Biomaterials 35 (2014) 720–726

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

The use of gadolinium-carbon nanostructures to magnetically enhance stem cell retention for cellular cardiomyoplasty

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ARTICLE INFO

Article history:
Received 2 August 2013
Accepted 2 October 2013
Available online 20 October 2013

Keywords:
Single-walled carbon nanotube
Cellular cardiomyoplasty
Gadonanotube
Mesenchymal stem cell
Nanotechnology

ABSTRACT

In this work, the effectiveness of using Gadonanotubes (GNTs) with an external magnetic field to improve retention of transplanted adult mesenchymal stem cells (MSCs) during cellular cardiomyoplasty was evaluated. As a high-performance T_1-weighted magnetic resonance imaging (MRI) cell tracking label, the GNTs are gadolinium-loaded carbon nanotube capsules that render MSCs magnetic when internalized. MSCs were internally labeled with either superparamagnetic GNTs or colloidal diamagnetic lutetium (Lu). *In vitro* cell rolling assays and *ex vivo* cardiac perfusion experiments qualitatively demonstrated increased magnetic-assisted retention of GNT-labeled MSCs. Subsequent *in vivo* epicardial cell injections were performed around a 1.3 T NdFeB ring magnet sutured onto the left ventricle of female juvenile pigs ($n = 21$). Cell dosage, magnet exposure time, and endpoints were varied to evaluate the safety and efficacy of the proposed therapy. Quantification of retained cells in collected tissues by elemental analysis (Gd or Lu) showed that the external magnet helped retain nearly three times more GNT-labeled MSCs than Lu-labeled cells. The sutured magnet was tolerated for up to 168 h; however, an inflammatory response to the magnet was noted after 48 h. These proof-of-concept studies support the feasibility and value of using GNTs as a magnetic nanoparticle facilitator to improve cell retention during cellular cardiomyoplasty.

1. Introduction

Over the past decade, interest in cellular cardiomyoplasty for treating injured cardiac tissue has grown exponentially. Of the various cell types used for cellular cardiomyoplasty, adult bone marrow-derived mesenchymal stem cells (MSCs) are particularly attractive because of their allogeneic use, therapeutic potential, and ease of isolation and expansion [1]. Numerous preclinical and clinical studies have shown the benefits of transplanted adult bone marrow cells such as MSCs in cardiac repair [2,3]. However, the retention and survival of transplanted cells in the heart are severely limited by muscle contraction and blood flow at the injection site, which may reduce the benefits achieved with current cell transplantation approaches. In preclinical and clinical studies, immunohistochemical and radiolabeling techniques have shown that only 5–15% of transplanted MSCs remain in the heart, regardless of the model, delivery method, and dosage level used [4–6].

In order to address these challenges in cellular cardiomyoplasty, several biochemical approaches have been studied to improve the retention and survival of transplanted cells in the myocardium. These include transgenically enhancing protein secretion, conditioning cells *in vitro* to improve survival, exploiting endogenous mechanisms to increase homing, and targeting tissues with antibodies and proteins to increase cell adhesion and engraftment [7,8]. However, the clinical translation of such methods may be difficult.

To help cells withstand the mechanical challenges in the heart, physical methods have been developed such as transplanting cells embedded in hydrogels, delivering cell sheet fragments, or using 3D constructs [9–11]. One physical approach that has been clinically translatable and effective in improving cell homing and retention is the magnetic targeting of superparamagnetic iron oxide-labeled cells to grafts, stents, and cardiac tissue under an external magnetic field [12–14]. An additional benefit of using iron-oxide particles is their performance as a T_2-weighted cellular label

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http://dx.doi.org/10.1016/j.biomaterials.2013.10.013

for magnetic resonance imaging (MRI), which enables noninvasive, real-time visualization of the transplanted cells. However, internally labeling cells with iron-oxide particles usually requires the use of polycationic transfection agents, many of which have been shown to affect MSC differentiation and are considered cytotoxic when used in high concentrations [15,16]. Furthermore, the darkening effects of T_2-weighted agents may not be ideal for enhancing visual details, as compared to T_1-weighted MRI agents, which brighten images.

The Gadonanotubes (GNTs) are a high-performance T_1-weighted MRI contrast agent and an effective cellular magnetic probe that may have the potential to address the limitations of cell therapy. These gadolinium (Gd^{3+}) ion-containing carbon nanocapsules possess the highest recorded T_1-weighted relaxivity to date at a clinically relevant field, with a value of 170 mM^{-1} s^{-1} per Gd^{3+} ion (37 °C, 1.5 T) [17,18]. It was also recently established that the empty carbon nanocapsule component of the GNTs can serve as a T_2-weighted contrast agent, due to residual metal oxide catalyst found within the carbon nanotube capsule sidewall [19]. Previous studies demonstrated that the Gd^{3+} ion clusters within the GNTs remained stable and intact after exposure to physiological challenges, which alleviates concerns of heavy metal ion loss that other Gd^{3+}-based contrast agents may exhibit [18]. Moreover, the nanoscale length and unusually high aspect ratio of GNTs allow them to be rapidly internalized by mammalian cells, such as breast cancer cells, MSCs, and macrophages, without the need of a transfection agent [20–22]. Recent studies have shown that GNTs do not affect the viability, differentiation potential, or phenotype of MSCs when used as an intracellular MRI label [21]. In addition to being a biocompatible and effective MRI cell-labeling agent, the inherently magnetic GNTs, when internalized by MSCs, can render the cells magnetically attracted to external magnetic fields. This characteristic enables the magnetic retention of the GNT-labeled MSCs in cardiac tissue for cellular cardiomyoplasty.

Although over 2000 stem cell-based clinical trials are currently underway [23], the retention and survival of transplanted cells at target sites remain major challenges. As such, innovative and translatable strategies to improve cell retention and survival within the heart, which should lead to better therapeutic outcomes, are highly desirable for cellular cardiomyoplasty. In the present proof-of-concept study, *in vitro*, *ex vivo*, and *in vivo* experiments were conducted to examine the magnetically driven, therapeutic potential of GNTs as a T_1-weighted magnetic nanoparticle facilitator for improving transplanted cell retention during cellular cardiomyoplasty. Cell dosage, magnetic exposure time, and endpoints were varied to determine the safety and efficacy of the proposed therapy in porcine models.

2. Materials and methods

2.1. Cell culture and labeling

MSCs were isolated from the bone marrow of male pigs as described elsewhere [24] and were grown in alpha-modified minimum essential medium (αMEM) containing 10% FBS and 1% antibiotic supplement (200 mM L-glutamine, 10,000 units/mL penicillin, and 10 mg/mL streptomycin) at 37 °C (95% relative humidity in 5% CO_2). MSCs were expanded by two successive passages (P) at 2 × 10^3 cells/cm^2 and then frozen at P_2 in cryovials in 10% DMSO/90% FBS. At appropriate times, cells were thawed and expanded once (P_3) before labeling. All labeling studies were performed with P_3 MSCs grown to 70% confluence in tissue culture flasks (175 cm^2). Three MSC cultures were separately labeled as described below; unlabeled cells were used as control cells for the *in vitro* studies. Cell concentrations were verified by a Beckman Coulter MultiSizer 3.

2.2. GNT-labeled MSCs

GNTs were produced and suspended in an aqueous solution of Pluronic F108 (0.17%, BASF) as previously reported [21]. MSCs were then incubated in the GNT-labeling solution (27 μM Gd^{3+}) for 24 h. Cells were isolated from excess GNTs in solution by density gradient centrifugation. Briefly, Histopaque 1077 (25 °C, Sigma–Aldrich) was slowly added to the bottom of the tube at a 1:2 ratio (Histopaque:cell suspension). The sample was centrifuged at 400× g for 20 min. GNT-labeled MSCs located at the interface of the αMEM and Histopaque phases were isolated, washed with PBS, and centrifuged at 285× g for 10 min.

2.3. USPIO-labeled MSCs

USPIO nanoparticles (125 μL) (Molday ION(−); 10 mg Fe/mL; Biophysics Assay Laboratory, Inc., Worcester, MA) were diluted with 400 μL cell culture grade water. To this solution, 12.5 μL poly-L-lysine (10 mg/mL; Biophysics Assay Laboratory, Inc.) was added. The USPIO-poly-L-lysine conjugate solution was gently mixed by using a vortex, incubated at 25 °C for 20 min, and stored at 4 °C. Before cell labeling, the solution was mixed again, incubated at 25 °C for 20 min, diluted in 12 mL αMEM, and thoroughly mixed. The SPIO-poly-L-lysine solution was added to 12.5 mL αMEM into each cell culture flask to yield a final concentration of 50 μg Fe/mL and 5 μg/mL poly-L-lysine. After incubation for 24 h, cells were washed thoroughly with PBS and were lifted by using trypsin-EDTA for 5 min. The cell suspension was then passed through a 70 μm nylon filter to remove large cell aggregates.

2.4. Lu-labeled MSCs

MSCs were incubated in 2 v/v% colloidal Lu (20 nm; BioPAL CellTrackTM; Biophysics Assay Laboratory, Inc.) for 24 h in αMEM. Cells were washed thoroughly with PBS and were lifted by using trypsin-EDTA for 5 min. The cell suspension was then passed through a 70 μm nylon filter to remove large cell aggregates.

2.5. In vitro magnetic cell retention assay

GNT-labeled MSCs, USPIO-labeled MSCs, and unlabeled MSCs were separately prepared at 5 × 10^5 cells/mL in running buffer (10 mM Tris, 103 mM NaCl, 24 mM $NaHCO_3$, 5.5 mM glucose, 5.4 mM KCl and 2 mg/mL bovine serum albumin [BSA], pH 7.4). Cells were examined in a modified parallel-plate flow-fluid shear stress rolling assay [25]. Briefly, 24 × 50 mm slides were cut from 15 × 100 mm polystyrene Petri dishes. The slides were washed with PBS, blocked with 2% BSA for 2 h at 25 °C and assembled in a parallel-plate flow chamber. To assess the magnetic retention of MSCs, a 1.3 T ring NdFeB magnet (RX4C2, K&J Magnetics) was affixed to the slide. Cells were injected into the flow chamber, and running buffer was drawn through the chamber at a constant force of 1 dyne/cm^2 for 5 min by using a computer-controlled syringe pump (Harvard Apparatus). The number of adherent cells remaining was recorded by digital microscopy (VI-470 charge-coupled device video camera; Optronics Engineering) at 20× on an inverted Nikon DIAPHOT-TMD microscope. The buffer solution that was passed through the chamber was collected and centrifuged to recover cells that were not magnetically retained in the chamber. The recovered cells were fixed with 2% paraformaldehyde in PBS and analyzed by flow cytometry (FACS Calibur BD) with the use of a forward and side scatter gate to distinguish live cells. The number of gated events was used to quantify recovered cells (% of cells not retained in the chamber by the magnet), which was used to determine the amount of magnetically adherent cells (% of cells magnetically retained in the chamber).

2.6. Ex vivo perfusion study

Separate 0.2 mL transepicardial bolus cell injections (100 × 10^6 cells/mL in 2% FBS) of the following cell populations were administered approximately 5 mm into the left ventricle of an excised bovine heart: (1) GNT-labeled MSCs with a 1.3 T cylindrical NdFeB magnet (D66SH; K&J Magnetics) held in place over the injection site, (2) GNT-labeled MSCs without a magnet, and (3) unlabeled MSCs. (Refer to Fig. S1 in the Supplementary Data.) The heart was perfused with cold saline solution for 72 h, maintained in 10% formalin for 24 h, and prepared for histopathologic analysis. A 1.7 cm slice perpendicular to the long axis of the heart was obtained approximately 6 mm above and 6 mm below the suture marks. The portion of wall containing each injection site was removed from the slice and sectioned into 5 levels. Adjacent tissue sections were stained with hematoxylin and eosin.

2.7. In vivo retention study

For *in vivo* studies, animals were cared for in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines of the Texas Heart Institute. GNT-labeled MSCs and Lu-labeled MSCs were separately suspended in 2% FBS and maintained on ice in sterile polystyrene tubes until injection. Three aliquots (0.2–2 × 10^6) of each cell population were also collected in glass scintillation vials for elemental analysis. A left thoracotomy was performed on female juvenile domestic pigs ($n = 21$) under general anesthesia. A sterilized 1.3 T NdFeB ring magnet (RX4C2; K&J Magnetics) was sutured with prolene stitches onto the anterior wall of the left ventricle. Cells were gently pipetted before transepicardial injections, which were completed with a 21-gauge butterfly needle around the inner and outer perimeters of the ring magnet approximately 5 mm into the tissue. The pigs were divided into 7 groups (Groups A-G; $n = 3$ per group) in which the cell doses, magnetic exposure times, and study endpoint times varied (see Table S1 in the Supplementary Data for details). After cell injections, the implanted magnet remained in the animals for the entire duration of the study in Groups A-C: pigs in Group A were maintained on the