Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

Analysis of time-course gene expression profiles of a periodontal ligament tissue model under compression

Yu Li [a,*], Meile Li [a], Lijun Tan [a], Shengbin Huang [b], Lixing Zhao [a], Tian Tang [a], Jun Liu [a], Zhihe Zhao [a,*]

[a] Department of Orthodontics, State Key Laboratory of Oral Diseases, West China School and Hospital of Stomatology, Sichuan University, PR China

[b] Department of Prosthodontics, State Key Laboratory of Oral Diseases, West China School and Hospital of Stomatology, Sichuan University, PR China

ARTICLE INFO

Article history:
Accepted 7 October 2012

Keywords:
Periodontal ligament cell
Gene expression
Compressive force
Orthodontic tooth movement
In vitro model
3-D culture

ABSTRACT

Objective: We recently reported establishment of a periodontal ligament (PDL) tissue model, which may mimic the biological behaviour of human PDL under static compression in orthodontic tooth movement (OTM). In the present study, we aimed at investigating the time-course gene expression profiles of the PDL tissue model under compression.

Design: The PDL tissue model was established through 3-D-culturing human PDL cells (PDLCs) in a thin sheet of porous poly lactic-co-glycolic acid (PLGA) scaffolds, which was subjected to 25 g/cm^2 static compression for 6, 24 and 72 h respectively. After that, its gene expression profiles were investigated using microarray assay, followed by signalling pathway and gene ontology (GO) analysis. Real-time RT-PCR verification was done for 15 identified genes of interest. The cell proliferation alteration was detected through EdU labelling.

Results: (1) Among the genes identified as differentially expressed, there were numerous osteoclastogenesis inducers (including CCL20, COX-1, COX-2, RANKL, PTHrP, IL-11, IL-8, etc.), osteoclastogenesis inhibitors (including IL-1Ra, NOG, OPG, etc.), and other potential bone remodelling regulators (including STC1, CYR61, FOS, etc.). (2) According to analysis of the microarray data, the most significant pathways included *Cytokine–cytokine receptor interaction* (containing CCL20, RANKL, IL-11, IL-8, etc.), *MAPK* (containing FGF7, FOS, MAP3K8, JUN, etc.) and *Cell cycle* (containing CDK1, CCNA2, etc.); the most significant GOs included *Cell–cell signalling* (containing CCL20, STC1, FGF7, PTHrP, IL-11, IL-8, etc.), *Extracellular space* (containing CCL20, IL-1Ra, NOG, PTHrP, IL-11, IL-8, etc.) and *Microtubule-based movement* (containing KIF11, KIF23, etc.). (3) After prolonged compression, cell proliferation was significantly inhibited.

Conclusion: The present findings have expanded our understandings to the roles that PDL plays under static compression in OTM.

* *Corresponding authors at*: Department of Orthodontics, West China Stomatology Hospital, Sichuan University, 14#, 3rd Section, South Renmin Road, 610041 Chengdu, PR China. Tel.: +86 028 85503645; fax: +86 028 85582167.

E-mail addresses: yuli@scu.edu.cn (Y. Li), zhzhao@scu.edu.cn (Z. Zhao).

0003–9969/$ – see front matter
http://dx.doi.org/10.1016/j.archoralbio.2012.10.006

1. Introduction

Periodontal ligament (PDL) is a unique connective tissue sandwiched between two mineralized tissues, alveolar bone and tooth cementum, functioning as a cushion to dissipate and translate the mechanical stimuli transferred from the tooth. The essential role of PDL has been accepted in the regulation of mechano-induced bone remodelling, both during mastication and during orthodontic tooth movement (OTM).[1] However, it is not yet clear how PDL responds to mechanical stimuli biochemically, which results in a series of remodelling of the PDL itself and the adjacent alveolar bone.

Great efforts have been made to answer this issue, particularly through investigating the gene expression alteration in PDL cells (PDLCs) after various mechanical stimulation in vitro or in vivo. In the past years, an innovation is that a variety of high-throughput techniques have been employed to provide comprehensive information on this issue. Human PDLCs were subjected to cyclic tension up to 16 h in vitro, followed by oligonucleotide array analysis, and significant changes of mRNA accumulation for 122 genes were shown.[2] PDLCs were stretched for 6 h, and then the cDNA arrays specific for NF-κB and apoptotic pathways were used to detect altered gene expression in these pathways.[3] PDLCs were simulated by cyclic tension for up to 24 h and 16 genes were identified as differentially expressed, detected with a real-time RT-PCR array containing 78 genes implicated in osteoblast differentiation and bone metabolism.[4]

On the other hand, three-dimensional (3-D) cell culture approach has drawn increasing interest in recent years, as it can better mirror the authentic environment the cells live in, and therefore may result in experimental data more consistent with in vivo. Regarding the mechano-induced biochemical responses of PDL, several innovative studies have been carried out, in which PDLCs were 3-D-cultured in collagen gel and analyzed using microarray to identify the differential gene expression profiles under static[5,6,7] or intermittent[8] compression.

Though collagen gel has been widely used for 3-D cell culture, recent studies reported using synthetic polymers as scaffold for 3-D culture of PDLCs. Rat PDLCs were seeded and 3-D-cultured in poly lactic-co-glycolic acid (PLGA) scaffolds, and the cell proliferation and migration were observed.[9] The tooth–ligament interfaces were delicately reconstructed in a 3-D model of biomimetic hybrid scaffolds, combined of poly caprolactone (PCL), poly glycolic acid (PGA) and tooth dentin.[10] In our recent study,[11] a PDL tissue model was established through 3-D-culturing human PDLCs in a thin sheet of porous PLGA scaffolds, which has been shown to desirably simulate the biological behaviours of human PDL under static compression in OTM.

In the present study, therefore, we were going to investigate and analyze the time-course gene expression profiles of this newly established PDL tissue model under static compression, in order to provide new insights into the biomechanical behaviours of PDL.

2. Materials and methods

2.1. Synthesis of the PLGA scaffolds

PLGA polymers (10^5 g/mol Mw), with a lactide/glycolide ratio of 75:25, were mixed with sucrose particulates (80–120 μm) at a volume ratio of 15:85. The mixture (200 mg) was put in a square mould (2 cm × 2 cm) and subjected to pressure of 5 MPa for 60 s to form a thin sheet. After that, the PLGA sheets were put in a CO_2 (5.5 MPa) reaction kettle at normal temperature for 48 h. Then, the samples were immersed in ddH2O for another 48 h, during which the water was changed every 6–8 h to remove the sucrose. Finally, the PLGA sheets, 2 cm × 2 cm large, 300 μm thick, with 85% porosity and 80–120 μm average pore size, were dried to a constant weight in vacuum, packaged and sterilized by Gamma ray (25 kGy).[11] Pilot studies had been carried out to determine the optimal parameters, including the lactide/glycolide ratio, the pore size and porosity (data not shown).

2.2. Culture of the PDL tissue model

Primary human PDLCs were cultured using an enzyme digestion facilitated tissue explants method. The periodontally healthy and non-carious human premolars were extracted from young donors (10–14 yrs old) for orthodontic reasons. The PDL tissue was obtained by scrapping the middle third of the root, minced into 1-mm pieces, and subjected to 0.3% collagenase type I (Sigma) at 37 °C for 45 min. After that, the precipitate from centrifugation was transferred to a culture flask, and cultured with the complete media in a humidified atmosphere of 5% CO_2 at 37 °C. The complete media contained Dulbecco's modification of Eagle's medium (DMEM, Invitrogen), supplemented with 10% fetal calf serum (GIBCO), 100 units/ml penicillin, 100 μg/ml streptomycin and 200 mM glutamine. Several human PDLC lines were established and the 4–6th passage cells were used for 3-D culture. The procedures were approved by the Bioethics Committee of Sichuan University, China.

For the 3-D cell culture, the PLGA sheets were put in a 6-well plate (Falcon), one in each well. A stool made of stainless steel orthodontic wire was seated on the PLGA sheet (Fig. 1A), in order to prevent it from floated by the media. Suspension of PDLCs (1×10^5 cells in 2 ml medium) was dripped into each well to immerse the PLGA sheet (Fig. 1B). After incubated at 37 °C for 24 h, the PLGA/PDLC constructs were displaced to another 6-well plate with the medium changed, so as to exclude interference from the cells 2-D cultured on the bottom of the well.

2.3. Microscopic observation

Four days after cell implantation, the PDL tissue models, i.e., the cultured PLGA/PDLC constructs, were stained by 0.01% acridine orange in dark, observed under a fluorescence inverted microscope (Leica DMI6000B). Furthermore, the sample was fixed with 2.5% glutaraldehyde solution overnight, dehydrated with gradient ethanol, treated with hexamethyl-