Matrix Modulus-induced Myogenic Differentiation of Rat Mesenchymal Stem Cells in Thermosensitive Hydrogels

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Statement of Purpose: Mature skeletal muscle cells generally lack regeneration capacity [1]. External stem cells that can differentiate into functional skeletal muscle cells are used for skeletal muscle repair and regeneration [1]. However, the stem cells delivered into the skeletal muscle tissue often experience low level of survival and differentiation, due to harsh environment like ischemia in the injured area. In order to augment cell survival, injectable hydrogels have been used as cell carriers. The hydrogels may protect the cells from the attack by apoptotic cytokines resulted from ischemia. To differentiate cells in the hydrogels, approaches like growth factor induction have been tried. In recent years, people have found that stem cells can differentiate into different cell lineages simply by the influence of matrix stiffness [2]. In this study, we investigated if bone marrow derived mesenchymal stem cells (MSCs) can differentiate into skeletal muscle cells in a new family of thermal sensitive and injectable hydrogels by controlling the hydrogel stiffness.

Methods: Hydrogels were synthesized via free radical polymerization method as previously described [3]. In short, stoichiometric amount of NIPAAm, acrylic acid and macromer hydroxyethyl methacrylate-polym(hydroxybutyrate) (HEMA-HB) were polymerized using benzoyl peroxide as an initiator. The HEMA-HB macromers were synthesized via ring-opening polymerization of 2-hydroxybutyrate. Number of HB units in the macromer was controlled to be 2, 4 and 6, respectively. The polymers synthesized using these macromers were abbreviated as HB2, HB4 and HB6, respectively. Rat mesenchymal stem cells (MSCs) were pre-stained with a live cell tracker CMFDA, mixed with hydrogel solution (20 wt% in DPBS), and then incubated under normal culture conditions. The effect of matrix modulus on MSC differentiation was evaluated after 1, 7, and 14 days of culture. Cells were characterized in terms of dsDNA content, gene expression (by real time RT-PCR) and protein experession (by immunohistology).

Results: All three hydrogels have number average molecular weight between 10 and 13kDa, with PDI around 1.3. The thermal transition temperatures are between 15°C and 24°C. The hydrogel solutions have a fast gelation rate (5-7s). By tailoring the length of HB in the hydrogels, hydrogels with different modulus were obtained (40.1±3.7 kPa for HB2, 20.0±2.6 kPa for HB4, and 11.1±3.5 kPa for HB6). These hydrogels have similar water content. MSC proliferation in hydrogels was quantified by dsDNA content. Cell number significantly increased in all three hydrogels during the 2-week culture period, demonstrating the hydrogels support MSCs growth. This is consistent with live cell images.

The myogenic differentiation of the capsulated MSCs was investigated by real time RT-PCR and immunohistology. MSCs in HB4 with modulus of around 20 kPa showed the highest expression of myogenic mature markers of MHC, Desmin, Myogemin, MyoD1 and MEF2. The gene expression results were consistent with the protein expression. Myosin heavy chain (MHC), a crucial component of muscle tissues responsible for contraction, was expressed by most MSCs seeded in HB4, while those in HB2 and HB6 almost showed no sign of MHC expression.

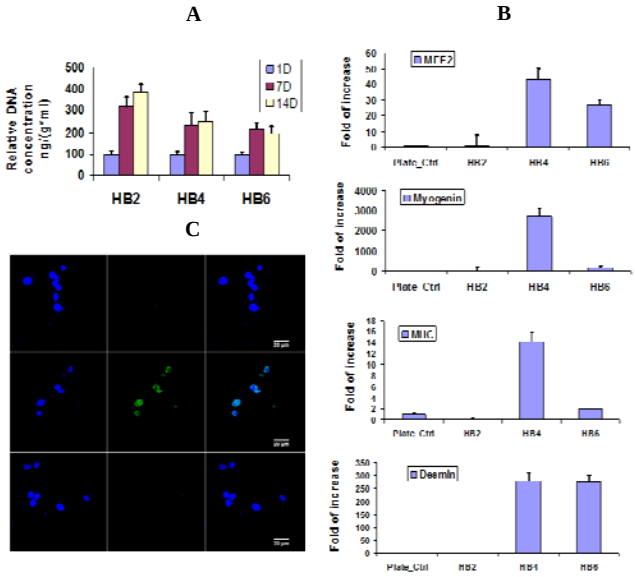

Figure 1. A. dsDNA concentration; B. Gene expressions; C. Protein expression (MHC) of MSCs seeded in hydrogels after 2 weeks of culture.

Conclusions: Matrix modulus has been recognized as a critical biomechanical factor on the fate of cells including proliferation, migration and differentiation [2]. In this work, a family of hydrogels with different modulus was synthesized by controlling the length of HB side chain in the repeating unit. The MSCs showed modulus dependent differentiation in these hydrogels. The 20kPa hydrogel most significantly stimulated MSCs differentiated into skeletal muscle cells [4]. These hydrogels have the potential to serve as MSC carriers for functional muscle regeneration.

References:

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