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Human gingival fibroblast feeder cells promote maturation of induced pluripotent stem cells into cardiomyocytes

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ABSTRACT

The use of human induced pluripotent stem (iPS) cells has been investigated in multiple regenerative medicine studies. However, although methods for efficient differentiation of iPS cells into heart tissues have been devised, it remains difficult to obtain cardiac tissue with high contractility. Herein, we established a method for differentiating iPS cells into highly contractile cardiomyocytes (CMs), and demonstrate that the use of human gingival fibroblasts (HGFs) as a feeder cells promotes maturation of iPS-derived CMs (iPS-CMs) *in vitro*. After CM differentiation of iPS cells, iPS-CMs showed increased mRNA expression of the CM specific maker cardiac troponin T (cTnT) in the absence and presence (on-feeder condition) of cocultured HGFs, and decreased expression of pluripotent markers was observed under both conditions. Protein expression of cTnT was also observed in immunocytochemical analyses, although on-feeder CMs showed comparatively robust sarcomere structure and significantly stronger contractility than feederless cardiomyocytes, suggesting that HGF feeder cells facilitate CM differentiation of iPS cells.

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1. Introduction

Although heart failure is a serious medical problem, cardiac transplantation is limited by the poor availability of donor hearts [1]. Thus, stem cells are increasingly considered as novel therapies for heart failure [2]. Although many methods have been devised to achieve cardiomyocyte (CM) differentiation of induced pluripotent stem (iPS) cells, poor differentiation efficiencies and safety issues of xeno-transplantation remain obstacles to therapeutic application [2]. Previous studies demonstrate that cross-talk between CMs and non-CMs is important for subsequent electromechanical functions of CMs [3,4]. In particular, Ieda et al. showed that cocultured cardiac fibroblasts promote CM proliferation [5], suggesting that fibroblasts contribute factors that enhance the differentiation of iPS cells and likely influence maturation of CMs.

Exogenous feeder cells are extensively used to maintain the pluripotency of stem cells [6], but contamination with xeno factors remains problematic for autologous transplants of cultured CMs. Because human gingival fibroblasts (HGF) can be harvested from patients using minimally invasive surgical procedures, we investigated their use as feeder cells (Fig. 1A–C) and clarified their effects on CM differentiation of iPS cells.

2. Materials and methods

2.1. Acquisition of HGF

HGFs were isolated from patients after tooth extraction in Okayama University Hospital orthodontic clinic. Patients were 16 years of age or older and those suffering from periodontal disease were excluded. All HGF handling and experimental procedures were approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences and Okayama University Hospital. Written informed consent was obtained from all subjects prior to participation in the study. HGFs were isolated from gingival tissue pieces of about 2 mm^3 that had been surgically removed and washed four times in phosphate-buffered saline (PBS) supplemented with 4-μg/ml gentamicin and 50-μg/ml amphotericin B. Subsequently, tissues were minced, placed in tissue culture dishes and incubated in DMEM-low glucose

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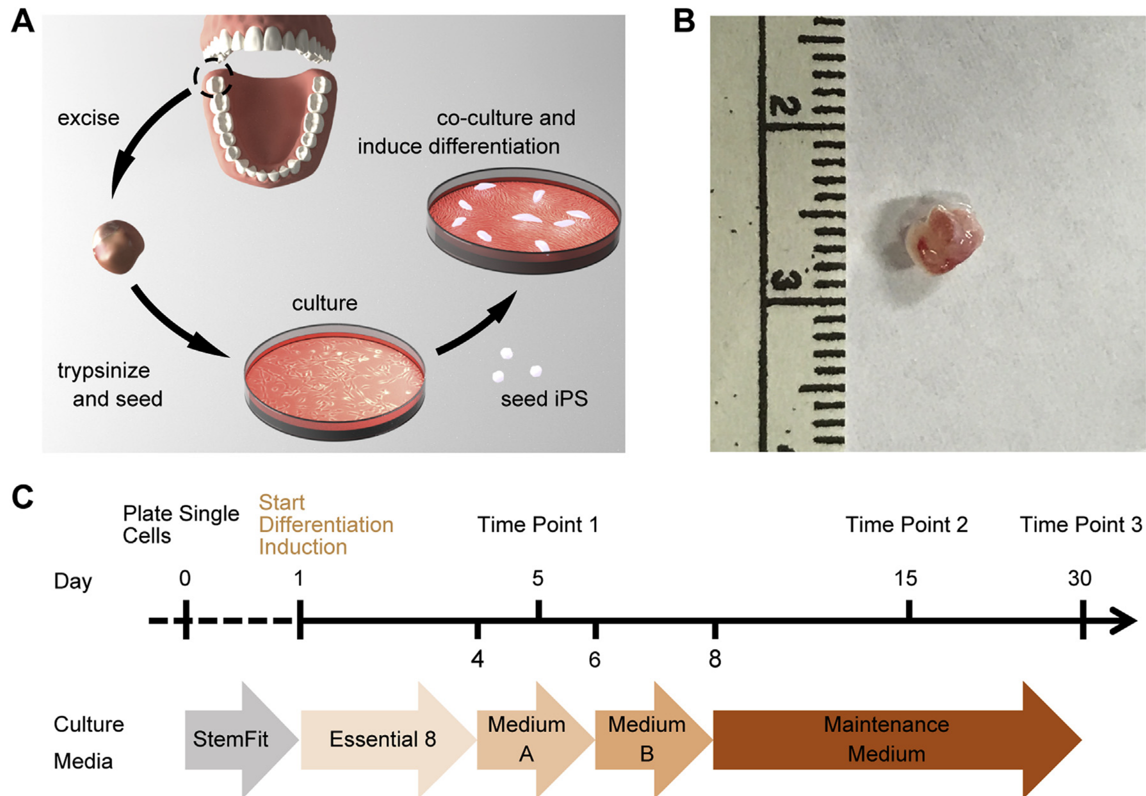

Fig. 1. Protocols for acquisition of human gingival fibroblasts (HGF) and cardiomyocyte (CM) differentiation of iPS cells. (A) Schematic of protocols for acquisition of human gingival tissue and isolation and culture of HGF. (B) Harvested human gingival tissue fragment. (C) Schematic of the culture process for cardiac differentiation and three time points at which immunocytochemical and qPCR analyses were carried out.

medium (Thermo Fisher Scientific, MA, USA) supplemented with 10% fetal bovine serum (FBS), 1-mol/l-HEPES Buffer Solution (Nacalai tesque, Kyoto, Japan), and Penicillin-Streptomycin Solution (Wako, Osaka, Japan) in a humidified incubator containing 5% CO_2 at 37 °C. Confluent HGFs from explants were periodically trypsinised and subcultured using 0.2% trypsin in PBS. All experiments were performed using HGFs between 3rd and 22nd passages.

2.2. iPS cells maintenance

Human iPS cells (201B7 cells of dermal fibroblast origin) were purchased from RIKEN (Tsukuba, Japan) and were cultured in StemFit AK02 N medium (Ajinomoto, Tokyo, Japan) on 6-well culture plates coated with laminin (iMatrix-511, Nippi, Tokyo, Japan), which was diluted to 0.5-μg/ml in PBS prior to coating plates with 2.0 ml/well at 37 °C for 1 h. After coating, the iMatrix-511 solution was removed and iPS cells were seeded without drying. Human iPS cells were passaged according to the protocol supplied by the manufacturer of the medium. Briefly, spent medium was aspirated and cells were washed in PBS. Subsequently, 800 μl of TrypLE Select (Life Technologies, Carlsbad, CA, USA) was added and incubated for 7 min in a humidified incubator containing 5% CO_2 at 37 °C. TrypLE Select reagent was then removed and cells were washed again in PBS, and 1 ml of StemFit AK02 N medium containing Y-27632 (10 μM) was added. Cells were then removed using a cell scraper, were collected in 15-ml centrifuge tube, and were then seeded on iMatrix-511 coated 6-well culture plates at a density of 1.3×10^5 cells/well. The following day, culture medium was aspirated and replaced with StemFit AK02 N medium without Y-27632. Media were changed on days 1, 2, 5 and 6. All experiments were performed using iPS cells between 7th and 20th passages.

2.3. Induction of cardiac differentiation of iPS cells

Differentiation protocols were performed using 96-well plates, and HGF cells were used as feeder cells during CM differentiation of iPS cells. HGFs were seeded at 15,750 cells/well for feeder conditions. To produce feederless conditions, iMatrix-511 was diluted to 2.4 μg/ml with PBS and aliquots of 0.2 ml/well were added to wells and incubated at 37 °C for 1 h. Subsequently, iPS cells were seeded onto on-feeder and feederless 96-well plates at a density of 2.0×10^5 cells/well. One day after seeding, cells were cultured in Essential 8 medium (Thermo Fisher Scientific, MA, USA) for 3 days, and the medium was changed every day. Subsequently, iPS cell differentiation was initiated using PSC cardiomyocyte Differentiation Kits (Thermo Fisher Scientific, MA, USA) according to the manufacturer's instructions. Briefly, spent medium was aspirated and slowly replaced with pre-warmed Cardiomyocyte Differentiation Medium A. Two days later, the medium was aspirated and slowly replaced with pre-warmed Cardiomyocyte Differentiation Medium B, and after two days incubation the medium was again replaced with pre-warmed Cardiomyocyte Maintenance Medium. Subsequently, cells were incubated in a CO_2 incubator at 37 °C and Cardiomyocyte Maintenance Medium was replaced every other day. Cardiac differentiation of iPS cells was then evaluated using immunocytochemistry and quantitative PCR analyses at days 5, 15 and 30. Contractility of iPS cells was evaluated using video image-based analyses on day 30.

2.4. Immunocytochemistry

Expression levels of pluripotent and cardiac markers were determined using immunocytochemical analyses. Prior to

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