Alveolar Ridge Split And Augmentation Using Recombinant Human Bone Morphogenic Protein (rh-BMP-2) And Inorganic Xenograft

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Abstract

Background: The initial stage of restoration of missing teeth and, accordingly, their functional or aesthetic value, is dental implantation, which requires adequate volume (at least 10mm height and 6 mm – width) and quality of surrounding bone tissue. In case of its deficiency, different methods of bone augmentation are used, among which is the splitting of the alveolar ridge with simultaneous dental implant placement.

Aim: The aim of the studies is to improve the result of augmentation during bone splitting, with osteoconductive xenograft by using a bone morphogenic protein of an osteoinductive nature, during which bone formation occurs faster, with the formation of a full-fledged structure.

Methods: When the bony ridge is split with a piezoelectric instrument, it is divided into buccal and lingual (palate) plates. Its indication is the presence of a narrow alveolar ridge (less than 6 mm, but not less than 3 mm). An implant is placed between these two plates simultaneously with a bone graft material. The graft with its osteoconductive properties, represented by an inorganic components - hydroxyapatite and tricalcium phosphate, is enriched with recombinant human bone morphogenic protein (rhBMP-2), giving the graft an osteoinductive character, i.e. Stimulates osteogenesis and accelerates bone consolidation. RhBMP is obtained by modifying the transfected gene of the Escherichia Coli.

Results: Granular graft together with bone morphogenic protein ensures full-fledged bone formation by maintaining own buccal and lingual (palatal) bone plates, which is the best condition for dental implant integration. Bone morphogenic protein is not only a growth factor of bone cells, but also of blood vessels. After this procedure, quantitatively and qualitatively better bone is obtained. The combination of rh-BMP and mineral graft accelerates the process of bone formation and maturation and establishes a normal bone structure.

Conclusions: In case of small horizontal (bucco-lingual) dimension of the bone, splitting the alveolar ridge is the best method, which provides the maximum amount of bone around the implant. At the same time, the filling of the bone-free space with osteoiductive and osteoconductive bone substitution material leads to quantitatively and qualitatively perfect bone formation in the shortest possible time. (**TCM-GMJ December 2024; 9 (2): P66-P73)**

Key words: Alveolar ridge splitting, bone augmentation, Bone Morphogenic Protein, osteoinduction, osteoconduction, piezosurgery

Introduction

The best way to rehabilitate partial or complete edentulism is dental implantation with orthopedic constructions placed on it. A necessary condition for achieving a perfect and successful result with this method of treatment is the presence of quantitatively and qualitatively perfect bone tissue around the implant, both in vertical and horizontal dimensions, because implantation is based on such a phenomenon as osteointegration, during which bone grows on the surface of the implant, and is biologically connected to it. Clinical studies have shown (established) that at least 1-1.5 mm of bone must be present on the buccal and palatal (lingual) sides to ensure long-term bone coverage of the implant (1, 2)

As a result of tooth extraction, trauma, periodontal disease or congenital defect, the alveolar bone undergoes changes, in particular, it decreases in size – in height and width (3). Therefore, the condition of the toothless alveo-

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lar ridge in some cases may be unfavorable for implant placement (4). Most bone loss occurs in the horizontal dimension, mainly on the buccal (facial) side of the ridge (5, 6

This resorptive process results in narrowing of the ridge predominantly on the buccal side and it is shifted to the lingual (palatal) position (7).

Residual ridge size decreases most rapidly in the first 6 months, when approximately 0.90–3.6 mm of buccal bone and 0.4–3.0 mm of lingual bone are resorbed, although resorptive activity continues at a slower rate throughout life, leading to a reduction in jaw bone size (8,9,10).

The narrowing of the alveolar ridge width reduces the ability of the bone to support the implant, especially in the buccal and lingual bone plates. That is why the implantation of the implant in a narrow ridge is carried out using a special surgical procedure called the technique of splitting and dividing the alveolar ridge, with its further augmentation. The method, first introduced by Tatu in 1986 (11) and subsequently re-introduced by Scipione in 1990 (12, 13), involves splitting the bony ridge into cheek and tongue plates, then widening it with special osteotomes, after which an implant is placed in the intraosseous space by the "sandwich" principle. In 1994, Sommers (14) described this technique, which is based on the viscoelastic properties of bone, applying pressure to the buccal and lingual plate with osteotomes. The effectiveness of splitting and expanding the ridge has been confirmed by a number of clinical and histological studies ().

The use of osteotomy makes the bone surrounding the implant more compact without losing it (15). This technique has been refined using different instruments and tools such as chiesels, osteotomes and piezoelectric devices. Today, a bone replacement substance is inserted between the bone plates and covered with a resorbable collagen membrane.

The advantage of the ridge splitting technique compared to other augmentation methods is the absence of the need for bone donor site, low risk of graft and membrane exposure, short treatment time due to the simultaneous use of the implant and augmentation (16, 17, 18, 19, 20, 21). However, this method is not universal for treating all types of bone deficiency. The minimum bone width required for bone splitting is 3 mm, with at least 1-1,5 mm of cancellous bone between the 2 cortical plates, which maintains a normal blood supply to the split bone (22, 23, 24)(**Fig.1**).

The maxillary bone is pliable, it can be easily manipulated to improve its quality (compaction and corticalization) and expanded to the desired width [15]. The success of this technique depends on maintaining the integrity of the labial (cheek) bone. The periosteum, due to its elasticity, allows the expansion and manipulation of the bone, acts as a barrier membrane, ensures the healing of bone microfractures due to the unaltered (undamaged) blood circulation. Due to the preservation of periosteum integrity, the splitting of the alveolar ridge may be considered as the so-called fracture of greenstick of the buccal bone. This technique is more easily performed on the upper jaw compared to the lower jaw, because the upper jaw bone is more porous, mainly represented by D2, D3 and D4 bones, which are relatively easy to manipulate. However, the mandible is mostly represented by D1 and D2 density bone, which involves more difficulty in manipulating it, due to the thick cortical bone. A number of authors have discussed different splitting techniques [25,26], in which the ridge osteotomy is combined with an adjacent vertical osteotomy, thereby achieving a "green stick fracture" of the buccal plate. After widening the space between the buccal and lingual bony plates by more than 2 mm, a bone graft material is inserted (two-stage treatment) [27] and an implant is placed together with the graft (one-stage treatment).

Bony Augmentation. The issue of filling the space surrounding the implant and between the bone plates after splitting deserves special attention. Traditionally, auto-allo- or xenograft is used, during which the graft plays the role of a biological scaffold, represented by biphasic calcium phosphate, which is the mixture of hydroxyapatite (HA) and β-tricalcium phosphate (36, 37, 38).

In recent years, there is an increasing demand to use osteogenesis stimulating agents together with bone graft or as an alternative to it. Natural signaling proteins play an important role in embryogenesis, and organogenesis. Such a factor in the body is bone morphogenic protein, which (BMP) is a member of the transforming growth factor (TGF-b) superfamily, participates in the stem cell proliferation and differentiation, regulates bone balance by controlling the differentiation of osteoblasts and osteoclasts (39-41).

Nowadays, approximately 20 members of the BMP family have been identified. BMP is a dimeric molecule consisting of two polypeptide chains linked by a single disulfide bond. Based on the structural similarity of BMP amino acid sequences, BMP family members are generally divided into four categories: BMP2/4; BMP5/6/7/8; BMP9/10; and BMP12/13/14. (42, 43) (Liu et al., 1995; Gomez-Puerto et al., 2019).

Notably, BMP-2 and BMP-7 can significantly increase osseointegration (44, 45) (Dent-Acosta et al., 2012; Dolanmaz et al., 2015). Therefore, the Food and Drug Administration (FDA) has approved the use of two factors containing recombinant human BMP (rhBMP)-2 and rhBMP-7 for the treatment of several orthopedic diseases such as open fractures, non-healing fractures, spine and maxillofacial bone defects (46) (Cecchi et al., 2016). The development of recombinant technology has made it possible to clone the DNA sequence (cDNA) and synthesize recombinant human BMP protein (rhBMP). This, in turn, created an opportunity for the production of a highly purified BMP protein preparation (47). Subsequently, a number of studies with rhBMP have demonstrated the potential of rhBMP-2 as a safe and effective alternative to autogenous bone grafts (48–52) based on its osteoinductive propety.

Based on a number of studies, recombinant human bone morphogenic protein-2 (rhBMP-2), which is obtained by transfected gene modification of the Escherichia Coli (ErhBMP-2), together with inorganic bone replacement

components have been recommended to use. It accelerates the ossification process by controlled proliferation and differentiation of osteoblasts from progenitor cells and promotes the biosynthesis of bone matrix. So, if the mineral components of the graft have an osteoconductive property, which implies the growth of natural bone on its surface, after the use of bone morphogenic protein, the graft acquires osteoinductive ability, i.e. It can stimulate osteogenesis, induces the proliferation of osteoblasts from mesenchymal stem cells (MSC). i.e. BMP is the factor responsible for osteoinduction. The primary osteoinduction mechanism of BMP-2 is the differentiation of mesenchymal stem cells (MSCs) into osteoblasts. By binding to specific receptors, BMP-2 activates signaling pathways and ultimately activates osteogenic genes to differentiate MSCs into osteoblasts (53). Differentiated osteoblasts form bone matrix and secondarily deposit calcium phosphate by secreting alkaline phosphatase for bone formation. Activated osteoblasts are embedded in the formed bone and act as osteocytes, which are responsible for the bone structure and its supporting and supporting ability (54).

Experiments using bone grafts containing rhBMP-2 revealed a better regenerative outcome compared to grafts containing only mineral components. Osteoconductive scaffold and osteoinductive protein fusion trials were performed. Osteoinductive protein such as recombinant human bone morphogenic protein-2 (rhBMP-2) induces the differentiation of mesenchymal stem cells and preosteoblasts into osteoblasts and promotes the migration of osteoblastic cells (55,56). In terms of interactions with the immune system, the risks posed by rhBMP-2 are low (57). In addition, the bone regeneration ability of rhBMP-2 is improved by carrier materials (58,59), which are readily available and easy to use. According to studies on heterogeneous bone graft as a carrier (transporter), the space was sufficiently provided and the graft proved to be an excellent carrier of osteoinductive proteins (59). The combined use of bone graft materials and rhBMP-2 promotes the regeneration of mature bone because rhBMP-2 has the potential to improve bone regeneration (60) (**Fig.2**). Thus, using rhBMP-2 overcomes the poor osteoinductive property of the mineralized graft (**Fig.3**).

The carrier of bone morphogenetic proteins (BMPs) should be a scaffold (framework) for bone-forming cells, and should also be biocompatible so that it can be replaced by newly formed bone without any adverse tissue reaction (61,62). Although an ideal carrier should be biodegradable, it should also be able to maintain its integrity over time to allow sufficient maturation of newly formed bone (63).

The ability to maintain space for BMP carrier is a critical factor in bone formation and maturation (64-66). Macroporous biphasic calcium phosphate (MBCP), which is a biphasic mixture of hydroxyapatite and β-tricalcium phosphate in a ratio of 60:40, is the carrier for BMP. Micropores (MBCP) on the surface may represent a site for precipitation of biological precipitate (67), and macropores - a site for binding BMP-2 (rhBMP 2) due to its high affinity for calcium phosphate. It provides space for new bone to mature (68).

Using different types of augmentation methods, different results were obtained (revealed) in a comparative study of the healing time of skull fractures: In the placebo group and in the case of using only the granular graft, 2 weeks after the operation, only the formation of connective tissue and a minimal amount of new bone tissue was observed, active bone formation occurred only from the 8th week (**Fig.4**). In the case of using recombinant bone morphogenic protein (rh-BMP) and biphasic calcium phosphate particle (CPP) or rh-BMP + biphasic calcium phosphate block graft (CPB), a large amount of new bone was formed after 2 weeks, and after 8 weeks, the quantitative formation of bone was actually completed and its maturation, and at the same time, even the formation of bone marrow took place (69).

It can be concluded that CPP and CPB together with ErhBMP-2 enhance and accelerate new bone formation, and CPP and CPB seem to be suitable carriers for rhBMP-2, which not only produce bone formation in a short time, but also it provides a structurally perfect tissue morphology.

Methods

Alveolar ridge splitting technique. After passing through the bony crest and the gingival sulcus the incision under local anesthesia, the complete mucoperiosteal flap is reflected. A horizontal osteotomy, carried out with a piezotome, divides the alveolar ridge into 2 parts: buccal and lingual. Two additional vertical osteotomies, carried out on the cheek bone plate, are connected to the horizontal osteotome (28, 29, 30, 31). This technique is particularly important in the mandible because the mandibular cortical plate is less elastic and prone to fracture. The bone of the upper jaw is more elastic than the lower jaw, and therefore more flexible, so the vertical osteotomy of the bone is not necessary in some cases. Afterwards, the space between the two plates is expanded by means of chisels and bone spreaders, and the implant socket is prepared with bone compressive and expanding instruments to the desired depth in the undivided (unsplit) part of the bone. The use of non-cutting bone exspanders of increasing diameter is appropriate for the gradual densification of cancellous bone without its removal. Dental implants are placed in the prepared space with optimal torque, after which the space between bone plates and implants is filled with bone substitute material; We used recombinant human bone morphogenic protein-2 (rhBMP-2), which is obtained by transfected gene modification of the Escherichia Coli (ErhBMP-2), together with inorganic bone replacement components. The graft and bone defect are covered with a collagen membrane. The soft tissue is closed by suture (32 -35).

Case 1: The patient, a 52-year-old woman. There are 14, 15 secondary edentulism. The teeth were extracted 7 years ago. Clinically, attention is drawn to the toothless defect in the area of teeth 14 and 15 (**Fig.5**); In order to restore the chewing function, a dental implant placement procedure

was planned. The conducted radiographic examination revealed an alveolar bone with a width of 3 mm in the part of the ridge, in its middle part – 3,4 mm, the height of the bone from the ridge to the bottom of the upper jaw cavity – 18,4 mm; Bone type according to density - D3 (**Fig.6**). The presence of a narrow alveolar ridge was established, which is a challenge for conventional dental implantation. Alveolar ridge splitting procedure with subsequent bony augmentation was planned.

After local buccal infiltration and great palatal nerve block anesthesia with 4% Articaine, midcrestal and sulcular incisions are made, the mucoperiosteal flap was reflected with a periosteal elevator to expose the bony ridge (**Fig.7,8**). A 4 mm deep horizontal cut of the bone (osteotomy incision) was made along the ridge with a piezoelectric osteotome (**Fig.9**). By avoiding pressure on the buccal plate and maintaining tactile digit support on the buccal plate to, it bisects the ridge crest and divides the cortical plates, the osteotomy preparation was directed to the palatal plate. A vertical release osteotomy was avoided because of the elasticity of the maxillary bone. Then, a thin tapered bur was used to deepen the initial osteotomy in the desired depth according to the implant length. With a special chisel, the distance between the buccal and palatal plates was widened provided periosteum remains intact, and with the use of bone screw spreaders, cancellous bone densification and the formation of the implant bed were performed without bone loss (**Fig.10, 11**). Two implants (Cowellmedi; Korea) size 3.5x12 mm were inserted into the implant site with an optimal twisting force (torque) of 35 N/cm (**Fig.12**). Bone morphogenic protein with rhBMP-2 xenograft () was applied between the buccal and palatal bone plates and between the implants to provide complete bone support for the implants (**Fig.13**). The graft was covered with a resorbable collagen membrane (**Fig.14**), and the wound was closed with a simple interrupted suture for a period of 4 months (**Fig.15**).

Case 2: A 45-year-old male patient with secondary edentulism of teeth 35, 36, 37. A narrow alveolar ridge covered with keratinized gingiva was probably noted by clinical examination (**Fig.16**), which was confirmed by cone-beam computed tomography (**Fig.17**). Alveolar ridge width – 4 mm. The distance from the bony ridge to the mandibular canal – 16 mm, bone density D2. Under local infiltration anesthesia (4% articaine) a mid-crestal and sulcular incisions were made on the buccal and lingual gingiva (**Fig.18**). Then the mucoperiosteal flap was reflected. A horizontal cut (osteotomy incision) was made on the crestal bone (osteotomy depth 5 mm) with 2 additional vertical bone incisions on the buccal plate (due to the thick cortical wall) with a piezoelectric device saw (**Fig.19, 20**). These 2 additional vertical cuts were created at the mesial and distal end of the horizontal incision. Osteotomes of increasing size were used for the progressive lateralization of the buccal plate and bone spreaders and expanders of increasing size for densification of canciolous bone (**Fig.21**). An undivided portion of bone was prepared with initial and pilot drills to the desired depth (12 mm) and then widened by the expanders to so as to expand the base of the bone in V shape and achieve primary implant stability. (The initial length of the osteotome was prepared approximately 3 mm deeper than the desired implant length, which was followed by insertion of successive larger diameter osteotome of 0.5 mm shorter than the preceding instrument, so as to expand the base of the bone in V shape). Two implants 4.0x10 mm and one implant 4,5x8 mm (Cowellmedi, Korea) were placed subcrestal (1,5 mm) in the prepared space (**Fig.22**). The space between the implants was filled with a mixture of recombinant human morpogenic protein rhBMP and mineral xenograft after placing the cover screw (**Fig.23**). The graft was covered with an absorbable collagen membrane (**Fig.24**). A simple interrupted suture was applied to the wound (**Fig.25**). The patient was recalled after four months of implant placement for healing abutment placement. The healed gingival collar around implant showed healthy peri-implant keratinized mucosa.

Results

The described technique of splitting the alveolar ridge together with augmentation with a mixture of bone morphogenic protein and xenograft is one of the best ways to rehabilitate a narrow alveolar ridge (>3 mm) with the method of dental implantation and prosthetic construction.

The midcrestal and sulcular incisions leave the periosteum intact which after the bone ridge splits and expansion creates the similar phenomenon as green stick fracture, avoiding bone sequestration.

The osteotomy incision carried out by a piezoelectric device on the bone ridge provides minimal bone loss, as the thickness of the piezo saw is 0.3-0.5 mm, and moreover, the piezosurgical device is based on controlled frequency ultrasound vibration, which ensures a precise incision (cut) on the bone.

Due to the fact that the implant is placed between the buccal and lingual (palatal) bony plates after splitting, it has bilateral natural bone support, the alveolar ridge is maintained by the buccal-lingual walls, forming for bone graft a solid, retaining barrier, which reduces the risk of its resorbtion.

Drilling used during the splitting procedure is minimal; Osteotomes, bone spreaders, or compression expanders used to prepare the cancellous bone make the relatively loose one more compact, thereby improving the quality of the bone surrounding the implant apically and laterally for further osteointegration without losing it.

The simultaneous use of splitting and augmentation reduces the overall treatment time.The nature of the graft, which is a mixture of recombinant human bone morphogenic protein (rh-BMP2) and xenograft (combination of hydroxyapatite and beta-tricalcium phosphate), gives it not only an osteoconductive property, but also an osteoinductive one, i.e. stimulates bone formation. Bone morphogenic protein is not only a growth factor of cells, but also of blood vessels. After this procedure, quantitatively and qualitatively better bone is obtained. The combination of rh-BMP and mineral graft accelerates the process of bone

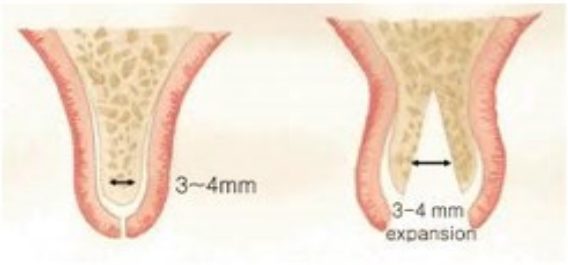

Fig.1 Ridge splitting of narrow bone for dental implant placement

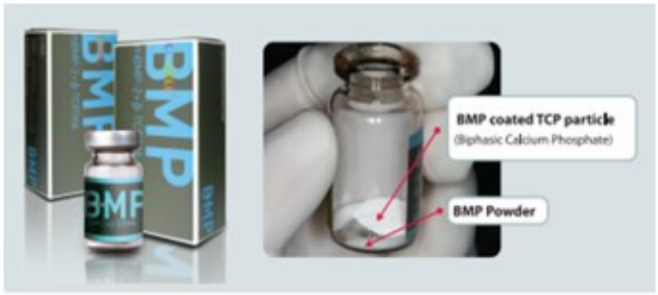

Fig.2 Bone Morphogenic Protein with Biphasic Calcium Phosphate

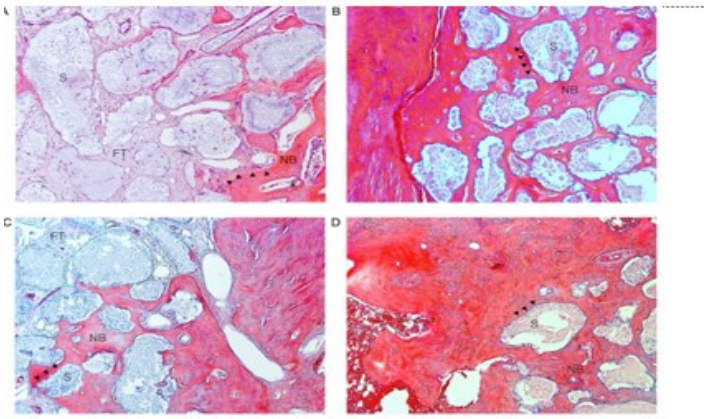

Fig. 3. Representative photomicrographs of new bone formation after bone augmentation. (A) BCP group at 4weeks. (B) BCP-ErhBMP-2 group at 4weeks. (C) BCP group at 8weeks. (D) BCP-ErhBMP-2 group at 8 weeks; NB, new bone; FT, fibrous tissue; S, scaffold; arrowheads indicate osteoblastic cell lining.

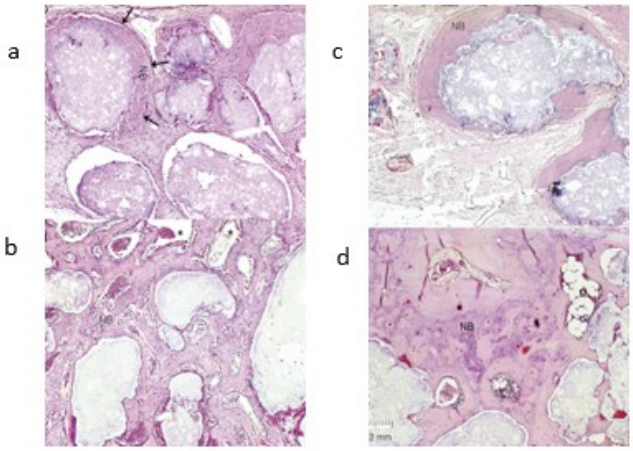

Fig.4 Representative photomicrographs of the CPP groups at 2 weeks (a), 8 weeks (c) and the ErhBMP-2/CPP groups at 2 weeks (b), 8 weeks (d). Arrowheads indicate defect margin; arrows indicate osteoblastic cell lining. *, Blood vessel; NB, new bone.

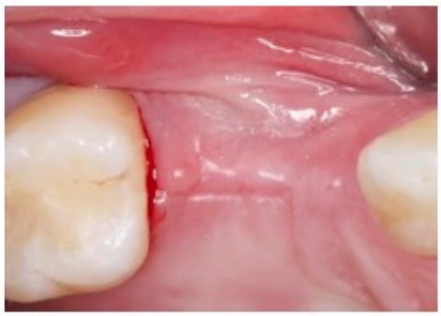

Fig. 5 Presurgical condition of surgical field

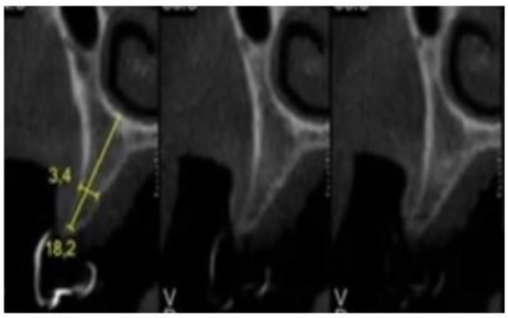

Fig.6 Presurgical radiograph (CT-scan)

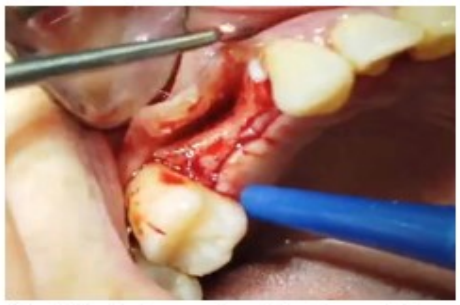

Fig. 7. Incision and reflection of the mucoperiosteal flap

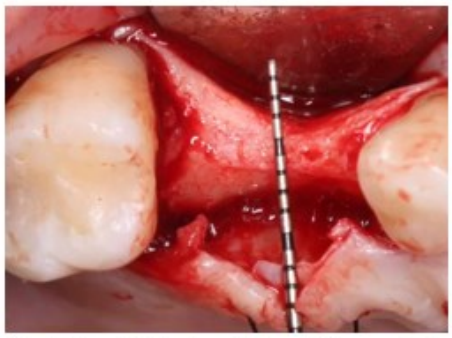

Fig. 8 Insufficient width of the crestal bone for dental implant placement

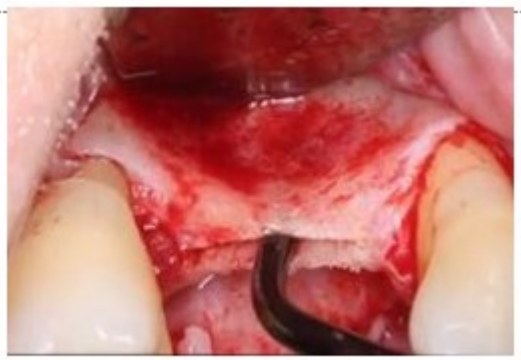

Fig.9 Ridge split with piezotome

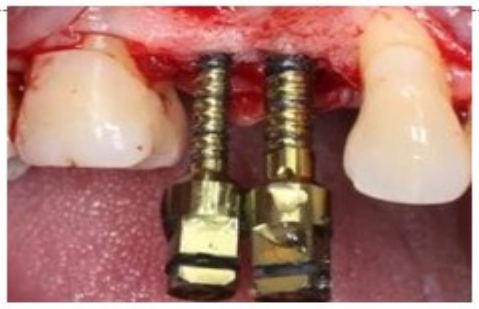

Fig. 10 Using bony expanders to expand the bone and compress the canciolus bone

formation and maturation and establishes a normal bone structure.

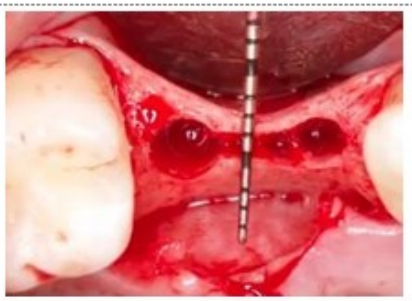

Fig.11 Ridge after split and implant bed preparation. Ridge split without vertical release

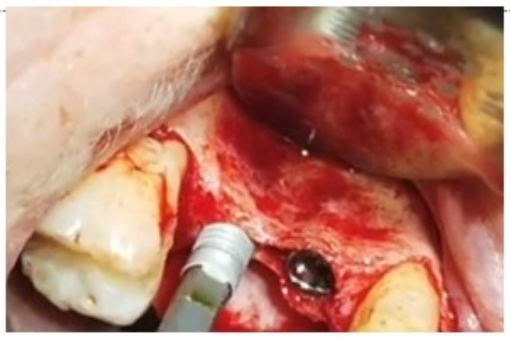

Fig. 12 Dental implant placement between the split bony plates

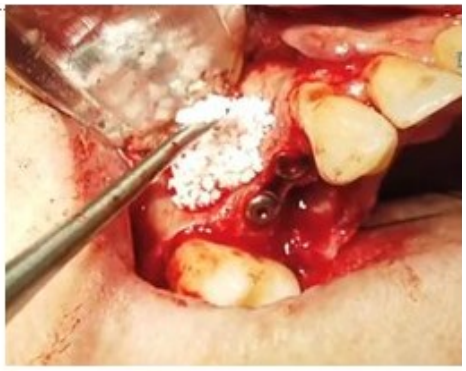

Fig. 13 Augmentation with BCP + BMP

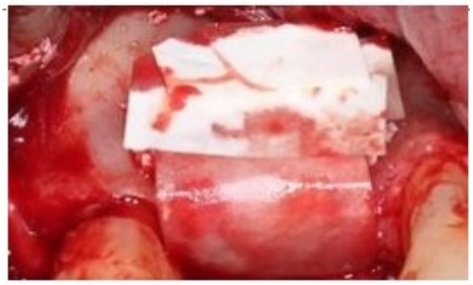

Fig. 14 Collagen membrane covering graft material

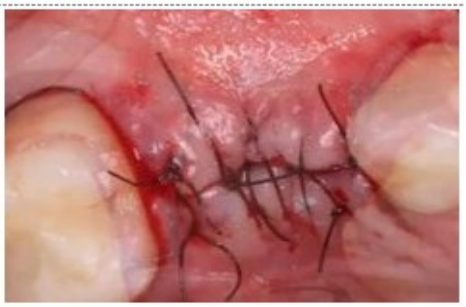

Fig.15 Suturing of surgical field

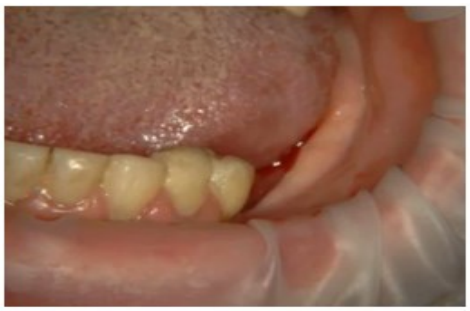

Fig. 16 Presurgical condition of surgical field

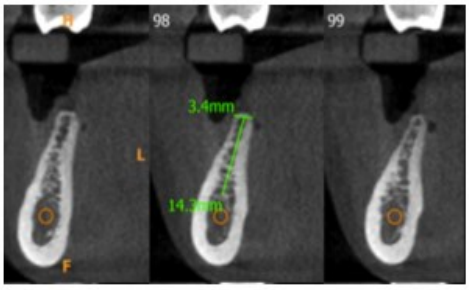

Fig.17 Presurgical radiograph

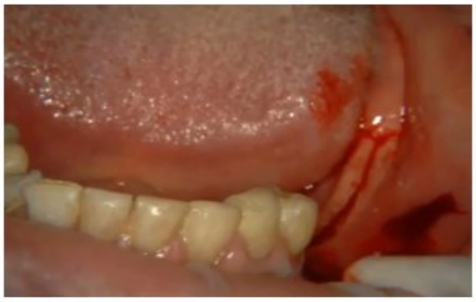

Fig. 18 Midcrestal incision

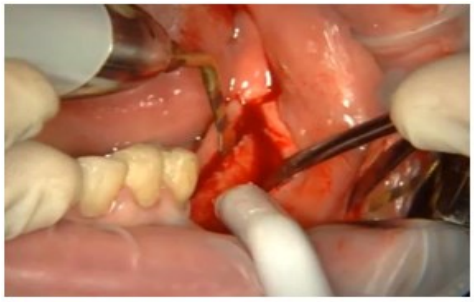

Fig.19 Ridge split using Piezotome

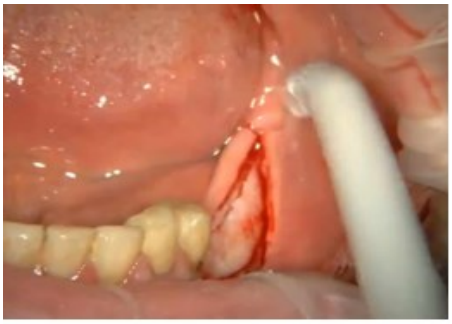

Fig.20 Ridge after split

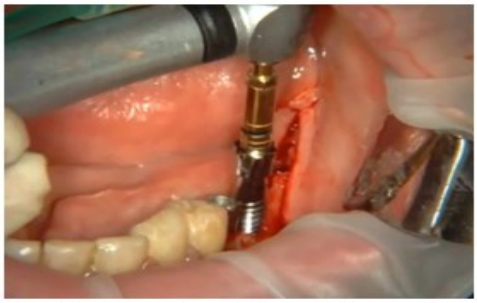

Fig.21 Expansion of the split bone with the bony expanders

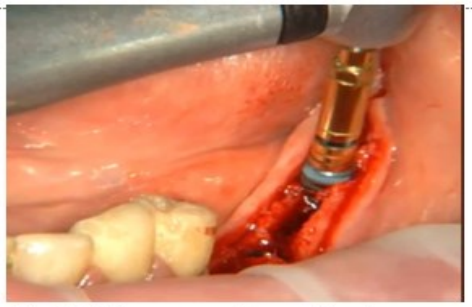

Fig.22 Placement of dental implants between buccal and lingual cortical plates

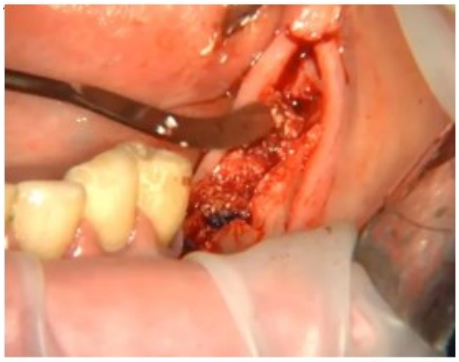

Fig.23 Bone augmentation with graft material: BCP + BMP

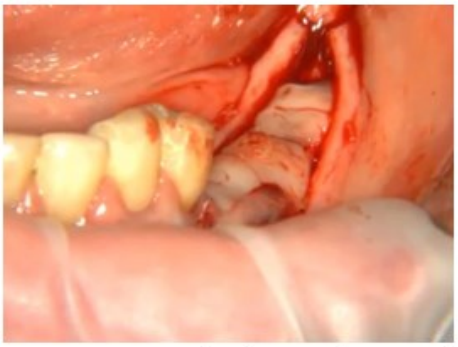

Fig.24 Covering the bone graft material with collagen membrane

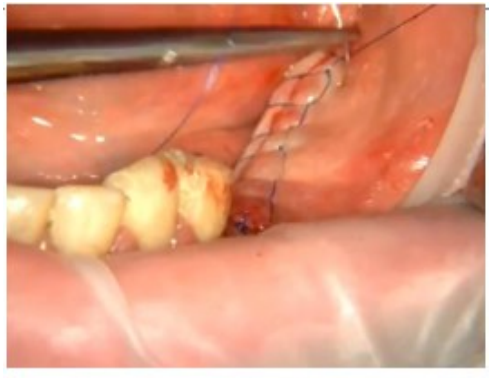

Fig.25 Continous locking suture

Conclusion

Splitting the narrow alveolar ridge and placing an implant or several implants between the resulting two bony plates (buccal and lingual) is the best solution to surround the titanium artificial root with natural bone as much as possible. Augmentation of the space between plates and implants with recombinant human bone morphogenic protein and xeno- or allograft ensures quantitatively and qualitatively perfect bone, which is so important for successful integration of the implant and its long-term support.

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