Available online at www.sciencedirect.com

SciVerse ScienceDirect

British Journal of Oral and Maxillofacial Surgery 52 (2014) 501–506

BRITISH Journal of Oral and Maxillofacial Surgery

www.bjoms.com

Fundamental study of application of umbilical cord mesenchymal stem cells to the periodontium to aid healing after autotransplantation of teeth

Yunpeng Li [a,1,2], Rui Hou [a,1,2], Yibo Wang [b,3], Bin Lu [a,2], Junrui Zhang [a,2], Xinghua Feng [a,2], Yanpu Liu [a,**], Qiang Cao [a,*]

[a] *State Key Laboratory of Military Stomatology, Department of Oral and Maxillofacial Surgery, School of Stomatology, The Fourth Military Medical University, Xian, Shaanxi 710032, China*

[b] *Department of Stomatology, 121 Infantry Division of PLA, Guilin, Guangxi, China*

Accepted 2 April 2014

Available online 1 May 2014

Abstract

After autotransplantation of teeth the healing of periodontal tissue regulates the patient's prognosis. Umbilical cord mesenchymal stem cells (UCMSC) have shown excellent pluripotent and proliferation potential. In the present study we investigated the characteristics and developmental capability of osteogenic differentiation to find out whether human UCMSC promote periodontal healing. UCMSC were obtained by primary culture and identified using flow cytometry. Flow cytometry, real-time polymerase chain reaction (PCR), Western blotting, assays of alkaline phosphatase activity, and alizarin red staining were used to assess the potential for hUCMSC to proliferate and differentiate in vitro. Both dentine and predifferentiated or undifferentiated cells were transplanted subcutaneously onto the backs of immunodeficient mice to mimic periodontal tissue healing in vivo. The result showed that hUCMSC were readily obtained, and expressed numerous mesenchymal stem cell markers. Expression of stemness markers decreased notably during osteogenic differentiation. Through investigation of different time points, we found that the osteogenic procedure could be activated and detected at day 7. In the in vivo experiments, the predifferentiated hUCMSC showed increased ability to form cementum-like deposits surrounded by fibroblast-like tissue on the surface of the dentine. In conclusion, the potential for proliferation and differentiation, and the ability to form cementum-like tissue, suggest that hUCMSC are promising candidates as a source of mesenchymal stem cell for sources of periodontal healing after autotransplantation of teeth.

Keywords: Mesenchymal stem cells; Umbilical cord; Wharton's jelly; Tooth autotransplantation; Periodontal ligament healing; Osteogenic differentiation

Introduction

Autotransplantation of teeth is a treatment that involves movement of a tooth from its original site to another one to recover its physiological function. In cases of early loss of teeth from trauma, caries, or because they are congenitally absent, autotransplantation of teeth (compared with implantation) preserves dentition using a natural tooth rather than a mechanical prosthesis, which can be used for all patients regardless of age or socioeconomic status, and has a success rate of 90%,[1] suggesting that it may be potentially beneficial for tooth loss.

* Corresponding author. Tel.: +86 29 84772534; fax: +86 29 8477 6097.

** Corresponding author. Tel.: +86 29 84772531; fax: +86 29 8477 6097.

E-mail addresses: leonidban@hotmail.com (Y. Li), hourui@fmmu.edu.cn (R. Hou), yibowanglucky@126.com (Y. Wang), lubin@fmmu.edu.cn (B. Lu), zhangjr8@fmmu.edu.cn (J. Zhang), fengxh@fmmu.edu.cn (X. Feng), liuyanpu@fmmu.edu.cn (Y. Liu), caoqiangfmmu@163.com (Q. Cao).

[1] These authors contributed equally to this study and share first authorship.

[2] Tel.: +86 29 8477 2534; fax: +86 29 8477 6097.

[3] Tel.: +86 29 8477 6109; fax: +86 29 8477 6097.

http://dx.doi.org/10.1016/j.bjoms.2014.04.001

There are many factors that influence the prognosis of an autotransplanted tooth, such as extraoral time, adaptability between the recipient bone and root surface, age, and others.[2–5] All these factors can be attributed to the adequacy of periodontal tissue and its healing. The ideal condition is healing of the periodontal ligament. A physiological structure (cementum/periodontal ligament/alveolar bone) can be formed to achieve normal function, so improvement of the periodontal condition to achieve healing of the periodontal ligament is a key factor in the increase in the success rate of autotransplantation.

Periodontal ligament stem cells (PDLSC) exist in the periodontium, and may play an important part in periodontal healing,[6] which can be used to improve the periodontal status and the success rate of autotransplantation. However, the source of PDLSC is limited because the cells can be obtained only from a planned operation. Bone marrow mesenchymal stem cells (BMMSC) can be used as substitute mesenchymal stem cells (MSC) and are multipotent adult stem cells.[7] Nevertheless, the harvesting of bone marrow is invasive. It is therefore necessary to explore other substitute stem cells for practical application.

Umbilical cord mesenchymal stem cells (UCMSC) may be a good choice for this purpose. Wharton's jelly contains many fibroblast-like mesenchymal cells that have the capacity for self-renewal and multipotent differentiation,[8] and resemble many properties of BMMSC. In addition, the stem cells harvested from the umbilical cord are abundant at the cell source, are easy to acquire, present no ethical difficulties, and have little immunogenicity.[9] All these characteristics are suitable for use in the autotransplantation of teeth.

We have used in vitro experiments to investigate the stem cell markers and osteogenic properties of hUCMSC. Based on previous findings, we used in vivo experiments to evaluate the formation of roots and periodontal tissue. The findings may provide guidelines towards the increase in the success rate of the autotransplantation of teeth.

Materials and methods

Culture and identification of cells

All human umbilical cords were obtained from the Department of Obstetrics and Gynaecology, Xijing Hospital, the Fourth Military Medical University. The mothers provided written informed consent for the study, which was approved by the ethics committee of the hospital. The hUCMSC were isolated from the Wharton's jelly in the umbilical cord of the newborn baby as described previously.[8] The cells from passages 3 to 8 were used in the experiments.

The antibodies for identification of the cells were all bought from eBioscience® (San Diego, CA, USA), and the manufacturer's instructions were followed strictly. The related markers (CD29, CD44, CD146, CD105, SOX2, CD34, and CD45) were analysed using a flow cytometer.

Induction of osteogenic differentiation

The cells were incubated in osteogenic differentiation medium (Dulbecco's modified Eagle's medium (DMEM, HyClone, Logan, UT, USA) containing 10% foetal bovine serum (HyClone), ascorbate-2 phosphate 50 μg/ml (Sigma–Aldrich, St Louis, MO, USA), dexamethasone 10 nmol/L (Sigma–Aldrich) and β-glycerophosphate 10 mmol (Sigma–Aldrich)). The medium was changed every 2 days. The cells were collected on days 0, 7, 14, and 21 for the following studies.

Alizarin red staining and assay of alkaline phosphatase activity

The calcification was tested on day 21 after osteogenic differentiation using 1% alizarin red s solution (Sigma–Aldrich). The alkaline phosphatase activity (U/pg protein) was measured using the AKL/ALP Test Kit (Najing Jiancheng Bioengineering Institute, Jiangsu, China) at every time point according to the manufacturer's instructions. It was measured and calculated through absorption at 520 nm.

Change in stemness markers during osteogenic differentiation

The following antibodies were used: anti-human CD146 fluoroescein isothiocyanate (FITC), and anti-human/mouse SOX2 eFluor® 660 (eBioscience). The changes in SOX2 and CD146 were evaluated at every time point using a flow cytometer following the protocols *Staining cell surface antigens for flow cytometry* and *Staining intracellular antigens for flow cytometry* (eBioscience).

Real-time quantitative polymerase chain reaction

Total RNA was extracted from hUCMSC at every time point using the Total RNA Kit I (Omega, Norcross, GA, USA) according to the manufacturer's instructions. A total RNA 1 μg was converted to cDNA using the PrimeScript RT Master Mix Perfect Real Time Kit (TAKARA, Kyoto, Japan). For real-time PCR we used the SYBR Premix Ex Taq II (TAKARA) and ABI 7500 Real Time PCR system. The cycling conditions were 40 cycles in two steps. An initial denaturation step at 95 °C for 90 s was followed by denaturation at 95 °C for 15 s and annealing-extension at 60 °C for 34 s. The dissociation stage was 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. The primers were designed by TAKARA, and the sequences are shown in Table S1.