MEDICAL SCIENCE MONITOR

HYPOTHESIS

e-ISSN 1643-3750
© Med Sci Monit, 2014; 20: 624-627
DOI: 10.12659/MSM.890057

Received: 2013.11.21
Accepted: 2014.01.03
Published: 2014.04.16

The root canal system: A channel through which we can seed cells into grafts

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Source of support: Nation Nature Science Foundation of China (304164175)

Bone tissue engineering is bringing hope to patients with jawbone defects, but this technology works well only for small- to moderate-sized jawbone defects. For large segmental jawbone defects, it is difficult to form the functional vascular networks within the graft due to limited diffusion of nutrition and uneven distribution of seed cells. From the standpoint of bionics, seed cells should be continuously transmitted into the graft to replace the necrotic cells during the entire process of bones regeneration. However, the existing one-time inoculation method (OIM) fails to achieve this goal because it is almost impossible to re-open the wound and inoculate cells into grafts that have already been implanted into the body. Inspired by the anatomical structure of jawbones, we hypothesize that the root canal in teeth of jawbones could be used as a channel through which seed cells could be delivered into the graft. Therefore, the multiple-times inoculation method (MIM) could be achieved via the root canal system if defects are located on the maxillofacial bones with teeth. Both osteogenesis and vascularization would be promoted to a large extent because the engineered construct has a limitless supply of seed cells and growth factors.

MeSH Keywords: Bone Tissue Engineering • Seed Cells • Dental Pulp Cavity

Full-text PDF: http://www.medscimonit.com/download/index/idArt/890057

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Background

The reconstruction of jawbone defects caused by accidents, violence, and tumors is the most urgent issue for maxillofacial orthopedic doctors[1]. To better repair jawbones injuries, it is important to gain insight into the biological properties of natural bone and to mimic the process of bone regeneration. Bone is a highly organized connective tissue that provides mechanical support for important organs. There are 3 types of cells in natural bone: osteocytes, osteoblasts, and osteoclasts [2]. The other organic components of bone are extracellular matrix (ECM), which combine with growth factors to trigger the cellular signaling pathways. These synergistic processes play an important role in bone formation [3]. Bone regeneration runs through different stages, requiring interactions among various kinds of cells, growth factors, and ECM [4,5]. For example, osteoblasts regulate deposition of osteoid and mineral nucleation in the bone formation process. Osteoclasts secrets hydrogen ions and acid proteases in the bone resorption process [6]. Throughout all the above-mentioned processes, stem cells in the bone marrow cavities need to be activated continuously to replace the lost cells [7].

As a promising approach for jawbones reconstruction, more and more synthetic composite scaffolds are receiving attention and are widely used in craniofacial research [3]. Unfortunately, to date, translating lab research results into clinical application presents a series of formidable challenges. One challenge is the inability to reconstruct the functional micro-vascular system in the middle part of the scaffold, especially large ones [8,9]. After the cell-containing hybrid materials have been implanted into the body, nutrient deficiencies and/or hypoxia frequently occur in the inner region of the bone tissue substitutes due to limited diffusion within the graft [10]. Before establishment of a pre-vascularized graft, survival of seed cells within the graft mainly depend on the infiltration of nutrition and oxygen diffused from the surrounding normal tissue [11–13]. Hence, cells necrosis often occurs at the center of scaffold in response to exhaustion of the pre-seeding cells, which can result in decreased bone tissue formation [14]. Consequently, the currently used inoculation method, in which seed cells are delivered into the scaffold one time *in vitro*, cannot meet the need for jawbone regeneration in seed cells.

Another crucial but often neglected obstacle is uneven distribution of seed cells in scaffold. Uniform cell-seeding could build the foundation for uniform tissue formation [15]. However, our previous study demonstrated that OIM at least partially results in low cells densities in the center and high cells densities at the surface of the scaffolds [15,16].

Overall, uniform distribution and a limitless supply of seed cells cannot be achieved by OIM. This prompted us to design a new inoculation approach in which cells can be seeded into a graft multiple times, even when the graft has already been implanted into the body.

The Hypothesis

Using the multiple-times inoculation method may simulate the bone repair and healing processes better than the OIM. According to previous studies, however, it is impossible to inoculate seed cells into the scaffolds when the wound is closed [16]. How can we inoculate seed cells into the scaffolds *in vivo* without re-opening the wound? Our hypothesis is that MIM could be achieved by transmitting seed cells though the root canal system of teeth if defects are located on the maxillofacial bones. The root canal is used as the pipeline in this hypothesis (Figure 1). Thus, we can seed a limitless supply of cells and growth factors into the 3-dimensional bone grafts with MIM and fully mimic the complex cellular environment of maxillofacial bones.

Testing the Hypothesis

We want to use different kinds of experimental methods to realize this hypothesis. In animal experiments, beagle dogs with critical-size defects will be used in this study. Briefly, after the dog is anesthetized with intravenous injection of pentobarbital sodium at a dose of 40 mg/kg body weight, an approximately 1-cm submandibular incision was made to expose the mandible, and a rectangular critical-size defect is carefully created on the lower margin of the mandible, until the apical third of the mandibular first premolar and canine are exposed. Then, the apical foramens are enlarged for cells transportation, followed by augmentation, preparation, and cleaning of the root canal. Finally, the scaffold is implanted and anchored in the defect with the tooth roots inserted into the scaffold (Figure 1A). The scaffolds should be processed before implantation so that these personalized scaffolds can be adapted to individual characters of defects and the location of tooth roots. At 1, 2, and 3 months after the first implantation, BMSCs tagged with green fluorescence protein (GFP) would be transmitted into the scaffolds in defects through the root canal system. After completion of cell seeding, root canals need to be temporarily filled with mineral trioxide aggregate (MTA) under sterile conditions. Six months after the first implantation, the mandibles of all animals would be harvested to determine the source of regenerative cells by analyzing the GFP fluorescence with confocal microscopy. Furthermore, new bone formation and absorbance of scaffold would be assayed by immunohistochemical staining of osteopontin, osteonectin, osteocalcin, and collagen type II.

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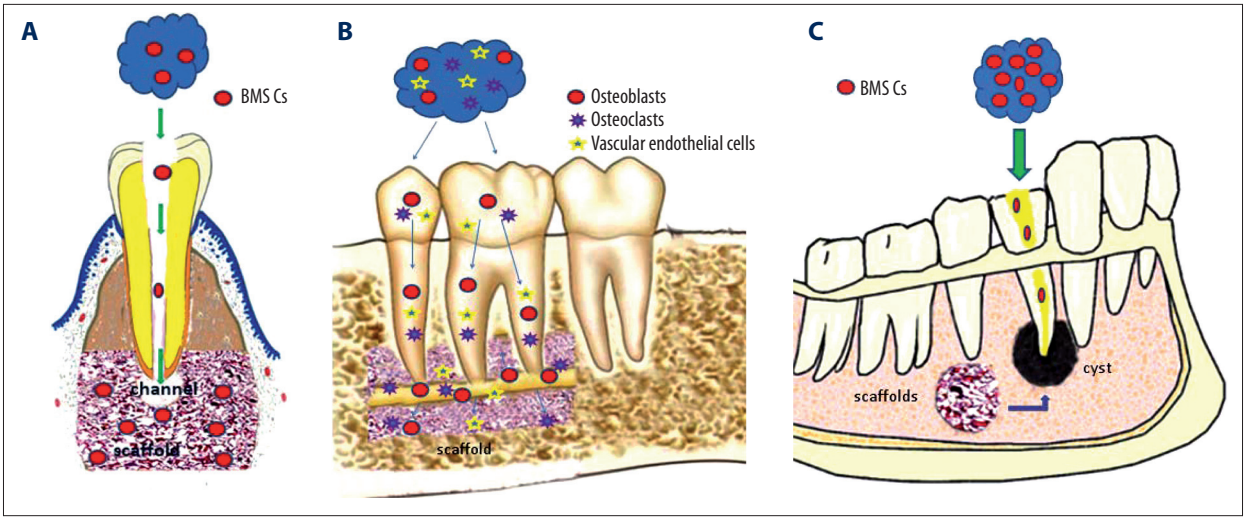

Figure 1. (**A**) Schematic diagram of the hypothesis: After preparing the root canal system, the scaffold is implanted into the defect beneath the teeth. The exposed roots of the teeth need to be inserted into the scaffolds. (**B**) Various types of cells, such as osteoblasts, osteoclasts, and vascular endothelial cells could be seeded into the constructs, depending on the needs of the bone regeneration process. (**C**) Treatment of apical cyst with multiple-times inoculation method. After preparing the root canal and curettage of the cyst, the scaffold in the shape of the defect is transplanted and anchored in the defect area, and seed cells and growth factors can be transmitted into the cell-construct through the prepared canal.

Evaluation of the Hypothesis

This novel strategy may become a major breakthrough in reconstruction of jawbone defects if the hypothesis proves to be practical. Compared with conventional OIM, root canal-assisted MIM could solve at least 2 problems in bone tissue engineering:

1. During the entire bone regeneration period, a continuous supply of seed cells could be transmitted into the graft to replace the apoptotic cells. The inoculation of a large number of seed cells results in more bone formation when compared with medium and low doses [17]. However, transplanted seed cells die quickly or migrate out of the defect site [18]. Boukhechba et al. demonstrated that newly transplanted BMSCs did not survive more than 3 weeks *in vivo* [19]. Consequently, seed cells inoculated *in vitro* with the OIM are far too few. Although the wound is closed, cells and growth factors could still be continuously transmitted into the scaffolds through the root canal at the given time points.
2. We can provide the graft with diverse types of seed cells depending on the need at different stages in jawbones healing. At the stage of bone resorption dominated by osteoclasts, we can transmit more osteoclasts into the graft. On the contrary, it is favored to increase the number of osteoblasts around the defect in the process of bone formation. We can also seed vascular endothelial cells into the graft during the angiogenesis process (Figure 1B).
3. This strategy would be especially applicable to the treatment of large apical cysts. As shown by Figure 1C, the root canal can be used as the cell-transmitting channel, as the apical part is located in the middle of the circular defect caused by the apical cyst (Figure 1C). Furthermore, root canal therapy is supposed to be performed according to normal treatment procedures for apical cysts. After preparing the root canal and curettage of the cyst, the custom-designed construct in the shape of the defect is transplanted into the jaw bone, and seed cells and growth factors can be transmitted into the cell-construct through the prepared canal.

Nevertheless, there is a limitation existed in this hypothesis. The prerequisite of the hypothesis is that there are teeth on the top of the defect (Figure 1A, 1B). Therefore, the hypothesis only applies for segmental jawbones defects.

The Difficulties in the Realization of Hypothesis

This novel design may achieve the multiple-times inoculation of exogenous cells into a jawbone graft with the help of the root canal system. However, there are 2 challenges need to be resolved.

The first problem hindering the realization of this hypothesis is the difficulty in keeping the channels in scaffolds and the root canal system unblocked. After implantation of the cell-scaffold compound, blood would penetrate, clog, and block the channels of the scaffold and root canal of teeth, which would prevent inoculation of seed cells into the scaffold. A possible solution is to temporary fill the channels of scaffold and root canals of teeth with a biocompatible material. Thus,

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by removing the temporary sealing materials in the root canal, we could open a gateway linking the graft *in vivo* with the outer environment and inoculate cells into the scaffold again.

The conditions that the filling material must meet are biocompatibility, toughness, and non-absorbability. According to previous studies, silicone gel is now the most widely-used implant material in plastic surgery [20,21] due to its good biocompatibility, toughness, and non-absorbability [22]. Consequently, it is reasonable to speculate that silicone gel may have the basic conditions to become the above-mentioned implant material. In this hypothesis, we propose to shape the silicone gel, forming a long strip based on the form of the channels in scaffold and teeth.

Another problem is that the leakage of bacteria and infective material in the oral cavity would cause the infection of the cell-scaffold compound in the course of opening the root canal, disturbing the temporary material and inoculating of seed cells. Moreover, hard bacterial plaques in the root canal system, such as *Enterococcus faecalis*, may also cause infection, and root canal irrigation and normal sterilization cannot stop the spread [23,24]. Consequently, infection control is vital and may be achieved by the following steps:

1. Screen the experimental objects strictly and exclude the animals with poor hygiene, necrotic pulp, and periapical disease.
2. Follow the aseptic principles during and after the operation.
3. Perioperative and postoperative use of antibiotics.

Consequence

Currently, in many places such as China, maxillofacial defects caused by car accidents, tumors, and trauma, are very common problems. When the relevant literature was reviewed, no research mentioning multiple-times inoculation of seed cells into scaffolds was found. In this article, our hypothesis aims to fill this gap and pave the way for future studies in bone tissue engineering. If the hypothesis is proved to be effective, the outcome of the proposed study will improve the efficiency of cell seeding, increase the number of pre-seeding cells, and eventually enhance the re-growth of jawbone tissue.

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