Acta Biomaterialia 35 (2016) 215–227

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Scaffold-free parathyroid tissue engineering using tonsil-derived mesenchymal stem cells

Yoon Shin Park [a,b,c,1], Ji-Young Hwang [d,1], Yesl Jun [d,e], Yoon Mi Jin [a,b], Gyungah Kim [a,b], Ha Yeong Kim [a,b], Han Su Kim [b,f], Sang-Hoon Lee [d,e,g,*], Inho Jo [a,b,*]

[a] Department of Molecular Medicine, School of Medicine, Ewha Womans University, Seoul 158-710, Republic of Korea
[b] Ewha Tonsil-derived mesenchymal Stem cells Research Center (ETSRC), School of Medicine, Ewha Womans University, Seoul 158-710, Republic of Korea
[c] School of Life Science, College of Natural Sciences, Chungbuk National University, Cheongju 361-763, Republic of Korea
[d] Department of Biomedical Engineering, College of Health Science, Korea University, Seoul 136-713, Republic of Korea
[e] KU-KIST Graduate School of Converging Science & Technology, Korea University, Seoul 136-713, Republic of Korea
[f] Department of Otorhinolaryngology – Head & Neck Surgery, School of Medicine, Ewha Womans University, Seoul 158-710, Republic of Korea
[g] Department of Bio-convergence Engineering, College of Health Science, Korea University, Seoul 136-713, Republic of Korea

ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form 27 February 2016
Accepted 1 March 2016
Available online 2 March 2016

Keywords:
Tonsil-derived mesenchymal stem cells
Spheroid
Parathyroid hormone
Hypoparathyroidism
N-cadherin

ABSTRACT

To restore damaged parathyroid function, parathyroid tissue engineering is the best option. Previously, we reported that differentiated tonsil-derived mesenchymal stem cells (dTMSC) restore *in vivo* parathyroid function, but only if they are embedded in a scaffold. Because of the limited biocompatibility of Matrigel, however, here we developed a more clinically applicable, scaffold-free parathyroid regeneration system. Scaffold-free dTMSC spheroids were engineered in concave microwell plates made of polydimethylsiloxane in control culture medium for the first 7 days and differentiation medium (containing activin A and sonic hedgehog) for next 7 days. The size of dTMSC spheroids showed a gradual and significant decrease up to day 5, whereafter it decreased much less. Cells in dTMSC spheroids were highly viable (>80%). They expressed high levels of intact parathyroid hormone (iPTH), the parathyroid secretory protein 1, and cell adhesion molecule, N-cadherin. Furthermore, dTMSC spheroids-implanted parathyroidectomized (PTX) rats revealed higher survival rates (50%) over a 3-month period with physiological levels of both serum iPTH (57.7–128.2 pg/mL) and ionized calcium (0.70–1.15 mmol/L), compared with PTX rats treated with either vehicle or undifferentiated TMSC spheroids. This is the first report of a scaffold-free, human stem cell-based parathyroid tissue engineering and represents a more clinically feasible strategy for hypoparathyroidism treatment than those requiring scaffolds.

Statement of Significance

Herein, we have for the first time developed a scaffold-free parathyroid tissue spheroids using differentiated tonsil-derived mesenchymal stem cells (dTMSC) to restore *in vivo* parathyroid cell functions. This new strategy is effective, even for long periods (3 months), and is thus likely to be more feasible in clinic for hypoparathyroidism treatment. Development of TMSC spheroids may also provide a convenient and efficient scaffold-free platform for researchers investigating conditions involving abnormal calcium homeostasis, such as osteoporosis.

1. Introduction

Hypoparathyroidism mainly occurs when parathyroid glands fail to secrete sufficient parathyroid hormone (PTH) as a largely unavoidable consequence of parathyroidectomy (PTX) followed by thyroidectomy. Since PTH plays a pivotal role in calcium homeostasis in the body [1,2], low PTH production and secretion leads to a lack of calcium in blood and bones [3]. Severe and chronic hypocalcemia can be fatal.

* Corresponding authors at: Department of Bio-convergence Engineering, College of Health Science, Korea University, Seoul 136-713, Republic of Korea (S.-H. Lee). Department of Molecular Medicine, School of Medicine, Ewha Womans University, Seoul 158-710, Republic of Korea (I. Jo).
E-mail addresses: dbiomed@korea.ac.kr (S.-H. Lee), inhojo@ewha.ac.kr (I. Jo).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.actbio.2016.03.003
1742-7061/

Several different methods have been used clinically in the treatment of hypoparathyroidism. These include 1) autografting of parathyroid tissues discarded from patients during surgery, an approach associated with uncertainty regarding whether or for how long the graft tissue will survive in the body; 2) daily intake of multiple mega-doses of calcium (1–9 g/day every 6 h) and vitamin D (1.25–5 mg/day) for a remarkably long time, which can provoke hypercalciuria, resulting in kidney problems and vitamin toxicity; and 3) daily injection of the synthetic PTH, teriparatide (brand name, Forteo®), but a maximum of ~2 years is recommended to avoid side effects. At present, the combination of autografting of parathyroid tissues together with multiple high doses of vitamin D and calcium is the most common therapeutic approach [4]. These limitations highlight the need for the development of new therapies, such as direct replacement of functional parathyroid cells without the need for multiple high doses of vitamin D and calcium. In this context, the use of stem cells has recently received considerable attention because of its therapeutic potential for use in tissue engineering and clinical applications [5]. It was previously reported that human embryonic stem cells (hESC) [6] and transdifferentiated thymic stromal cells [7] could be used for the *in vitro* regeneration of parathyroid-like cells. However, these cells have critical limitations for clinical use, including ethical issues associated with the use of hESC and the considerably long time ($\geqslant$10 weeks) required for transdifferentiation of thymic stromal cells into PTH-secreting parathyroid-like cells. To overcome these limitations, we recently introduced human tonsil-derived mesenchymal stem cells (TMSC) for *in vivo* parathyroid tissue regeneration [8]. However, the therapeutic potential of TMSC against hypoparathyroidism in this previous study was limited; differentiated TMSC (dTMSC), but not undifferentiated, control TMSC (cTMSC), resembled parathyroid cells *in vitro* and, more importantly, they only restored parathyroid functions *in vivo* when embedded in a scaffold, Matrigel. Problems associated with scaffolds themselves have been a recurring clinical issue in tissue regeneration.

Tissue regeneration using scaffold-free conditions has recently been highly recommended for safe and long-term clinical applications [9,10]. To this end, we previously established a novel method for three-dimensional (3D) spheroidal culture of various types of cells to manufacture organoids for tissue regeneration [11,12]. For example, rat hepatocytes engineered in a 3D spheroid system have been previously reported to exhibit greater advantages for liver regeneration [12–14]. Another example is the enhanced viability and *in vivo* function of spheroidal cultures of adipose tissue-derived MSC (AD-MSC) and pancreatic islets in an animal model of type I diabetes mellitus [15,16]. Although detailed underlying mechanisms have not yet been elucidated, cells within spheroids appear to exert concerted effects on one another.

On the basis of our previous studies, we here investigated whether a scaffold-free engineering of 3D organoids using dTMSC can surmount requirements for a Matrigel scaffold to restore *in vivo* parathyroid cell functions. Our current results clearly showed that implantation with self-assembled dTMSC spheroids significantly increases survival rates and the levels of serum intact PTH (iPTH) and ionized calcium (iCa^{2+}) in a PTX rat model, suggesting the greater likelihood of applying a scaffold-free system to parathyroid regeneration in a clinical setting.

2. Materials and methods

2.1. Isolation and culture of TMSC

TMSC were isolated and cultured as previously described [8,17]. Briefly, a total of five patients less than 10 years of age undergoing tonsillectomy (3 boys and 2 girls; mean age, 6.2 years) were recruited at random from consecutive patients presenting between April 2012 and December 2012 at the Department of Otorhinolaryngology–Head and Neck Surgery, Ewha Womans University Medical Center (EWUMC, Seoul, Republic of Korea). Informed written consent was obtained from legal guardians of all patients participating in this study, and the study protocol was approved by the institutional review board of EWUMC (IRB No. ECT-11-53-02).

Isolated tonsillar tissue was chopped and digested with collagenase type I (Invitrogen Corp., Carlsbad, CA, USA) and DNase (Sigma–Aldrich, St Louis, MO, USA) for 30 min at 37 °C. Digested tissue was filtered through a wire mesh and washed with RPMI-1640 medium containing normal human serum, after which adherent mononuclear cells were obtained by Ficoll-Paque (GE Healthcare, Little Chalfont, UK) density gradient centrifugation. Cells were then cultured in high-glucose (4500 mg/L) Dulbecco's Modified Eagle Medium (DMEM-HG; Welgene Inc., Gyeongsan, Republic of Korea) containing 10% fetal bovine serum (FBS; Invitrogen). After 48 h, non-adherent cells were discarded and adherent mononuclear cells (hereafter called TMSC) were cultured. All TMSC used in this study were between passage 3 and 9.

2.2. Differentiation into parathyroid-like cells

TMSC were differentiated into parathyroid-like cells using the modified Bingham protocol [18], as previously described [8,17]. Briefly, cells were incubated in 3D culture conditions in the absence or presence of differentiation medium for the indicated times (0, 7, and 14 days) (Fig. 1). Differentiation medium contained activin A (100 ng/mL, R&D Systems Inc., Minneapolis, MN, USA) and soluble sonic hedgehog (Shh; 100 ng/mL, R&D Systems Inc.) and was changed every other day.

2.3. Fabrication of concave molds

Polydimethylsiloxane (PDMS; Dow Corning, Midland, MI, USA)-based concave molds were fabricated using soft lithography techniques with a PDMS membrane and SU-8 mold plates, as previously described [19,20]. Briefly, vacuum pressure was applied to an acrylic chamber to deflect the SU-8 prepolymer on the PDMS membrane to form convex SU-8 structures. The concave PDMS microwell structure was fabricated using the SU-8 plate as a master mold. In this study, we used concave microwell molds 500 μm in diameter, 250 μm in depth and 500 μm apart at a density of 100 wells/cm^2 (StemFIT 3D; MicroFIT, Seonnam, Republic of Korea).

2.4. Formation and culture of TMSC spheroids

All microwell plates were pre-coated with 3% (w/v) bovine serum albumin (BSA, Sigma–Aldrich) before use to minimize cell attachment. After TMSC were dispersed into a single-cell

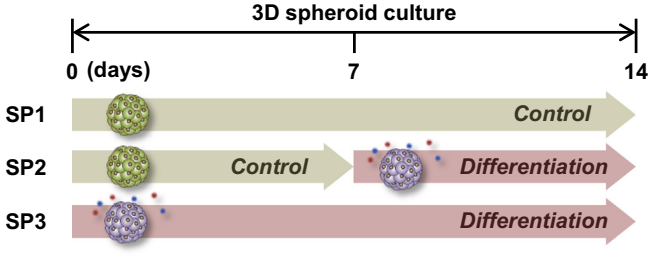

Fig. 1. Three experimental groups for constructing TMSC spheroids. TMSC were cultured in the absence or presence of differentiation culture medium for the indicated times under 3D condition. TMSC spheroids are classified into three groups as follows: SP1 (TMSC in control culture medium for all 14 days); SP2 (TMSC in control medium for the first 7 days, followed by differentiation medium for the second 7 days); SP3 (TMSC in differentiation medium for all 14 days).