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Self-assembling peptide hydrogel enables instant epicardial coating of the heart with mesenchymal stromal cells for the treatment of heart failure

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A B S T R A C T

Transplantation of mesenchymal stromal cells (MSCs) is an emerging therapy for the treatment of heart failure. However, the delivery method of MSC is currently suboptimal. The use of self-assembling peptide hydrogels, including PuraMatrix® (PM; 3-D Matrix, Ltd), has been reported for clinical hemostasis and in research models. This study demonstrates the feasibility and efficacy of an advanced approach for MSC-therapy, that is coating of the epicardium with the instantly-produced PM hydrogel incorporating MSCs (epicardial PM-MSC therapy). We optimized the conditions/procedure to produce “instant” 2PM-MSC complexes. After spreading on the epicardium by easy pipetting, the PM-MSC complex promptly and stably adhere to the beating heart. Of note, this treatment achieved more extensive improvement of cardiac function, with greater initial retention and survival of donor MSCs, compared to intramyocardial MSC injection in rat heart failure models. This enhanced efficacy was underpinned by amplified myocardial upregulation of a group of tissue repair-related genes, which led to enhanced repair of the damaged myocardium, *i.e.* augmented microvascular formation and reduced interstitial fibrosis. These data suggest a potential for epicardial PM-MSC therapy to be a widely-adopted treatment of heart failure. This approach may also be useful for treating diseases in other organs than the heart.
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1. Introduction

Transplantation of mesenchymal stromal/stem cells (MSCs) has been developed as a promising new approach for various diseases that are difficult to treat with current treatments, including graft-versus-host disease, autoimmune diseases, and heart failure [1–3]. In addition to their capacity for anti-inflammation and tissue repair, MSCs have an advantage as a potential donor for stem cell therapy in the utility for allogeneic cell transplantation. Although autologous stem cells are immunologically most appropriate, cells from aged patients with multiple comorbidities have compromised therapeutic efficacy and reduced *in-vitro* expansion ability [4]. Furthermore, the use of autologous stem cells requires an invasive biopsy from the patient and takes protracted culture for expansion, and quality control of each cell product, which imposes significant logistic, economic, and timing constraints [5]. As such, the use of allogeneic MSCs would allow the development of an MSC-bank, enabling an “off-the-shelf” supply of quality-assured MSCs with reduced costs. The most common source for MSCs is bone marrow. However, adipose-tissue, fetal-membrane, amnion membrane, and cord blood are also considered to be promising sources [6–9].

The potential of MSC transplantation for heart failure has been extensively reported in animal models [10,11]. While MSCs may not offer clinical benefits via differentiation to cardiomyocytes, they are able to secrete growth factors, cytokines, chemokines, exosomes, and microRNAs, which stimulate intrinsic self-repair systems to favor recovery of viable but failing myocardium [5,12]. Despite this, clinical trials of MSCs for heart disease to date reported only “modest (if preliminary) effects” [5]. One solution to

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overcome this limitation may lie in refining the method of cell delivery to the heart. Intramyocardial, intravenous and intracoronary injection techniques are currently used, but all of these methods result in poor donor cell survival [13–16]. Epicardial placement (not injection) is an alternative route for cell delivery to the heart. We have reported that epicardial placement of MSCs in the form of a "cell-sheet", produced in temperature-responsive dishes markedly improved donor cell survival and amplified therapeutic effects compared to intramyocardial injection in rat models of acute MI and heart failure [17,18]. As a result, this technique enhanced repair of the damaged myocardium in association with amplified upregulation of reparative factors and augmented cardiac function compared to intramyocardial injection. Furthermore, epicardial placement is free from the risks of coronary embolism and arrhythmogenesis [14–17]. Other reports demonstrated the efficacy of alternative epicardial placement methods, including the use of fibrin glue or pre-made tissue-engineered contracts [19,20].

Self-assembling peptide hydrogels may have the potential to achieve more effective epicardial placement of MSCs on the heart. On exposure to salt, this fully-synthetic material assembles into nanofibers on a scale similar to the extracellular matrix, forming a histocompatible and bioresorbable hydrogel. PuraMatrix® (PM; 3-D Matrix, Ltd.) is one of the most extensively studied among this type of hydrogel. PM consists of 99% water and amino acids (1% w/v; sequences of Arginine-Alanine-Aspartic Acid-Alanine) [21,22]. Under physiological conditions, the peptide component of PM self-assembles into a 3-dimensional hydrogel that exhibits a highly organized nanometer scale fibrous structure with an average pore size of 50–200 nm [22]. The soluble material can be spread onto organs *in-vivo* and will subsequently form a hydrogel. Morphology and rheological properties of PM gel have been already reported in details [22–25]. In particular, this gel has been suggested to be effective for hemostasis during surgery [26]. We hypothesized that such controllable gelation, adhesiveness, and easy handling characteristics of PM both pre- and post-gelation will realize an advanced method of epicardial placement, that is epicardial "coating" with a PM-MSC complex which is instantly produced in the operating room at the time of surgery. This technique negates the need for not only labor/cost-demanding GMP-production and transportation of cell-sheets or pre-made constructs, but also expensive GMP-cell culture facility in the treatment hospital. Instead, it is proposed that ready-to-use MSCs will be delivered by the hub cell processing center to each hospital. Such an approach should enable the use of this technique by any cardiac surgeon in any hospital.

This study therefore aimed to firstly optimize a protocol to instantly produce the PM-MSC complex that can be used clinically during surgery by applying it directly onto the heart with a simple application procedure. Secondly we aimed to investigate the feasibility and efficacy of the use of the instantly-produced PM-MSC complex in epicardial "coating" of the heart in clinically-relevant animal models.

2. Materials and methods

All studies were performed with the approval of the institutional ethics committee and the Home Office, UK. The investigation conforms to the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health Publication, 1996). All in-vivo and in-vitro assessments were carried out in a blinded manner.

2.1. Collection of MSCs

2.1.1. Rat fetal membrane-derived MSCs

Fetal membrane-derived mesenchymal stem/stromal cells (MSC) were collected from wild type pregnant Lewis or Sprague-Dawley rats (pregnant day 19–20; purchased from Charles River, UK) and expanded following a reported protocol [27,28]. Collected cells were placed in 25 cm^2 flasks (Nunc) with an initial plating concentration of approximately 1×10^6 cells/cm^2, and cultured in αMEM (Gibco) with 10% inactivated fetal bovine serum (FBS) containing L-glutamine (200 mM; Gibco), penicillin (100 U/ml) and streptomycin (100 mg/ml; Sigma), at 37 °C in a humidified atmosphere containing 95% air/5% CO_2. (incubator: Binder, Germany). The culture medium was aspirated and changed every 48–72 h without additional washing. When cell confluency reached 80–90%, cells were passaged by detachment using 0.25% Trypsin/0.2% EDTA (Sigma). Plating concentrations for subsequent passages were approximately 1×10^4 cells/cm^2.

2.1.2. Rat bone marrow-derived MSCs

Bone marrow-derived MSCs were collected from the bone marrow of the tibias and femurs of male Lewis rats (100–150 g; Charles River UK) and expanded as we have described previously [17,18,29]. Collected cells were cultured in αMEM with 20% inactivated FBS containing L-glutamine, penicillin and streptomycin under the same conditions as above.

2.1.3. Human amnion-derived MSCs

Human amnion-derived MSCs were collected as previously described [8]. The Ha-MSCs were isolated from fetal membranes of healthy donor mothers after the delivery (cesarean section) with written informed consent obtained. 1×10^6 of cryopreserved Ha-MSCs were defrosted and plated in 75 cm^2 flasks (Nunc). αMEM with 15% inactivated FBS containing L-glutamine, penicillin and streptomycin was used for cell cultivation under the same conditions as above.

2.2. Characterization of MSCs

At each passage, cell numbers and viability were counted by Countess Cell Counter (Invitrogen) with Trypan blue staining and doubling time was estimated.

2.2.1. Cell surface marker detection by flow-cytometric analysis

For cell-surface marker characterization using flow-cytometry, 1×10^6 MSCs were stained with 1:100 dilution of fluorescein isothiocyanate-conjugated anti-CD34 (Santa Cruze, USA), CD45 (Chemicon; Hampshire, UK), CD90 (Abcam, Cambridge, UK) or Alexa 647-conjugated anti-CD29 (Biolegend, London, UK) antibodies. Corresponding isotype-matched control antibodies were used for negative controls. All antibodies were used at 1:100 dilution following instructions stipulated by the company's guidelines. Samples were analyzed using the Dako Cyan flow-cytometer (Dako Cytomation, UK).

2.2.2. Osteogenic and adipogenic differentiation assay

MSCs were plated on 24-well plates and subjected to adipogenic or osteogenic differentiation medium. Adipogenic differentiation medium was α-minimal essential medium (α-MEM) supplemented with 100 μM isobutyl methylxanthine (Sigma-Aldrich, UK), 60 μM indomethacin (Fluka; Dorset, UK), 1 μg/ml insulin (Sigma-Aldrich), and 0.5 μM hydrocortisone (Sigma-Aldrich), while osteogenic differentiation medium was α-MEM supplemented with 0.1 μM dexamethasone (Sigma-Aldrich), 10 mM β-glycerophosphate (Sigma-Aldrich), and 0.05 mM ascorbic acid (Sigma-Aldrich).

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