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Osteogenic potential of platelet-rich plasma in dental stem-cell cultures

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Abstract

The purpose of this study was to analyse the potential of platelet-rich plasma (PRP) culture media to induce osteogenic differentiation of periodontal ligament stem cells and dental pulp stem cells compared with four other methods of culture. Both types of cell were collected from 35 healthy patients and cultured in five different media (Dulbecco's modified eagle's medium (DMEM); DMEM and melatonin; DMEM and PRP; DMEM and ascorbic acid 200 μmol; DMEM and L-ascorbate 2-phosphate 50 μmol). Cells were characterised by flow cytometry. Alizarin Red stain, alkaline phosphatase stain, and the expression of collagen type 1 (Col-1), runt-related transcription factor (RUNX2), osteoprotegerin, and osteopontin (quantified by qRT-PCR) were used to detect the osteogenic profile in each culture. Flow cytometry showed that both types of stem cell were a homogeneous mixture of CD90(+), CD105(+), STRO-1(+), CD34 (−), and CD45 (−) cells. Dental pulp stem cells that were cultured with PRP showed the best osteogenic profile (RUNX2 $p = 0.0002$; osteoprotegerin $p = 0.001$). The group of these stem cells that showed the best osteogenic profile was also cultured with PRP (osteoprotegerin $p = 0.001$). Medium five (with L-ascorbate 2-phosphate 50 μmol added) showed an increase in all osteogenic markers for periodontal ligament stem cells after PRP, while the best culture conditions for osteogenic expression of dental pulp stem cells after PRP was in medium four (ascorbic acid 200 μmol added). These results suggested that culture in PRP induces osteogenic differentiation of both types of stem cell, modulating molecular pathways to promote bony formation.

Keywords: Bone regeneration; dental pulp stem cells; periodontal dental ligament stem cells; Platelet-rich plasma; cell culture

Introduction

Treatment with stem cells is one of the most promising strategies for the repair and regeneration of tissues and organs.[1] Although adult stem cells are not totipotent, they can renew themselves for the lifetime of the organism, and can differentiate into many types of mature cells.[2]

Mesenchymal stem cells (MSC), which are present in bone marrow and in adipose and connective tissue,[3] have been identified in dental pulp and periodontal ligaments. Stem cells interact with signal molecules to differentiate into specific line cells. Cells derived from dental pulp and periodontal ligament express MSC markers including CD105, CD73, CD90, CD146, and STRO-1 but do not express CD45 and CD34 cell surface markers, and can also differentiate into osteoblasts and odontoblasts.[4]

Stem cells from dental pulp and periodontal ligaments are a good option for bony regeneration in the treatment of periodontitis and for the reconstruction of deficient alveolar bone for dental implants.[5] They have also been used for reconstruction of bony defects with autologous platelet-rich

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plasma (PRP) but their efficacy for improving tissue regeneration is controversial.[6] MSC derived from umbilical cord, adipose tissue, and bone marrow, combined with PRP induce osteogenesis in vitro,[7] and in vivo, dental pulp stem cells with PRP showed osteogenic potential around dental implants. However, to our knowledge, the response of both types of cell cultured in a PRP medium for osteogenic differentiation in vitro has not been studied. Furthermore, they have the potential for mineralisation in response to appropriate pharmacological induction, but we do not yet know which specific media will influence appropriate tissues for bony regeneration.

The purpose of this study therefore was to analyse the potential of PRP culture media (compared with four other methods of culture) to induce the osteogenic differentiation of stem cells from dental pulp and periodontal ligaments.

Material and methods

Cell isolation

Sixty human impacted third molars were collected from 35 healthy patients (aged between 18 and 30 years) after they had given written informed consent according to the protocol approved by the Ethics Committee of Dentistry, Pontificia Universidad, Javeriana, Bogotá.

The teeth were immersed in Dulbecco's modified eagle's medium (low-glucose DMEM, Sigma-Aldrich, St Louis, USA) and immediately transported to the cell culture laboratory for processing. Periodontal ligament was gently removed from the middle third to the apical segment, by washing the teeth with 5.25% sodium hypochlorite. To obtain the dental pulp, we used a zekrya bur in a high-speed handpiece to section the teeth (while irrigating them with saline solution) and a sterile endodontic excavator.[8] Both types of stem cell were then dissolved separately in a solution containing Collagenase/Dispase® 4 mg/ml (Roche Diagnostics, Indianapolis, USA) for 30 minutes at 37 °C.

Flow cytometry

We used flow cytometry to characterise the stem cells. A total of 1×10^5 dental pulp stem cells were suspended in PBS 300 μl for analysis in FACS (Calibur™, BD Bioscience, San Jose, USA). The same procedure was applied to periodontal ligament stem cells. The commercial monoclonal antibodies CD90, CD105, CD45, CD34, and STRO-1 (Rochem Biocare, Bogotá, Columbia) were used to characterise the cells. Background staining for antibodies was used to show negative cell lines.

Cell culture

The cells were grown in DMEM at 37 °C in 5% carbon dioxide supplemented with antibiotics (penicillin 100 U/ml and streptomycin 100 g/ml; Invitrogen, Carlsbad, USA) and 10% fetal bovine serum (Gibco™, ThermoFisher Scientific, USA). The cells were then treated with 0.3% trypsin (Invitrogen) and cultured. The medium was changed every four days until the cells reached confluence. Each culture was analysed in passages three to four.

Osteogenic differentiation

Both types of stem cells (1×10^4 cells/cm^2) were seeded on 90-well culture plates, and five different media for osteogenic differentiation were used (Table 1). PRP was obtained from 25 healthy patients using a technique described by Gonshor in 2002.[9] The cultured flask of each type of cell was randomly divided for osteogenic differentiation into five groups at 14 days and five groups at 21 days. The negative control group was cultured in low-glucose DMEM (Sigma-Aldrich), and the Clonetics™ Normal Human Osteoblasts System (Lonza, Walkersville, USA) was used for positive control. Reverse transcription quantitative real-time PCR (qRT-PCR) was used for differentiation.

Cultures were stained with Alizarin Red (Sigma-Aldrich) on days 14 and 21 to show deposits of calcium phosphate. Alkaline phosphatase activity was measured by a colorimetric assay using an assay kit (104-LS, Sigma-Aldrich) according to the manufacturer's instructions.

Table 1
Composition of media used for osteogenic differentiation.

Medium	Composition
1 (negative control medium)	Dulbecco's modified Eagle's medium (low-glucose DMEM, Sigma-Aldrich)
2	Low-glucose DMEM (Sigma-Aldrich); 10% bovine serum (Gibco™); 1% antibiotic (Invitrogen); dexamethasone 100 nmol (Sigma-Aldrich), ascorbic acid 200 μmol (Sigma-Aldrich), β-glycerophosphate 10 mmol (Sigma-Aldrich); melatonin 50 μmol (Sigma-Aldrich)
3	DMEM and 10% platelet-rich plasma (PRP)
4	Low-glucose DMEM (Sigma-Aldrich); 10% bovine serum (Gibco™); 1% antibiotic (Invitrogen); dexamethasone 100 nmol (Sigma-Aldrich); ascorbic acid 200 μmol (Sigma-Aldrich); β-glycerophosphate 10 mmol (Sigma-Aldrich)
5	Low-glucose DMEM (Sigma-Aldrich); 10% bovine serum (Gibco™); 1% antibiotic (Invitrogen); dexamethasone 100 nmol (Stemcell Technologies); L-ascorbate-2 phosphate 50 μmol (A2-P)(Stemcell Technologies); β-glycerophosphate 10 mmol (Stemcell Technologies)