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Microcapsules engineered to support mesenchymal stem cell (MSC) survival and proliferation enable long-term retention of MSCs in infarcted myocardium

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ABSTRACT

The limited efficacy of cardiac cell-based therapy is thought to be due to poor cell retention within the myocardium. Hence, there is an urgent need for biomaterials that aid in long-term cell retention. This study describes the development of injectable microcapsules for the delivery of mesenchymal stem cells (MSCs) into the infarcted cardiac wall. These microcapsules comprise of low concentrations of agarose supplemented with extracellular matrix (ECM) proteins collagen and fibrin. Dextran sulfate, a negatively charged polycarbohydrate, was added to mimic glycosaminoglycans in the ECM. Cell viability assays showed that a combination of all components is necessary to support long-term survival and proliferation of MSCs within microcapsules. Following intramyocardial transplantation, microcapsules degraded slowly *in vivo* and did not induce a fibrotic foreign body response. Pre-labeling of encapsulated MSCs with iron oxide nanoparticles allowed continued cell-tracking by MRI over several weeks following transplantation into infarcted myocardium. In contrast, MSCs injected as cell suspension were only detectable for two days post transplantation by MRI. Histological analysis confirmed integration of transplanted cells at the infarct site. Therefore, microcapsules proved to be suitable for stem cell delivery into the infarcted myocardium and can overcome current limitations of poor cell retention in cardiac cell-based therapy.

1. Introduction

Delivery of adult stem cells, especially mesenchymal stem cells derived from various sources, has been investigated extensively for tissue regeneration and repair [1,2]. However, administration of MSCs into motile/contractile tissue, such as the myocardium has resulted in less than optimal outcomes. Although literature on delivery of stem cells in animal studies have reported improvement in functional cardiac recovery, the translation to the clinic has been more challenging [3,4]. Whilst clinical studies have reported some convincing data, no significant improvement has been shown following MSC treatment [5]. One of the major limitations of cellular therapy for cardiac repair has been poor retention of stem cells in the myocardium, post-implantation [6,7]. Due to the contractile nature of the tissue, the injected cells are invariably flushed out through the injection site or squeezed through damaged vein walls and out of the affected tissue. Furthermore, continuous tissue

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movement impairs cell attachment and integration into the host tissue, resulting in progressive cell death [6,7].

As practiced with other tissue engineering applications, the issue of cell delivery into the myocardium can be aided with the use of a suitable encapsulation biomaterial [8,9]. Therefore, using microcapsules with small diameters of around 100 μm offer a number of advantages. They allow for optimal mass transport (oxygen, nutrients, growth factor exchange) and are small enough to be injected into the myocardium, thus allowing for a relatively easy cell delivery [10]. In comparison to other moldable hydrogels, such as fibrinogen, the microcapsules show superior mechanical stability, allowing for better protection of injected cells from constant tissue movement, and hence, better long-term retention of cells within the host tissue [11].

Historically, microcapsules were employed for the delivery of allogeneic or xenogeneic cells. Designed to be non-degradable and semipermeable, these microcapsules were meant to protect encapsulated cells from the host immune system [12]. Material of choice was often alginate [12]. Unfortunately, alginate is known to be immunogenic due to protein impurities in the polysaccharide hydrogel [13], and has been shown like many other biomaterials, to induce a foreign body response (FBR) [12,14,15]. The chronic inflammation phase of the FBR is characterized by a high influx of macrophages [16]. These macrophages are heterogeneous and can range from classically activated (inflammatory) M1 macrophages to alternatively activated (anti-inflammatory, wound-healing) M2 macrophages [17]. The macrophage phenotypes and ratios strongly depend on biomaterial properties and adsorbed proteins [18–20]. The extent to which a fibrotic capsule around the biomaterial is formed depends on the cytokines secreted by macrophages around the biomaterial, which in turn is dependent on the macrophage phenotypes and their ratios (M1:M2) present. It was observed that the M1:M2 macrophage ratio is directly proportional to the thickness of the fibrous capsule, indicating that M2 macrophages around the implant can augment the exclusion of the biomaterial from the surrounding tissue [21,22]. The induction of a foreign body response can be interpreted as a lack of biocompatibility, as fibrosis around the implant will lead to hypoxia and necrosis, resulting in a failure of the constituent cells to implant [15,23].

It is essential that microcapsules be customized for specific cell-types and the tissue into which they are delivered. The aim of the present study was to deliver bone marrow derived MSCs into the rat cardiac wall (post infarction), that are encapsulated in degradable microcapsules; thus, supporting their long-term cell survival and allowing histointegration of MSCs. Magnetic Resonance Imaging was also employed for the long-term tracking of labeled encapsulated cells *in vivo*.

Agarose as a biomaterial is not degradable enzymatically within tissue, but has been shown to be phagocytized by macrophages [24]. Hence, in contrast to other enzymatically degradable materials, agarose persists longer within the host tissue, thus allowing for a slow and gradual release of cells. Similar to alginate, agarose is a polysaccharide derived from seaweed, but is neutral in charge [25]. It has previously been reported that neutrally charged polymers induce lower FBR than charged ones [26]. Nevertheless, agarose has been reported to induce a FBR at high concentrations [24]; therefore lower concentrations of agarose are necessary to reduce a FBR. Since agarose was found to be insufficiently immunoprotective, and also allowed extrusion of encapsulated cells due to its large pores, it was previously dismissed for uses in allogeneic or xenogeneic cell transplantation [14]. Interestingly, it's exactly these properties that make agarose an ideal candidate for MSC delivery into the contracting myocardium.

Furthermore, addition of ECM proteins such as collagen I and fibrin into the microcapsules, provides a matrix for encapsulated cells, supporting cellular attachment [14]. Anchorage and interaction of cells with the surrounding microenvironment are essential for a proper arrangement of the cytoskeleton, affecting cell signaling, metabolism and ensuring proper cell function [27]. In addition, both collagen I and fibrin can polymerize to form a gel, increasing the stability of the capsule [28]. Fibrin is an early matrix component produced during wound healing, is pro-angiogenic and provides further wound-healing signals to the encapsulated cells [29]. Supplementation of ECM proteins into hydrogel microcapsules has shown to improve the overall cell survival; however, a decrease in cell viability was still observed over time in these studies [30,31].

Therefore, it may be argued that supplementation with ECM proteins might not be sufficient to ensure long-term survival of encapsulated cells. In addition to proteins, the ECM also contains glycosaminoglycans (GAGs), which interact with ECM proteins and cell receptors, sequester and present growth factors and actively take part in cell-microenvironment signaling [32]. Although various GAGs possess specialized functions, they are all net-negatively charged polycarbohydrates with a protein core [32]. In the present study, dextran sulfate (DxS), an off-the-shelf negatively charged polycarbohydrate, was added to the microcapsules as a substitute for GAGs. It has previously been demonstrated that ECM deposited in the presence of DxS enhanced the proliferative capacity of MSCs, while retaining their differentiation capacity [33].

Microcapsules synthesized with different combinations of the components outlined above were tested for their ability to support long-term survival of MSCs whilst retaining their functional properties. The most optimal combination of these biomaterials has been examined for its suitability for *in vivo* MSC delivery.

2. Materials & methods

2.1. Cell culture

Human bone marrow MSCs (ATCC), rat bone marrow MSCs (isolated from rat femur) and IMR-90 fibroblasts (ATCC) were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C and 5% CO_2 in a humidified incubator.

2.2. Composition of biomaterial mixture

Low temperature gelling agarose (Sigma) was dissolved and autoclaved in PBS at a stock concentration of 2% (w/v). Dextran sulfate (pK Chemicals, Denmark) with a molecular weight of 500 kDa was prepared as a stock solution of 10 mg/ml in deionized water. A $CaCl_2$ dihydrate solution (2M) was prepared in deionized water. Collagen I (bovine skin, Advanced Biomatrix) was neutralized using 80% (v/v) collagen stock solution 10% (v/v) in x10 PBS and 10% (v/v) 0.1 M NaOH. Final neutralized collagen stock had a concentration of 2.48 mg/ml. Fibrinogen (bovine plasma, Sigma Aldrich, USA) stock was prepared fresh every time at a concentration of 40 mg/ml in PBS. Thrombin (Sigma Aldrich, USA) aliquots were stored at a concentration of 2 mg/ml at −20 °C.

MSCs or fibroblasts were encapsulated at a concentration of 0.5–1 × 10^6 cells in 100 μl biomaterial. For this purpose cells were harvested and the exact number of cells per sample was collected in a pellet. All components of the biomaterial mixture were brought up to and maintained at 37 °C just before mixing. PBS or collagen I and/or fibrinogen were warmed to 37 °C first, and then pre-warmed agarose was

Table 1
Composition of microcapsule material.

	PBS/Col I/Fibrin	Agarose	DxS (10 mg/ml)	$CaCl_2$ (2M)
PBS	80 μl	20 μl	0.1–10 μl	2 μl
Collagen I (2 mg/ml)	80 μl	20 μl	0.1–10 μl	2 μl
Fibrinogen (20 mg/ml)	40 μl PBS/40 μl Fibrinogen	20 μl	0.1–10 μl	2 μl
Fibrinogen (20 mg/ml) Collagen I (1 mg/ml)	40 μl Col I/40 μl Fibrinogen	20 μl	0.1–10 μl	2 μl