Biomechanical Evaluation of Implant Osseointegration After Guided Bone Regeneration With Different Bone Grafts

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Abstract: The aim of this study was to compare the biomechanical osseointegration of titanium implants after guided bone regeneration (GBR) with a hydroxyapatite graft, deproteinized bovine bone graft, human-derived allograft, and calcium sulfate bone graft. Thirty-two female Sprague Dawley rats were divided into four groups, each containing eight (n = 8) rats: hydroxyapatide (HA), deproteinized bovine bone graft (DPBB), allograft (ALG), and calcium sulfate. Bone defects were created in the tibia of the rats, which were grafted with HA, DPBB, ALG, or CP bone grafts for the purpose of GBR. Ninety days after surgery, machine-surfaced titanium implants were inserted into the area where GBR had been undertaken. After 90 days of the surgical insertion of the implants, the rats were sacrificed, the implants with surrounding bone tissue were removed, and biomechanical osseointegration (N/cm) analysis was performed. No statistically significant differences were found among the groups in osseointegration (N/cm) three months after the GBR procedures ($P > 0.05$). According to the biomechanical results, none of the grafts used in this study was distinctly superior to any of the others.

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For successful implant treatment, sufficient bone should be present in the implantation sites to ensure satisfactory implant placement, aesthetics, and function. Adequate and healthy bone tissue is required in the area where the implant is placed in order to obtain a good long-term prognosis for osseointegrated implants. However, bone loss or insufficiency remains a major challenge for osseointegration, for reasons such as certain systemic and periodontal diseases, trauma, and tumors. Various strategies, such as bone grafting techniques, alveolar distraction, and guided bone regeneration (GBR) have been applied to re-adjust the lost bone and ensure that the implant is fully integrated and maintained during functional loading. GBR is one of the most frequently used, most successful, and best-documented methods for alveolar bone reconstruction and the treatment of peri-implant bone deficiencies. It is estimated that 40% of implants used in treatment for toothlessness, needed of GBR.[1–7] The survival rate of implants placed in areas where bone augmentation is applied varies between 79% and 100%, and most of the studies show a more than 90% survival rate after at least 1 year of function.[6] The survival rate for implants placed after the horizontal or vertical GBR procedure has been reported as 95%.[8] Today, different types of bone grafts are used in combination with membrane (absorbable and not absorbable) in the GBR technique.[1]

The primary types of bone graft materials are autogenous bone, allografts, xenografts, and alloplasts. All graft materials have one or more of three mechanisms of action, known as osteogenesis, osteoinduction, and osteoconduction. Autogenous bone obtained from the patient forms new bone by means of the above three mechanisms of action. Allografts collected from cadavers have osteoconductive and possibly osteoinductive properties, but they are not osteogenic. Xenografts / alloplasts are typically only osteoconductive.[2] The most commonly used graft materials are reportedly deproteinized bovine mineral (53.0%) and autogenous bone particles (32.5%).[9]

An important issue discussed in the literature on clinical studies is whether bone tissue generated after GBR can remain in the long term without loss. In experimental GBR studies carried out in rodents, it has been reported that the newly formed bone is slightly resorbed in the short term but remains stable, even though it shows a small amount of resorption in the long term. In these studies, the researchers performed GBR beyond the skeletal system, using a rigid barrier. However, information in the literature about the bone connection of implants inserted in newly acquired bone tissue is limited.[10]

In our study, unlike other studies, the implants were placed in areas where GBR was applied. The bone implant connection of

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bone structure was evaluated in the area where GBR was applied. The aim of this study was to investigate the bone implant connection of titanium implants and new bone tissue by a biomechanical method using new bone tissue generated by GBR and different graft materials in rat tibia.

MATERIAL AND METHODS

Subjects and Experimental Design

All surgical and experimental procedures were performed at Firat University Experimental Research Center, Elazig, Turkiye. This study was approved by Firat University Animal Experiments Local Ethics Committee (Protocol Number: 2016/31). All of the animals used in the experimental stage were provided from the same center. Recommendations in the Helsinki Declaration regarding the protection of laboratory experimental animals were strictly followed.

Thirty-two female, 280- to 350-g, 3-month-old Sprague Dawley rats were used. For standardization of the study, all subjects were in the same estrus period. The rats were kept in a humidity- (55%) and temperature-controlled room ($22 \pm 2^{\circ}C$) in a 12-hour light/dark cycle. All rats were placed in standard cages in groups of four animals and provided with normal diet and water and ad libitum feeding. The rats were divided randomly into four equal groups ($n = 8$ in each group): hydroxyapatite (HA), allograft (ALG), bovine source xenograft (DPBB), and calcium phosphate (CP). After 90 days post-GBR procedures, implants were inserted in bone tissue obtained with the GBR in the rat tibia, and 90 days after the surgical placement of the titanium implants, the rats were sacrificed and the osseointegration of the implants was analyzed biomechanically.

Surgical Procedures

All animals were fasted for 8 hours before surgery and general anesthesia. All surgical procedures were performed under general anesthesia under sterile conditions. As a general anesthetic, xylazine hydrochloride (Rompun; Bayer, Germany; 10 mg / kg) and ketamine hydrochloride (Ketasol; Richter Pharma, Austria; 40 mg/kg) were administered intra-muscularly. Local anesthetics were also applied to the rats for hemostasis of the wound area by an infiltrative injection of mepivacaine hydrochloride (0.3 ml/kg, 2% scandicaine epinephrine 1: 100,000; Septodont, France). The area to be surgically treated was washed with povidone iodine after shaving. After a 1.5 cm incision was made in the tibial crest with a scalpel blade No. 15, the proximal part of the tibia was accessed with the periosteum elevator. Bone sockets with a diameter of 4 mm were created in each tibia, and these sockets were filled with different bone grafts. At the end of the 90-day healing period, 4 mm-long, 2.5 mm-diameter titanium implants were placed into the new bone tissue obtained by reaching the metaphyseal parts of the subjects' tibia under anesthesia with an incision (Fig. 1 A, B). After the implants were placed, flaps were closed using absorbable threads (4/0 vicryl; Ethicon, Inc., Somerville, NJ, USA) for soft tissues and monofilament suture (Nylon 4.0; Ethicon, Inc.) for skin. Postoperatively, the rats were monitored daily for signs of pain, opening, infection, limited movement, loss of appetite, and weight loss. Antibiotics (50 mg/kg penicillin) and analgesic (0.1 mg/kg tramadol hydrochloride) were administered intramuscularly once a day for 3 days in order to prevent postoperative infection and pain. All subjects were sacrificed after a 12-week recovery period. The implants were taken for biomechanical analysis together with the surrounding bone tissues.

Biomechanic Analyses

A reverse torque test was performed on samples obtained from sacrificed animals after the 12-week osseointegration period. Biological samples were prepared immediately after removal of the block bone fragment tibia containing the implants. Samples were kept in liquid solution (10% buffered formalin) and evaluated immediately to avoid dehydration. All implants were embedded in polymethylmethacrylate blocks. After the special reversing part was screwed on to the implants, the digital torque tool (Tonichi STC400CN, Buffalo Grove, IL) for each implant was fixed, and a counterclockwise force was applied manually, slowly but gradually increasing. The procedure was completed with the implant starting to rotate inside the bone socket. The highest torque value (Ncm) obtained on the digital torque screen at the time of breaking was automatically recorded (Fig. 2).

Statistical Analyses

Statistical analysis was performed with SPSS 23.0 for Windows software (USA). Data for each group are expressed as mean ± standstandard deviation. Differences between the groups were detected using one-way ANOVA. Tukey's HSD (honestly significant difference) test was used to determine the group that caused these differences, and $P < 0.05$ was considered statistically significant.

RESULTS

Implants that were not properly inserted into the bone were excluded from the analysis. In reverse torque analysis, the torque values (Ncm) formed were HA: 5.49 ± 0.55, DPBB: 6.2 ± 1.1, ALG: 5.34 ± 0.64, and CP: 5.16 ± 0.91. In reverse torque analysis, no statistical difference was found between the groups (Supplementary Digital Content, Table 1, http://links.lww.com/SCS/B886).

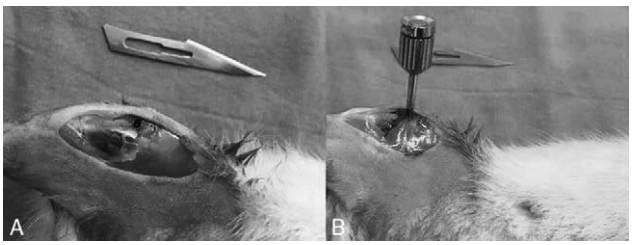

FIGURE 1. (A) Surgical preperation of the titanium implant sockets and (B) after insertion of the titanium implants in bone sockets.

FIGURE 2. Reverse torque analysis of the titanium implants (Tonichi STC400CN, Buffalo Grove, IL).

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DISCUSSION

Guided bone regeneration using bone graft is the process of increasing the bone level in anatomically and functionally deficient areas. Bone augmentation in dental surgery is performed by procedures such as sinus augmentation, socket grafting, and increasing alveolar bone height. The purpose of the new bone is to provide stability and support for the dental implant. As new regenerated bone occurs, the bone is replaced with graft, or it merges with the graft to form new bone that supports the dental implant.[11]

Cha et al suggested that 50.3% of patients required a bone graft during dental implant surgery.[12] In addition, 77% of bone augmentation is reportedly done for esthetic reasons, especially in dental implants applied in the anterior maxillary region.[13–15] In the present study, the osseointegration of titanium implants applied to the tibia of the rats, allowing bone healing after applying different graft materials to bone defects, as frequently used in clinical applications (HA, DPBB, AL, and CP), was evaluated by a biomechanical method.

In the treatment of bone defects with GBR, autografts, allografts, demineralized bone matrix, xenograft (bovine), calcium sulfate, calcium phosphate, and HA, known as substituted bone grafts, are used.[16] In the present study, HA, human-sourced ALG, DPBB graft, and CP were applied. In previous animal experiment studies, it has been reported that a 3-month period after defect grafting is sufficient for recovery, angiogenesis, and new bone formation.[17–19] In our study, implants were placed 3 months after grafting.

Currently autogenous bone grafts remain the best quality among graft materials in bone surgery. Although autogenous grafts are the gold standard in the GBR applications too in dental medicine, they are not always preferred, for reasons such as requiring a second surgical procedure, the risk of resorption of the bone graft, the potential for causing nerve injuries, the limited amount of the graft, loss of patient comfort after surgery, and the risk of infection. Deproteinized bovine bone graft, biphasic calcium phosphate, hydroxyapatite, and human-derived allografts can be used as an alternative to an autogenous graft in GBR.[1,2,20–24] Some studies indicate that autogenous bone graft application does not significantly increase the success rate of dental implants and dentures. However, although autogenous bone grafts are not statistically significantly superior to other bone grafts, their use is recommended, because they generate more osteointegration, entail less recovery time, and are more compatible with natural bone.[20,25]

In a study by Artas et al, in which they investigated GBR with a rat calvarial model, applying HA, DPBB, human-derived allogenic bone graft, and calcium sulfate graft materials in peri-implant GBR, they reported no difference in new bone formation in histological and immunohistochemical examination.[10]

Papageorgiou et al, having conducted a systematic review of randomized clinical studies on bone graft material applications performed in dental implants, suggested that there was no significant difference in new bone formation occurring in graft materials.[26] Chavda et al compared non-autogenous bone grafts with autogenous grafts, concluding that non-autogenous bone grafts are an alternative in graft applications.[27] In systematic examinations and meta-analyses of studies in which bone graft materials were used in sinus-lifting procedures, autogenous grafts have been reported to produce more new bone formation compared to xenografts, allografts, or alloplasts.[28] However, the use of non-autogenous grafts as an alternative may be appropriate because of lower morbidity and more availability of the materials.[29]

In several studies in the literature comparing xenografts and allografts, a slower osseointegration occurred in the xenograft, as well as a lower bone junction rate, radiolucent areas, and local complications.[16,30,31] However, in our study, when xenografts and allografts were compared according to biomechanical parameters, higher values (N/cm) were obtained in the osseointegration bovine xenograft (bovine sources) graft, but the differences were not statistically significant. In an experimental animal study, Miron et al compared HA-derived synthetic bone grafts with autogenous bone, showing that the HA-derived grafts promoted new bone formation with excellent stability and new bone regenerative properties. They suggested that, thanks to their content and structure, HA bone grafts slowly dissolve and are gradually displaced by bone tissue.[30] In the present study, HA-derived synthetic bone graft was compared with human-sourced allograft ALG, bovine-sourced graft DPBB, and CP graft and, although it had the second highest value (N/cm), the difference was not statistically significant.

Wood and Mealey compared demineralized freeze-dried bone allograft with demineralized freeze-dried bovine allograft in terms of the quality of newly formed bone and determined the percentage of newly formed bone, residual graft material and connective tissue by histological analysis. They stated that demineralized freeze-dried bone allograft had a significantly higher percentage of newly formed bone percentage.[32] Kotsakis et al compared bovine bone mineral and calcium phosphosilicate putty bone substitutes with each other and with a natural healing control group. The findings of this study suggested that after 5 months of observation, both bovine bone mineral and putty bone helped reduce alveolar bone height loss compared to natural recovery, but could not find a statistically significant difference.[33] In one study, used a natural clot and corticocancellous pig bone graft to fill the sockets after extraction. As a result of the study, they suggested that graft materials help prevent bone loss after extraction.[34]

In our study, 4 different graft materials were evaluated in the same study (hydroxyapatide (HA), deproteinized bovine bone graft (DPBB), allograft (ALG), and calcium sulfate). Unlike previous studies, graft materials were placed in the area where the defect was applied. After the ossification, implants were applied and osteointegrations of different bone materials were compared in the regions where bone augmentation was performed. This study was specifically designed to guide us about the osteointegration of bone materials applied after immediate loading. As a result of the analysis, no statistically significant difference was found in the osteointegration of the graft materials.

CONCLUSION

Our analyses showed that the highest osseointegration value was realized in DPBB graft material, followed by HA, ALG, and CP, in that order No statistically significant difference was found between the grafts in terms of biomechanical bone implant connection values. This shows that, although autogenous grafts are accepted as the gold standard in bone augmentation, we think that other graft materials can be used in patients who cannot obtain autogenous grafts. Since there is no significant difference between graft materials, both cost and effectiveness should be evaluated for the patient and the most appropriate graft material should be selected. More studies are needed to obtain more precise results.

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