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The use of injectable spherically symmetric cell aggregates self-assembled in a thermo-responsive hydrogel for enhanced cell transplantation

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ABSTRACT

Typical cell transplantation techniques involve the administration of dissociated cells directly injected into muscular tissues; however, retention of the transplanted cells at the sites of the cell graft is frequently limited. An approach, using spherically symmetric aggregates of cells with a relatively uniform size self-assembled in a thermo-responsive methylcellulose hydrogel system, is reported in the study. The obtained cell aggregates preserved their endogenous extracellular matrices (ECM) and intercellular junctions because no proteolytic enzyme was used when harvesting the cell aggregates. Most of the cells within aggregates (with a radius of approximately 100 μm) were viable as indicated by the live/dead staining assay. After injection through a needle, the cell aggregates remained intact and the cells retained their activity upon transferring to another growth surface. The cell aggregates obtained under sterile conditions were transplanted into the skeletal muscle of rats via local injection. The dissociated cells were used as a control. It was found that the cell aggregates can provide an adequate physical size to entrap into the muscular interstices and offer a favorable ECM environment to enhance retention of the transplanted cells at the sites of the cell graft. These results indicated that the spherically symmetric cell aggregates developed in the study may serve as a cell delivery vehicle for therapeutic applications.

1. Introduction

Cell transplantation via local intramuscular injection is promising for therapeutic application in ischemic and degenerative disorders of both skeletal and cardiac muscles [1,2]. Prior to cell transplantation, a large scale of the desired cell types must be expanded *in vitro* on tissue culture polystyrene (TCPS) dishes. Upon confluence, detachment of the cultured cells from TCPS dishes often requires using a proteolytic enzyme [3]. The use of enzyme, however, commonly dissociates the cultured cells and disrupts their extracellular matrices (ECM) and integrative adhesive agents [4].

Additionally, following injection of the dissociated cells, retention of the transplanted cells at the sites of the cell graft is frequently limited [5]. We speculated that the dissociated cells injected into muscles might not have a sufficient physical size to entrap into the muscular interstices and the transplanted cells do not have a temporary matrix to which they can attach, thus causing the cell loss. To overcome these problems, an approach, using multicellular aggregates with the preservation of endogenous ECM self-assembled in a thermo-responsive methylcellulose (MC) hydrogel system, is developed in the study to enhance the engraftment of cells.

MC is a water-soluble polymer derived from cellulose, the most abundant polymer in nature. Aqueous MC undergoes a sol–gel reversible transition upon heating or cooling [6,7]. This unique thermo-responsive behavior of MC has made it as a promising functional hydrogel for various biomedical applications [8,9]. It was shown in our previous study that the hydrated surface of the MC hydrogel is hydrophilic and neutrally charged [9]. Such kind of culture surface can effectively inhibit the protein adsorption and the attachment of cells onto substrates [10], thus prompting aggregation of the seeded cells.

In this study, construction of spherically symmetric aggregates of mesenchymal stem cells (MSCs) with a relatively uniform size in the MC hydrogel system is reported. MSCs are of significant interest as a renewable source of therapeutically useful cells; they retain the ability to differentiate into various types of tissue cells and contribute to the regeneration of a variety of mesenchymal tissues including muscle and adipose [11,12]. The morphology and activity

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of the obtained cell aggregates, before and after injection through a needle, were examined *in vitro*. Additionally, transplantation of cell aggregates in the skeletal muscle of a rat model via local intramuscular injection was investigated. The dissociated MSCs were used as a control.

2. Materials and methods

2.1. Preparation of the cell aggregate culture system

Aqueous MC solutions (12% by w/v) were prepared by dispensing the weighed MC powders (Sigma–Aldrich, St. Louis, MO, USA) in heated water with the addition of phosphate buffered saline (PBS, 5.0 g/l) at 50 °C [8]. The prepared MC solution was autoclaved and then kept in a refrigerator at 4 °C for 24 h. The obtained homogeneous MC solution at 4 °C was poured into each well of a 96-well PCR plate (Applied Biosystems, Foster City, CA, USA) individually. Subsequently, the plate was pre-incubated at 37 °C for 2 h and an opaque gelled layer in each well was formed and the obtained hydrogel system was used to cultivate cell aggregates (Fig. 1).

2.2. Cultivation of cell aggregates

Bone-marrow MSCs were isolated from the femora and tibia of FVB green-fluorescent transgenic mice which were generated and bred at the National Health Research Institutes, Miaoli, Taiwan. The expression of enhanced green-fluorescent protein (EGFP) was under the control of the cytomegalovirus (CMV) enhancer and the chicken β-actin promoter, which was derived from an expression vector, pCAGGS [13]. The isolated MSCs were cultured in alpha-Modified Minimum Essential Medium (Gibco, Grand Island, NY, USA) supplemented with 20% fetal bovine serum (Hyclone, Logan, UT, USA) and 1% penicillin–streptomycin (Gibco). After reaching confluence, cells were dissociated from culture dishes with a 0.05% trypsin and then seeded in the prepared 96-well MC hydrogel system with a multichannel pipette at different cell densities (5.0×10^3, 1.0×10^4, 5.0×10^4, 1.0×10^5 or 2.0×10^5 cells/well) and cultivated for 24 h to form cell aggregates. Finally, the grown cell aggregates were colleted with a multichannel pipette and loaded in a syringe for cell transplantation (Fig. 1).

2.3. Characterization of cell aggregates

Cell aggregates forming in the 96-well MC hydrogel system incubated in a living chamber were imaged every 5 min using a fluorescent microscope (Axio Observer Z1, Zeiss, Jena, Germany). The diameters of the grown cell aggregates were measured using an Image-Pro Plus (Version 4.5, Media Cybernetics, Bethesda, MD, USA) software ($n = 7$ batches). The viability of cells in aggregates was investigated using a Live/Dead Viability kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Additionally, cell aggregates were trypsinized and subjected to trypan blue dye exclusion to determine total viable cells.

The morphology, endogenous ECM and integrative adhesive agents of cell aggregates, before and after injection through a needle, were examined. Briefly, cell aggregates were resuspended in 3 ml of culture medium, loaded in a syringe, injected through a 27-gauge needle and subsequently seeded onto a 12-well culture plate (Costa Corning, Cambridge, MA, USA). Changes in morphology of cell aggregates on the plate with time in a living chamber were investigated and photographed using a fluorescent microscope (Zeiss) or an inverted microscope (Eclipse TE200, Nikon, Tokyo, Japan).

Paraformaldehyde-fixed cell aggregates were prepared for immunofluorescent staining. The antibodies used were collagen type I (MP Biomedical, Solon, OH, USA), collagen type III (Chemicon, Temecula, CA, USA), fibronectin (Abcam, Cambridge, UK), laminin (Chemicon) and E-CAM (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Different Alexa Fluor secondary antibodies (Invitrogen) were used to obtain fluorescent colors. Cell aggregates were costained to visualize F-actins and nuclei by

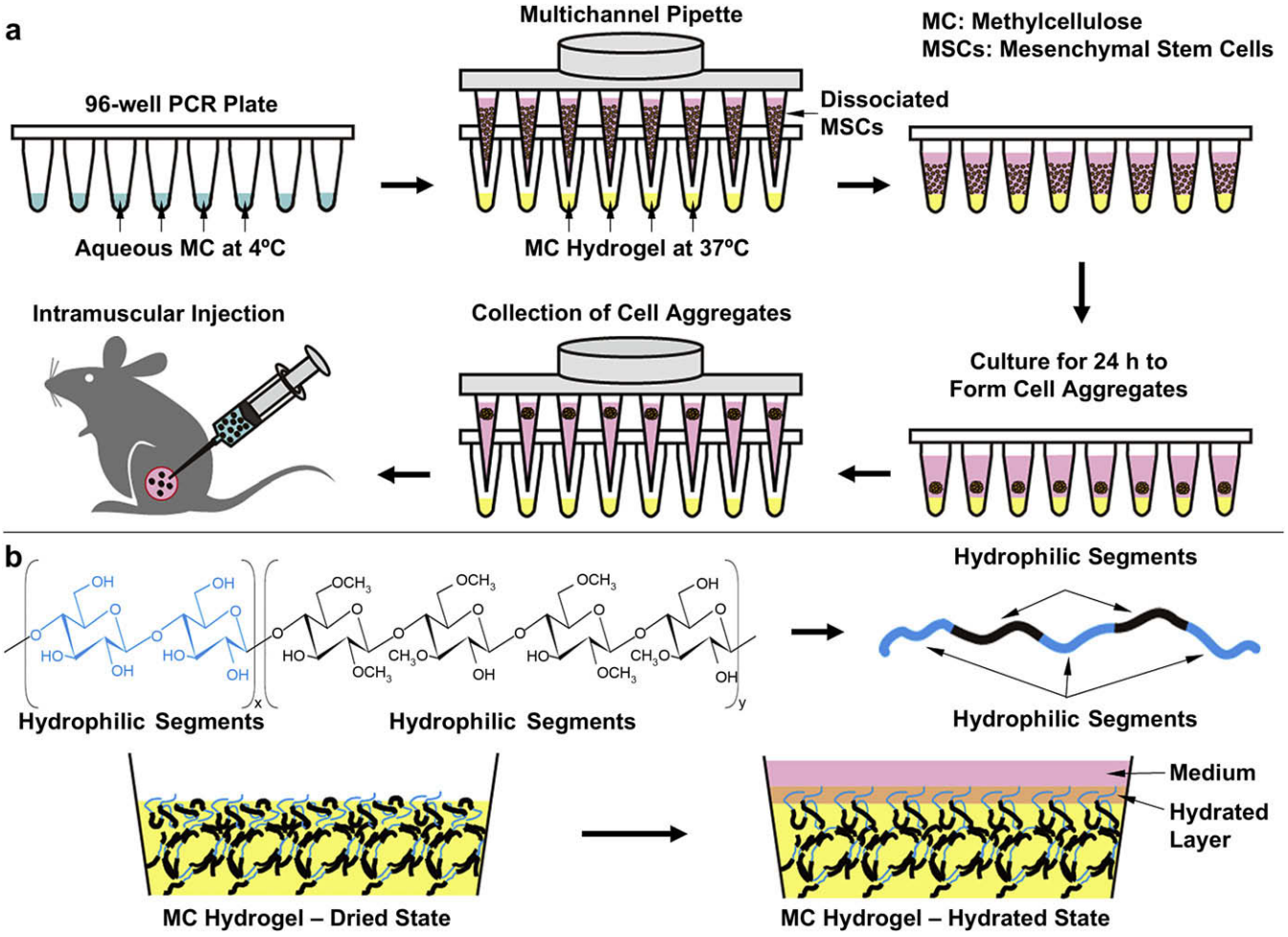

Fig. 1. (a) Schematic illustrations of the process used for the construction of spherically symmetric cell aggregates inherent with the endogenous extracellular matrices for local intramuscular injection; (b) Physical structures of the MC hydrogel formed in the wells of a 96-well PCR plate in the dried or hydrated states. MSC: mesenchymal stem cell; MC: methylcellulose.