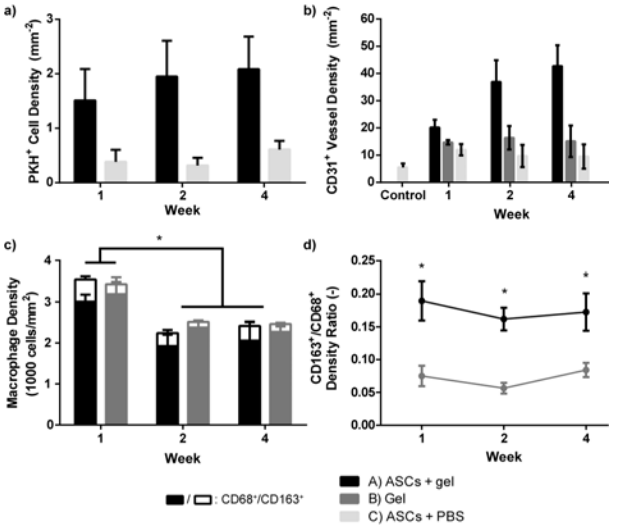

Figure 1: Density within muscle tissue: a) $PKH26^+$ rASCs, (b) $CD31^+$ vessels, (c) $CD68^+$ & $CD163^+$ cells, and (d) Ratio of $CD163^+/CD68^+$ cells.

Almost complete macrophage infiltration was observed in rASC-seeded hydrogels, while infiltration into unseeded hydrogels was limited to an average depth of 120 µm ± 14 µm even after 4 weeks.

Conclusions: These results demonstrate the effectiveness of the injectable, *in situ*-gelling hydrogel scaffold to improve cell retention and localization at the site of IM delivery over an extended period. Further, the presence of rASCs within the hydrogel alter the host response by enhancing angiogenesis around the scaffold, and by shifting the macrophage response towards a more pro-regenerative M2 phenotype. Future work will investigate the delivery of ASCs in a model of hindlimb ischemia to assess the efficacy of the treatment strategy based on limb perfusion and functional recovery.

References: [1]Raval, Z, *et al. Circ. Res.*, 2013, **112** (1288–1302). [2] Hou, D, *et al. Circulation*, 2005, **112** (I150-6).