

**Clinical and Biomimetic Strategies for
Dental Implantation in Insufficient
Bone Volume Conditions**

Yuanyuan Sun

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VRIJE UNIVERSITEIT

Clinical and Biomimetic Strategies for Dental Implantation in Insufficient Bone Volume Conditions

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CHAPTER 1

General Introduction



GENERAL INTRODUCTION

1. Dental implants and surrounding bone volume

Dental implants are medical devices surgically inserted into the jawbone, supporting and anchoring fixed or removable dental prostheses with enhanced stability and retention ¹. Their mounting applications for replacing missing teeth are attributed to their ability to provide natural-looking and long-lasting results ². "Osseointegration," the underlying biological principle of dental implants first introduced by Professor Per-Ingvar Brånemark in 1969 ^{3,4}, is significantly influenced by the volume of the surrounding bone of the implant surface.

In clinical practice, inadequate alveolar bone, resulting in compromised osseointegration, poses significant challenges to dental implant-supported repairs. Several factors can contribute to alveolar bone defects. Various genetic disorders are associated with severe alveolar bone atrophy ^{5,6}. Among these, Ectodermal Dysplasia (ED) ⁷ is the most prevalent condition, characterized by insufficient alveolar bone volume and missing teeth (Fig. 1). Moreover, anatomical variations, such as an enlarged mandibular nerve canal (Fig. 2) and expanded maxillary sinus (Fig. 3), also limit the available bone volume for promising osseointegration ⁸. Furthermore, some pathological conditions, including odontogenic cysts, tumors (Fig. 4), apical periodontitis, periodontal disease, and peri-implantitis, can accelerate alveolar bone resorption, ⁹. These pathological conditions present additional challenges during dental implant placement surgical procedures, potentially compromising the success of dental implant therapy.

Besides, after tooth extraction, the alveolar bone undergoes a series of physiologic remodeling processes that may significantly reduce its dimensions ¹⁰. A deficiency of alveolar bone volume can be classified as horizontal, vertical, or a combination defect ¹¹. Inadequate bone volume can reduce bone-to-impact contact and decrease primary stability, leading to poor osseointegration in the initial healing phase of dental implants ¹².

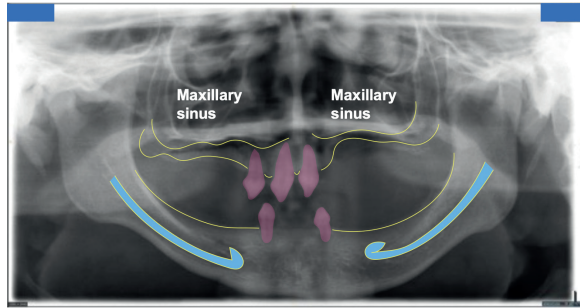


Figure 1. A digital panoramic radiograph of an ectodermal dysplasia syndrome patient with insufficient alveolar bone in the maxilla and mandible due to congenitally missing teeth.
Note: The original digital panoramic radiograph was from our clinic (unpublished data).

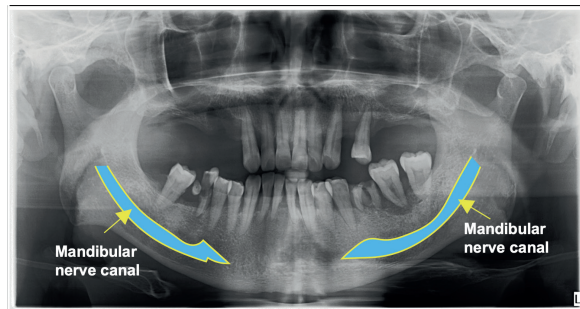


Figure 2. A digital panoramic radiograph of a 60-year-old patient with bilaterally enlarged mandibular nerve canals and severe alveolar bone defects in the mandible.
Note: The original digital panoramic radiograph was from our clinic (unpublished data).

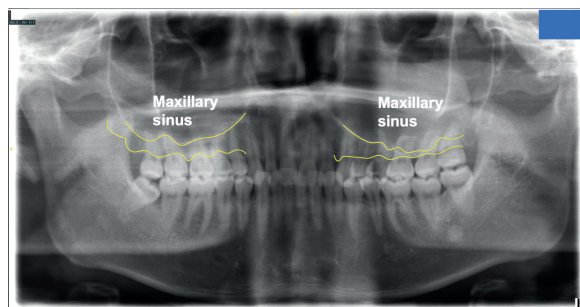


Figure 3. A digital panoramic radiograph of a 23-year-old patient with bilaterally enlarged maxillary sinus and severe alveolar bone defects in the maxilla.
Note: The original digital panoramic radiograph was from our clinic (unpublished data).

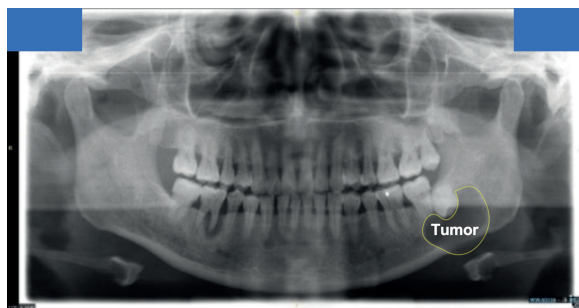


Figure 4. A digital panoramic radiograph of a patient with an alveolar bone defect caused by a tumor in the left mandible.

Note: The original digital panoramic radiograph was from our clinic (unpublished data).

2. Therapeutic approaches for dental implants in alveolar bone defect: strategies and considerations

Two primary clinical approaches for the placement of dental implants in the alveolar bone defect are applied: (A) implants placed simultaneously or following bone augmentation and (B) the utilization of the existing bone and graft-less approaches ^{13,14}.

Implants placed simultaneously or following bone augmentation

Bone grafting

Bone grafting has been widely used in clinical practice to repair bone defects that cannot heal spontaneously ¹⁵. This procedure involves the application of bone graft substitutes and barrier membranes, each of which plays a distinct role ^{15,16}. In detail, bone graft materials serve as fillers and scaffolds to promote cell adhesion, migration, and proliferation, which is essential for osteogenesis and osteoinduction¹⁷. Barrier membranes are concurrently used to provide space for bone regeneration and prevent the ingrowth of soft tissues during regenerative processes ¹⁸.

Ridge preservation

Rapid alveolar bone resorption occurs in the first 3 months following tooth extraction due to the absence of root support and a lack of nutrition from the periodontal ligament (Fig. 5A-B) ¹⁹. Usually, after tooth extraction, the alveolar ridge resorption is 11–22% in the vertical dimension at 6 months and 32% in the horizontal dimension at 3 months ²⁰. Ridge preservation can mitigate this resorption and maintains the alveolar bone volume after tooth extraction (Fig. 5C) ²¹. Ridge preservation ensures adequate bone volume around the dental implant (Fig. 5D), thus reducing the need for bone augmentation or Maxillary sinus floor elevation surgery ²².

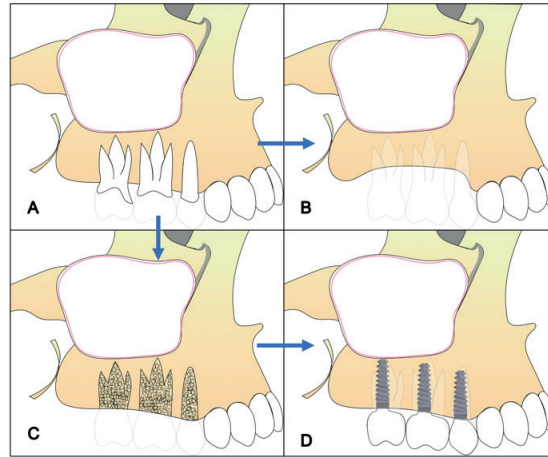


Figure 5. Schematic diagrams of the role of ridge preservation in retaining alveolar bone volume. A and B. Rapid alveolar bone resorption occurs in the first 3 months following tooth extraction due to the absence of root support and nutrition from the periodontal ligament. C and D. Ridge preservation can minimize the loss of ridge volume after tooth extraction. Ridge preservation ensures adequate bone volume around the implants.

Note: The schematic diagrams were drawn by Yuanyuan Sun (unpublished data).

Guided bone regeneration

Guided bone regeneration (GBR) typically involves using bone graft materials along with barrier membranes to address minor bone defects around dental implants, or combining them with autogenous bone to reconstruct larger bone defects.²⁴ The PASS principle (P: primary closure; A: angiogenesis; S: space maintenance; S: stability) is essential for successful GBR²⁵. A closed wound without tension and prevention of wound dehiscence critically impacts the success rate of GBR²⁶. Angiogenesis can promote new blood vessel formation from the pre-existing vascular network in the adjacent soft and suprapariosteal tissues and connect the bone-grafting material and adjacent bone marrow²⁷. This new blood vascular network can provide the nutritional elements, oxygen, immune system cells, mesenchymal stem cells, and growth factors required for bone regeneration²⁸. Tenting screws and barrier membranes play critical roles in space maintenance and stability during GBR²⁹. Another essential role of barrier membranes is tissue selectivity, which can prevent the soft tissue from growing into bone-grafting areas and influence bone regeneration³⁰. In the stable bone regeneration space, osteoconductive bone-grafting materials can provide structures or scaffolds that closely interface with the adjacent bone and promote osteoblast adhesion, proliferation, and formation of bone extracellular matrix³¹. Two results are obtained from a successful GBR procedure: high quality of bone and sufficient bone volume around the implant. Vertical bone augmentation is more complicated than horizontal bone

augmentation³². The treatment's effect is unpredictable due to limited osteogenic cell availability, taking a long time for vascularization, challenges in achieving primary closure, and an increased risk of wound dehiscence^{33,34}.

Maxillary sinus floor elevation

Maxillary sinus floor elevation, first described by Boyne and James in 1980³⁵, involves filling bone substitutes between the Schneiderian membrane and maxillary sinus floor to create sufficient bone volume for subsequent dental implant insertion (Fig.6). Various bone substitutes have been used for Maxillary sinus floor elevation, including autografts, allogeneic bone grafts, xenografts, and composite grafting materials, i.e., xenograft or synthetic biomaterials mixed with autogenous bone grafts³⁶. Comparing these filled materials, histomorphometric outcomes indicate that autografts outweigh their counterparts in new bone formation after maxillary sinus floor elevation³⁷. Although maxillary sinus floor elevation is one of the most studied augmentation procedures, and dental implant shows high survival rates inserted in the posterior maxilla following maxillary sinus floor elevation³⁸, this surgery approach is limited due to post-surgical complications, high cost, and complex procedures³⁹.

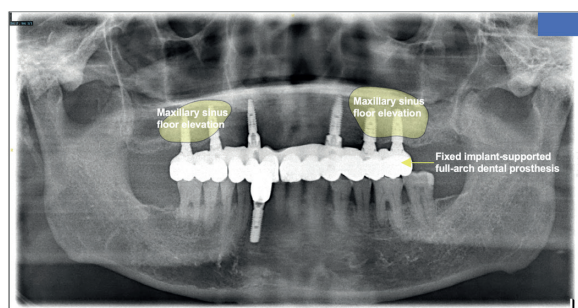


Figure 6. A digital panoramic radiograph of fixed implant-supported full arch restoration addressing a severe alveolar bone defect six months following maxillary sinus floor elevation.

Note: The original digital panoramic radiograph was from our clinic (unpublished data).

Bone graft biomaterials

Calcium phosphate bone substitutes have been widely used because they have proven effective in promoting bone regeneration⁴⁰. An ideal bone graft materials are expected to possess such characteristics: osteoconductivity, osteoinductivity, and osteogenesis⁴¹. At the graft site, bone can grow onto the surface or into the scaffold structure, and immature cells are recruited and transformed into preosteoblasts⁴². This ultimately leads to the formation of osteoblasts, which are crucial for bone regeneration and osteogenesis¹⁸.

Autogenous bone graft

Autogenous bone is considered the "gold standard" bone graft material due to its exceptional properties ⁴³. The osteogenic cells in the autogenous bone differentiate into osteoblasts when grafted onto a bone defect area ⁴⁴. Moreover, growth factors such as bone morphogenetic proteins (BMPs), platelet-derived growth factors, and transforming growth factor-beta (TGF- β) present in the autogenous bone can stimulate immature cell differentiation, proliferation, and matrix formation ⁴⁴. The autogenous bone also acts as a scaffold, promoting cell adhesion, migration, and proliferation ⁴⁴. This highly biocompatible graft material reduces the risk of immune rejection or adverse reactions, allowing for successful integration and bone healing ⁴⁵. However, some drawbacks, such as intraoral source availability, higher costs, pain associated with the procedure, and faster resorption, cannot be ignored. This restricts its widespread use for bone augmentation ⁴⁶.

Allogenic and xenogenous bone grafts

Allogenic and xenogenous bone grafts have also been used in implant dentistry and maxillofacial surgeries ⁴⁷. However, these products have limitations, including religious restrictions, risk of immunoreactions, and potential transmission of bacterial and viral diseases ⁴⁸. Allogenic bone grafts exhibit poor osteoinductive properties ⁴⁹, rendering them unsuitable for repairing severe bone defects ⁵⁰. Xenogenous bone grafts, such as deproteinized bovine bone (DBB) granules, exhibit a slower degradation rate after implantation into the alveolar bone defect area ⁵¹. Although it is beneficial for obtaining sufficient bone volume around the implant, bone regeneration takes a long time after bone grafting surgery ⁵².

Synthetic calcium phosphate substitutes

Synthetic calcium phosphate substitutes, such as hydroxyapatite (HA), β -tricalcium phosphate (β -TCP), biphasic calcium phosphate (BCP), and calcium phosphate cements (CPCs), have been extensively developed and utilized in implant dentistry over recent decades ⁵³. Compared to the autologous bone, synthetic calcium phosphate substitutes offer several advantages, such as ease of acquisition, avoidance of cross-infection concerns, and absence of religious restrictions ⁵⁴. HA, which has a structure and chemical composition similar to natural bone, has been employed in clinics since 1950s ⁵⁵. However, HA has certain limitations, including fragility and a slow degradation rate ^{56–58}. β -TCP, categorized as a bioceramic material, is frequently utilized in various medical and dental applications ⁵⁹. It closely resembles the natural bone in terms of chemical composition, mechanical properties, and resorption mechanisms, which are mediated by osteoclasts ⁵⁸. BCP, HA and β -TCP mixture at varied ratios, optimizes material properties for bone formation, such as resorption rate and mechanical strength ⁶⁰. A clinical study compared the stability of horizontal alveolar bone augmentation using different ratios of HA/ β -TCP (60/40 or 70/30); the 70/30 ratio significantly outperformed in maintaining facial thickness and preserving

stable horizontal dimensions of the augmented site⁶¹. The BCP has also demonstrated success in alveolar ridge reconstruction during maxillary sinus bone augmentation in humans⁶². CPCs, with advantageous properties, such as bioactivity, osteoconductivity, injectability, and moldability, have been promising delivery biomaterials for stem cells, drugs, and growth factors since their discovery in the 1980s⁶³. At body temperature, the dissolution-precipitation reaction is a unique characteristic of CPC, which can be injected into the bone defect site with minimally invasive application⁶³.

Although synthetic calcium phosphate substitutes have shown promise in clinical applications, the lack of osteoinductive properties limits their use in cases of severe bone defects⁶⁴. Hence, compared with autografts, these synthetic calcium phosphate substitutes have to be modified by combining osteoinductive cytokines to induce the differentiation of mesenchymal stem cells (MSCs) into preosteoblasts, ultimately increasing the potential for new bone formation.

Bone morphogenetic protein 2

Bone morphogenetic protein 2 (BMP-2), a member of the TGF- β superfamily, is a pivotal bone growth factor⁶⁵. This signaling molecule plays a critical role in bone formation and regeneration by inducing osteogenic differentiation of MSCs, transforming them into osteoblasts^{66,67}. These cells are responsible for bone matrix synthesis and subsequent bone formation⁶⁸. BMP-2 directly enhances osteoblast activity and promotes the production of critical proteins essential for bone tissue synthesis and mineralization, including type I collagen, alkaline phosphatase, and osteocalcin⁶⁹. Recombinant human bone morphogenetic protein 2 (rhBMP-2) is a synthetically created BMP-2 produced through genetic engineering techniques⁷⁰. It has been approved by the Food and Drug Administration (FDA) for bone regeneration and has been used in maxillary sinus augmentation and localized alveolar ridge augmentation⁴⁴. Because of its water-soluble property and relatively small molecular weight, rhBMP-2 can readily diffuse in bodily fluids⁷¹. Without a carrier, rhBMP typically remains effective at the intended bone healing site for only a brief period, often just a few hours⁷². Hence, the delivered carrier ensures that rhBMP-2 is released, localized, and sustained at the target site. Various carriers, including collagen sponges, calcium phosphate materials (HA and β -TCP), and synthetic polymers, have been employed for this purpose⁷³. The conventional approach involves the administration of rhBMP-2 molecules directly adsorbed by an FDA-approved carrier at a concentration of 1.5 mg/mL. However, this method has raised concerns owing to potential side effects, such as inflammation, radiculopathy, ectopic bone formation, activation of osteoclasts, bone loss, subsidence, urogenital complications, and wound-related problems, which are associated with high BMP-2 doses^{74,75}. Considering these concerns, there is an urgent need to limit the amount of BMP-2 used or create BMP-2 slow-release vehicle delivery systems that can achieve lower doses of rhBMP-2 release sustainably, locally, and stably. Such innovations can enhance safety and efficacy in clinical applications to promote bone formation.

Biomimetic calcium phosphate coatings as a slow delivery release system for Bone morphogenetic protein 2

Our research primarily focused on developing a novel drug delivery slowly release system, in which biomimetic calcium phosphate (BioCaP) coatings incorporated with biological agents such as BMP-2⁷⁶. This coating efficiently and uniformly accommodates low-dose BMP-2⁷⁷. The unique characteristic of this system is the gradual, cell-mediated degradation of the BioCaP coating, which results in the slow and consistent release of rhBMP-2⁷⁸. This mechanism effectively replicates the natural process of bone remodeling and mitigates the undesirable burst release of BMP-2 at high concentrations^{79–81}.

Deproteinized bovine bone granules coated with biomimetic calcium phosphate and bone morphogenetic protein 2

In previous studies, a sustained-release delivery system (*biomimetic calcium phosphate* coating incorporated with *bone morphogenetic protein 2*) was used to treat various bone defects *in vivo*. This BMP-2 functionalized DBB was used to repair critical-sized bone defects in sheep^{82,83} and test periodontal regeneration in a canine model of chronic periodontitis⁸⁴. Furthermore, DBB blocks (10 × 6 × 4 mm³) were biomimetically coated with BioCaP and BMP-2 and used to enhance vertical alveolar ridge augmentation with simultaneous implant placement in a canine model⁸⁵.

β-tricalcium phosphate coated with biomimetic calcium phosphate and bone morphogenetic protein 2

In the beginning of the year 2020, we introduced β-tricalcium phosphate as the foundational element in the bone morphogenetic protein 2 delivery system⁸⁶. This innovative composite material, denoted as BMP-2/BioCaP/β-TCP, was produced by Shanghai Rebone Biomaterials Co., Ltd. This production followed the ISO 13485 quality standards. A qualified organization, Weihai Desheng Technology Testing Co., Ltd., conducted a series of preclinical evaluations of the bone substitutes. These assessments encompassed cytotoxicity, sensitization, intracutaneous reactivity, pyrogenicity, acute systemic toxicity, chromosomal aberration, and bacterial reverse mutation testing, all of which were meticulously performed following rigorous ISO 10993 standards. To ascertain the optimal concentration of *Escherichia coli*-derived recombinant human bone morphogenetic protein 2 (ErhBMP-2), experiments were conducted utilizing a rat model with critically-sized bone defects (diameter of 8 mm). These investigations revealed that the ideal concentration of ErhBMP-2 for this application should fall within the range of 150–300 µg/g of BioCaP/β-TCP in this model⁸⁶. Based on preclinical studies, the efficacy and safety of this novel bone substitute (rhBMP-2/BioCaP/β-TCP) were examined for the first time in a human tooth-extraction-socket-healing model by Shanghai 9th People's Hospital. The results showed that although the new bone volume density in the rhBMP-2/BioCaP/β-TCP grafting material after ridge preservation was similar to that in the natural healing group 6 weeks later, the bone volume

maintenance in the rhBMP-2/BioCaP/ β -TCP group was better than that in the latter.

Calcium phosphate cements coated with biomimetic calcium phosphate and bone morphogenetic protein 2

Recently, the delivery of bone morphogenetic protein 2 using calcium phosphate cements as a vehicle system has been commercialized. The BMP-2 molecules were adsorbed at a concentration of 1088 $\mu\text{g/g}$ in this production. Our research led to the development of a novel BMP-2 sustained-release delivery system coated on the surface of CPC with a BMP-2 concentration of 239 $\mu\text{g/g}$ (**Chapter 5**). This system was applied to dogs' model with severe horizontal alveolar bone defects. Through this experiment, we aimed to investigate whether the rhBMP-2/BioCaP/CPC composite demonstrated comparable efficacy in large lateral bone augmentation with a lower BMP-2 dosage compared to higher BMP-2 dosage adsorbed on CPC. The experimental results indicated that whether BMP-2 was incorporated into CPC or adsorbed onto CPC, bone volume maintenance in these two groups was superior to that in the CPC and autologous bone groups.

Utilization of the existing bone and graft-less procedures

Various types of implants have been utilized to support prostheses, aiming to minimize treatment time and frequency for patients with a severely atrophic maxilla. This strategy leverages the existing bone volume while eliminating the need for bone grafting procedures. These include short implants, narrow-diameter implants, mini-dental implants, tilted implants, and extra-alveolar implants, such as zygomatic and pterygoid implants^{38,87–90}. These methods strive to optimize the use of available bone structures for effective prosthetic support.

Zygomatic and pterygoid implants

Two types of extra-alveolar implants, zygomatic and pterygoid implants, as alternative approaches to extensive bone augmentation combined with conventional implants, were positioned at specific angles to bypass the maxillary sinus (pterygoid implants) or traverse the sinus (zygomatic implants) and leverage the inherent characteristics of facial bone tissue^{91,92}. Remarkable bone densities in the zygomatic and pterygoid regions were classified as type I, in which almost the entire bone was composed of homogeneous compact bone, according to the Lekholm and Zarb classification⁹³. Consequently, zygomatic and pterygoid implants in these areas can provide exceptional stability and effectively support prostheses combined with conventional implants with or without bone grafting procedures^{94,95} (Fig. 7), which can decrease the need for maxillary sinus augmentation procedures in the posterior atrophic maxilla⁹⁶. Furthermore, the pterygoid implant placement surgery can be performed under local anesthesia.

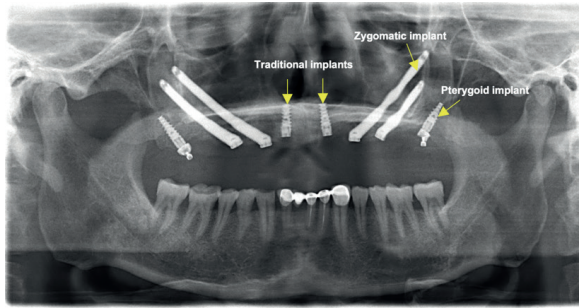


Figure 7. A digital panoramic radiograph of a patient treated with four zygomatic, two pterygoid, and two traditional dental implants.

Note: The original digital panoramic radiograph was from our clinic (unpublished data).

The pterygoid implant placement surgical technique involves traversing three distinct anatomical structures, maxillary tuberosity, pyramidal process of the palatine bone, and anchoring into the pterygoid process ⁹⁷. Thus, a high success rate of pterygoid implants hinges on several critical factors, including precise three-dimensional positioning, availability of high-quality and adequate bone surrounding the pterygoid implant, and the surgeon's extensive experience ⁹⁸.

Tilted implants

Tilted implant treatment is another strategy that is inserted into the posterior alveolar region and combined with axial implants in the anterior alveolar region for implant-supported restorations ⁹⁹ (Fig. 8). The tilted implant can avoid crucial anatomical structures, such as the mandibular canal, maxillary sinus, enlarged blood vessels, and alveolar bone defect area, and is embedded in the sufficient surrounding bone ¹⁰⁰. Compared with axial implants, there was not enough evidence to claim a higher incidence of biomechanic complications and more marginal bone loss in tilted implants ¹⁰¹.

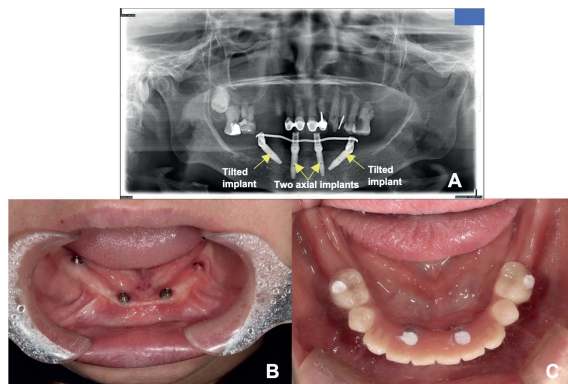


Figure 8. Four dental implants-supported restoration in a patient with a severe posterior mandibular bone defect. A. Digital panoramic radiograph showing the placement of four dental implants in the mandible, consisting of two tilted and two axial implants. The tilted implants are angled approximately 30–45 degrees relative to the occlusal plane. All implants are positioned anterior to the mental nerve in the mandible. **B.** Intraoral photograph showing the four implant abutments three months postoperatively. **C.** Intraoral photograph of a full-arch temporary denture supported by four implants.

Note: The original digital panoramic radiograph and intraoral photographs were from our clinic (unpublished data).

proliferation of stem and progenitor cells¹⁸⁷. As PRF is easy to handle¹⁸¹ in implant dentistry, PRF is usually used as a membrane or gel (Fig. 5)¹⁸⁸ or combined with deproteinized bovine bone mineral (DBBM)^{189–191}. In addition, a few studies have verified that GBR with PRF and CaP ceramics reduced the bone healing period and accelerated wound healing in bone defects^{192–195}. However, research suggested although PRF was superior to β -TCP in bone mineralization, it was inferior to maintaining the bucco-lingual volume¹⁹⁶.

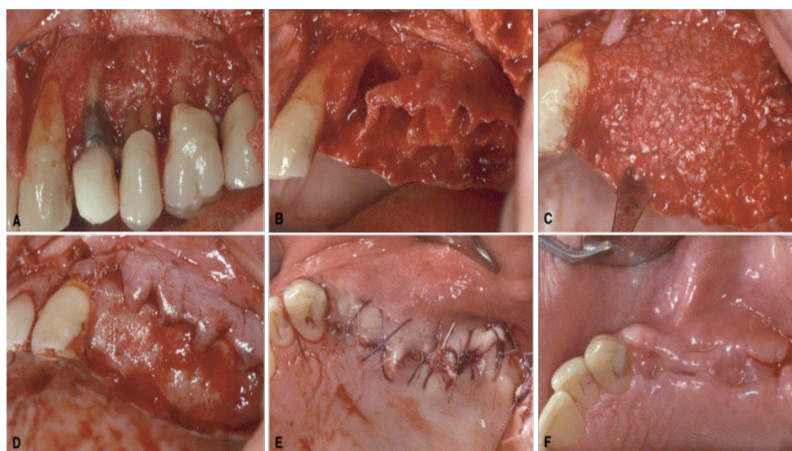


Figure 5. Clinical application of PRF as cover membranes in implant dentistry. **A**, Preoperative view. Periodontitis-caused bone defects; **B**, Teeth extraction. Impaired alveolar socket walls; **C**, Bone substitute filling; **D**, The use of PRF as cover membranes; **E**, A rapid epithelialization of the surface of the extraction sites; **F**, 48 Hour postoperatively. The wound was totally closed. (Reproduced from Choukroun et al.¹⁸⁸)

Compared to PRP and PRF, CGF is produced by centrifuging blood samples using alternating speeds. After centrifugation, blood is also separated into three layers: PPP, CGF, and RBC¹⁹⁷ (Fig. 4). Alternating rates lead to more GFs production in CGF than PRP and PRF^{198,199}. Moreover, CD 34⁺ stem cells that play a critical role in vascular maintenance, neovascularisation, and angiogenesis have only been discovered in CGF²⁰⁰. Hence, CGF demonstrates a potent effect on angiogenesis. The administration of CGF in implant dentistry is increasing, as it is reported that CGF can decrease symptoms and discomfort after GBR surgery²⁰¹.

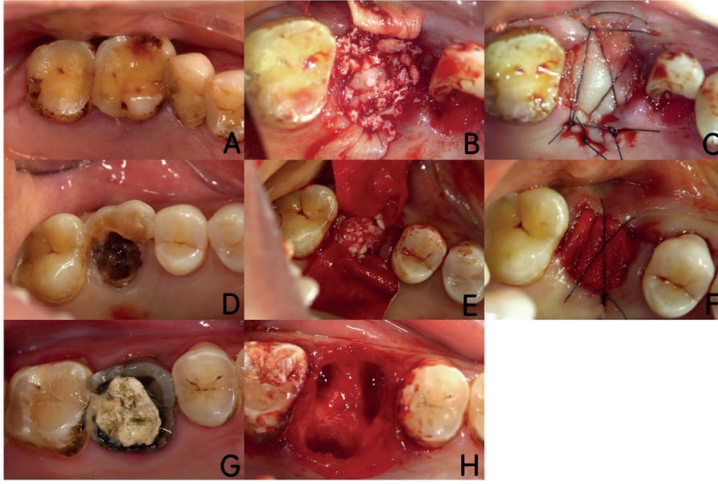


Figure 6. Clinical application of CGF for alveolar ridge preservation. **A**, Preoperative image for CGF/DBBM group; **B**, CGF/DBBM mixture implanted into the tooth socket; **C**, Covered with CGF membranes and sutured; **D**, Preoperative image for DBBM group; **E**, DBBM implanted into the tooth socket; **F**, Covered with Bio-Gide membranes and sutured; **G**, Preoperative image for control group; **H**, Nothing implanted (Reproduce from Lin et al.²⁰²). DBBM, deproteinized bovine bone mineral.

CGF's efficiency in bone regeneration has also been reported. A "sandwich" containing CGF, titanium mesh, and CaP materials was tested to repair severe alveolar bone defects. This sandwich simultaneously demonstrated outstanding potential for bone and soft tissue augmentation, which can significantly reduce dental implants loss and achieve a satisfactory esthetic outcome^{203,204}. In addition, CGF combined with DBBM was also used for alveolar ridge preservation and significantly promoted bone formation (Fig. 6)²⁰⁵. However, in another clinical study, the CGF membrane combined with CaP bone substitutes did not promote bone formation in contrast to bone substitutes²⁰⁶. Thus, the efficiency of CGF and its combination with CaP biomaterials need a more systematic assessment.

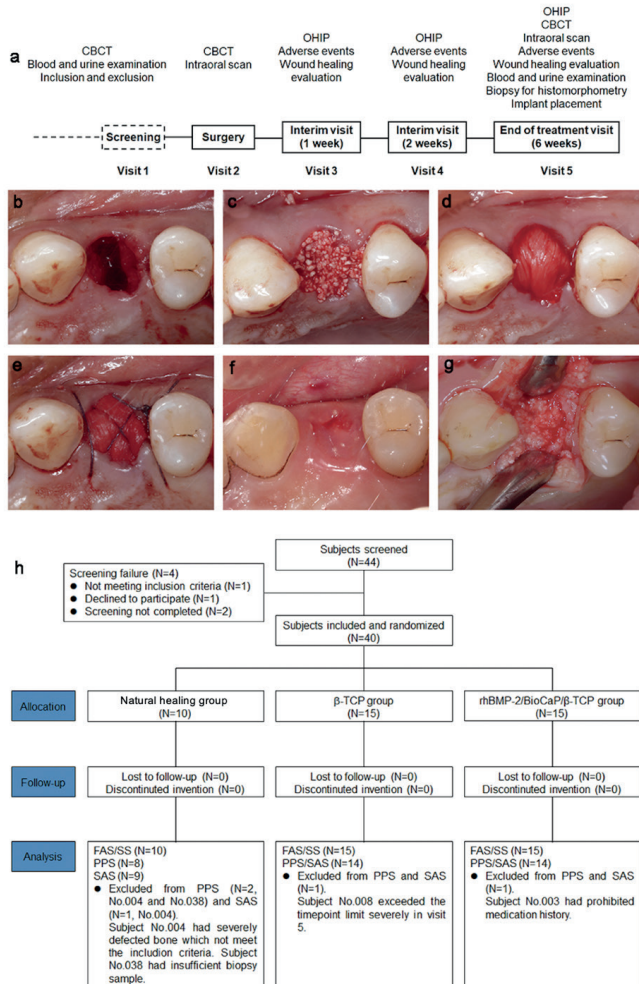


Figure 2. Five visits in trial procedures were planned and carried out (a). Representative intra-oral photographs of the standardized procedures of socket preservation surgery are shown in b-e (b, tooth extraction; c, bone substitute filling; d, membrane covering; e, suturing). The soft and hard tissue healing six weeks after surgery are shown in f & g, respectively. The CONSORT flow chart of the trial is shown in h.

2.7 Measurements of efficacy and safety outcomes

The primary outcome of the efficacy analysis was new bone volume density in the biopsy sample six weeks after surgery. The secondary outcomes included (1) the unmineralized tissue volume density in the biopsy sample, (2) the residual material volume density in the biopsy site, (3) the bone width and height changes measured by CBCT scans, (4) the soft tissue surface sectional area and width changes measured by intra-oral scans, and (5) the number of bone augmentation procedure required during dental implant placed. The safety outcomes contained (1) the soft tissue

- Patients who have an allergy to the investigational product.
- Female patients who are pregnant or nursing or refuse to take any contraception.
- Patients whose compliance could be improved by the investigator.
- Patients who have participated in or are participating in clinical trials of other medical devices or drugs within the 30 days before Day 0.

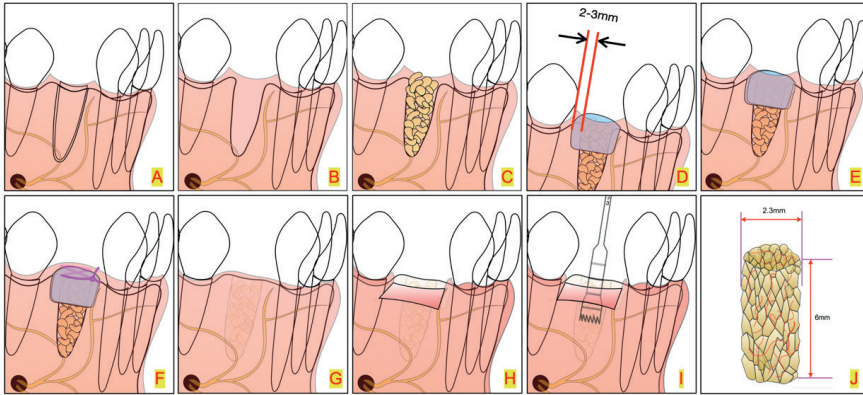


Figure 1. Schematic of clinical trial procedure. (A) The patients were randomly divided into rhBMP-2/BioCaP/ β -TCP, β -TCP, and natural healing groups; (B) tooth extraction, (C) the tooth socket filled with either rhBMP-2/BioCaP/ β -TCP or β -TCP in relative groups, (D) the first layer of collagen membrane covered the alveolar sockets and 2-3mm over the socket edge), (E) the second collagen membranes, (F) sutured, (G) two weeks later, took out the suture, (H) After six weeks, soft tissue flap releasing, (I) a trephine drill (outer diameter 3 mm, inner 2.3 mm diameter) is used to obtain the biopsy, (J) collected biopsy (2.3mm in diameter $\times$ 6mm in height).



Figure 2. Dental implant surgery and biopsy collection. (A) Trephine drill with biopsy. (B) implant site preparation. (C) implant insertion