Regenerative Therapy 2 (2015) 24–31

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original article

Effect of osteogenic differentiation medium on proliferation and differentiation of human mesenchymal stem cells in three-dimensional culture with radial flow bioreactor

Itsurou Nishimura [a], Ryuichi Hisanaga [c], Toru Sato [c], Taichi Arano [a], Syuntaro Nomoto [c], Yoshito Ikada [d], Masao Yoshinari [b, *]

[a] Department of Crown and Bridge Prosthodontics, Division of Oral Implant Research, Oral Health Science Center, Tokyo Dental College, Chiba, Japan
[b] Division of Oral Implant Research, Oral Health Science Center, Tokyo Dental College, Chiba, Japan
[c] Department of Crown and Bridge Prosthodontics, Tokyo Dental College, Chiba, Japan
[d] Division of Life Science, Nara Medical University, Kashihara, Japan

ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form
3 September 2015
Accepted 11 September 2015

Keywords:
Human mesenchymal stem cells
Radial flow bioreactor
Threedimensional culture
Osteogenic differentiation medium
Proliferation
Differentiation

ABSTRACT

Human mesenchymal stem cells (hMSCs) are multipotent cells, and have been expanded and differentiated into several kinds of mesodermal tissue in vitro. In order to promote bone repair, enhancement of the proliferation and differentiation of hMSCs into osteoblasts in vitro is recommended prior to therapeutic delivery. However, for clinical applications, it is still unclear which method is more advanced for tissue engineering: to transplant undifferentiated cells or partially differentiated stem cells. Therefore, the present study aimed to investigate how osteogenic differentiation medium (ODM) affects hMSCs cultured in a 3D scaffold using a radial-flow bioreactor (RFB) besides cell growth medium (GM). To produce precultured sheets, the hMSCs were first seeded onto type 1 collagen sheets and incubated for 12 h, after which they were placed in the RFB for scaffold fabrication. The culture medium was circulated at 3 mL/min and the cells dynamically cultured for 1 week at 37 °C. Static cultivation in a culture dish was also carried out. Cell proliferations were evaluated by histological analysis and DNA-based cell count. Alkaline phosphatase (ALP) activity, immunocytochemical analysis with BMP-2, and osteopontin on the hMSCs in the collagen scaffold were performed. After 14 days of ODM culture, a significant increase in cell number and a higher density of cell distribution in the scaffold were observed after both static and dynamic cultivation compared to GM culture. A significant increase in ALP activity after 14 days of ODM was recognized in dynamic cultivation compared with that of static cultivation. Cells that BMP-2 expressed were frequently observed after 14 days in dynamic culture compared with other conditions, and the expression of osteopontin was confirmed in dynamic cultivation after both 7 days and 14 days. The results of this study revealed that both the proliferation and bone differentiation of hMSCs in 3D culture by RFB were accelerated by culture in osteogenic differentiation medium, suggesting an advantageous future clinical applications for RFB cell culture and cell transplantation for tissue engineering.

1. Introduction

Large bone defects caused by trauma, inflammation, tumors, or congenital abnormalities are often treated with autologous or allogeneic bone grafts. Implantation of autologous bone grafts is the most popular treatment method due to their high performance in terms of osteogenesis, the only drawback being their limited availability due to donor site morbidity. Allogeneic bone grafts are less attractive because of the risk of immunogenicity, donor-to-host transmission of disease (e.g., HIV), and graft failure as a consequence of the reduced osteoinductivity of allograft bone [1].

Recently, cell-based tissue engineering has drawn much interest as an alternative to these approaches, offering the potential for the

* Corresponding author.
E-mail address: yosinari@tdc.ac.jp (M. Yoshinari).
Peer review under responsibility of the Japanese Society for Regenerative Medicine.

http://dx.doi.org/10.1016/j.reth.2015.09.001

creation of bioartificial tissues and even whole organs. Human mesenchymal stem cells (hMSCs) are multipotent cells, can be readily isolated from adult donors with little damage, and are inducible osteoprogenitor cells, making them the cell of choice in bone tissue engineering and regeneration [2,3]. However, the amount of hMSCs harvested from donor tissue is limited to that which is to be applied to clinical treatment. Static cultivation of MSCs on porous scaffolds and maturation is the simplest method of building a cell-scaffold complex for in vivo implantation [4]. Several studies have reported low seeding efficiencies and non-uniform cell distributions within scaffolds owing, in part, to the manual- and operator-dependent nature of the process [5–7]. Moreover, because of a lack of influence from shear stress and mechanical loading, efficient osteoinduction in static culture seems difficult to achieve. Accordingly, specialized dynamic culture systems, called bioreactors, have been utilized in bone tissue engineering. Such a dynamic three-dimensional (3D) culture system may represent more of a physiological environment than a dish and demonstrate that fluid flow is an important component for seeding and culturing BMSCs in 3D environments [8–11]. This increased interest in tissue engineering has led to the development of various types of equipment for the construction of bioreactors, including spinner flasks, rotating wall vessels, and direct perfusion bioreactors, all of which have been extensively investigated in bone tissue engineering [12,13].

The radial-flow bioreactor (RFB) has shown the ability to maintain an even cell culture environment by radial provision of the medium, enabling the construction of comparatively larger tissues [14–17]. To enable even distribution of oxygen, culture medium is pumped from the periphery to the center of the chamber under low shear stress.

As one approach of promoting bone repair, the enhancement of proliferation and differentiation of hMSCs towards osteoblasts in vitro is recommended prior to therapeutic delivery [4]. In a previous study, it was reported that preosteoblast-like cells and hMSCs were expand uniformly over a 3D scaffold under dynamic cultivation using an RFB, and the cellular characteristics of the hMSCs were not changed in comparison to static cultivation in DMEM without bone differentiation medium [14,18]. However, for clinical applications, it is still unclear which method is more advantageous for tissue engineering: to transplant undifferentiated or, to some extent, differentiated stem cells.

Therefore, the present study aimed to investigate how osteogenic differentiation medium (ODM) affects hMSCs cultured in 3D scaffolds by a radial-flow bioreactor (RFB).

2. Materials and methods

Fig. 1 is a summary of the study protocol.

2.1. Culture of human MSCs

hMSCs derived from human bone marrow (PT-2501; Lonza Walkersville, MD, USA) and donated by a 19-year-old male were passaged 5 times for use in this study. Dulbecco's Modified Essential Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (Sigma–Aldrich) and 100 units/mL penicillin-streptomycin (Gibco) was used as growth media (GM) for static and dynamic cultivation as well as for preculture. On the other hand, GM supplemented with 50 nM dexamethasone, 0.2 mM ascorbic acid, and 10 mM b-glycerophosphate was used as an osteogenic differentiation medium (ODM) for static and dynamic cultivation.

A cell suspension containing 5.0×10^5 cells was seeded into 75 cm^2 flasks, and 20 mL fresh culture medium was added to each flask. Cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2. The culture medium was changed every 3 days. After 1 week of cultivation, before reaching confluence, the cells were harvested by trypsin treatment and seeded onto type 1 collagen sheets (Gunze) (pore size, 70–110 μm; porosity, 80%–95%; diameter, 12 mm; thickness, 3 mm).

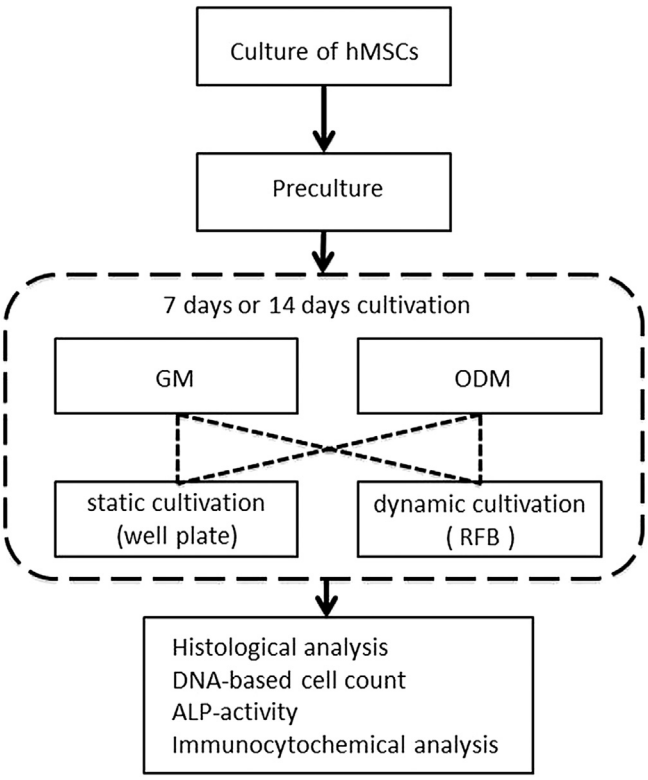

Fig. 1. Flowchart of the present study.

2.2. Preculture

To optimize appropriate pre-culture method for initial cell attachment with high rate of cell density to the collagen sheets, a pre-culture assay was performed that involve turning over the sheets according to the previous study [14,18]. Briefly, type 1 collagen sheets were placed in a 12-well plate and a cell suspension (80 μL) containing 1.0×10^5 cells was seeded onto them. The sheets were then incubated in a humidified atmosphere at 37 °C with 5% CO_2 for 6 h. Next, the sheets were turned over, and a further 80 μL cell suspension containing 1.0×10^5 cells was added before a further 6 h incubation. (The final cell seeding density was 2.0×10^5 cells per sheet.)

2.3. Dynamic cultivation

Fig. 2 shows the RFB (Able) and RFB cell culture system used. To form a scaffold, three precultured sheets were placed in the RFB in layers after incubation for 12 h (6 h + 6 h). The temperature (37 °C), pH (7.4), and dissolved oxygen (DO, 6.86 ppm) in the medium reservoir were controlled and monitored. The medium volume was maintained at 100 mL. After commencement of culture, the medium was changed every day from the third day onward. The medium flow rate was set at 3 mL/min. Culture was carried out for 7 days and 14 days in each of the GM and ODM, as shown in Fig. 1.