Biomaterials 61 (2015) 339–348

Contents lists available at ScienceDirect

Biomaterials

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

Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction

Roberto Gaetani [a, b, c, *, 1], Dries A.M. Feyen [a, 1], Vera Verhage [a], Rolf Slaats [a], Elisa Messina [b], Karen L. Christman [c], Alessandro Giacomello [b], Pieter A.F.M. Doevendans [a, d], Joost P.G. Sluijter [a, d, *]

[a] Dept. of Cardiology, DH&L, University Medical Center Utrecht, Utrecht, The Netherlands
[b] Dept. of Molecular Medicine, Cenci-Bolognetti Foundation, Pasteur Institute, "Sapienza" University of Rome, Rome, Italy
[c] Dept. of Bioengineering and Sanford Consortium for Regenerative Medicine, University of California, San Diego, La Jolla, USA
[d] Interuniversity Cardiology Institute of the Netherlands (ICIN), Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 16 April 2015
Accepted 3 May 2015
Available online 28 May 2015

Keywords:
Cardiac progenitor cells
Tissue printing
Cardiac tissue engineering
Cardiac regeneration
Heart failure

ABSTRACT

Cardiac cell therapy suffers from limitations related to poor engraftment and significant cell death after transplantation. In this regard, *ex vivo* tissue engineering is a tool that has been demonstrated to increase cell retention and survival. The aim of our study was to evaluate the therapeutic potential of a 3D-printed patch composed of human cardiac-derived progenitor cells (hCMPCs) in a hyaluronic acid/gelatin (HA/gel) based matrix. hCMPCs were printed in the HA/gel matrix (30×10^6 cells/ml) to form a biocomplex made of six perpendicularly printed layers with a surface of 2×2 cm and thickness of 400 μm, in which they retained their viability, proliferation and differentiation capability. The printed biocomplex was transplanted in a mouse model of myocardial infarction (MI). The application of the patch led to a significant reduction in adverse remodeling and preservation of cardiac performance as was shown by both MRI and histology. Furthermore, the matrix supported the long-term *in vivo* survival and engraftment of hCMPCs, which exhibited a temporal increase in cardiac and vascular differentiation markers over the course of the 4 week follow-up period. Overall, we developed an effective and translational approach to enhance hCMPC delivery and action in the heart.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

An ischemic event in the myocardium has dire consequences for the heart since the death of cardiomyocytes within the infarcted area leaves the heart with less contractile elements. This shifts the pumping burden to the remaining viable myocardium, which can have deleterious consequences for patients suffering from the disease, potentially leading to the development of heart failure. Current therapeutic approaches do not provide patients with ways to repair the organ and instead focus on limiting secondary damages to slow down the progression of the disease [1,2]. Therefore, end-stage heart failure patients often require heart transplantation, for which donor hearts are in short supply and carry risks of rejection [3]. Although the existence of endogenous stem cell populations in the heart has been documented [4,5], these cells are unable to sufficiently repair the injury and restore the function of the heart.

The need to replenish the heart with new myocytes is of critical importance to avoid the chronic manifestation of the disease. In this regard, stem cell transplantation therapy offers a new therapeutic avenue to create *de novo* myocardium either *in vitro* or *in vivo*. A wide variety of stem cells have shown the ability to differentiate into cardiomyocytes. Of these, the multipotent Sca1+ cardiac-derived cardiomyocyte progenitor cells (CMPCs) offers a desirable combination of a patient-specific cell source with cardiogenic potential, both *in vitro* as *in vivo* [6,7]. Besides direct

* Corresponding authors. Dept. of Cardiology, University Medical Center Utrecht, Heidelberglaan 100, 3508 GA, Utrecht, The Netherlands.
E-mail addresses: R.gaetani@umcutrecht.nl (R. Gaetani), J.Sluijter@umcutrecht.nl (J.P.G. Sluijter).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biomaterials.2015.05.005
0142-9612/© 2015 Elsevier Ltd. All rights reserved.

involvement in tissue repair, the plethora of factors secreted by CMPCs can also activate endogenous stem cell pools, thereby making it a well suited cell type for the implementation of cardiac regenerative strategies [8,9].

Although promising results have come out of pre-clinical and clinical studies [10–12], cardiac stem cell therapy still suffers from inefficient delivery, engraftment, and differentiation of cells in the myocardium [13–15]. Furthermore, during the progression of heart failure, extracellular matrix is also modified and replaced by scar tissue. Therefore, combining procedures aiming at regenerating both myocardial cells and the extracellular matrix could improve the effectiveness of cellular therapy. For this reason, hybrid therapies that include biomaterials and cells are being developed as potentially new therapeutic approaches for repairing myocardial tissue [15,16]. We recently showed that tissue printing technology can be used with alginate in combination with CMPCs to create, *in vitro*, a cardiogenic patch with precise pore size and microstructure, which allows for better cell viability over time [17].

Here, we improved this tissue engineering approach by creating a cardiogenic scaffold of hCMPCs and a hyaluronic acid/gelatin (HA/gel) based biomaterial. The new biomaterial enhanced cell attachment and survival, and is suitable for tissue printing technology applications. We were able to build a customized patch that can harbor CMPCs without affecting their growth and differentiation potential. Furthermore, we demonstrated the *in vivo* applicability of the printed patch in a murine model of MI. The transplanted biocomplex sustained cell viability, leading to excellent cell survival and engraftment. Lastly, mice which received the scaffold showed improved cardiac function after MI.

2. Methods and materials

2.1. CMPC isolation and culture

Human fetal CMPCs were isolated by magnetic cell sorting based on Sca-1 positive selection and propagated as previously described [7]. Briefly, cells were plated at 0.1% gelatin coated wells in growth medium consisting of 25% EGM-2 (3% EGM-2 single quotes (Cambrex) in EBM-2 (Cambrex)) and 75% M199 (BioWhittaker), 10% FBS (Hyclone), 1× MEM non-essential amino acids (BioWhittaker) and 1× penicillin/streptomycin. Differentiation medium consisted of 50% IMDM (GIBCO), 50% HamF12 GlutaMAX-1 (GIBCO), 1× MEM nonessential amino acids, 2% horse serum (GIBCO), 1× insulin-transferrin-selenium (GIBCO), 1× penicillin/streptomycin. 5-azacytidine (5 μM; Sigma), L-ascorbic acid (10^{-4} M; Sigma) and TGF-β (1 ng/ml; Peprotech) were added according to our previously published protocol [7]. Standard informed consent procedures and prior approval of the ethical committee of the University Medical Center Utrecht were obtained.

2.2. Tissue preparation by 3D printing

A Bioscaffolder tissue printer (Sys + Eng, Gladbeck, Germany), resolution 5 μm/step, working area 200 × 150 × 90 mm) was used to print a mixture of gelatin and hyaluronic acid matrix (HyStem matrix; Sigma) with or without CMPCs. Scaffold architecture was determined and converted to a computer-aided design (CAD) file and then combined with specific material settings to a numerical control (NC) code (Rhinoceros software), which directs the robotic arm of the 3D printer. HyStem matrix was prepared according to the manufacturer's protocol. CMPCs were combined with the matrix to a final concentration of 30×10^6 cells per mL. Directly after addition of the thiol reactive crosslinker, the biocomplex was loaded into a syringe and 3D-printed under sterile conditions in a laminar flow-cabinet. The scaffold was composed of 6 perpendicular layers, and each layer was made of 7 strands printed at a distance of 2.5 mm from each other. The construct had a final size of 2 × 2 cm. This patch was cultured up to 28 days for *in vitro* analysis and for 7 days in growth medium prior to *in vivo* transplantation. Differentiation assays were started 1 week after printing.

2.3. Live/dead assay

To determine cell viability of printed CMPCs, scaffolds were cut (0.5 cm × 0.5 cm), washed with PBS and subsequently stained with 2 μM calcein AM and 4 μM ethidium bromide (EthD-1) solution (Invitrogen), as described by the manufacturer. After staining, the tissue was washed again and analyzed by confocal microscope analysis as described before [17].

2.4. qPCR

Total RNA was isolated from the scaffolds and heart cryosections using Tripure isolation reagent (Roche), according to manufacturer's protocol. To homogenize the scaffolds/tissue, microbeads were added to tripure samples and mixed by a tissue homogenizer. cDNA was synthesized by using iScript cDNA synthesis Kit (Bio-rad) and quantitative RT-PCR amplification was detected in a MyIQ single-color real-time polymerase chain reaction system using iQ SYBR Green Supermix (Bio-Rad) and specific primers (see Suppl. Table 1) as described before [18].

2.5. Animal experiments

All experiments were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals*, with prior approval by the Animal Ethical Experimentation Committee, Utrecht University.

2.6. Mouse model and matrix application

Female NOD-SCID mice, aged 10–12 weeks, were anesthetized (Fentanyl 0.05 mg/kg; dormicum 5 mg/kg; domitor 0.5 mg/kg) and MI was induced by ligation of the left anterior descending (LAD) coronary artery as described earlier [19]. In this study, the mice were divided into three groups; control (LAD ligation only), empty matrix (HyStem matrix without CMPCs), and CMPCs matrix (HyStem matrix with CMPCs). Directly after coronary ligation, a 0.2 × 0.2 cm part of the printed construct was cut and applied epicardially with the aid of fibrin glue (1 μl of each component; Sanquin).

2.7. MRI measurements

Cardiac parameters were determined prior to MI, and at 3 and 28 days post-MI for each mouse. End-Diastolic Volume (EDV), End-Systolic Volume (ESV), Ejection fraction (EF), cardiac output (CO) and stroke volume (SV) were measured by high-resolution magnetic resonance imaging (MRI; 9.4 T, Bruker Biospin), as described earlier [20]. Analysis was performed using Q-mass for mice digital imaging software (Medis) by a blinded investigator.

2.8. Bioluminescent imaging (BLI)

CMPCs were transduced with a lenti-viral construct, containing pLV-CMV-luc-GFP as previously described [21] and used to monitor printed CMPCs survival upon *in vivo* transplantation. For detection of luciferase activity, mice were treated with 125 mg/kg of D-luciferin sodium salt (Promega) in PBS via intra-peritoneal injection, 15 min prior to measurement. The detection of omitted

Download English Version:

https://daneshyari.com/en/article/6485590

Download Persian Version:

https://daneshyari.com/article/6485590

Daneshyari.com