Available online at www.sciencedirect.com

ScienceDirect

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

Platelet-rich plasma enhanced umbilical cord mesenchymal stem cells-based bone tissue regeneration

Yong Wen [a,b], Weiting Gu [c], Jun Cui [d], Meijiao Yu [a,b], Yunpeng Zhang [a,b], Cuizhu Tang [a,b], Pishan Yang [a,b,*], Xin Xu [a,b,*]

[a] School of Stomatology, Shandong University, Jinan, PR China
[b] Shandong Provincial Key Laboratory of Oral Biomedicine, Jinan, PR China
[c] Qilu Hospital, Shandong University, Jinan, PR China
[d] Jinan Stomatologic Hospital, Jinan, PR China

ARTICLE INFO

Article history:
Accepted 3 July 2014

Keywords:
Umbilical cord mesenchymal stem cells (UC-MSCs)
Platelet-rich plasma (PRP)
Bone regeneration
Tissue engineering

ABSTRACT

Objectives: To evaluate the effects of platelet-rich plasma (PRP) on the proliferation and differentiation of umbilical cord mesenchymal stem cells (UC-MSCs) and explore the possibility that PRP combined with UC-MSCs may be useful for bone tissue regeneration *in vivo*.

Methods: The proliferation potential of UC-MSCs was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The pluripotent differentiation capacity and alkaline phosphatase (ALP) expression were further determined by ALP staining. The expression of osteoblast-associated genes was evaluated by real-time PCR. In addition, rat critical-sized calvarial defects were examined to evaluate bone regeneration *in vivo*.

Results: PRP enhanced UC-MSC proliferation, and 10% PRP caused the strongest ALP and Alizarin red staining. At 7 days, the expression levels of ALP, Collagen 1 (COL-1) and Runt-related transcription factor 2 (RUNX2) in the PRP group were higher than those in the FBS group. Newly regenerated bone was observed in the defect areas, and PRP combined with UC-MSCs can accelerate bone regeneration at an early stage.

Conclusions: Our current data suggest that UC-MSCs may be utilized in alternative stem cell-based approaches for the reconstruction and regeneration of bone defects, and PRP combined with UC-MSCs can enhance bone regeneration *in vivo*.

1. Introduction

Bone tissue engineering can reduce the expense and risks of using autografts or allografts and can also generate new bone tissue with good biological function and mechanical qualities.[1,2] The concept of bone tissue engineering is based on three elements: scaffolds, cells and growth factors.[3,4] Mesenchymal stem cells (MSCs) are an attractive cell source for tissue-engineered bone and stem cell-based bone regeneration and

* *Corresponding authors at*: School of Stomatology, Shandong University, No. 44-1, Wenhua Xi Road, Jinan, Shandong 250012, PR China. Tel.: +86 531 88382923; fax: +86 531 88382923.
E-mail addresses: wenyong@sdu.edu.cn (Y. Wen), yangps@sdu.edu.cn (P. Yang), xinxu@sdu.edu.cn (X. Xu).
http://dx.doi.org/10.1016/j.archoralbio.2014.07.001
0003–9969/

have already been used in clinical trials.[5] Bone mesenchymal stem cells (BMSCs) have been successfully used to repair damaged skeletal tissue or large bone defects[6–9]; however, their differentiation potential and number in the marrow decreases significantly with ageing.[10] Moreover, the harvesting procedure is painful and invasive, which may lead to complications and morbidity.[11] Because of the disadvantages associated with BMSCs, it is necessary to find new alternative sources of MSCs that function as well as BMSCs but overcome these limitations.[12]

Recently, we isolated MSCs from various tissues, including adipose tissue,[13] umbilical cord tissue,[12] skeletal muscle,[14] amniotic fluid[15] and umbilical cord blood.[16] Among these sources, human umbilical cord tissue is routinely discarded as clinical waste, which makes it an ideal candidate cell source for bone tissue engineering. Moreover, the number of fibroblast colony-forming units is significantly higher in human umbilical cord mesenchymal stem cells (hUC-MSCs), and these cells have a faster proliferation rate in monolayer culture.[17,18] Therefore, we hypothesize that umbilical cord tissue may provide a large number of cells in short time period to meet the needs of bone tissue engineering.

Platelets were thought to be rich sources of growth factors, including platelet-derived growth factor (PDGF), vascular endothelial growth factor, and transforming growth factor-β (TGF-β).[19,20] TGF-β and PDGF-AB are typically present in the highest amounts, promoting the healing of soft tissue and bone through stimulation of collagen production to improve wound strength and the initiation of callus formation.[21,22] Platelet rich plasma (PRP) has been applied to promote bone healing and was developed as a novel material for bone regeneration. Transplantation of BMSCs and PRP shortened the treatment period and reduced the associated complications.[23] Autologous platelet-rich plasma has been shown to be safe, reproducible, and effective in mimicking the natural process of bone and wound healing.[24]

In this study, we examined the effects of PRP on proliferation and compared the osteogenic differentiation capacity of UC-MSCs treated with PRP compared to foetal bovine serum (FBS). Furthermore, we applied PRP combined with UC-MSCs in a rat model of critical-sized calvarial defects to evaluate their effects on bone regeneration *in vivo*.

2. Materials and methods

2.1. Isolation and characterization of UC-MSCs

UC-MSCs were isolated in a manner similar to that described by others.[25] Briefly, the arteries and vein were removed from umbilical cord fragments, washed and incubated in 1% type I collagenase (Sigma) for 20 min at 37 °C. A cell pellet was obtained by centrifugation ($300 \times g$) for 5 min, and 1×10^6 UC-MSCs were collected and plated in a 100 mm Petri dish. UC-MSCs were cultured in complete α-MEM (Gibco) containing 10% FBS (Hyclone), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma) in a tissue culture dish at 37 °C with 5% CO_2. The medium was changed every 2–3 days.

2.2. Flow cytometric analysis

Approximately 5×10^5 cells were incubated with specific phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated mouse monoclonal antibodies against human CD29, CD105 (Biolegend), CD34, CD44 (eBioscience), and CD90 (R&D Systems). Flow cytometry data were analyzed using CellQuest (BD Biosciences) analysis software.

2.3. Osteogenic differentiation

UC-MSCs were incubated in 6-well plates (8×10^3 cells/cm^2) overnight and exposed to osteogenic induction medium supplemented with α-MEM containing 10% FBS (Hyclone), 0.1 mM dexamethasone (Sigma), 10 mM β-glycerophosphate (AlfaAesar), 50 mM ascorbate-2-phosphate (Sinopharm Chemical Reagent Co.) and 100 U/ml penicillin/streptomycin (Sigma). The medium was changed every 3–4 days. After 3 weeks later, mineralization was detected by Alizarin red.

2.4. Adipogenic differentiation

UC-MSCs were incubated in 6-well plates (8×10^3 cells/cm^2) in α-MEM growth medium, allowed to adhere overnight, and replaced with adipogenic induction medium supplemented with α-MEM, containing 10% FBS, 1 μM dexamethasone (Sigma), 200 μM indomethacin (Sigma), 10 μM insulin (Sigma), 0.5 mM isobutyl-methylxanthine (IBMX, Sigma) and 100 U/ml penicillin/streptomycin (Sigma). After 2 weeks, Oil Red O staining was used to detect the formation of oil droplets.

2.5. Preparation of activated platelet-rich plasma

Two whole blood samples were derived from a healthy volunteer. PRP was prepared by a traditional two-step centrifugation procedure.[26] Briefly, whole blood was initially centrifuged at $220 \times g$ for 15 min. The superstratum of yellow plasma was removed to another tube and subsequently centrifuged at $220 \times g$ for 15 min. After centrifugation, the platelets accumulated at the bottom, with the platelet-poor plasma (PPP) accumulating on top. To separate PRP from PPP, the PPP was drawn off. The platelets were activated by thrombin activators, and the mixture was allowed to undergo maximal clot retraction at 4 °C overnight prior to centrifugation at $3000 \times g$ for 10 min. The superstratum (rich in growth factors released from PRP) was collected and stored at −70 °C.

2.6. UC-MSC proliferation in 2D culture

Cell proliferation analysis was performed using the MTT assay. UC-MSCs were seeded in 96-well plates, and the optical density (OD) of the plates was determined with a microplate reader (Bio-Rad Model 550, Hercules, CA, USA) at a wavelength of 490 nm.

2.7. Alkaline phosphatase activity of UC-MSCs

UC-MSCs seeded in 24-well plates were induced in osteogenic induction medium. The level of ALP activity was determined on day 7 by absorbance measurements at 405 nm using