• Research Article •

Alveolar bone defect repair using autologous bone marrow mesenchymal stem cells combined with platelet-rich fibrin

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Cite this article: *Li SH, Dai XW, Zhang WL, Chen C, Wu PL. Alveolar bone defect repair using autologous bone marrow mesenchymal stem cells combined with platelet-rich fibrin. Zhongguo Zuzhi Gongcheng Yanjiu. 2016;20(1): 3-7.*
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Abstract
BACKGROUND: Alveolar bone deficiency will not meet aesthetic and functional requirements for dental implants.
OBJECTIVE: To observe the repair effect of passage 3 autologous bone marrow mesenchymal stem cells (BMSCs) and platelet-rich fibrin (PRF) on alveolar bone defects in rabbits.
METHODS: Twenty-seven New Zealand rabbits were randomly divided into BMSCs/PRF group, PRF group and model group (*n*=9 per group). The left mandible incisors were extracted in all the rabbits under general anesthesia. BMSCs/PRF group was immediately implanted BMSCs/PRF composite into the alveolar socket, PRF group only implanted PRF, and model group implanted nothing.
RESULTS AND CONCLUSION: In the model group, the alveolar crest and alveolar mucosa become sunken notably and narrowed. In the BMSCs/PRF and PRF groups, the thickness of alveolar bone wall, alveolar bone width, alveolar bone height difference, and bone mineral density were all increased, especially in the former group. In addition, the trabecular arrangement was better in the BMSCs/PRF groups than the model and PRF group. Our findings indicate that alveolar socket filling with composite of BMSCs and PRF can achieve preservation of alveolar bone width and height after tooth extraction in rabbits.
Subject headings: Mesenchymal Stem Cells; Fibrin; Platelets; Tissue Engineering
Funding: the National Natural Science Foundation of China, No. 81460103; the Youth Science Fund Project of Xinjiang Uygur Autonomous Region, No. 2013211B53

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Accepted: 2015-11-24
http://www.crter.org

INTRODUCTION

Dental implant restoration has a high demand for alveolar bone mass in edentulous ridges. If there is not enough alveolar bone mass, it is unable to meet the aesthetic and functional requirement of dental implantation. However, the alveolar socket has an inevitable decrease in the width and height after tooth extraction, in particular at the labial side of the front teeth[1-4], thus increasing the difficulty of treatment or losing the opportunity for the implant restoration. How to preserve the alveolar bone mass of alveolar sockets and to avoid alveolar bone defect after tooth extraction is becoming a hot spot of research.

With the development of gene technology, materials science and molecular biology, the treatment of alveolar bone defect has been developed. Composite materials made of porous scaffolds, seed cells and cytokines using tissue engineering technology can be used for defect repair[5-6]. Bone marrow mesenchymal stem cells (BMSCs) not only have potential of multi-directional differentiation and self-replication, but also have no rejection after transplantation[7-8]. Platelet-rich fibrin (PRF) is the second generation platelet concentrate product after platelet rich plasma (PRP) was found by Choukroun in 2000. PRF contains a great amount of cytokines that can be released for over 1 week. Moreover, PRF has good three-dimensional structure which can promote cell migration and proliferation. PRF provides an appropriate environment and scaffold for seed cells growth which is conducive to wound healing[9-13]. The purpose of this study was to observe the repair effect of the BMSCs/PRF on alveolar bone defects of New Zealand rabbits after tooth extraction.

MATERIALS AND METHODS

Design

A randomized controlled animal experiment.

Time and setting

This experiment was completed at the Stem Cell and Animal Laboratory of Xinjiang Medical University, China from March to September in 2014.

Materials

Twenty-seven New Zealand white rabbits, 2 months old, weighing 2.7–3.2 kg, were provided by the Experimental Animal Center of the First Affiliated Hospital to Xinjiang Medical University (license No. IACUC-20140127004).

Methods

PRF preparation

10 mL blood sample was collected from the ear vein of each rabbit. Then, the blood sample was centrifuged for 15 minutes at 3 000 r/min. After centrifugation, the blood sample was divided into three layers and the middle layer was PRF.

Isolation, cultivation and identification of autologous BMSCs in vitro

Under aseptic conditions, 5 mL bone marrow was taken from the bilateral femurs of rabbits and then rinsed with DMED solution (Thermo Fisher Biochemical Products Co., Ltd., Beijing, China) and centrifuged at 1 500 r/min for 5 minutes followed by DMEM culture (10% fetal bovine serum, 100 U/mL penicillin, streptomycin 100 mg/mL) in 37 ℃, 5% CO_2 incubation under saturated humidity conditions. After 48 hours, the medium was exchanged first, the cell suspension was discarded. Then, the culture medium was changed every 2 days. Cell growth and morphology were observed using inverted microscope (Leica Microsystems IR GmbH, Germany). The cells were subcultured and expanded at the proportion of 1:2. In this study, passages 2–3 cells were used.

In vitro culture of autologous BMSCs with PRF

Passages 2–3 BMSCs were labeled with 4,6-acetyl 2-2-phenyl indole (0.02 g/L; Santa Cruz Biotech, Dallas, Texas, USA) overnight, washed with 10×PBS, digested and resuspended. BMSCs and PRF were co-cultured at an identical density of 6×10^7/mL cells onto 24-well plates. Nutrient solution (2 mL) was added and incubated at 37 ℃, 5% CO_2. Then, the medium was changed every 48 hours. BMSCs and PRF were mixed in culture bottles and observed under electron microscopy (Leica Microsystems IR GmbH).

Modeling and intervention

Twenty-seven rabbits were randomly divided into three groups: BMSCs/PRF group, PRF group and model group (n=9 per group). Under general anesthesia induced by 3% pentobarbital sodium injection (1 mL/kg), the left mandibular incisor was extracted aseptically from each rabbit. Width and height of the alveolar socket were measured. Adjacent incisor was cut as reference plane. The BMSCs/PRF group was implanted with autologous BMSCs (1.2×10^6, 20 μL) and PRF (4 mm× 6 mm). The PRF group was filled with PRF(4 mm×6 mm) and the model group filled with nothing. The wound was closed by suture with 4-0 silk. After surgery, each rabbit was injected gentamicin sulfate (1 mg/kg, Anyang, Henan, China), three times a day, totally for 3 days. Each rabbit was caged individually and had free access to food and water. Three animals of each group were killed under overdose 3% pentobarbital anesthesia at 4, 8 and 12 weeks.

Morphological observation

The width and height of the alveolar bone at extracted sites were measured.

Radiographic observation

Rabbit's mandible was fixed in 10% formalin as specimens and underwent computerized tomography. Alveolar bone density was observed after X-ray imaging.

Histological evaluation

The specimen was observed under inverted microscope after decalcification, embedding, sectioning. Hematoxylin-eosin and toluidine blue staining were used to observe osteoblast and trabecular structure.

Main outcome measures

The alveolar bone width, height and mineral density were measured. Osteoblast and trabecular structure were observed.

Statistical analysis

All data are expressed as mean±SD. Intergroup comparison was determined by analysis of variance, followed by Fisher post hoc test and paired Student t-test, when appropriate. A value of $P < 0.01$ was regarded as statistically significant.

RESULTS

Quantitative analysis of experimental animals

During the process of tooth extraction, there were two dead rabbits under anesthesia. Another two rabbits were supplemented and the experiment was continued.

Morphological observation

After 8 weeks, the labial bone plate was absorbed a little in the BMSCs/PRF group, but obviously in the PRF and model groups. After 12 weeks, the alveolar bone of BMSCs/PRF group was intact morphologically and similar to the adjacent alveolar bone as compared with the PRF group. The labial alveolar bone of model group was collapsed obviously.

Measurement of alveolar bone specimens

The percentage of width loss was lowered markedly in the BMSCs/PRF group as compared with the PRF and model groups ($P < 0.01$; **Table 1**). The model group had the highest average loss among the three groups ($P < 0.01$; **Table 2**).

X-ray observation

At 4 weeks, flocculent high-density area was observed at

Table 1 Effect of bone marrow mesenchymal stem cells (BMSCs)/plasma-rich fibrin (PRF) composite on alveolar bone width after tooth extraction ($\bar{x}\pm s$, n=27, mm)

Group	4 weeks	8 weeks	12 weeks	F	P
BMSCs/PRF	−0.20±0.02	−0.32±0.07	−0.58±0.07	15.52	0.01
PRF	−0.25±0.05	−0.44±0.04	−0.60±0.02	42.11	0.01
Model	−0.39±0.07	−0.58±0.08	−0.85±0.05	114.01	0.01

Table 2 Effect of bone marrow mesenchymal stem cells (BMSCs)/plasma-rich fibrin (PRF) composite on alveolar bone height after tooth extraction ($\bar{x}\pm s$, n=27, mm)

Group	4 weeks	8 weeks	12 weeks	F	P
BMSCs/PRF	−0.74±0.20	−1.08±0.15	−1.43±0.12	14.06	0.01
PRF	−0.92±0.03	−1.22±0.02	−1.54±0.02	314.06	0.01
Model	−1.22±0.02	−1.55±0.03	−2.63±0.04	489.19	0.01

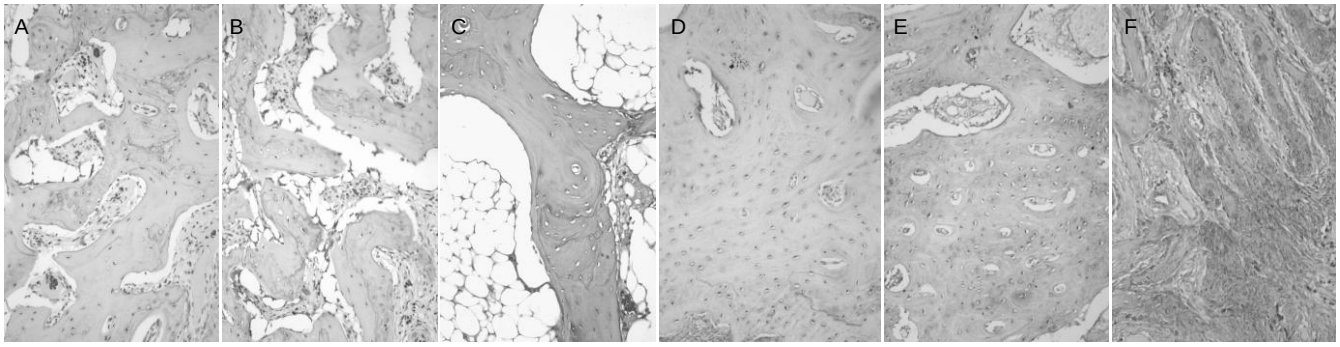

Figure 2 Effect of BMSCs/PRF composite on the alveolar bone morphology in the alveolar socket after tooth extraction
Note: (A−C) Hematoxylin-eosin staining (×20); (D−F) Toluidine blue staining (×20). (A) and (D): BMSCs/PRF group; (B) and (E): PRF group; (C) and (F): model group. The BMSCs/PRF group showed better trabecular arrangement than the PRF and model groups. BMSCs: Bone marrow mesenchymal stem cells; PRF: platelet-rich fibrin.

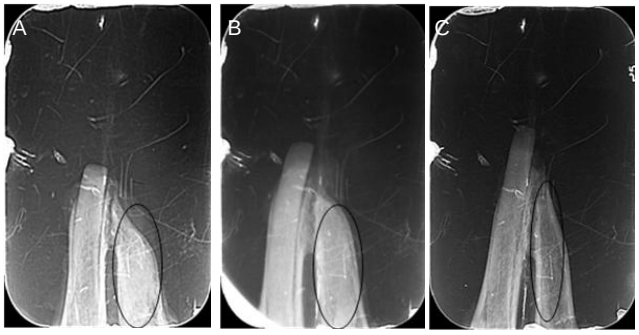

Figure 1 Effect of BMSCs/PRF composite on the alveolar bone morphology in the alveolar socket after tooth extraction (X-ray observation)
Note: (A) BMSCs/PRF group; (B) PRF group; (C) model group. Circles indicate the alveolar sockets. Compared with the PRF and model groups, the thickness of alveolar bone wall, alveolar bone width and height difference and bone mineral density were greater in the BMSCs/PRF group. BMSCs: Bone marrow mesenchymal stem cells; PRF: platelet-rich fibrin.

the surgical site of BMSCs/PRF group, and clearly larger low-density areas in the PRF and model groups.
At 8 weeks, bone healing and increased bone density were observed in the BMSCs/PRF group. The PRF group showed flocculent high-density and low bone density. The model group displayed low-density image and clear boundaries with the surrounding bone.

At 12 weeks, compared with the PRF and model groups, the BMSCs/PRF group showed roughly normal bone density and no significant difference between surrounding boundaries (**Figure 1**).

Histological observation
At 4 weeks, BMSCs/PRF group showed blood capillary, bone marrow tissue and more osteoblasts than the PRF and model groups.

At 8 weeks, some new trabeculae were seen in the BMSCs/PRF group, but not obvious in the PRF group. In addition, more fibrous tissues were visible in the model group.

At 12 weeks, the bone trabeculae were arranged regularly in the BMSCs/PRF group. PRF group showed a small amount of new bone tissues that were scattered. The model group displayed fibrous callus and irregular bone trabeculae (**Figure 2**).

DISCUSSION

To maintain the original volume and architecture of the alveolar bone is essential for aesthetic and functional reconstruction using dental implants[14]. Bone tissue engineering offers a simple way to conserve the labiolingual and mesiodistal alveolar bone after tooth extraction[15]. BMSCs can be isolated, cultured and differentiated into bone cells, nerve cells, fat cells, heart cells under certain inductions *in vitro*[16-18]. Therefore, autologous rabbit BMSCs were chosen as seed cells in this experiment.

PRF belongs to a new generation of platelet concentrate that has been used widely to accelerate soft tissue and hard tissue healing. It can be an immune regulation node with inflammation control and reduce postoperative infections as a surgical additive[19]. Additionally, the slow polymerization of fibrin during PRF preparation creates a fibrin network very similar to the natural one with incorporation of the platelets in

the fibrin meshes, which leads to efficient cell migration and proliferation[20]. Studies have demonstrated that PRF significantly promotes BMSCs proliferation and differentiation into osteoblast-like cells and nerve cells in a dose-dependent manner[21]. Thus, PRF was selected as a scaffold that provides a variety of cytokines in this experiment.

In this study, BMSCs were selected as seed cells, which worked together with PRF to repair alveolar bone defects. The width and height of the rabbit's alveolar bone showed different changes in the three groups at 4, 8 and 12 weeks post-operation. But the height difference value of the BMSCs/PRF group was lower than that of the PRF and model groups, and there was a significant difference. The PRF provided a framework for BMSCs colonization in the alveolar socket, which promoted BMSCs proliferation and differentiation into osteocytes and accelerated healing of the alveolar socket after tooth extraction.

Histological results showed that the BMSCs/PRF group had higher osteogenic activity and more regular bone trabecular arrangement than the PRF and model groups at 4, 8 and 12 weeks post-operation. The amounts of bone tissues and cells were higher in the BMSCs/PRF group than the PRF group. The model group had a few of fibrous connective tissues. Therefore, BMSCs/PRF composite has ability to improve new bone formation and exhibits good biocompatibility. Moreover, the composite can reduce the absorption of alveolar bone and promote wound healing.

Unfortunately, there are still some limitations resulting from many environmental factors that influence cellular differentiation in this experiment. It is necessary to create a more biomimetic environment[22]. Moreover, cellular differentiation needs to be assessed in different models to provide a comprehensive analysis of many factors that may contribute to this effect. At same time, observations have some limitations due to the limitation of time. However, statistically significant values have been found. PRF as a scaffold that produces a variety of cytokines will be better to promote the osteogenic differentiation of BMSCs in alveolar bone repair.

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自体骨髓间充质干细胞复合富血小板纤维蛋白促进拔牙窝骨愈合

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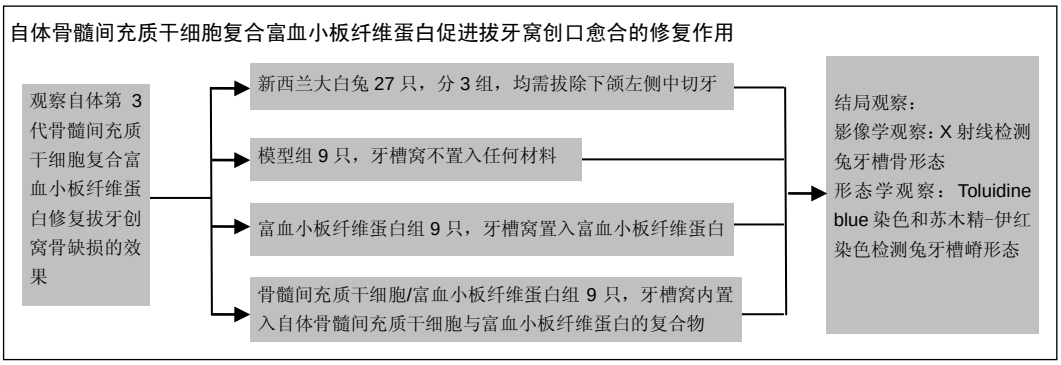

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文题释义：

富血小板纤维蛋白：2001 年由 Choukroun 等提出的区别于传统富血小板血浆技术的富血小板生物材料，被称为第 2 代的血小板浓缩物。具有良好的促进软硬组织再生的作用。

骨髓间充质干细胞：来源于发育早期的中胚层和外胚层，具有多向分化潜能、造血支持和促进干细胞植入、免疫调控和自我复制等特点。在体内或体外特定的诱导条件下，可分化为脂肪、骨、软骨、肌肉、肌腱、韧带、神经、 肝、心肌、内皮等多种组织细胞，连续传代培养和冷冻保存后仍具有多向分化潜能。

摘要

背景：牙槽骨骨量不足将不能满足种植修复的审美和功能重建的要求。

目的：观察自体第 3 代骨髓间充质干细胞复合富血小板纤维蛋白修复新西兰兔拔牙创窝骨缺损的效果。

方法：将 27 只新西兰兔随机等分为模型组、富血小板纤维蛋白组和骨髓间充质干细胞/富血小板纤维蛋白组，均拔除下颌左侧中切牙。骨髓间充质干细胞/富血小板纤维蛋白组牙槽窝内植入自体骨髓间充质干细胞与富血小板纤维蛋白的复合物，富血小板纤维蛋白组牙槽窝植入富血小板纤维蛋白，模型组牙槽窝不植入任何材料。

结果与结论：①模型组兔牙槽嵴及黏膜明显凹陷，宽度变窄；而富血小板纤维蛋白组和骨髓间充质干细胞/富血小板纤维蛋白组兔牙槽骨骨壁厚度、牙槽骨宽度、高度差值、骨密度增加，且骨髓间充质干细胞/富血小板纤维蛋白组牙槽骨骨壁厚度、牙槽骨宽度、高度差值、骨密度大于富血小板纤维蛋白组；骨髓间充质干细胞/富血小板纤维蛋白组骨小梁排列情况优于富血小板纤维蛋白组和模型组。②结果说明骨髓间充质干细胞复合富血小板纤维蛋白填充在拔牙窝内能够起到促进拔牙窝骨质生长，达到促进牙槽骨生长与修复的目的。

关键词：

干细胞；骨髓干细胞；富血小板纤维蛋白；牙槽骨缺损；骨髓间充质干细胞；拔牙位点；位点保存；增殖；国家自然科学基金

主题词：

间质干细胞；纤维蛋白；血小板；组织工程

基金资助：

国家自然科学基金(81460103)；新疆维吾尔自治区青年基金项目(2013211B53)

致谢：感谢新疆医科大学第二附属医院口腔科吴佩玲教授及口腔科全体科室工作人员对本实验的支持和帮助。

作者贡献：实验设计为李淑慧及陈诚，实验实施为戴晓玮、陈诚、张文丽，实验评估为吴佩玲，数据分析及文章撰写为李淑慧。吴佩玲审校。

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文章编号: 2095-4344(2016)01-00003-05

引用本文：李淑慧，戴晓玮，张文丽，陈诚，吴佩玲. 自体骨髓间充质干细胞复合富血小板纤维蛋白促进拔牙窝骨愈合[J].中国组织工程研究，2016，20(1):3-7.

(Edited by Wen SZ/Yu J/Wang L)