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3D printed complex tissue construct using stem cell-laden decellularized extracellular matrix bioinks for cardiac repair

Jinah Jang [a, 1], Hun-Jun Park [b, 1], Seok-Won Kim [a], Heejin Kim [b], Ju Young Park [c], Soo Jin Na [b], Hyeon Ji Kim [a], Moon Nyeo Park [a], Seung Hyun Choi [d], Sun Hwa Park [e], Sung Won Kim [e], Sang-Mo Kwon [d], Pum-Joon Kim [b, *], Dong-Woo Cho [a, **]

[a] Department of Mechanical Engineering, Pohang University of Science and Technology (POSTECH), 77 Cheongam-ro, Nam-gu, Pohang, Kyungbuk, 37673, South Korea
[b] Division of Cardiovascular Medicine, The Catholic University of Korea, College of Medicine, Cardiovascular Center, Seoul St. Mary's Hospital, 222 Banpodaero, Seocho-gu, Seoul, 06591, South Korea
[c] Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology (POSTECH), 77 Cheongam-ro, Nam-gu, Pohang, Kyungbuk, 37673, South Korea
[d] Medical Research Institute, Laboratory for Vascular Medicine and Stem Cell Biology, Department of Physiology, School of Medicine, Convergence Stem Cell Research Center, Immunoregulatory Therapeutics Group in Brain Busan 21 Project, Pusan National University, 20 Geumo-ro, Mulgeum-eup, Yangsan, 50612, South Korea
[e] Department of Otolaryngology-Head and Neck Surgery, Department of Biomedical Science, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul, 06591, South Korea

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ABSTRACT

Stem cell therapy is a promising therapeutic method for the treatment of ischemic heart diseases; however, some challenges prohibit the efficacy after cell delivery due to hostile microenvironment of the injured myocardium. 3D printed pre-vascularized stem cell patch can enhance the therapeutic efficacy for cardiac repair through promotion of rapid vascularization after patch transplantation. In this study, stem cell-laden decellularized extracellular matrix bioinks are used in 3D printing of pre-vascularized and functional multi-material structures. The printed structure composed of spatial patterning of dual stem cells improves cell-to-cell interactions and differentiation capability and promotes functionality for tissue regeneration. The developed stem cell patch promoted strong vascularization and tissue matrix formation *in vivo*. The patterned patch exhibited enhanced cardiac functions, reduced cardiac hypertrophy and fibrosis, increased migration from patch to the infarct area, neo-muscle and capillary formation along with improvements in cardiac functions. Therefore, pre-vascularized stem cell patch provides cardiac niche-like microenvironment, resulting in beneficial effects on cardiac repair.

1. Introduction

Ischemic cardiovascular diseases are the worldwide major cause of morbidity and mortality [1]. Chronic heart failure usually begins with initial coronary artery blockage and starts progressive cardiomyocyte loss caused by necrotic and apoptotic environments. To heal those coagulative necrosis, cell therapy has emerged as a promising method to achieve functional improvement. The cells are usually transferred via intracoronary delivery method; however, recent meta-analysis of clinical trials showed limited effect on improving cardiac functions because of the death of delivered cells, which resulted in the hostile microenvironment at the injured myocardium [2,3].

Towards the enhancement of therapeutic efficacy, there have been numerous approaches for promoting high cell survival via delivering of cells with functional biomaterials [4,5], pro-angiogenic factors [6,7], and genetically modified cells [8,9], or using sheet type carriers [10]. In particular, cell delivery through a functionalized patch system (e.g., cell sheet engineering) can be an interesting alternative to achieve the delivery of a larger number of

* Corresponding author.
** Corresponding author.
E-mail addresses: bjheart@catholic.ac.kr (P.-J. Kim), dwcho@postech.ac.kr (D.-W. Cho).
[1] J.J and H.-J.P were contributed equally to this work.

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cells at the desired site [11,12]. These cell patch system have revealed successful results, but low biophysical integration, and absence of organized vascular plexus within the platform remain as key barriers that must be overcome for a high level of functional repair for treating myocardial injury [13].

3D cell printing is a promising technology to produce precisely controlled 3D tissue or organ by mimicking both the outer shape and the inner architecture of native tissues [14]. Currently, the use of decellularized extracellular matrix (dECM) bioinks can recreate more complex biologically and biochemically relevant microenvironment, mimicking tissue specific ECM composition or resident cytokines. It enhances cellular functions, such as survival, maturation, differentiation, and migration, improving therapeutic effects after implanting the 3D printed tissue construct [15,16]. Seif-Naraghi and colleagues studied the therapeutic effects of injectable heart tissue derived dECM (hdECM) hydrogel via transendocardial injection in small and large animal models. Accordingly, the hdECM hydrogel increased the recruitment of endogenous cardiomyocytes in the infarct area and maintained cardiac function without inducing arrhythmias [3]. Moreover, the resident CPCs migrated toward the infarct from epicardium through triggering the WT1-based migration mechanism [17]. In previous research, we also have examined the use of a bioink composed of hdECM that could intensively mimic the native tissue microenvironment and enhance the stem cell differentiation into the specific lineage [15,16].

In this study, we developed a 3D pre-vascularized stem cell patch through spatial organization of cardiac progenitor/mesenchymal stem cells using 3D cell printing method. Major features of the prepared cardiac patch are the patterning of dual cells and the use of tissue-specific bioink. They can promote vascularization, prolonged cell survival and tissue remodeling process after transplanting the patch. Moreover, the pre-vascularized stem cell patch provides enhanced therapeutic effects, including decrease of cardiac remodeling and fibrosis, and promotional effects of cardiomyogenesis and neovascularization at the injured myocardium.

2. Materials and methods

2.1. Cell isolation and culture

Human c-kit + cardiac progenitor cells (hCPCs) were isolated from human infant-derived heart tissues after surgical procedures by following the protocol previously reported [18]. This protocol was approved by Institutional Review Board of the Pusan National University Hospital of Yangsan, Gyeongsangnam-do, Korea (Approval no. 2012-18). CPCs were cultured in Ham's F12 media supplemented with 10% fetal bovine serum (FBS, Life technologies, Grand Island, NY), 1 × penicillin/streptomycin, 2.5 mU/mL human erythropoietin (EPO, BioLegend, San Diego, CA), 10 mg/mL human recombinant basic fibroblast growth factor (bFGF, Peprotech, Rocky Hill, NJ), and 0.2 mM L-glutathione (Sigma-Aldrich, Saint Louis, MO). Human turbinate tissue derived mesenchymal stem cells (MSCs) were prepared following protocol previously described [19]. The procedure was approved by Institutional Review Board of the St. Mary's Hospital, The Catholic University of Korea. MSCs were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone, Logan, UT) supplemented with 10% FBS and 1 × penicillin/streptomycin. Human dermal tissue derived microvascular endothelial cells (ECs) were purchased (Lonza, Walkersville, MD) that were cultured in EGM-2MV medium (PromoCell, Heidelberg, Germany).

2.2. Preparation of hdECM bioink

Heart tissue from a 6-month-old Korea domestic pig was collected from a nearby slaughter house with supplier approval. The left ventricle from the complete porcine heart was isolated from the whole heart. The left ventricle was dissected and decellularized by following the protocol previously published [15]. In brief, tissues were minced and treated with 1% sodium dodecyl sulfate (Affimetrix, Santa Clara, CA) solution for 48 h followed by treatment with 1% triton × -100 solution (Bioneer, Dae-Jeon, Korea) for 1 h. The decellularized heart tissue was washed using PBS for at least 3 days to remove the residual detergent and then was sterilized using 0.1% peracetic acid (Sigma-Aldrich) solution in 4% ethanol for 4 h followed by washing several times with filter-sterilized PBS. After freeze-drying of the decellularized heart, the tissues were pulverized in liquid nitrogen using a mortar and pestle. 330 mg of dECM powder was digested in 10 mL of 0.5 M acetic acid solution (Merck Millipore, Billerica, MA) with 33 mg of pepsin powder (Sigma-Aldrich) at room temperature for 48 h. After solubilization, 1 mL of 10 × PBS was added to adjust ionic balance. To exclude the undigested particles, dECM solution was filtered through a 40 μm pore size mesh and then the resulting solution was stored at −20 °C for further examinations. Before conducting the printing experiment, the pH of dECM solution was adjusted to 7.4 through dropwise addition of sodium hydroxide solution while standing the conical tube in the ice bucket to avoid gelation of dECM bioink. We used three different bioink formulations for production of pre-vascularized stem cell patch (Table 1). VEGF were mixed at a concentration of 10 μg/mL to promote rapid vascularization of MSCs-laden heart tissue-derived decellularized extracellular matrix (hdECM) bioink, which is previously developed tissue specific bioink for printing tissues [15], supplemented with 0.02 w/v % of vitamin B2 for the post-crosslinking process [16].

2.3. Fabrication of patches

2.3.1. hdECM hydrogel patch

To investigate the effect of bioink on cardiac tissue regeneration, patch-type specimens were fabricated using hdECM bioink. The final concentration of the hdECM bioink was 20 mg/mL. Each gel patch was designed as a disk with a diameter of 8 mm and height of 0.5 mm.

2.3.2. Structures for in vitro maturation test

The effect of hdECM on the maturation of cardiac progenitor cells was investigated by using the printed structure with Bioink I (Table 1). As a comparison group, collagen bioink was selected (Therafill®, Sewon Cellontech Co., Ltd.), and the final concentration was 2 w/v %. We printed each hdECM and collagen bioink in a disk shape at a dispensing speed of 0.4471 μl/s and a feed rate of 100 mm/min with 26-gauge nozzle, and the structures were 8 mm in diameter and 0.5 mm in thickness. The printed structures were cultured in Ham's F12 media supplemented with 10% FBS, 1 × penicillin/streptomycin, 2.5 mU/mL of EPO, 10 mg/mL of bFGF, and 0.2 mM of L-glutathione.

2.3.3. Structures for in vitro vascularization test

We tested the vascularization capability of the selected cell source by using the printed structure with Bioink II (Table 1). The bioink was extruded at a dispensing speed of 0.4471 μ l/sec and a feed rate of 100 mm/min with the 26-gauge nozzle. The structure was 0.2–0.25 mm in diameter and 10 mm in length. The printed structures were cultured in Endothelial Cell Growth Medium MV2 (Promocell, Heidelberg, Germany) for 5 days.

2.3.4. Stem cell patches for in vivo test

As a supporting layer, polycaprolactone (PCL; MW 43,000–50,000; Polysciences Inc., Warrington, PA, USA) was used