Tissue and Cell 53 (2018) 61–67

Contents lists available at ScienceDirect

Tissue and Cell

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

The addition of human iPS cell-derived neural progenitors changes the contraction of human iPS cell-derived cardiac spheroids

Yosuke Mukae[a], Manabu Itoh[a], Ryo Noguchi[a], Kojiro Furukawa[a], Ken-ichi Arai[b], Jun-ichi Oyama[c], Shuji Toda[d], Koichi Nakayama[b], Koichi Node[c], Shigeki Morita[e,*]

[a] Department of Thoracic and Cardiovascular Surgery, Faculty of Medicine, Saga University, Saga, Japan
[b] Department of Regenerative Medicine and Biomedical Engineering, Faculty of Medicine, Saga University, Saga, Japan
[c] Department of Cardiology, Faculty of Medicine, Saga University, Saga, Japan
[d] Department of Pathology & Microbiology, Faculty of Medicine, Saga University, Saga, Japan
[e] Department of Cardiovascular Surgery, Kyushu Medical Center, A Hospital of National Hospital Organization, Fukuoka, Japan

ARTICLE INFO

Keywords:
Human iPS cell
Cardiomyocyte
Neural progenitor
Spheroid

ABSTRACT

Background: We havebeen attempting to use cardiac spheroids to construct three-dimensional contractilestructures for failed hearts. Recent studies have reported that neuralprogenitors (NPs) play significant roles in heart regeneration. However, theeffect of NPs on the cardiac spheroid has not yet been elucidated.
Objective: This studyaims to demonstrate the influence of NPs on the function of cardiac spheroids.
Methods: Thespheroids were constructed on a low-attachment-well plate by mixing humaninduced pluripotent stem (hiPS) cell-derived cardiomyocytes and hiPScell-derived NPs (hiPS-NPs). The ratio of hiPS-NPs was set at 0%, 10%, 20%,30%, and 40% of the total cell number of spheroids, which was 2500. The motionwas recorded, and the fractional shortening and the contraction velocity weremeasured.
Results: Spheroidswere formed within 48 h after mixing the cells, except for the spheroidscontaining 0% hiPS-NPs. Observation at day 7 revealed significant differencesin the fractional shortening (analysis of variance; $p = 0.01$). The bestfractional shortening was observed with the spheroids containing 30% hiPS-NPs.Neuronal cells were detected morphologically within the spheroids under aconfocal microscope.
Conclusion: Theaddition of hiPS-NPs influenced the contractile function of the cardiacspheroids. Further studies are warranted to elucidate the underlying mechanism.

1. Introduction

Despite significant advances in therapeutic modalities, cardiovascular disease remains the leading cause of death worldwide (World Health Organization, 2004; Ford and Capewell, 2011; Committee for Scientific et al., 2011). Many patients with severe heart failure are unable to undergo heart transplantation or left-ventricular assist device because of the limited number of donor organs and socio-economic restrictions. Cardiac regeneration therapy is a potential alternative for treating end-stage heart disease. Stem cell-derived cardiomyocytes have great therapeutic value for heart failure, such as in treating ischemic heart disease (Ortak et al., 2008). In particular, induced pluripotent stem (iPS) cells (Takahashi et al., 2007; Yu et al., 2007) are expected to be a useful source of cells for autogenous transplantation (Pfannkuche et al., 2009).

With regard to the direct injection of cardiac cells into failed myocardium, although a number of animal experiments have shown some improvements following such a procedure (Laflamme et al., 2007; Shiba et al., 2016), the application is limited by a very low retention rate, poor nutritional supply from the tissue, and a lack of a proper anchoring matrix. Usage of three-dimensional scaffolds has been proposed as an alternative approach in cardiac regenerative therapy, but the scaffold is usually made of foreign materials, which can be a source of inflammation and/or infection (Jawad et al., 2008).

We have been developing a scaffold-free bio-fabrication technology using spheroids as a unit to construct three-dimensional (3D) structures (Itoh et al., 2015; Noguchi et al., 2016). Spheroid formation is a well-known phenomenon based on the nature of cell aggregation. We have already succeeded in fabricating 3D tubular structure made of spheroids with fibroblasts and endothelial cells using a “Bio-3D printer”-based system to construct alternative small-caliber vascular prostheses (Itoh et al., 2015). We have also shown that spheroids made mainly of

* Corresponding author at: Department of Cardiovascular Surgery, Kyushu Medical Center, A Hospital of National Hospital Organization, 1-8-1 Jigyohama, Chuo-ku, Fukuoka, 810-8563, Japan.
E-mail address: morita.shigeki.mu@mail.hosp.go.jp (S. Morita).

https://doi.org/10.1016/j.tice.2018.05.002
Received 17 November 2017; Received in revised form 14 April 2018; Accepted 4 May 2018
Available online 05 May 2018
0040-8166/

cardiomyocytes can fuse together and beat synchronously (Noguchi et al., 2016). Three-dimensional cardiac structures are expected to be transplanted and improve the cardiac function in heart failure models in the future.

Many scaffold-free cell sheets have been developed with the goal of improving only the cardiac function after animal transplantation of the grafts, due to the paracrine effects (Masumoto et al., 2014; Kawamura et al., 2013). However, our aim is to improve the heart contraction directly by transplanting a self-beating cardiac patch. As such, the spheroids in these patches need a strong contractile ability. To improve the function and effectiveness of the structure, we must incorporate additional as-yet-undetermined cell components other than human iPS cell-derived cardiomyocytes (hiPS-CMs).

In the present study, we focused on the application of neurons to heart regeneration therapy. Recent studies have shown that nerves regulate cardiomyocyte proliferation and heart regeneration in zebrafish and mouse hearts (Mahmoud et al., 2015). In experimental models of myocardial infarction, nestin-positive cells differentiate into cardiomyocytes and typical neural crest-derived cells, including neurons, glia, and smooth muscle cells. A subpopulation of nestin cells may directly contribute to myocardial regeneration (Béguin et al., 2009; Galli et al., 2003; Tomita et al., 2005).

We herein report the influence of human iPS-derived neural progenitors (hiPS-NPs) on the contractile function of cardiac spheroids.

2. Material and methods

2.1. Protocol

ReproCardio2 and ReproNeuro (ReproCELL Inc., Yokohama, Japan) as cell sources were thawed as indicated (ReproCELL, 2015) and used as hiPS-CMs and hiPS-NPs, respectively, to construct spheroids. All spheroids were fabricated on a spindle-shaped 96-well ultra-low attachment plates (PrimeSurface, Sumitomo Bakelite CO., Ltd., Tokyo, Japan) by mixing cardiomyocytes and neural progenitors, with mixing rates containing neural progenitor cells of 0%, 10%, 20%, 30%, or 40%, and the total cell number was set at 2500 cells as depicted in Fig. 1. Sixteen spheroids were constructed and analyzed for each group, with the exception of the pure cardiomyocyte group (because of inadequate spheroid formation). The 96-well plates were placed in an incubator under 5% CO_2 at 37 °C.

The culture medium was made by mixing a medium for cardiomyocyte and neural cells at a 1:1 ratio. The medium used for cardiomyocyte was made using IMDM (Sigma-Aldrich, St. Louis, USA) with 0.4% human albumin (Wako, Osaka, Japan), 1% Penicillin-Streptomycin-Amphotericin B Suspension (×100) (Wako), 2 mM L-glutamine (Wako), 0.5 mM L-carnitine (Wako), and 0.001% 2-mercaptoethanol (Thermo Fisher Scientific K.K., Yokohama, Japan) (Minami et al., 2012). The culture medium for neural medium was made using DMEM/Ham's F12 (Wako), solution with 5% knockout Serum Replacement (Thermo Fisher), 1% Penicillin-Streptomycin-Amphotericin B Suspension (×100) (Wako), 1% 10 mM NEAA (Wako), 0.001% 2-mercaptoethanol (Thermo Fisher), 0.1 μM Retinoic Acid (Wako), 0.7 mg/ml Ascorbic Acid (Wako), and N-2 supplement (Thermo Fisher); the medium was modified by referencing previous reports (Kamiya et al., 2011; Mak et al., 2012). These media were refreshed every other day.

2.2. Motion recording and analyses

Spheroid images were obtained under a microscope (BZ-X710; Keyence, Osaka, Japan), at four days after mixing the cells on 96-well ultra-low attachment plates.

The motion of the spheroids was visualized under a microscope (BZ-X710; Keyence), which was kept at 37 °C, in the phase contrast imaging mode and recorded for 30 s as a video file. An off-line analysis was performed to measure the beating rate, shortening fraction, and the shortening velocity of the two most contracting points, as mentioned in Fig. 3, using a software program (BZ-H3A, BZ-H3M; Keyence) as described previously (Noguchi et al., 2016), as well as the contraction rate. The contraction rates of each group of spheroids were followed for four weeks. The maximum and minimum length between two points and the contraction velocity of all spheroids were measured at day 7 after spheroid formation by tracing the two most contractile peripheral points (Fig. 3). Fractional shortening was calculated from the difference between the dots divided by the maximum length.

2.3. Immunofluorescence

Samples were prepared at day 14 after mixing the cells on 96-well ultra-low attachment plates. They were fixed in 4% paraformaldehyde phosphate-buffered solution (Wako) for 16 h at 4 °C, blocked in Carbo-Free Blocking Solution (VECTOR LABOLATORIES INC., California, USA) for 15 min and permeabilized by 0.2% TritonX-100 (Wako) for

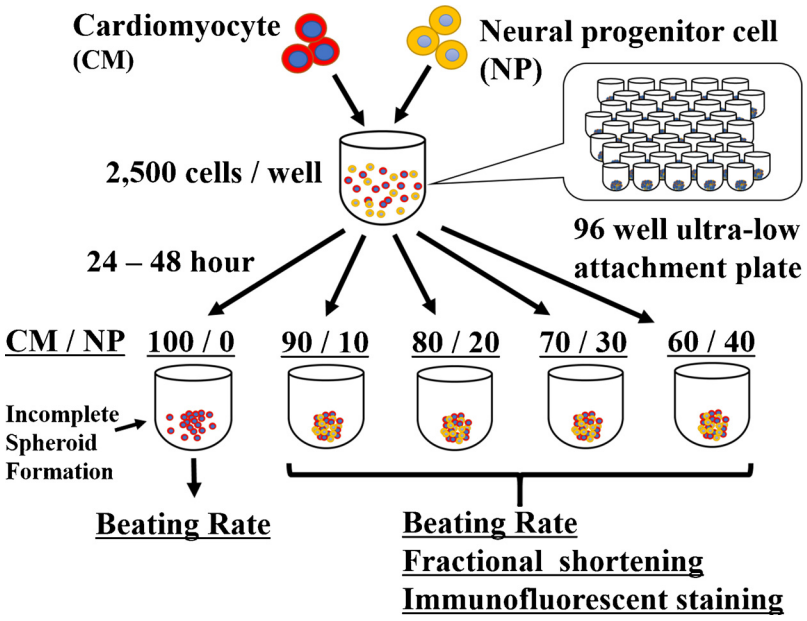

Fig. 1. The protocol of this study. A total of 2,500 cardiomyocytes with or without neural progenitor cells were mixed in each well of 96-well ultra-low attachment plate. In all wells cells aggregated to become spheroid, except for the wells which did not contain neural progenitor cells. These spheroids underwent for measuring beating rate, fractional shortening and immunofluorescent staining. CM, cardiomyocyte; NP, neural-progenitor cell.