Biomaterials 33 (2012) 4069–4077

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Injectable PLGA porous beads cellularized by hAFSCs for cellular cardiomyoplasty

Chieh-Cheng Huang [a,1], Hao-Ji Wei [b,1], Yi-Chun Yeh [a,1], Jiun-Jie Wang [c,d], Wei-Wen Lin [e], Ting-Yin Lee [a], Shiaw-Min Hwang [f], Sung-Wook Choi [g], Younan Xia [h,**], Yen Chang [b,***], Hsing-Wen Sung [a,*]

[a] Department of Chemical Engineering, National Tsing Hua University, Hsinchu 30013, Taiwan
[b] Division of Cardiovascular Surgery, Veterans General Hospital, Taichung, and College of Medicine, National Yang-Ming University, Taipei, Taiwan
[c] Department of Medical Imaging and Radiological Sciences, Chang Gung University, Taoyuan, Taiwan
[d] Department of Medical Imaging and Intervention, Chang Gung Memorial Hospital, Linkou, Taiwan
[e] Division of Cardiology, Veterans General Hospital, Taichung, Taiwan
[f] Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan
[g] Department of Biotechnology, The Catholic University of Korea, Gyeonggi-do, Republic of Korea
[h] Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine; School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA

ARTICLE INFO

Article history:
Received 20 January 2012
Accepted 9 February 2012
Available online 3 March 2012

Keywords:
Microfluidic device
Cellularized bead
Cell delivery
Myocardial infarction
Myocardial tissue engineering

ABSTRACT

Cellular cardiomyoplasty has been limited by poor graft retention after cell transplantation. To ensure good retention of the engrafted cells, a microfluidic device was used to fabricate spherical porous beads of poly(D,L-lactic-co-glycolic acid) as a platform for cell delivery. The beads thus obtained had a relatively uniform size, a highly porous structure, and a favorably interconnected interior architecture, to facilitate the transportation of oxygen and nutrients. These porous beads were loaded with human amniotic fluid stem cells (hAFSCs) to generate cellularized microscaffolds. Live/dead assay demonstrated that most of the cells in the porous constructs were viable. The hAFSCs that were grown in beads formed a complex three-dimensional organization with well-preserved extracellular matrices (ECM) according to their porous structure. Retention of the administered beads was clearly identified at the site of engraftment following an experimentally induced myocardial infarction in a rat model. The results of echocardiography, magnetic resonance imaging, and histological analyses suggest that the transplantation of hAFSC beads into an infarcted heart could effectively maintain its gross morphology, prevent successive ventricular expansion, and thereby improve the post-infarcted cardiac function. Immunofluorescent staining revealed that the microenvironment that was provided by the infarcted myocardium might offer cues for the induction of the engrafted hAFSCs into angiogenic and cardiomyogenic lineages. Our results demonstrate that the cellularized beads with endogenously secreted ECM were of sufficient physical size to be entrapped in the interstitial tissues following transplantation, thereby benefiting the infarcted heart.

1. Introduction

Myocardial infarction (MI) is one of the most common causes of mortality globally [1,2]. Following MI, the myocardium suffers irreversible injury, and the ensuing ventricular remodeling causes thinning of the injured wall and dilation of the ventricular cavity, leading to progressive contractile dysfunction and finally heart failure [2,3]. Stem cell-based therapy is believed to be an attractive solution for cardiac repair. Emerging evidence suggests that directly administering stem cells can improve cardiac performance after MI [1,3,4]. Various stem cell sources, such as mesenchymal stem cells (MSCs), embryonic stem cells (ESCs) and induced pluripotent stem (iPS) cells, have been proposed as candidates for cell transplantation [5–10]. Although the injection of such stem cells has been demonstrated to improve the function of an infarcted heart, MSCs can induce vasculogenesis but not necessarily cardiomyogenesis [11], whereas the use of ESCs or iPS cells in an undifferentiated state carries the risk of uncontrolled differentiation [3,12].

Human amniotic fluid stem cells (hAFSCs) have been considered as an alternative cell source for myocardial regeneration [13–15].

* Corresponding author. Tel.: +886 3 574 2504; fax: +886 3 572 6832.
** Corresponding author.
*** Corresponding author.
E-mail addresses: younan.xia@bme.gatech.edu (Y. Xia), ychang@vghtc.gov.tw (Y. Chang), hwsung@che.nthu.edu.tw (H.-W. Sung).
[1] The first three authors (C.C. Huang, H.J. Wei, and Y.C. Yeh) contributed equally to this work.

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2012.02.024

Derived from amniotic fluid, hAFSCs can be obtained without ethical concerns and are associated with lower risk of teratoma formation [16,17]. Additionally, hAFSCs possess the ability of self-renew and can be induced to differentiate into cells of types that represent various embryonic germ layers [16–18]. Our previous studies have demonstrated that hAFSCs have the potential to differentiate into cardiomyogenic and endothelial lineages [13–15], and thus may be ideal candidates for cellular cardiomyoplasty.

Despite its proven ability to improve the function of an infarcted heart, cellular cardiomyoplasty is limited by poor graft retention following the delivery of the cells [2,19]. A significant proportion of the transplanted cells leak out through the hole that is made by the puncturing needle or enter systemic circulation [20,21]. To prevent loss of cells, various injectable hydrogels, formed from native or synthetic polymers, have been co-implanted with the cells [8,19,22,23]. Upon injection, the hydrogels undergo an *in situ* sol–gel transition and thus embed the transplanted cells intramuscularly. As a major drawback, the cells encapsulated within the interior of a bulk hydrogel may lack a sufficient supply of oxygen or nutrient, resulting in massive cell death and a non-uniform cell distribution [24].

To solve the aforementioned problem, a microfluidic device was adopted to fabricate spherical porous beads of poly(D,L-lactic-co-glycolic acid) (PLGA) with a uniform physical size for cell delivery (Fig. 1). PLGA has been extensively evaluated as a carrier of drugs or cells [25,26]. As a microscaffold, the fabricated porous beads had large pores with a highly interconnected structure, which facilitated the effective mass transfer of oxygen and nutrients, providing an optimal environment for cell loading. Additionally, microbeads with a controlled physical size could be better entrapped by the muscular interstices following transplantation.

In this study, the porous beads were loaded with hAFSCs to generate cellularized microscaffolds for cellular cardiomyoplasty. The distribution and viability of hAFSCs within the porous beads together with their secreted extracellular matrices (ECM) and adhesion molecules were investigated *in vitro*. Direct intramyocardial injection of the hAFSC beads was conducted in a rat model to investigate the capacity of the beads to improve heart function after an experimentally induced MI. Empty porous beads and dissociated hAFSCs obtained by traditional trypsinization were used as controls.

2. Materials and methods

2.1. Preparation and characterization of PLGA porous beads

PLGA porous beads were prepared using a fluidic device that comprised a poly(vinyl chloride) (PVC) tube (1/32 in. i.d. × 3/32 in. o.d.), a glass capillary (0.5 mm i.d. × 0.9 mm o.d.), and a 26-gauge needle [27]. The device with two-way flow channels was fabricated by inserting the needle and the capillary tube into the PVC tube, which was then sealed with epoxy adhesive. The water-in-oil emulsion was prepared by emulsifying aqueous gelatin (2 g, 7.5 wt%) and poly(vinyl alcohol) (PVA, 1 wt%) in a PLGA solution (6 g, 2 wt%) using a homogenizer at 20,000 rpm for 3 min. Subsequently, the prepared emulsion was introduced as a discontinuous phase into the fluidic device, in which an aqueous PVA solution (1 wt%) served as the continuous phase. The flow rates of the discontinuous and continuous phases were maintained at 0.05 and 2 mL/min, respectively (Fig. 1).

The water-in-oil-in-water (W–O–W) droplets, formed at the tip of the needle, flowed along the capillary tube into ice-cold water (collection phase) and were gently stirred overnight to remove the organic solvent by evaporation. To remove the residual gelatin, the acquired beads were gently stirred for 3 h in a warm water bath. The resultant beads were washed with deionized water three times and collected for further application [27]. Additionally, PLGA porous beads that contained super paramagnetic iron oxide nanoparticles (SPION) were prepared to observe their intramuscular retention in the following animal study. The SPION was

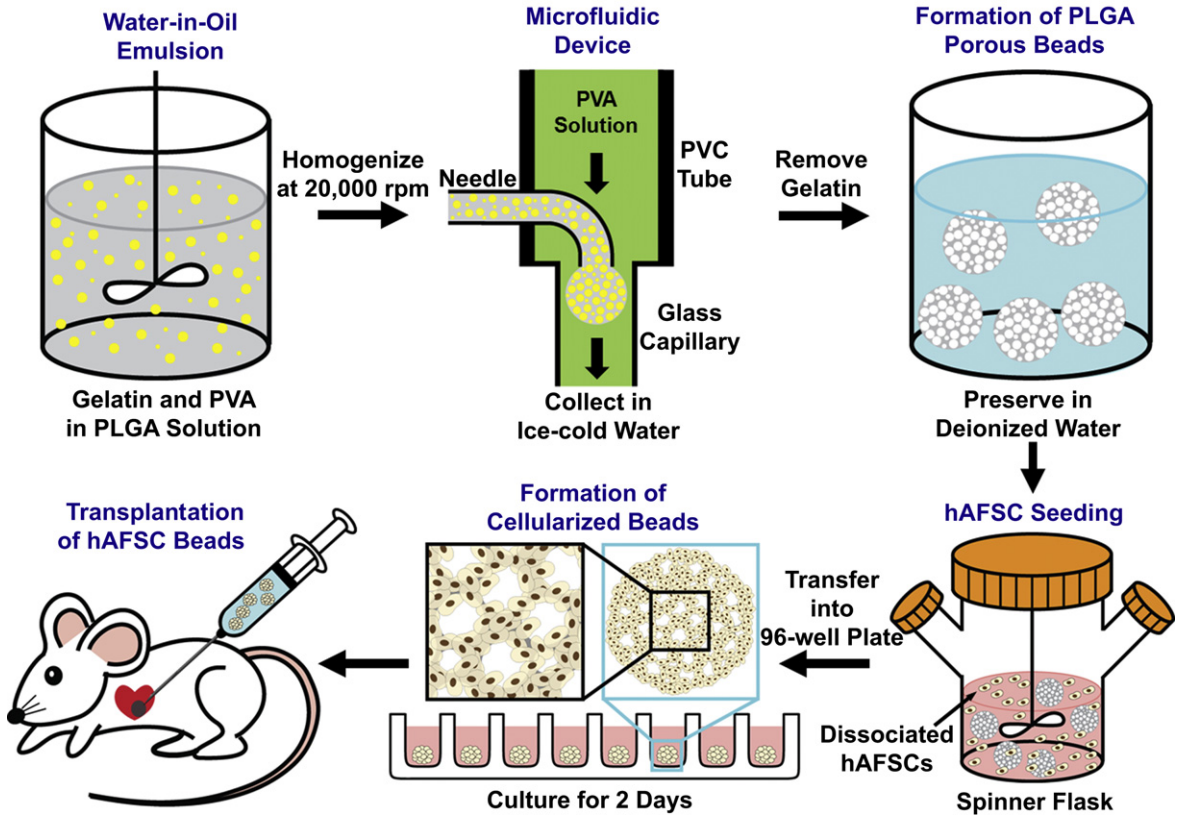

Fig. 1. Schematic illustrations showing how to fabricate PLGA porous beads and then cellularize them with hAFSCs for cellular cardiomyoplasty. A microfluidic device was employed to prepare the PLGA porous beads. For cell seeding, dissociated hAFSCs and porous beads were suspended in a spinner flask and incubated for 12 h. Subsequently, the hAFSC-inoculated beads were individually transferred into the wells of a 96-well culture plate and cultured for two additional days. Finally, the obtained hAFSC beads were collected, loaded in a syringe, and used for intramyocardial injection.