Biomaterials 33 (2012) 5317–5324

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Functional performance of human cardiosphere-derived cells delivered in an *in situ* polymerizable hyaluronan-gelatin hydrogel

Ke Cheng [a], Agnieszka Blusztajn [b], Deliang Shen [a,c], Tao-Sheng Li [d], Baiming Sun [a], Giselle Galang [a], Thomas I. Zarembinski [e], Glenn D. Prestwich [f], Eduardo Marbán [a], Rachel R. Smith [b,*], Linda Marbán [b,*]

[a] *The Cedars-Sinai Heart Institute, Los Angeles, CA 90048, USA*
[b] *Capricor, Inc., 8700 Beverly Blvd, 1099 Davis Building, Los Angeles, CA 90048, USA*
[c] *Department of Cardiology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, PR China*
[d] *Department of Stem Cell Biology, Nagasaki University Graduate School of Biomedical Science, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[e] *BioTime, Inc. Alameda, CA, 1301 Harbor Bay Parkway, Alameda, CA 94502, USA*
[f] *Department of Medicinal Chemistry and Center for Therapeutic Biomaterials, University of Utah, Salt Lake City, UT 84108, USA*

ARTICLE INFO

Article history:
Received 8 February 2012
Accepted 1 April 2012
Available online 3 May 2012

Keywords:
Acute myocardial infarction
Biodegradable polymers
Hyaluronan
Cardiac stem cells

ABSTRACT

The vast majority of cells delivered into the heart by conventional means are lost within the first 24 h. Methods are needed to enhance cell retention, so as to minimize loss of precious material and maximize effectiveness of the therapy. We tested a cell-hydrogel delivery strategy. Cardiosphere-derived cells (CDCs) were grown from adult human cardiac biopsy specimens. *In situ* polymerizable hydrogels made of hyaluronan and porcine gelatin (*Hystem®-C™*) were formulated as a liquid at room temperature so as to gel within 20 min at 37 °C. CDC viability and migration were not compromised in *Hystem-C™*. Myocardial infarction was created in SCID mice and CDCs were injected intramyocardially in the infarct border zone. Real-time PCR revealed engraftment of CDCs delivered in *Hystem-C™* was increased by nearly an order of magnitude. LVEF (left ventricular ejection fraction) deteriorated in the control (PBS only) group over the 3-week time course. *Hystem-C™* alone or CDCs alone preserved LVEF relative to baseline, while CDCs delivered in *Hystem-C™* resulted in a sizable boost in LVEF. Heart morphometry revealed the greatest attenuation of LV remodeling in the CDC + *Hystem-C™* group. Histological analysis suggested cardiovascular differentiation of the CDCs in *Hystem-C™*. However, the majority of functional benefit is likely from paracrine mechanisms such as tissue preservation and neovascularization. A CDC/hydrogel formulation suitable for catheter-based intramyocardial injection exhibits superior engraftment and functional benefits relative to naked CDCs.

1. Introduction

Cardiovascular disease remains the leading cause of death and disability in Americans, claiming more lives each year than cancer, diabetes mellitus, HIV and accidents combined [1]. Ischemic heart disease is the predominant contributor to cardiovascular morbidity and mortality; ~1 million myocardial infarctions (MIs) occur per year in the United States while ~5 million patients suffer from chronic heart failure [2]. Death rates following MI have improved dramatically over the last four decades [3], but new approaches are nevertheless urgently needed for those patients who deteriorate and develop ventricular dysfunction [4]. Over the past ten years, stem cell transplantation has emerged as a promising therapeutic strategy for acute or chronic ischemic cardiomyopathy. Over the last six years, we have taken a unique cell therapy product, cardiosphere-derived cells (CDCs) from proof-of-concept animal studies [5–13] to a recently completed phase I clinical trial. Data from our clinical trial (CADUCEUS, NCT00893360 at clinicaltrials.gov) indicates that CDCs augment cardiac function and reduce scar size in mild to moderate ischemic cardiomyopathies [14]. However, CDCs face the same fate as most other cell types, that is extremely low retention rates in the heart shortly after delivery, which certainly cripples the efficiency and efficacy of cell therapies in general [15]. In fact, the vast majority of cells delivered into the heart by conventional means are lost within the first 24 h [16]. Methods are needed to enhance retention.

* Corresponding authors. Tel.: +1 310 423 2104; fax: +1 310 423 7551.
E-mail addresses: rsmith@capricor.com (R.R. Smith), lmarban@capricor.com (L. Marbán).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2012.04.006

The blooming of biomaterial and tissue engineering research opens the door for a new paradigm of cell therapy [17]. Injectable biomaterial gels are particularly appealing as they are amenable to minimally-invasive delivery and capable of enhancing cell engraftment by providing a temporary scaffold [18]. We have previously reported that injection of platelet gel alone or platelet gel spiked with CDCs ameliorates cardiac dysfunction in rats with myocardial infarction [19,20]. Despite of its appearing autologous nature (i.e. derived from the same animals/patients who receive the gel), platelet gel usually contains numerous components that vary from patient to patient. Also, the gel needs to be freshly prepared from the patients in advance to injection and cannot be offered in an "off the shelf" fashion. Therefore, we started look for a commercially-available and chemically-defined biomaterial that can enhance the therapeutic benefit of our CDC products. One among various biomaterial choices, hyaluronan is a glycosaminoglycan component of the extracellular matrix of all connective tissues, making it an attractive scaffold [21]. Hyaluronan-based hydrogels can be formulated with varying gelation times depending on the concentrations of the individual monomers, making them suitable for catheter delivery and *in situ* polymerization. *Hystem*®-*C*™ (BioTime Inc.) is a hyaluronan-based hydrogel crosslinked using thiol-reactive poly(ethylene glycol) diacrylate and covalently linked to thiolated collagen to aid cell attachment. The base product is chemically-defined and nonimmunogenic and the collagen is porcine derived. It has been demonstrated that *Hystem-C*™ promotes tissue repair in various organ systems [22]. However, its utility in cardiac applications has yet to be explored.

In the present study, we developed and tested a cell-biomaterial strategy which embeds human CDCs within the *Hystem-C*™ hydrogel. In a mouse model of myocardial infarction, we compared the functional benefits of this CDC/hydrogel combination product with those seen using CDCs or hydrogel alone.

2. Methods

2.1. Human CDC culture

Percutaneous endomyocardial heart biopsies were obtained from the right ventricular aspect of the septum in patients during clinically-indicated procedures with IRB approval and informed consent from the patients. Cardiosphere-derived cells (CDCs) were derived as described [13]. Briefly, heart biopsies were minced into small fragments. After brief digestion with collagenase, the tissue fragments were cultured as "explants" on dishes coated with 20 μg/ml fibronectin (BD Biosciences). Within 1–2 weeks, stromal-like flat cells, and phase-bright round cells, emerged from the tissue fragments and became confluent. These cardiac-derived cells were harvested using 0.25% trypsin (Gibco), and then cultured in suspension as self-aggregated cardiospheres on poly-D-Lysine (20 μg/mL; BD Biosciences). CDCs were grown by seeding cardiospheres on fibronectin-coated dishes and passaged twice as described [13]. All cultures were incubated in 5% CO_2 at 37 °C, using IMDM basic medium (Gibco) supplemented with 20% FBS (Hyclone), 1% penicillin/streptomycin, and 0.1 mM 2-mercaptoethanol.

2.2. Flow cytometry analysis

The phenotype of CDCs was investigated by flow cytometry analysis. Briefly, cells were incubated with FITC, PE, or APC-conjugated antibodies against CD45, CD90, CD105, CD117 (c-kit), and DDR2 (ebiosciences Inc., San Diego, California) for 30 min. Isotype-identical antibodies served as negative controls. Quantitative analysis was performed using a CYAN-ADP flow cytometer with Summit 4.3 software (Beckman Coulter, Brea, California).

2.3. Hyaluronan-based hydrogel

Glycosan *HyStem*® (BioTime Inc., Alameda, CA) is a hyaluronan-based hydrogel crosslinked using thiol-reactive poly(ethylene glycol) diacrylate. Extracellular matrix (ECM) proteins such as collagen can be blended with hyaluronan to aid cell attachment. One such formulation utilizes thiolated denatured porcine collagen which can be covalently crosslinked to thiolated hyaluronan to create a cell-compatible hydrogel (*Hystem*®-*C*™) [23]. All materials were prepared according to the manufacturer's instructions.

2.4. CDCs in hyaluronan-based hydrogel

Human CDCs were incorporated within the hydrogels during the crosslinking process prior to gelation. The final aqueous cell solution passed readily through a 30-gauge needle (used for injections in small animal studies) with no appreciable loss of material. Gelation occurred within 20 min of mixing all components. CDC viability within the *Hystem* and *Hystem-C*™ hydrogels was first examined in a 96 well format by an *in vitro* cell viability assay (Cell Counting Kit-8, Dojindo) at Day 4 and Day 7 after seeding. Since the final goal is to deliver CDCs that can survive and migrate out of the hydrogel to regenerate the infarcted myocardium, we next tested the *in vitro* migratory potential of CDCs incorporated within the two hydrogels (*Hystem* and *Hystem-C*™). A transwell plate setup allowed for cell migration through pores into the lower chamber where they could be detected. Calcein-labeled CDCs (10,000 cells/μL) were incorporated and fetal bovine serum (FBS) served as a chemo-attractant in the lower chamber. As the CDCs migrated from the upper to the lower chamber, fluorescence (RFU) increased.

2.5. Animal model

Acute myocardial infarction was created in adult male SCID-beige mice (10–12 weeks old), as described [12,13]. Briefly, after general anesthesia and tracheal intubation, mice were artificially ventilated with room air. A left thoracotomy was performed through the fourth intercostal space and the left anterior descending artery (LAD) was ligated with 9-0 prolene under direct visualization. The mice were then subjected to intramyocardial injections with a 30-gauge needle at two to four points in the infarct border zone. CDCs were added to the hydrogels immediately prior to injection, allowing for gelation to occur primarily *in situ*. For the acute retention study, CDCs were labeled with DiI and suspended at 10,000 cells/μL (total 1.5×10^5 CDCs) in PBS, *Hystem*™, or *Hystem-C*™ ($n = 4$–5 mice for each group). All animals were sacrificed 24 h after injection for qPCR and histological analysis of cell retention. For the long-term functional study, 4 groups were compared: 1) Control: intramyocardial injection of 15 μL PBS ($n = 8$); 2) *Hystem-C*™ only: intramyocardial injection of 15 μL *Hystem-C*™ hydrogel ($n = 8$); 3) CDCs only: intramyocardial injection of 1.5×10^5 CDCs in 15 μL PBS ($n = 8$); 4) CDCs + *Hystem-C*™: intramyocardial injection of 1.5×10^5 CDCs in 15 μL *Hystem-C*™ hydrogel ($n = 8$). Animals were followed for 3 weeks.

2.6. Cell engraftment assay by quantitative PCR

Animals were sacrificed and their hearts excised to obtain an actual measurement of the number of cells engrafted. Real-time PCR experiments using the human-specific repetitive Alu sequences were conducted [24]. The whole heart was weighed and homogenized. Genomic DNA was isolated from aliquots of the homogenate corresponding to 30 mg of myocardial tissue, using the DNAeasy minikit (Qiagen), according to the manufacturer's protocol. The TaqMan® assay (Applied Biosystems) was used to quantify the number of transplanted cells with the human Alu sequence as template (Alu forward, 5′-CAT GGT GAA ACC CCG TCT CTA-3′; Alu reverse, 5′-GCC TCA GCC TCC CGA GTA G-3′; TaqMan probe, 5′-FAM-ATT AGC CGG GCG TGG TGG CG-TAMRA-3′, Applied Biosystems). For absolute quantification of cell number, a standard curve was constructed with samples derived from multiple log dilutions of genomic DNA isolated from the same human CDC isolates that were used for the animal experiments. All samples were spiked with 50 ng of mouse genomic DNA to control for any effects this may have on reaction efficiency in the actual samples. All samples were tested in triplicates. The result from each reaction, number of human cells in 50 ng of genomic DNA, was expressed as the number of engrafted cells per heart, by first calculating the cell number in the total amount of DNA corresponding to 30 mg of myocardium and then extrapolating to the total weight of each heart.

2.7. Heart morphometry

For morphometric analysis, mice were euthanized at 3 weeks and the hearts were explanted and frozen in OCT compound ($n = 3$–5 mouse hearts per group). Cryo-sections every 100 μm (5 μm thickness) were prepared. Masson's trichrome staining (6 sections per heart, collected at 400 μm intervals) was performed as described [5]. Images were acquired with a PathScan Enabler IV slide scanner (Advanced Imaging Concepts, Princeton, NJ). From the Masson's trichrome-stained images, morphometric parameters including viable tissue in infarct area and infarct wall thickness were measured in each section with NIH ImageJ software. Measurements were averaged for each heart.

2.8. Echocardiography

Mice underwent echocardiography 3 h (baseline) and 3 weeks after surgery using Vevo 770™ Imaging System (VISUALSONICS™, Toronto, Canada) ($n = 7$–8 mice per group). With general anesthesia, the hearts were imaged two-dimensionally in long-axis views at the level of the greatest left ventricle diameter. Left ventricle ejection fraction (LVEF) was measured with VisualSonics V1.3.8 software from 2D long-axis views taken through the myocardial infarction area. The echocardiography images were obtained and analyzed blindly by an experienced study staff.