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Single-cell hydrogel encapsulation for enhanced survival of human marrow stromal cells

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ABSTRACT

Inadequate extracellular matrix cues and subsequent apoptotic cell death are among crucial factors currently limiting cell viability and organ retention in cell-based therapeutic strategies for vascular regeneration. Here we describe the use of a single-cell hydrogel capsule to provide enhanced cell survival of adherent cells in transient suspension culture. Human marrow stromal cells (hMSCs) were singularly encapsulated in agarose capsules containing the immobilized matrix molecules, fibronectin and fibrinogen to ameliorate cell-matrix survival signals. MSCs in the enriched capsules demonstrated increased viability, greater metabolic activity and enhanced cell-cytoskeletal patterning. Increased cell viability resulted from the re-induction of cell-matrix interactions likely via integrin clustering and subsequent activation of the extracellular signal regulated MAPK (ERK)/mitogen activated protein kinase (MAPK) signaling cascade. Proof of principle in-vivo studies, investigating autologous MSC delivery into Fisher 344 rat hindlimb, depicted a significant increase in the number of engrafted cells using the single-cell encapsulation system. Incorporation of immobilized adhesion molecules compensates, at least in part, for the missing cell-matrix cues, thereby attenuating the initial anoikis stimuli and providing protection from subsequent apoptosis. Thus, this single-cell encapsulation strategy may markedly enhance therapeutic cell survival in targeted tissues.

1. Introduction

The aim of cell-based ischemia repair is to compensate for the loss of function due to insufficient muscle mass both in the heart, in the case of myocardial infarction (MI), and in ischemic limbs in cases of peripheral vascular disease (PVD) [1]. Many strategies being pursued to restore muscle function involve repopulating the ischemic region with exogenously delivered cells, either by direct injection or intravascular delivery [2,3]. In the case of MI, the first attempts to repopulate the scarred aftermath of myocardial infarction were over fifteen years ago [4,5]. Since then, interest in cell transplantation for the prevention and treatment of heart failure as well as PVD has flourished, leading to multiple animal studies and clinical trials [6,7]. Despite the developments in cell transplantation, tissue regeneration is significantly limited by death of transplanted cells. Critical factors currently impeding cell viability and organ retention include: (1) injection site cell leakage, and clearance by the vasculature and lymphatic system; (2) mechanical insult during injection and delivery; (3) inadequate extracellular matrix cues at the site of transplantation; and (4) subsequent apoptotic and necrotic cell death [7,8–18].

The first stress the cells encounter is lack of matrix support, which essentially begins even prior to transplantation. When cells that normally grow in adherent conditions are forced into suspension; as in the case of adherent cell-based therapeutic strategies, a pathway of cell death termed anoikis is initiated [19]. Anoikis, the Greek word for 'homelessness', is defined as programmed cell death induced by loss of matrix attachments [21–29]. The attachment of cells to components of the extracellular matrix (ECM) is primarily regulated by integrins (heterodimeric transmembrane cell-surface receptors for ECM proteins), which play a key role in mediating survival signals, hence providing protection from anoikis [23–27]. Integrins associate with a number of proteins on the cytoplasmic side of the plasma membrane, forming cell–ECM complexes that supply a structural link between the ECM and the cytoskeleton and act as a scaffold for signaling molecules [20]. Several studies have addressed the significance of selected proteins such as fibronectin and fibrinogen in mediating survival signals, all showing different results depending on the cell type in question and the anoikis models chosen [23–27]. The intracellular mechanisms of anoikis can involve loss of signaling through a number of

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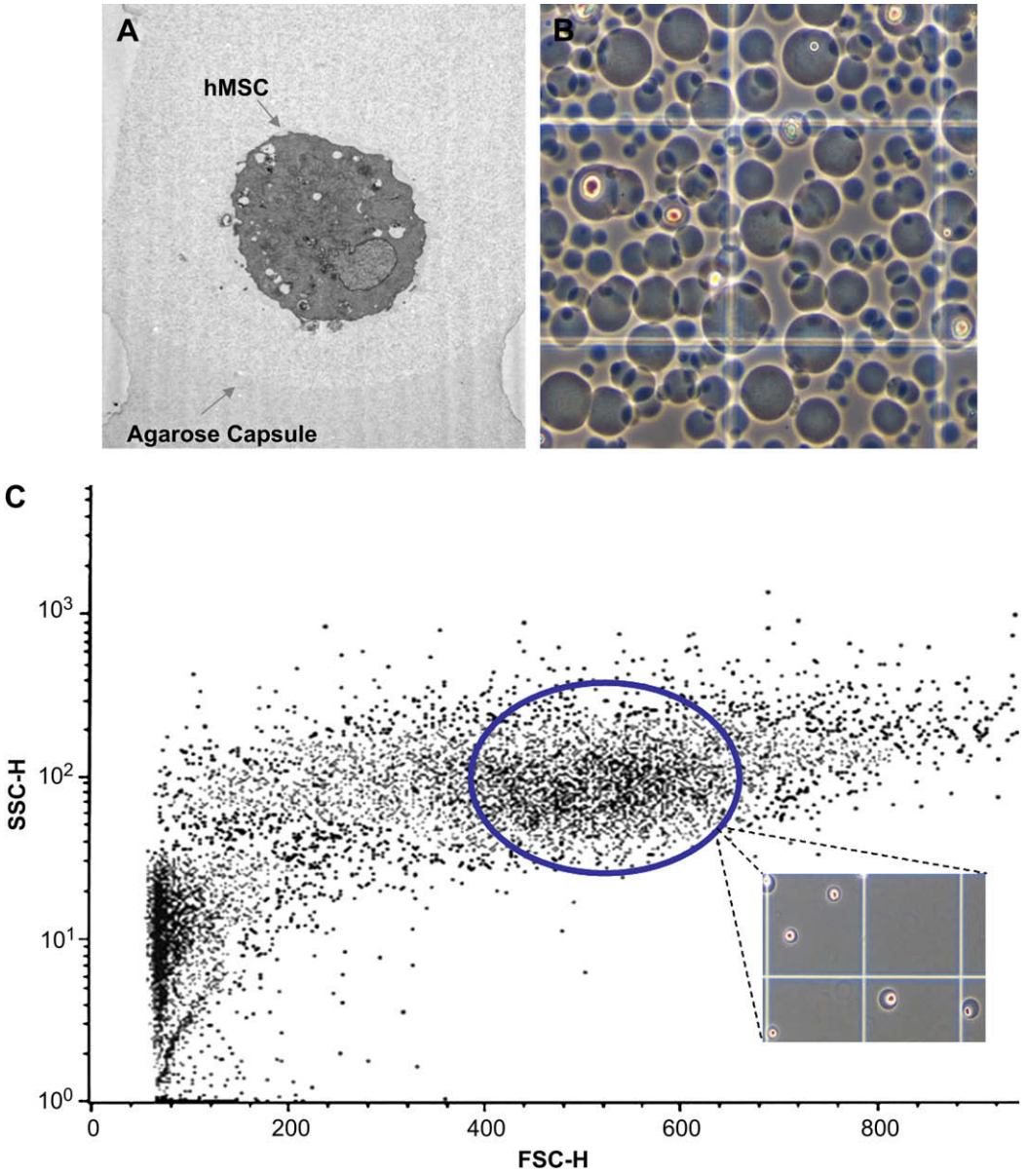

Fig. 1. Single-cell encapsulation of human marrow stromal cells. (A) Transmission electron microscopy of a singly encapsulated cell in an agarose capsule. (B) Phase contrast light microscopy image of singly encapsulated cells (20×). (C) Representative flow cytometry profile of human marrow stromal cells with the *x*-axis representing the forward scatter of light indicative of cell size and the *y*-axis representing the side scatter of light indicative of cell granularity. The singly encapsulated cells are gated and the insert is a phase contrast light microscopy image of the sorted singly encapsulated cell population (10×).

pathways including: focal adhesion kinase (FAK), adaptor protein Shc, integrin linked kinase (ILK), phosphoinositide-3-OH kinase (PI3K)/protein kinase B (PKB/Akt), extracellular signal regulated MAPK (ERK)/mitogen activated protein kinase (MAPK), and jun N-terminal kinases [28–40]. The pathway that predominates in anoikis prevention will be a function of both the cell type in question and the specific cell-matrix interactions.

The objectives of this study are three fold. Firstly, we assess the fate of human marrow stromal cells (hMSCs) in a three-dimensional single-cell agarose hydrogel anoikis model. Secondly, we investigate whether we can manipulate the hydrogel 'capsule' to provide the necessary extracellular matrix-cell signaling resulting in attenuation of anoikis and enhancement of hMSC viability. And finally, we examine if our single-cell encapsulation system will ameliorate the retention of syngeneic rat marrow stromal cells in the rat hindlimb.

2. Methods

2.1. Cells and cell culture

Cryopreserved human marrow stromal cells (hMSCs) were kindly provided by Tulane Centre for Gene Therapy (New Orleans; LA) and cultured according to the specific protocols provided. Briefly, cells were cultured in human marrow cell culture media containing Alpha Minimum Essential Media, αMEM (Invitrogen Canada Inc., Burlington; Canada), supplemented with 16.5% fetal bovine serum, FBS (Atlanta Biologicals Inc., Lawrenceville, GA, USA), 1% L-glutamine (Invitrogen Canada Inc., Burlington; Canada) and 1% penicillin/streptomycin solution, P/S (Invitrogen Canada Inc., Burlington; Canada). Cells were incubated at 37 °C, in a humidified 5% CO_2 environment in T-75 cm^2 flasks with media replacement every three days. All experiments were carried out on cells between passages 3 and 5.

2.2. Single-cell encapsulation

Human marrow stromal cells (1.0×10^6–1.5×10^6) were re-suspended in approximately 200 μL of hMSC culture media and mixed with 400 μL of 4% ultra low