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The effect of encapsulation of cardiac stem cells within matrix-enriched hydrogel capsules on cell survival, post-ischemic cell retention and cardiac function

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ABSTRACT

Transplantation of *ex vivo* proliferated cardiac stem cells (CSCs) is an emerging therapy for ischemic cardiomyopathy but outcomes are limited by modest engraftment and poor long-term survival. As such, we explored the effect of single cell microencapsulation to increase CSC engraftment and survival after myocardial injection. Transcript and protein profiling of human atrial appendage sourced CSCs revealed strong expression the pro-survival integrin dimers αVβ3 and α5β1- thus rationalizing the integration of fibronectin and fibrinogen into a supportive intra-capsular matrix. Encapsulation maintained CSC viability under hypoxic stress conditions and, when compared to standard suspended CSC, media conditioned by encapsulated CSCs demonstrated superior production of pro-angiogenic/cardioprotective cytokines, angiogenesis and recruitment of circulating angiogenic cells. Intra-myocardial injection of encapsulated CSCs after experimental myocardial infarction favorably affected long-term retention of CSCs, cardiac structure and function. Single cell encapsulation prevents detachment induced cell death while boosting the mechanical retention of CSCs to enhance repair of damaged myocardium.

1. Introduction

While mechanical interventions and pharmaceuticals have reduced the mortality associated with myocardial infarction, survivors are often left with significant damage and chronic heart failure [1]. Recently, transplantation of *ex vivo* proliferated cardiac stem cells (CSC) has garnered attention as a promising means of improving left ventricle function while reducing infarct size [2,3]. Despite these developments, the full capacity of CSCs to repair myocardium is limited by modest retention immediately after transplant into the vascular heart which, in part results in very low long term survival [4,5]. This notion is supported by several recent papers demonstrating early CSC engraftment predicts later cardiac recovery from myocardial infarction while methods targeted towards boosting the early retention of transplanted cells enhance cells-mediated cardiac repair [6–9]. These measures address the challenge to acutely retain injected cells within a vascular organ by reducing mechanical extrusion from the myocardium and clearance through lymphatic or venous drainage [10,11]. But once cells are acutely retained in damaged myocardium, ongoing cell death ensues as the suspended cells have lost vital integrin-dependent attachments to the extracellular matrix (ECM)(12) which reduces stimulation of pro-survival pathways and results in detachment-induced programmed cell death (or anoikis) [13,14].

The objective of this work is to increase the survival of *ex vivo* proliferated CSCs during the suspension period that precedes cardiac transplantation to boost acute engraftment. Molecular profiling of the CSC adhesion molecules will permit the rationale design of a supportive three-dimensional hydrogel capsule to increase acute retention of transplanted cells and militate against anoikis. We hypothesize that encapsulation will improve acute retention of CSCs by preventing mechanical clearance from the heart while protecting against anoikis. Ultimately, our goal is to provide cocooned cells with the opportunity to survive, mobilize and repair infarcted myocardium.

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2. Methods

2.1. Patients and cell culture

Human CSCs were cultured from the atrial appendages obtained from patients undergoing clinically-indicated surgery using established methods (Fig. 2A) [15]. Briefly, tissue sections were minced and digested prior to being placed in cardiac explants media (CEM; Iscove's Modified Dulbecco's Medium (Invitrogen), 20% Fetal Bovine Serum (FBS; Invitrogen), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen), 2 mmol/l L-glutamine (Invitrogen) and 0.1 mmol/l 2-mercaptoethanol (Invitrogen). After seven days in culture, the heterogeneous population of cells that spontaneously emigrated from the plated tissue was harvested using mild trypsinization (0.05% trypsin; Invitrogen). Circulating angiogenic cells (CACs) were isolated from peripheral blood samples donated by patients undergoing clinically indicated coronary angiography as previously described [16]. Using density-gradient centrifugation (Histopaque 1077; Sigma–Aldrich), mononuclear cells were isolated and cultured in endothelial media (EBM-2, 2% FBS, 50 ng/ml human vascular endothelial growth factor (VEGF), 50 ng/ml human insulin-like growth factor-1 and 50 ng/mL human epidermal growth factor; Clonetics). One week later, CACs were harvested and used for experimentation. Cell viability was quantified using a colorimetric WST-8 dehydrogenase assay (CCK-8; Dojindo). A trypan blue stain was used to identify cell death (Sigma–Aldrich).

2.2. Cell encapsulation

Human CSCs were harvested and suspended in media and mixed with low melt agarose (Sigma–Aldrich). The agarose was supplemented with human fibronectin (Sigma–Aldrich) or human fibrinogen (Sigma–Aldrich) as indicated. To form capsules, the cell/matrigel mixture was added drop-wise to agitated dimethylpolysiloxane (Sigma–Aldrich) and then rapidly cooled using a combination of cold HBSS and ice. The mixture was then centrifuged and capsules were filtered from the coalesced hydrogel using a 100 μm filter (Fischer Scientific) and were re-suspended in appropriate media for testing. The capacity of immobilized matrix proteins to incorporate into matrigel capsules was verified using Oregon-green conjugated fibrinogen (Invitrogen). Fibrinogen was visualized in empty capsules under the fluorescent microscope (Zeiss Observer.A1 Axio) at 470 nm to demonstrate protein localization (Fig. 2D).

2.3. Integrin profile expression

The admixture of CSCs harvested from the plated tissue was sorted using a FACSAria flow cytometer (BD Biosciences) with isotype matched immunoglobulin antibodies used as controls (c-kit, 9816-11, Southern Biotech; CD90 (BD Biosciences). Total RNA was extracted using PARIS™ RNA extraction kit (Invitrogen) and treated with 2U DNase I (Invitrogen) for 15 min at room temperature to eliminate genomic DNA. From 0.5 μg total RNA, first strand cDNA synthesis was performed using GoScript™ reverse transcriptase (Promega) and 0.5 μg random hexamer primers (Integrated DNA Technologies) for 1 h at 40 °C. With gene specific primers designed using DNAMAN software (Lynnon Biosoft) and primer3 (v.0.4.0; Rozen and Skaletsky, 2000), target gene mRNA levels were assessed by RT-qPCR using BRYT Green GoTaq qPCR Master Mix (Promega) and a LightCycler 480 Real-Time PCR system (Roche). Relative changes in mRNA expression of target genes were determined using the Δ-ΔCt method normalized against *18S* and Gapdh [17]. Western blot analysis was used to look at protein expression of integrins in c-Kit+/CD90-, c-Kit-/CD90- and c-Kit-/CD90- subpopulations. Representative bands shown were traced from different blots; densitometry was normalized to the corresponding *Gapdh* from each blot.

2.4. Pro-survival gene expression

RNA was extracted from CSCs after 48 h of culture in adherent, encapsulated or suspended conditions using TRIzol (Invitrogen). Activation of attachment mediated pro-survival pathways (AKT, ERK, and JNK) was quantified by RT-qPCR expression of downstream targets (*BCL-2, FOS,* and *JUN*) using qPCR Master Mix (Roche) and a LightCycler 480 Real-Time PCR system (Roche). Relative changes in mRNA expression of target genes were determined using the Δ − ΔCt method normalized to Gapdh [17].

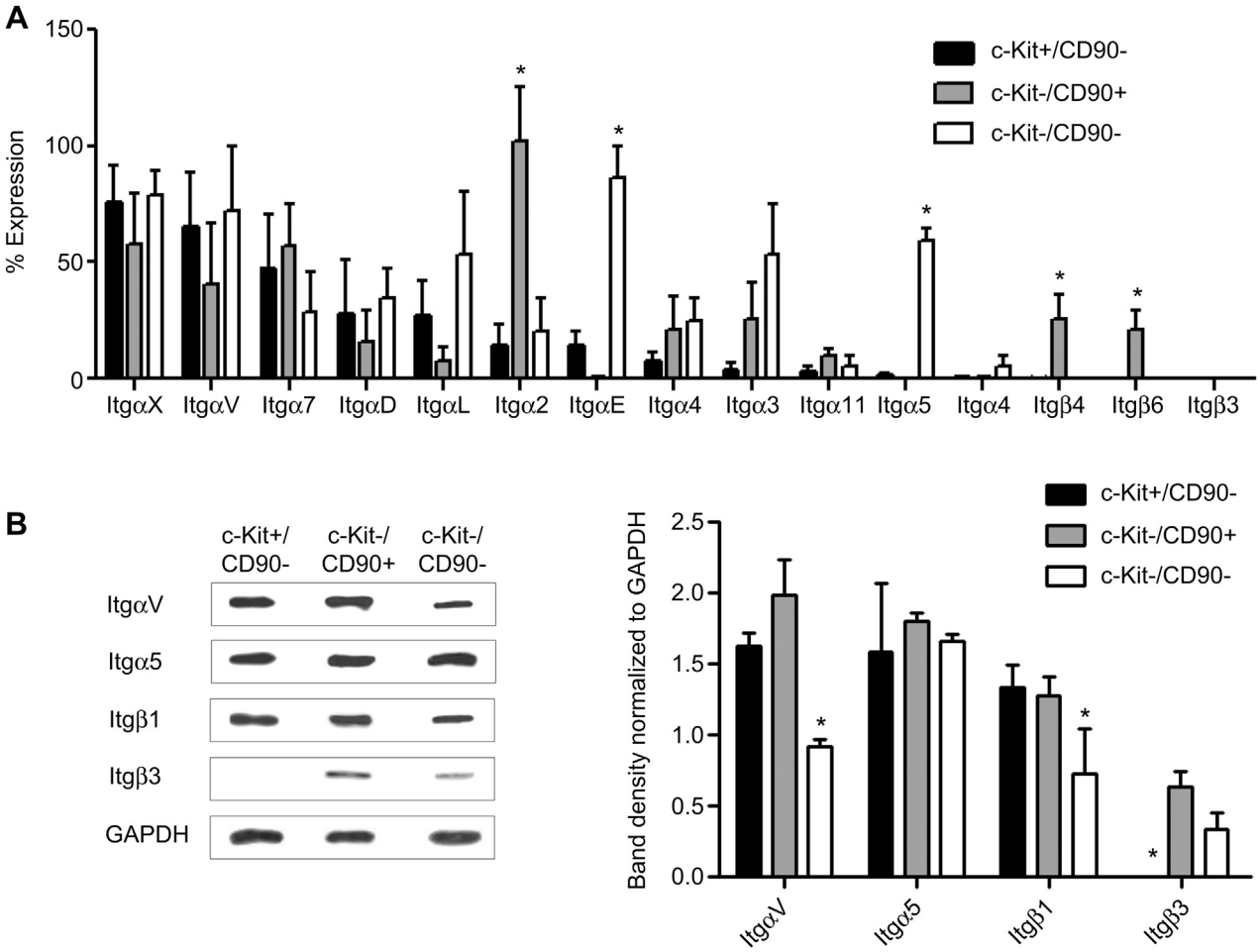

Fig. 1. Surface expression of CSCs integrin. (A): qPCR analysis of integrin expression on relevant sub-populations within the CSC admixture. Data is presented as the percent of total gene expression of CSC integrins normalized to *Gapdh*; $n = 3$. (B): Western Blot analysis with corresponding densitometry graph; $n = 3$.