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Original Article

The osteogenesis effect of rhBMP2-loaded calcium phosphate cements in repairing dental extraction sockets

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Abstract: Purpose: After extracting impacted mandibular third molars (IMM3), the resulting bone loss at the distal surface of the distal root of mandibular second molars (MM2) is responsible for the poor stability of MM2. This study aimed to identify the clinical osteogenesis effect of recombinant human bone morphogenetic protein-2 (rhBMP-2)-loaded calcium phosphate cements (CPCs) and rhBMP-2 delivery systems (rhBMP-2/CPCs, named CPCII) on bone loss repair at the distal surface of the MM2 distal root after IMM3 extraction. Methods: Written informed consent was obtained from every participant whose IMM3 needed extraction. The impact of IMM3 on both sides was basically identical. From April 2014 to March 2016, extraction of IMM3 was performed in 9 patients (5 males/4 females, 26-42 years old). One side was randomly selected as the experimental group, and CPCII systems were implanted into the distal surface of the distal root in dental extraction sockets. The wounds on the other side were sutured and allowed to heal naturally (be treated as the control group). New bone formation in the alveolar fossa was detected 3 and 12 months after the operation by cone-beam computed tomography (CBCT) to measure the distance from the cementoenamel junction (CEJ) to the crest of the alveolar ridge (CAR). Results: The CAR-CEJ distance on the test side was less than that on the control side ($P<0.5$). Conclusion: The quantity of new bone formation in the experimental group was greater than that in the control group. CPCII systems have osteogenic potential in the healing process of tooth extraction sockets.

Keywords: Recombinant human bone morphogenetic protein-2 (rhBMP-2), calcium phosphate cements (CPCs), rhBMP-2-loaded CPCs (rhBMP-2/CPCs, CPCII), impacted mandibular third molar (IMM3), mandibular second molars (MM2)

Introduction

IMM3 may damage the overall periodontal health distal to MM2, and the extraction of IMM3 seems to be beneficial for MM2. However, residual pockets after the surgical extraction of IMM3 are independent risk factors for the periodontal health of MM2 [1]. Intraoral bone defects possess a high self-regenerative capacity. Factors such as the extent of bone loss, the presence of bony walls, a closed healing environment, space provision and mechanical wound stability substantially influence healing/regeneration [2]. Residual bone defects are common after the surgical removal of IMM3 [3].

Bone morphogenetic protein-2 (BMP-2) is a growth factor that affects the transcription of osteogenesis-related genes that can promote bone formation, regeneration and repair and has been used clinically [4, 5]. Recombinant human bone morphogenetic protein-2 (rhBMP-2) can be loaded into scaffolds and released from scaffolds for bone tissue regeneration [6].

Scaffolds with osteoinductive/osteoconductive properties are necessary for bone regeneration in bone tissue engineering. CPCs possess excellent biocompatibility and osteogenic induction ability. These properties make them widely used as bone graft substitutes in dentistry and orthopedics, and they have been developed

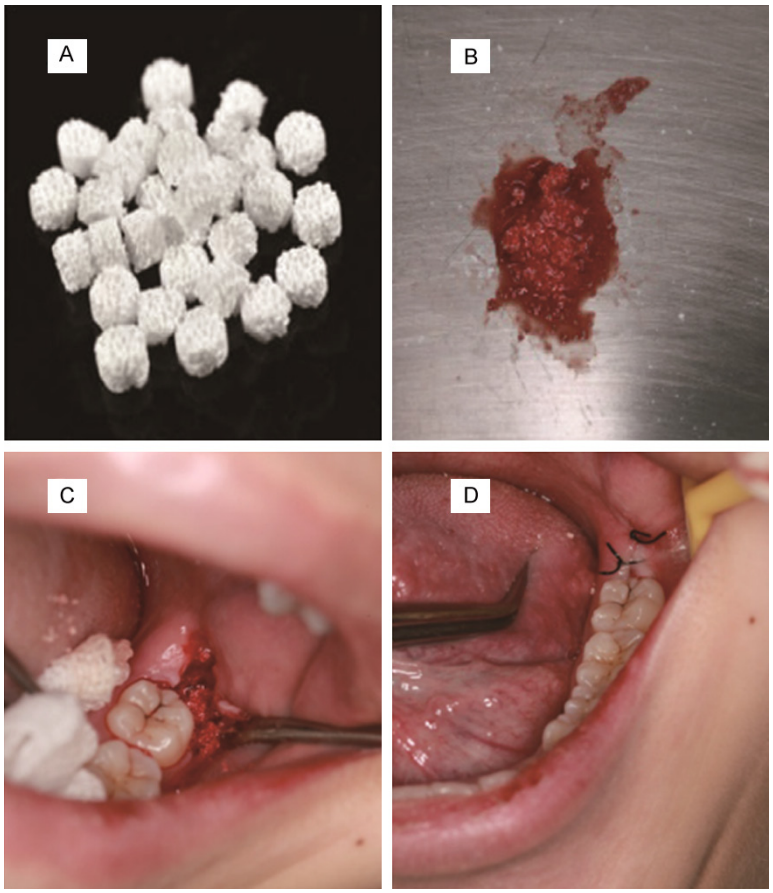

Figure 1. The surgical removal of IMM3 and the CPCII system were implanted into the extraction socket. A: CPCII; B: CPCII mixed with autologous blood; C: Implantation of CPCII in the extraction socket; D: Suture of the extraction socket.

into a controlled release system, releasing BMP cytokines to mediate osteogenesis [7-10].

The osteogenesis effect of the controlled release rhBMP-2-loaded CPC system (rhBMP-2/CPCs, CPCII) has been generally established in vivo and in vitro [11]. However, the clinical effect of CPCII in repairing dental extraction sockets is not clear. The osteogenesis effect of CPCII in repairing dental extraction sockets was clinically evaluated in this study.

Materials and methods

Materials and reagents

CPCII systems were purchased from Shanghai Rebone Biomaterials Co., Ltd. (Shanghai, China). Lidocaine hydrochloride for injection was purchased from Shanghai Hefeng Pharmaceutical Co., Ltd. (Shanghai, China). The images from the CBCT scans were obtained from KaVo 3D exam vision (Imaging Sciences International, PA, USA).

CPCII systems were implanted in dental extraction sockets

The study was approved by the Department of Science and Education of Shanghai East Hospital affiliated with Tongji University Hospital, and all participants signed the informed consent agreement. Nine patients with bilaterally symmetrical IMM3 were enrolled in this study. One side of each patient was randomly assigned as the control or test side (9 cases, 9 controls and 9 tests). The inclusion criterion was horizontal IMM3. Each patient was informed of the surgical purpose, surgical protocol, recovery period, and possible complications, and a consent form was signed.

Local anesthesia with 2% lidocaine was used to anesthetize the tongue, buccal nerve and inferior alveolar nerve. Surgical extraction of IMM3 was performed, and CPCII systems were implanted into the tooth extraction fossa closing the distal surface on the distal root of M2 on the test side. Natural healing after the extraction of IMM3 without the use of CPCII systems was performed on the negative control (NC) side. Then, the tooth extraction sockets were sutured (**Figure 1**). On the control side, IMM3 were extracted, and extraction wounds were sutured. The same antibiotic was administered to both sides. After 10 days, we removed the sutures on both sides.

After 3 and 12 months, the CEJ-CAR distances were measured by CBCT scans to evaluate new bone formation on the distal surface of the distal root in MM2.

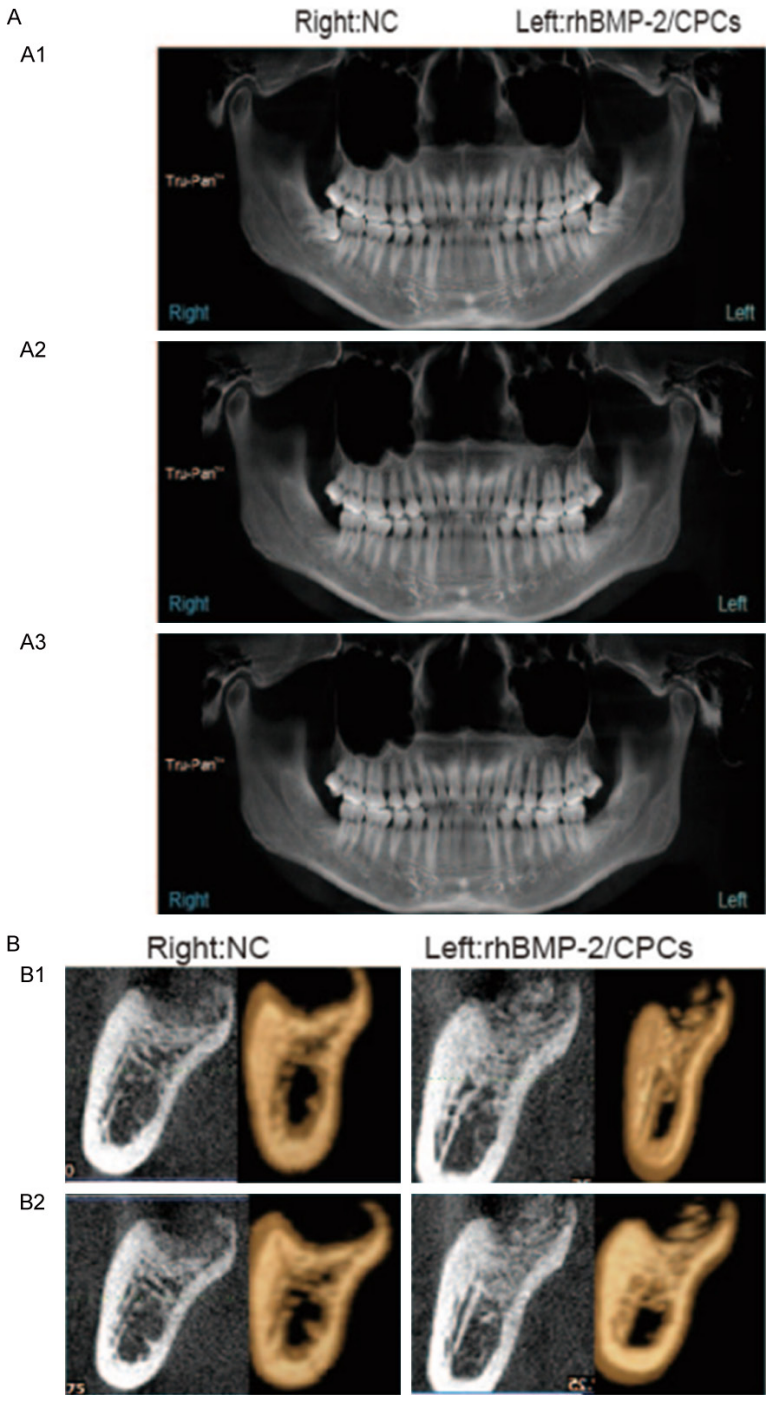

Figure 2. CBCT image analysis of CPCII implantation in the extraction socket after the extraction of IMM3. A: Panoramic radiograph. B: The coronal image and 3D reconstruction views of the CBCT image. A1: Before the extraction of IMM3. A2, B1: Three months post-extraction with/without CPCII implantation.javascript:void(0). A3, B2: Twelve months post-extraction with/without CPCII implantation. Right: negative control (NC); Left: extraction socket post-extraction with CPCII.

Statistical analysis

Data analysis was carried out with SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Differences within groups in all assays were tested by ANOVA and Dunnett's t test. $P<0.05$ was considered statistically significant.

Results

Clinical observation

The sockets showed no signs of infection on either the test or control side 3 and 12 months after the operation according to the CBCT scans (**Figure 2**).

The evaluation of bone loss in dental extraction sockets

As shown in **Figure 3**, the distance from the CEJ to the alveolar crest was lower on the test side than on the control side. This means that there was less bone defect on the test side than on the control side.

Discussion

Surgical extraction of IMM3 is one of the most common operations in the oral clinic. Horizontal IMM3 are usually deeply impacted with large bone resistance, leading to extraction difficulty. In addition, horizontal IMM3 are occasionally located below the cervical lines of MM2, producing the risk of adjacent tooth trauma. Moreover, the extraction of IMM3 is responsible for bone defects distal to the adjacent M2 [1, 12].

To repair bone defects after extracting IMM3, many graft materials are used for implantation into the tooth extraction socket. Of these graft materials, autogenous bone transplantation is still considered the gold standard for repairing bone defects by biological reconstruction. Autogenous bone can promote new bone regeneration and mineralization.

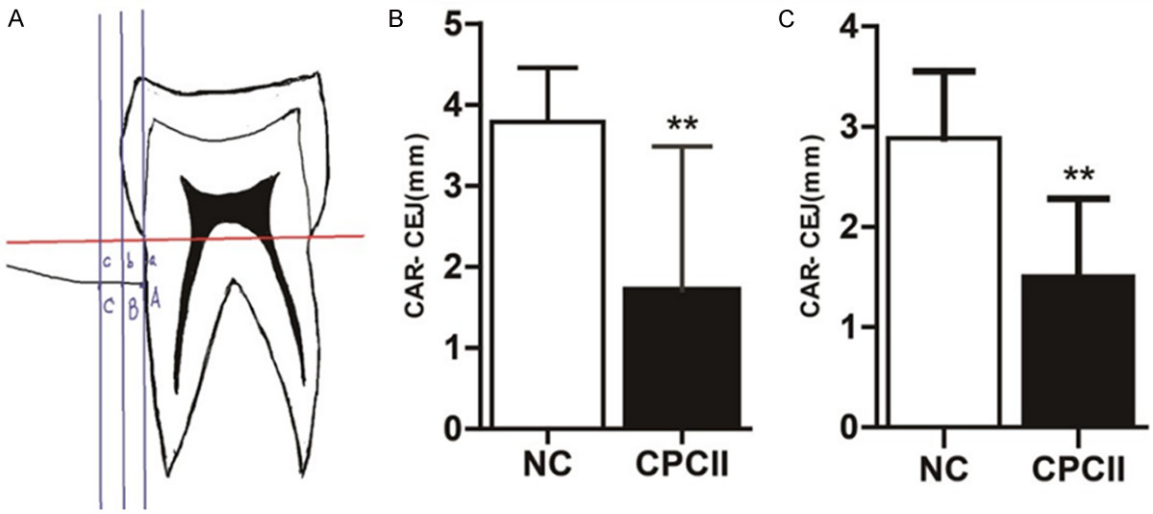

Figure 3. Evaluation of bone loss in dental extraction sockets after the operation. A: Taking CBCT and using the manual function of the KaVo eXam Vision measurement software system, a three-dimensional image of the mandible can be obtained, which can be divided into the transverse, coronal and sagittal planes. The three red, green and blue datum lines of the three planes are placed in the position of bisector M2. On the coronal plane, the height of alveolar bone was measured. The meso-distal enamel-cementum boundary of MM2 was taken as the horizontal datum line, and the meso-alveolar crest junction of MM2 was selected as the measurement of point A, with an interval of 1 mm in turn. The corresponding alveolar crest's highest points were selected as measurement points B and C. The distance from points A, B and C to the horizontal datum line, i.e., the length of line segments a, b and c, was measured, and the average was taken as the distance between the alveolar crest and the enamel-cementum boundary. B: Three months after the extraction of IMM3. C: Twelve months after the extraction of IMM3.

However, autogenous bone grafts may continue to be reabsorbed during healing, and the amount of bone available is limited. Therefore, the development of bone substitutes is urgent to reduce or even replace autologous bone as grafting material. Although CPCs have a low degradation rate and lack macroporosity, they are commonly used as bone substitute materials [13-15].

rhBMP-2 and CPCs can better fuse with a rare complication when a lower dose of rhBMP-2 is used [14]. Moreover, rhBMP-2 has osteoinductive capacity and has been used in clinical practice. The use of rhBMP-2 does not enhance residual graft resorption in vertical bone augmentation procedures and can augment bone height [15]. According to imaging and clinical observations, CPC-loaded rhBMP-2 implanted into tooth extraction sockets to repair the bone defects of extracted IMM3 is feasible. However, we do not have histological or quantitative assessments because it is impossible for patients to receive specimens for reoperation. Surgical removal of IMM3 often results in infection in the mandible, and rhBMP2 administration may lead to inflammation and clinical failure [12, 16, 17]. However, in our study, all patients showed good healing results without infection, which may be associated with the use of antibiotics after surgery. Collectively, these results corroborated our clinical observation that delivery systems (RhBMP-2/CPCs) provide an effective strategy for repairing bone defects after the extraction of IMM3. However, how to control the BMP-2 clinical dose and improve the osteogenesis ability of CPCs are major challenges in bone regeneration for the local use of RhBMP-2/CPCs in bone tissue engineering to obtain better clinical outcomes [18, 19]. Regarding the extensive use of rhBMP-2/CPCs in oral clinics, further studies with more participants and long-term follow-up durations are necessary.

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This study was approved by the ethics committee of Shanghai East Hospital Affiliated with Tongji University (No. PKJ2013-Y12). Notices about automatic opt-in consent for the study for data collection and methods for opting-out were posted in the hospital, as approved by the Ethics Committee of the university. Participants were informed that there was an option for an opt-out of this retrospective research at any time by documenting the refusal of consent using the forms available.

Disclosure of conflict of interest

None.

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