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Cell density, dimethylsulfoxide concentration and needle gauge affect hydrogel-induced bone marrow mesenchymal stromal cell viability

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Abstract

Mesenchymal stromal cells (MSCs) have shown potential therapeutic benefits for a range of medical disorders and continue to be a focus of intense scientific investigation. Transplantation of MSCs 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. Hydrogel-seeded bone marrow (BM)-MSCs offer improved viability in response to mechanical forces caused by syringe needles, cell density and dimethylsulfoxide (DMSO) concentration, which in turn, will help to clarify which factors are important for enhancing biomaterial-induced cell transplantation efficiency and provide much needed guidance for clinical trials. In this study, under the control of cell density ($<2 \times 10^7$ cells/mL) and final DMSO concentration (<0.5%), hydrogel-induced BM-MSC viability remained >82% following syringe needle passage by 25- or 27-gauge needles, providing improved cell therapeutic approaches for regenerative medicine.

Key Words: *cell density, cell transplantation, cell viability, cryopreserved cells, dimethylsulfoxide, hyaluronan hydrogel, mesenchymal stromal cells*

Introduction

Stem cell therapy is the introduction of progenitor cells into a tissue or organ as a means to offer treatment for various diseases and injuries [1]. Mesenchymal stromal cells (MSCs) have become a popular source for stem cell therapy because they have differentiation and immunologic features [2], and they offer feasibility and safety in clinical trials [1]. Enhancement of both autologous and allogenic transplanted cell survival has become a vital and rapidly expanding area of investigation [3], with good manufacturing practices (GMPs) required to offer optimal defined quality and safety in cell transplantation.

Furthermore, enriching stem cell therapy is the use of biomaterials as a vehicle for implantation of cells into local tissue for regenerative purposes [4]. Within the past four decades, chemically and physically diverse hydrogels have emerged to become standard materials in regenerative biology due to their unique biocompatibility. Hyaluronic acid (HA)-based hydrogels have been found to be a synthetic biomaterial [5] that can protect encapsulated cells from inflammation and surrounding macrophages [6] and to provide a biocompatible environment for cell attachment, survival, migration, growth and proliferation [7]. In addition to the mechanical protection provided by encapsulated hydrogels, other factors for optimal cell viability outcomes involve cell-seeding density as well as cryopreservation techniques.

Proper freezing, transport and cryopreservation of stem cells are crucial for viability, safety and efficiency in cell therapy [8], with recent cell treatments more often involving cryopreserved cells due to the ease of storage, transportation and large-scale supplementation.

Dimethyl sulfoxide (DMSO) has widespread applications as a drug injectate solvent, cryoprotectant and differentiating agent [9]; however economical, this has been shown to adversely affect cell viability, morphology, differentiation, and gene expression in a dose-dependent manner [10,11]. Furthermore, numerous studies have contested the GMP of conventional 10% DMSO for long-term cell storage, elucidating a safe alternative at 5% [12,13]. DMSO concentration in freezing medium, cell freezing procedures and period of cell storage time [14] have all been extensively scrutinized, however, to date, no studies have reported on the effects of DMSO on MSC viability when used as an injectable solvent for cell transplantation following clinically relevant delivery models.

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Previous investigations have provided initial insight into important biological concerns for enhancing the efficiency of cell therapy, however, further study is required to identify important parameters for enhancing biomaterial-induced cell transplantation efficiency and to provide current GMPs for clinical trials. The goal of the present study was to evaluate the effects of several cell delivery factors on the survival of clinical-grade current good manufacturing practice (cGMP) MSCs for hydrogel-induced cell treatment after passage through clinically relevant 25-gauge (25G) or 27-gauge (27G) syringe needles. These parameters included the following: (1) cell-seeding density and (2) concentration of DMSO in final hydrogel-cell solution. Results of the present study should provide clinically relevant and necessary benchmarks for cell transplantation clinical trials.

Methods

HA hydrogel preparation

HyStem-C is a low-salt HA-gelatin hydrogel (Biotime Inc.) that was obtained by mixing 1 mL 1.4% (wt/vol) Glycosil with 75 uL 1.0% (wt/vol) Gelin-S and cross-linking this mixture with 8.2% (wt/vol) Extralink poly (ethylene glycol) diacrylate (PEGDA). The final concentration of HyStem-C is 1.2% Glycosil, 0.06% Gelin-S and 0.8% PEGDA. All components were dissolved in Lactated Ringer's solution (pH 7.3–7.4) in a cell culture hood to ensure sterility. At room temperature, HyStem-C casts in about 5 min.

Cells and three-dimensional cell culture

Clinical-grade primary human bone marrow (BM)-MSCs were produced, expanded and tested by Waisman Biomanufacturing at the University of Wisconsin-Madison, using a manufacturing process and quality control test methods that are similar to those used for cGMP protocols for human clinical trials. BM aspirates were obtained from a 22-year-old female donor, and BM-MSCs were isolated using the Ficoll-Paque gradient method as previously described [15]. Once isolated, MSCs were cultured in alpha minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum (FBS) and 1X Glutamax. Cell cultures were maintained at 37°C in a humidified incubator with 5% CO^2 atmosphere and medium was changed once every 2 days; cell growth was monitored under phase-contrast light microscopy. Cells positively expressed common MSC-specific cell surface markers CD105, CD73 and CD90, and were negative for CD34, CD45, CD19 and CD14 [16].

Once the cells reached sub-confluence, cells were harvested with TrypLE, and expended into new flasks. For cryopreservation, BM-MSCs were resuspended in cold freezing medium containing PlasmaLyte, 10% serum albumin and 2.5% DMSO, aliquoted into cryovials and subsequently frozen by steps involving slowly decreasing temperatures to a final freeze point of -196°C. Cryopreserved cells were stored in liquid nitrogen. Passage six cells were used in all experiments.

NIH 3T3 cells, used as controls, initially emerged from a cell line established in 1962 at the New York University School of Medicine Department of Pathology, and have since become a widely accepted and standard fibroblast cell line. NIH 3T3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% calf bovine serum (CBS), 1X non-essential amino acid (all from Sigma), and cryopreserved for long-term storage in DMEM containing 10% CBS and 5% DMSO. Before use, cryopreserved cells were quickly thawed in a 37°C water bath with gentle agitation, and then mixed with hydrogel dressing.

For three-dimensional culture, cell suspensions were mixed with HyStem-C at one of four cell densities: 2×10^6, 4×10^6, 1×10^7 and 2×10^7 cells/mL. These cell densities were chosen because they represent densities that have been reported in the literature for tissue-specific injections. Cell-gel mixture (0.5 mL) was placed into the individual wells of a six-well plate with transwell permeable inserts (0.4 μm membrane pore size; Millipore Inc.) by pipette, 25G, 27G standard syringe needles and 27G longer ear, nose, throat (ENT) (27G ENT) needle. These gauges were chosen because they are common gauges used for injection of biomaterials. After gelation (gel thickness was approximately 0.5 mm), cell culture medium (DMEM-10% FBS or DMEM-10% CBS) was added above and below the gel. Cell plates were kept in an incubator at 37°C and 5% CO^2. Use of transwell plates allowed for completion of the cell viability assays. To test DMSO of frozen media on cell viability, DMSO concentration in final cell-gel mixture was controlled at 0.1, 0.5 and 1.0%.

Cell viability assay

Cell survival rates in HyStem-C were analyzed using the LIVE/DEAD Viability/Cytotoxicity Kit (Invitrogen), which is based on a cell-permeable dye for staining of live cells and a cell-impermeable dye for staining of dead and dying cells, subsequently characterized by compromised cell membranes. Live cells are distinguished by the presence of ubiquitous intracellular esterase activity as determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant calcein AM to calcein, which displays as an intense, uniform green fluorescence in live cells (ex/em 495 nm/515 nm). The red component (ethidium homodimer-1, EthD-1) is cell-permeant and, therefore,