Biomechanical Evaluation of the Osseointegration Levels of Implants Placed Simultaneously With Tibia, Femur, and Jaw Allogeneic Bone Grafts

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Abstract: In implant-supported prosthetic treatments, the jaw bones may not always have enough bone to accommodate implants. Allogeneic grafts can also be preferred as an alternative to autogenous grafts in cases of vertical and horizontal bone deficiencies. The aim of this study is to evaluate the osseointegration levels of lathe-faced titanium implants placed together with allogeneic bone tissue transplants obtained from tibia, femur, and mandible bones using a biomechanical method. Twenty-eight female Sprague Dawley rats were included in the study. The subjects were divided into groups where bone transplantations were performed together with implant placement from the tibia (n = 7), femur (n = 7), and lower jaw (n = 7) regions. Four rats (left and right) were used as donors. Grafts, along with implants, were surgically placed in the cortico-cancellous part of the metaphyseal parts of the tibia bones of rats. At the end of the 2-week experimental setup, all rats were killed, and the implants and surrounding bone tissues were subjected to biomechanical reverse torque analysis (N/cm), no statistical difference was detected in terms of bone-implant fusion between the groups in which the femur and tibia bones were transplanted ($P > 0.05$), while the bone-implant fusion value in the group in which the lower jaw bone was transplanted was found to be statistically higher than the femur and tibia bones ($P < 0.05$). Based on the limited results of this study, it can be thought that the lower jaw allogeneic jawbone has a higher osseointegration potential than allogeneic grafts obtained from the femur and tibia.

Key Words: Allogenic bone graft, bone implant connection, femur, mandible, osseointegration, tibia

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In recent years, the use of dental implants in partially and completely edentulous patients has become standard. Having sufficient bone volume to stabilize the implant and hold it successfully in the mouth after implant applications is vital for the implant.[1] However, there may be situations where there is not enough bone volume for implant applications. Examples of these conditions are jaw atrophy, osteomyelitis, cancers that cause jaw bone defects, advanced bone loss due to periodontitis, and tooth extraction and trauma. Sometimes, due to atrophy or defects in the alveolar bone, proximity to anatomic structures such as the alveolar nerve, maxillary sinus floor, or nasal floor makes implant applications difficult.[1–7]

When atrophic conditions are observed in the alveolar ridge, implants shorter than 8 mm can be used as an alternative treatment option. However, in cases where even short implants cannot be applied, it is necessary to resort to bone augmentation techniques.[3,4] Bone regeneration has been reported as a commonly used procedure for augmentation, often at dental implant sites.

Autogenous bone grafts are a material accepted as the gold standard in bone augmentation procedures in oral implantology and fracture treatments in the field of orthopedics. However, it may not be an ideal option in all cases involving bone augmentation procedures due to the need for a second operation site in the same individual and the limited amount of graft. Today, many types of graft materials, including allografts, xenografts, alloplasts, and bone morphogenic proteins, are used in clinical applications as alternatives to autogenous grafts.[2,5,6] Allogeneic grafts have become a frequently preferred graft material today because they eliminate these disadvantages of autogenous grafts. In addition, with the advances made on allogeneic bone grafts in recent years, it has the advantage that their osteogenic, osteoconductive, and osteoinductive effects are similar to autogenous bone grafts.[5] Although autogenous or allogeneic bone grafts are often obtained from intraoral areas, extraoral areas such as iliac, tibia, fibula, femur, calvarium, ribs can be preferred as an alternative.[6,7] Success rates vary when these bone grafts obtained from different regions are used in bone augmentation procedures.[6] In addition, there were cases

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where large mandibular defects have been successfully treated with bone morphogenetic protein alone, without using any graft material.[2,6]

The aim of this study is to examine the osseointegration levels of titanium implants placed together with allogeneic bone grafts taken from different regions (tibia, femur, and jawbone) by biomechanical method, using reverse torque analysis.

MATERIAL AND METHODS

Animals and Experimental Design

This experimental study was carried out on 25 female Sprague Dawley rats, at least 3.5 to 4 months old and 280 to 320 g in weight. The rats were obtained from Firat University Experimental Research Center, Elazig, Turkiye. The research protocol was approved by Firat University Animal Experiments Local Ethics Committee on 23 June 2022 with number 9348. All instructions and rules for experimental animal studies specified in the Declaration of Helsinki were strictly followed throughout the experimental setup. In this study, it was calculated that there should be a minimum of 7 rats in each group, according to statistical power analysis, in order not to allow unnecessary animal waste as stated in the Declaration of Helsinki. The minimum number of rats required for this study was determined by power analysis. Taking alpha hata: 0.05 and beta hata: 0.20, the minimum number of rats required for the study was calculated as 21. To ensure standardization, all rats were kept under the same conditions, in rooms with ventilation systems, and in special cage systems throughout the study. Care was taken to ensure that the female rats were in the same estrus period. To evaluate the osseointegration levels of the implants placed with allogeneic bone grafts obtained from different regions, the subjects were divided into 3 equal groups, with 7 rats in each group; femur allogeneic bone graft (FABG) ($n = 7$), tibia allogeneic bone graft (TABG) ($n = 7$), and jaw allogeneic bone graft (JABG) ($n = 7$). Allogeneic bone grafts were obtained on the right and left sides from 4 rats included outside the study groups (donor group, $n = 4$).

The rats used in this study are the same species, were born in the same period, and were inbred. With inbred breeding, genetically identical subjects were obtained. Therefore, no immunological or toxicological complications were encountered during the study.

Surgical Procedures

Obtaining Allogeneic Bone Grafts

Donor rats ($n = 4$) were first sacrificed, and 5 mm thick and 5 mm wide block grafts were obtained from the corticocancellous bones of the metaphyseal parts of the femur-tibia bones, right and left, and the ramus part of the lower jaw bones (Fig. 1).

Placement of Allogeneic Bone Grafts and Implants

The resulting grafts were placed tightly into the corticocancellous bone part of the metaphyseal part of the right tibia bones of the rats under deep anesthesia (10 mg/kg xylazine and 50 mg/kg ketamine). Surgical procedures were performed under sterile conditions. After the surgical area was shaved, it was washed with povidone-iodine and then covered with sterile drapes. After a ~15 mm incision made over the tibial crest using a scalpel number 15, the skin, soft tissues, and periosteum were dissected (Fig. 2). Titanium implants (Implance Dental Implant Systems, AGS Medical Corporation, Istanbul, Turkiye) with a turned surface, along with the bone grafts taken, were placed in

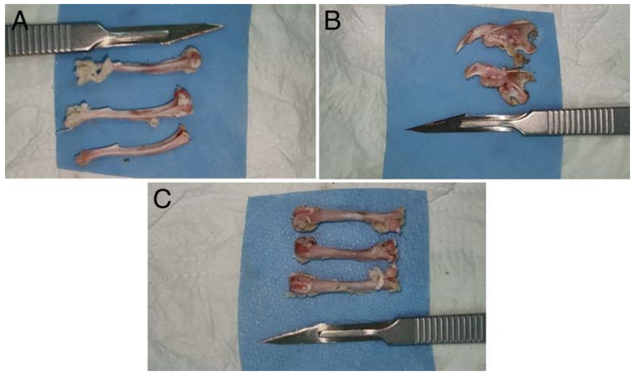

FIGURE 1. Bones were obtained from donor rats from which allografts were obtained. (A) Tibia, (B) mandible, and (C) femur.

the tibia bones of the subjects by ensuring initial tightness (Fig. 3).[7] Implant cavities were opened with a rotary tool at ~600 rpm with sterile saline cooling. After the implants were placed, the surgical area was sutured with 4-0 absorbable sutures. Antibiotics (50 mg/kg penicillin) and painkillers (0.1 mg/kg tramadol hydrochloride) were administered intramuscularly to the rats in all study groups for 3 days after the operations to prevent infection and pain. All surgical procedures were performed by researchers experienced in experimental animal studies to ensure standardization.

Biomechanical Analysis

After a 2-week osseointegration period, all subjects were sacrificed and taken together with the implants and surrounding bone tissues for analysis (Fig. 4). The implants were embedded in autopolymerizing acrylic with the bone tissue around them. The implants were subjected to reverse torque testing with a torque device (Mark 10, New York, USA) (Fig. 5). When reverse torque was applied to the implant, the force value at

FIGURE 2. An approximately 1.5 to 2 cm long incision was made in the tibia crest, and the metaphyseal part of the tibial bone was reached.

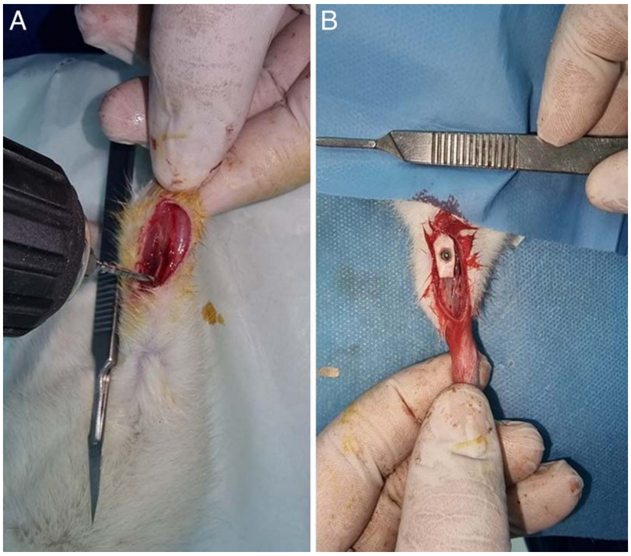

FIGURE 3. Opening the implant socket (A) and placing the implant with the transplanted bone into the bone socket (B).

which the implant made the first rotational movement within the bone was recorded. Data were obtained in N/cm and evaluated statistically. Care was taken to ensure that there was a 90-degree angle between the force application apparatus and the implant. The excised tissues were fixed in a 10% formalin solution. A rapid assessment was made to avoid possible dehydration of the samples. The samples were placed in polymethylmethacrylate blocks before analysis. All biomechanical reverse torque analysis procedures were performed by researchers experienced in experimental animal studies. While performing biomechanical analyses, care was taken to ensure that the researcher did not know which group the samples belonged to. Thus, the reverse torque data obtained was made more objective.

Statistical Analysis

While evaluating the findings obtained in the study, the IBM SPSS 22 version statistical program was used for statistical analysis. The Mann-Whitney *U* and Kruskall-Wallis tests were used for intergroup comparisons of parameters. Significance was evaluated at $P < 0.05$ level.

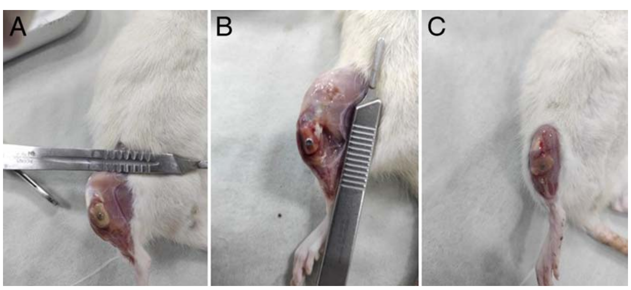

FIGURE 4. Transplanted bone tissue and implants after the end of the experimental setup. It is seen that healing is achieved and the bone and the recipient bed fuse without any connective tissue. (A) Mandible allogenic bone. (B) Tibia allogenic bone. (C) Femur allogenic bone.

FIGURE 5. Measuring the ossoeintegration levels of implants with a reverse torque device (Mark 10, New York, USA).

RESULTS

No complications were encountered during the experimental procedures, and all rats recovered without any problems. The biomechanical bone-implant connection levels of the groups are shown in Supplemental Table 1 (http://links.lww.com/SCS/G549).

The average bone-implant connection values of the JABG, FABG, and TABG groups are 2.34, 1.01, and 0.84 N, respectively. The bone-implant connection value of the JABG group was found to be statistically significantly higher than the FABG and TABG groups ($P < 0.05$). No statistical difference was detected between the FABG and TABG groups in terms of biomechanical bone-implant fusion ($P > 0.05$).

DISCUSSION

Nowadays, with the increasing demand for implant surgeries, the need for bone grafts and bone augmentation operations has also increased. Although autogenous bone grafts are the gold standard due to biocompatibility, they also have many disadvantages. In particular, the need for a second operation site and limited graft availability have led researchers to develop allogeneic bone grafts.[8] Since the spongiose and cortical bone ratios in allogeneic bone grafts obtained from different regions vary, the amount and quality of bone obtained in the region where they are transplanted also varies.[9] In fact, it has been shown that even implants placed in different locations on the same bone have different degrees of osteointegration.[10] Therefore, our aim in this study is to investigate the osteointegration levels of implants placed simultaneously with allogeneic bone grafts obtained from different regions using biomechanical tests. To the best of our knowledge, this is the first study to investigate jaw, tibia, and femur allogeneic bone grafts in the same study in terms of osteointegration levels of the implants.

The reverse torque test is a frequently used method in many studies, especially animal experiments, to evaluate the primary stability and osteointegration level of implants.[11–13] It is a test in which the force required to separate the bone contact formed by the filling of new bone between the grooves of the implant from the implant surface is measured. It provides quantitative results by comparing implants with different surface designs and the structural properties of the bone to which they are applied.[14] In our study, the osteointegration levels of implants placed simultaneously with different bone structures were evaluated using the reverse torque test. It was found that differences in the region where the bone was obtained affected the osteointegration level of the implants.

In their study, Jinno and colleagues placed 96 titanium and hydrophilic implants in the right and left tibia and femur bones of 48 rabbits and evaluated osteointegration using the reverse torque test at 2, 4, and 8 weeks following implantation. They could not find a statistical difference in terms of osteointegration levels between implants with both surface structures placed in the tibia and femur bones.[15] In our study, osteointegration levels of implants placed simultaneously with JABG, TABG, and FABG were investigated using the reverse torque test in the second week following implantation. The lack of statistical difference between the osteointegration levels of implants placed simultaneously with TABG and FABG is compatible with the study of Jinno and colleagues in their study where they applied implants to both the femur and tibia bones of 16 rabbits. Balatsouka and colleagues evaluated osteointegration using reverse torque analysis in the second and fourth weeks. As a result, they found the osteointegration levels of the femur and tibia bones to be similar and explained this situation with the similar cortical and cancellous bone contents of the 2 bones.[16] In their study they evaluated bone regeneration by placing 16 implants in the distal femur and proximal tibia spongy bone and tibial diaphyseal cortical bone of 2 sheep. Canciani and colleagues reported that the cortical bone showed a faster regenerative effect than the cancellous bone structure.[17] In our study, the bone-implant connection level of the JABG group was found to be statistically higher than the TABG and FABG groups. Bone-implant connection levels of the TABG and FABG groups did not show a statistically significant difference. We think that this may be due to the fact that the cortical bone density in the jawbone is higher than that of the tibia and femur bones, thus its regenerative activity is faster, and the cortical-spongious bone content of the tibia and femur bones is similar.

It is known that after dental implants are placed in the bone and primary stability is achieved, the healing process is primarily achieved by secondary stability as a result of the resorption of the old bone in contact with the implant surface and the apposition of new bone to these areas.[18,19] In a study in which they placed implants in the posterior mandibular region of 12 dogs and examined the healing of the bone on the implant surface, Rossi and colleagues compared the regions where cortical bone is dense with the trabecular bone regions. They reported that in implant areas where trabecular bone is dense, old bone is found at a lower rate, resorption is higher, and soft tissue is observed.[20] In their study examining the effects of old bone residues attached to the implant surface on the early healing period, Bosshardt and colleagues reported that, similar to Rossi and colleagues, there were more old bone residues in the cortical bone regions and that these bone residues increased the formation of new bone and increased the rate of mineralized bone in contact with the implant surface.[21] As a result of their study in which they investigated the osteointegration levels by applying implants to the tibia bones of 8 sheep, Morelli et al[22] reported that the contact of the implant with the cortical bone is essential for the osteointegration process. In our study, the bone-implant connection level of the JABG group was found to be statistically higher than the TABG and FABG groups. We think that this is due to the fact that cortical bone density is higher in JABG than in TABG and FABG.

The osteointegration process has an intramembranous ossification pattern that starts with the formation of woven bone in the first week following implantation and continues until the formation of lamellar bone.[23] In intramembranous ossification, mesenchymal cells differentiate into osteoblasts, and the bone matrix is synthesized directly. In endochondral ossification, a cartilage template is first formed, and then a bone matrix is synthesized according to this template.[24] In their study investigating the effects of intramembranous ossification in mandibular regeneration treatments, Ye and colleagues reported that intramembranous ossification is a process completed earlier than endochondral ossification, and if the healing in regenerative treatments is completed with the intramembranous process, the bone is ready to absorb mechanical forces at an earlier period.[25] In their study to develop bone regeneration techniques, Soares and colleagues showed that the mandibular bone has unique properties compared with other craniofacial bones and limb bones of the body. They reported that these differences arise from the fact that the mandibular bone originates from the neural crest, while the limb bones are of mesodermal origin, and that the mandible develops by intramembranous ossification, while other skeletal bones develop by endochondral ossification. They also reported that the higher collagen content and bone mineral density of the mandible than other skeletal bones due to its exposure to multidirectional forces play a role in more successful results in regenerative treatments.[26] In our study, the bone-implant connection level in the JABG group was found to be statistically higher than the TABG and FABG groups. We think that this is due to the fact that the jawbone heals with intramembranous ossification, while the femur and tibia bones heal with endochondral ossification, and the cortical bone density of the jawbone is higher.

In addition, when studies in the literature are examined, new bone formation can be achieved with bone morphogenetic protein applications without the need for grafts. Cicciù and colleagues successfully treated jaw defects due to bisphosphonate use and central giant cell granuloma with recombinant bone morphogenetic protein application. The above studies, unlike our study, indicate that jaw defects can be treated successfully without the need for any grafts.[27,28]

This study has some limitations. First, the molecular mechanisms of the relationship between allogeneic bone transplantation and the recipient's bone tissue have not been fully revealed due to the methods used in this study. Second, although in vivo studies are important for understanding the mechanisms of various pathologies and clinical scenarios, data from these studies are used to extrapolate to human clinical applications. Third, the survival rate of implant and remodeling mechanism of implant-bone contact, which is important for the clinical success of placed implants, could not be evaluated in this study. Fourth, supporting the data of this study, long bones such as tibia and femur have different osteogenic properties than jaw bones (mandible-maxilla), and therefore the clinical results of allogeneic transplants performed in the jaws may be different.[29]

CONCLUSION

Based on the limited results of this study, it was concluded that the ossification processes of allogeneic bone grafts obtained from jaw bone were completed earlier than grafts obtained from tibia and femur bones, and the bone-implant connection and the resulting bone quality were better. It is thought that this may be due to the healing of the jaw bone by intramembranous ossification and the higher cortical bone density. To evaluate the effects of allogeneic bone grafts on bone-implant fusion, more comprehensive experimental and clinical studies are needed in which various evaluation methods are applied to grafts obtained from different bone structures, using different implant surfaces, and at different time periods.

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REFERENCES

1. Cicciù M, Cervino G, Terranova A, et al. Prosthetic and mechanical parameters of the facial bone under the load of different dental implant shapes: a parametric study. *Prosthesis* 2019; 1:41–53
2. Herford AS, Tandon R, Stevens TW, et al. Immediate distraction osteogenesis: the sandwich technique in combination with rhBMP-2 for anterior maxillary and mandibular defects. *J Craniofac Surg* 2013;24:1383–1387
3. Tang CX, Du QH, Luo JY, et al. Simultaneous placement of short implants. 8mm) versus standard length implants (≥ 10 mm) after sinus floor elevation in atrophic posterior maxillae: a systematic review and meta-analysis. *Int J Implant Dent* 2022;8:45
4. Nisand D, Renouard F. Short implant in limited bone volume. *Periodontol 2000* 2014;66:72–96
5. Baldwin P, Li DJ, Auston DA, et al. Autograft, allograft, and bone graft substitutes: clinical evidence and indications for use in the setting of orthopaedic trauma surgery. *J Orthop Trauma* 2019;33:203–213
6. Cicciù M, Herford AS, Cicciù D, et al. Recombinant human bone morphogenetic protein-2 promote and stabilize hard and soft tissue healing for large mandibular new bone reconstruction defects. *J Craniofac Surg* 2014;25:860–862
7. Acikan I, Dundar S. Biomechanical examination of osseointegration of titanium implants placed simultaneously with allogeneic bone transfer. *J Craniofac Surg* 2022;33:350–353
8. Moraschini V, de Almeida DCF, Calasans-Maia MD, et al. Immunological response of allogeneic bone grafting: a systematic review of prospective studies. *J Oral Pathol Med* 2020;49:395–403
9. Ahmed GA, Ishaque B, Rickert M, et al. Allogeneic bone transplantation in hip revision surgery : Indications and potential for reconstruction. *Orthopade* 2018;47:52–66
10. Rong M, Zhu A, Guo Z, et al. The effects of early osseointegration in different implant sites in rabbit tibias. *J Mater Sci Mater Med* 2013;24:959–965
11. Yurttutan E, Dereci Ö, Karagöz MA. Biomechanical and histologic evaluation of osseointegration of titanium dental implants modified by various combinations of sandblasting, acid-etching, hydroxyapatite, and hyaluronic acid coating techniques. *Int J Oral Maxillofac Implants* 2023;38:583–590.
12. Tekin S, Dundar S, Demirci F, et al. Biomechanical and biochemical evaluation of the effect of systemic application of omeprazole on the osseointegration of titanium implants. *Int J Implant Dent* 2021;7:27
13. Qamheya AHA, Arisan V, Mutlu Z, et al. Thermal oxidation and hydrofluoric acid treatment on the sandblasted implant surface: a histologic histomorphometric and biomechanical study. *Clin Oral Implants Res* 2018;29:741–755
14. Tekin S, Dundar S, Demirci F, et al. Biomechanical and biochemical analyses of the effects of propranolol on the osseointegration of implants. *J Craniofac Surg* 2021;32:1174–1176
15. Jinno Y, Stocchero M, Galli S, et al. Impact of a hydrophilic dental implant surface on osseointegration: biomechanical results in rabbit. *J Oral Implantol* 2021;47:163–168
16. Balatsouka D, Gotfredsen K, Lindh CH, et al. The impact of nicotine on osseointegration. An experimental study in the femur and tibia of rabbits. *Clin Oral Implants Res* 2005;16:389–395
17. Canciani E, Ragone V, Biffi CA, et al. Understanding the role of surface modification of randomized trabecular titanium structures in bone tissue regeneration: an experimental study. *Medicina (Kaunas)* 2022;58:315
18. Botticelli D, Lang NP. Dynamics of osseointegration in various human and animal models—a comparative analysis. *Clin Oral Implants Res* 2017;28:742–748
19. Soto-Peñaloza D, Caneva M, Viña-Almunia J, et al. Bone-healing pattern on the surface of titanium implants at cortical and marrow compartments in two topographic sites: an experimental study in rabbits. *Materials* 2019;12:85
20. Rossi F, Lang NP, De Santis E, et al. Bone-healing pattern at the surface of titanium implants: an experimental study in the dog. *Clin Oral Implants Res* 2014;25:124–131
21. Bosshardt DD, Salvi GE, Huynh-Ba G, et al. The role of bone debris in early healing adjacent to hydrophilic and hydrophobic implant surfaces in man. *Clin Oral Implants Res* 2011;22:357–364
22. Morelli F, Lang NP, Bengazi F, et al. Influence of bone marrow on osseointegration in long bones: an experimental study in sheep. *Clin Oral Implants Res* 2015;26:300–306
23. Bosshardt DD, Chappuis V, Buser D. Osseointegration of titanium, titanium alloy and zirconia dental implants: current knowledge and open questions. *Periodontol 2000* 2017;73:22–40
24. Galea GL, Zein MR, Allen S, et al. Making and shaping endochondral and intramembranous bones. *Dev Dyn* 2021;250: 414–449
25. Ye X, He J, Wang S, et al. A hierarchical vascularized engineered bone inspired by intramembranous ossification for mandibular regeneration. *Int J Oral Sci* 2022;14:31
26. Soares AP, Fischer H, Aydin S, et al. Uncovering the unique characteristics of the mandible to improve clinical approaches to mandibular regeneration. *Front Physiol* 2023;14:1152301
27. Dundar S, Bozoglan A, Bulmus O, et al. Effects of restraint stress and high-fat diet on osseointegration of titanium implants: an experimental study. *Braz Oral Res* 2020;34:e008
28. Cicciù M, Herford AS, Juodžbalys G, et al. Recombinant human bone morphogenetic protein type 2 application for a possible treatment of bisphosphonates-related osteonecrosis of the jaw. *J Craniofac Surg* 2012;23:784–788
29. Herford AS, Cicciù M. Recombinant human bone morphogenetic protein type 2 jaw reconstruction in patients affected by giant cell tumor. *J Craniofac Surg* 2010;21:1970–1975

