Factors on the Cell Viability of Hydrogel Induced Human Mesenchymal Stromal Cell Therapy

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Statement of Purpose: Mesenchymal stromal cells (MSCs) have shown potential therapeutic benefits for a range of medical disorders and maintain a focus of intense scientific investigation. Transplantation of stem cells into injured tissue can improve wound healing, tissue regeneration and functional recovery. However, implanted cells rapidly lose their viability or fail to integrate into host tissue [1]; effects of induced biomaterials also disappear as they degrade. Optimization of cell therapy requires delivery to the target area without significant loss of cellular viability or function. Current tissue engineering techniques have incorporated biomaterial and cells to overcome these limitations. Hyaluronan hydrogel (HyStem-C) is a synthetic biomaterial that mimics the natural extracellular matrix component, hyaluronic acid [2-3], and can provide a biocompatible environment for cell attachment, survival, migration, growth and proliferation [4-6]. Therefore, treatment with HyStem-C seeded with cells may accelerate the formation of new tissue and improve the quality of this newly generated tissue, serving as a potential engineering tool for clinical regeneration applications. Unfortunately, there is paucity in the literature regarding factors that affect biomaterial/cell viability that may increase transplantation efficiency for tissue regeneration. In this study, we selected human bone marrow derived MSCs (BM-MSCs) to analyze cell viability in response to mechanism forces caused by syringe needles, cell density and dimethylsulfoxide (DMSO) concentration of cell-hydrogel solution. The purpose of this study is to clarify which factors are important for enhancing biomaterial-induced cell transplantation efficiency and provide much needed guidance for clinical trials.

Methods: Human BM-MSCs were isolated from human donors and stocked in 10% human albumin serum and 2.5% DMSO in liquid nitrogen. Injectable chemically modified HyStem-C was synthesized using a biocompatible, thiol-modified semisynthetic glycosaminoglycan analogous (HA-DTPH), thio-modified gelatin (gelatin-DTPH) and crosslinked by PEGDA [2]. For 3-diemnsional culture (3D), 0.5ml mixed solution of HyStem-C and cells at densities (1 x 10^6, 2 x 10^6 and 1 x 10^7 cells/ml) and DMSO concentrations (0.1%, 0.5% and 1.0%) were transferred onto transwell permeable inserts by 25 or 27-gauge syringe needle (25G or 27G needle) or pipet (control). To investigate cell survival rate in HyStem-C, cells were doubly stained by calcein AM and ethidium homodimer-1 (Live/Dead Viability/Cytotoxicity kit). Stained cells were imaged with Nikon E600 florescence microscope equipped Olympus CCD camera using green and red filters. Percentage of live and dead cells was determined with MetaMorph software for each condition in quadruplicate and analyzed with SAS statistical software.

Results: Cell viability was determined by simultaneous staining live cells (green) and dead cells (red) Fig. 1. 27G needle-delivered BM-MSCs showed lower survival than control pipet-delivered cells, however there is no statistical difference, suggesting that physical force caused by the 27G needle did not significantly affect cell viability. Cell viability at 48h were slightly higher than at 2h, revealing that the 27G needle could increase early apoptotic cells immediately post injection but with some recovery by 48 hours post injection. After 48-hour incubation, 25G- and 27G-needle-delivered cell viabilities were above 82% (82.2 to 87.4%, respectively) in the cell density range of 1x10^6 to 1x10^7 cells/ml HyStem-C (Fig.2). When the final DMSO concentration was increased to 1.0% (Fig. 3), 27G-needle-deliveried BM-MSCs displayed significantly lower survival rates (77.3%) compared to 0.1to 0.5% DMSO and 25G needle and pipet-delivered cells (p<0.01).

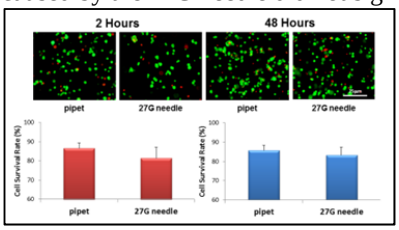

Figure 1. Fluorescent images of viable (green) and membrane damaged (red) BM-MSCs in HyStem-C and effect of physical force on cell viability after 2 and 48-hour incubation. Scale bars, 2.5µm.

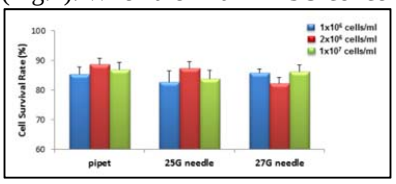

Figure 2. Effect of cell density in HyStem-C on BM-MSCs viability after 48-hour incubation

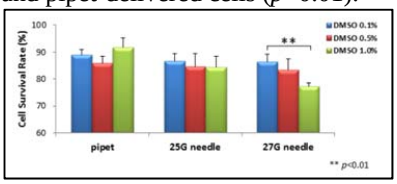

Figure 4. Effect of final DMSO Concentration on BM-MSCs viability after 48-hour incubation

Conclusions: Cryopreserved BM-MSCs survival rate in HyStem-C is important for hydrogel induced cell therapy. For hydrogel induced cell therapy, cryopreserved BM-MSCs induced by common clinical needles (25G or 27G) at cell densities (less than 1x10^7 cells/ml) and DMSO concentrations (less than 0.5%) may provide better cell therapeutic approaches for regenerative medicine. In this study, we have mainly focused on BM-MSCs viability after injection. Further work will focus on the induced MSCs function (such as differentiation and proliferation) in these common clinical cell therapy conditions.

References: 1. Molcanyi M, et al., J Neurotrauma. 2007;24:625-37. 2. Shu XZ, et al., Biomacromolecules. 2002;3:1304-11. 3. Shu XZ, et al., Biomaterials. 2003;24:3825-34. 4. Qian L, et al., Tissue Eng Part A. 2012;18:1652-63. 5. Chen X, et al., Acta Biomater. 2010;6:2940-8. 6. Johnson BQ, et al., Laryngoscope. 2010;120:537-45.

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