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Comparison of Bioabsorbable Calcified Triglyceride Bone Cement Versus Deproteinized Bovine Bone in Guided Bone Regeneration: Experimental Study

Yönlendirilmiş Kemik Rejenerasyonunda Biyoabsorbe Kalsifiye Trigliserid Kemik Simanı ile Deproteinize Sığır Kemiğinin Karşılaştırılması: Deneysel Çalışma

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ABSTRACT Objective: The aim of this study was to evaluate, for the first time, the effect of bioabsorbable calcified triglyceride bone cement (CTBC) on bone formation when used as an adjunct to guided bone regeneration (GBR) and compare it with the results obtained with deproteinized bovine bone (DBB). **Material and Methods:** Forty-eight rats were randomly divided into three equal-sized groups. Following incisions along with the inferior border of the mandible, full thickness flaps were elevated via an extraoral approach. Custom-made rigid, hemispherical teflon capsules were packed with CTBC, or DBB, or were left empty and placed facing the lateral surface of the mandibular ramus. At the postoperative 4th months, all rats were sacrificed and tissue samples were processed for decalcified histological evaluation of the newly formed bone, residual graft particles, and soft connective tissue within the space created by the capsules. **Results:** It was observed that the amount of mineralized bone formation in the capsules filled with CTBC and DBB was similar, and most of the capsules were filled with biomaterial particles embedded in the connective tissue. There was no significant difference between the 2 groups in terms of newly formed bone, graft particles or connective tissue ($p>0.05$). On the other hand, it was determined that a greater amount of new mineralized bone was formed in the control group compared to the CTBC or DBB groups ($p<0.05$). **Conclusion:** Grafting with CTBC or DBB did not make any difference when used as an adjunct to GBR.

Keywords: Guided bone regeneration;
deproteinized bovine bone;
calcified triglyceride bone cement

ÖZET Amaç: Mevcut çalışmanın amacı, yönlendirilmiş kemik rejenerasyonunda biyoabsorbe kalsifiye trigliserid kemik simanının (KTKS) etkisini ilk defa değerlendirmek ve sonuçlarını deproteinize sığır kemiği (DSK) ile karşılaştırmaktır. **Gereç ve Yöntemler:** Kırk sekiz sıçan rastgele 3 eşit büyüklükte gruba ayrıldı. Mandibula alt kenarı boyunca yapılan kesilerin ardından, ekstraoral yaklaşımla tam kalınlıkta flep kaldırıldı. Özel yapım sert, yarı küresel teflon kapsüller KTKS, DSK ile doldurulduu veya boş bırakıldı (kontrol) ve mandibular ramusun lateral yüzeyine bakacak şekilde yerleştirildi. Ameliyat sonrası 4. ayda tüm sıçanlar sakrifiye edilerek, greft alanlarından alınan doku örnekleri kapsüller tarafından oluşturulan boşluk içinde yeni oluşan kemik, rezidüel greft partikülleri ve yumuşak bağ dokusu açısından histolojik olarak değerlendirildi. **Bulgular:** KTKS ve DSK ile doldurulmuş kapsüllerde mineralize kemik oluşumunun benzer şekilde ve kapsüllerin büyük kısmının bağ dokusu içine gömülü biyomateryal partikülleri ile dolu olduğu gözlendi. Yeni oluşan kemik, greft partikülleri veya bağ dokusu açısından 2 grup arasında anlamlı bir farklılık bulunmadı ($p>0,05$). Buna karşılık, kontrol grubunda KTKS veya DSK grupları ile karşılaştırıldığında daha büyük miktarda yeni mineralize kemik oluştuğu belirlendi ($p<0,05$). **Sonuç:** KTKS veya DSK ile greftleme, yönlendirilmiş kemik rejenerasyonuna ek olarak kullanıldığında herhangi bir farklılık yaratmadı.

Anahtar Kelimeler: Yönlendirilmiş kemik rejenerasyonu;
deproteinize sığır kemiği;
kalsifiye trigliserid kemik simanı

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The guided bone regeneration (GBR) concept is based on preventing migration of surrounding soft tissues by creating spaces on the bone defect with various combinations of grafts and membranes while promoting the growth of osteogenic cells.[1] The ideal graft material for GBR is still controversial. Autogenous bone is still the gold standard for bone regeneration due to its biocompatibility, lack of antigenicity, and osteoconductive and osteoinductive properties.[2] Alloplastic bone substitutes, xenogenic, freeze-dried and demineralized freeze-dried bones have been used in GBR procedures.[3] Deproteinized bovine bone (DBB) is one of the most commonly used xenogenic bone substitutes. Its osteoconductive properties and good adaptation to bone tissue have been proved in various experimental and clinical studies.[4] DBB is used in combination with GBR in peri-implant bone deficiencies and sinus lift procedures.[5,6]

Calcified triglyceride bone cement (CTBC) is an odorless, exothermic calcified triglyceride cement and was introduced as an osteoconductive, adhesive and bioabsorbable material.[7,8] CTBC gets its feature from its three components. Two of these (prepolymer and polyol) are hydroxyl-terminated fatty acids derived from castor oil naturally. CTBC takes its porous structure from its third component, calcium carbonate, and this component gives the osteoconductive feature of CTBC.[7] Moreover, the material can be quickly and easily mixed, with no vapors or harmful residues. It can be used as graft or as bone adhesive agent according to its preparation choice. While the porous structure of CTBC guides osseointegration, its minimal exothermic reaction protects the adjacent tissue from necrosis.[9] Although many studies have been published on its use in cranioplasty and orthopedic surgery, no study has so far investigated its use in addition to GBR.[7,10,11] This study aimed to reveal the adjunctive effect of CTBC (Kryptonite™ bone cement, Doctors Research Group Inc, Southbury, CT) on GBR and compare it with results obtained with DBB (Gen-Os®, Tecnoss, Giaveno).

MATERIAL AND METHODS

ANIMALS

The study protocol was approved by Ondokuz Mayıs University Ethics Committee for Animal Experimentation (date: November 28, 2013; no: B.30.2.ODM.0.20.09.00-050.04-98). All experimental procedures were performed in accordance with the Laboratory Animal Care Committee of the Faculty of Medicine and the Declaration of Helsinki for experimental studies. Experimental stages of the study were performed in Ondokuz Mayıs University Experimental Animals Research Center Laboratory. The study included forty-eight, 3 months old Spraque-Dawley rats that were housed in separate cages (1 animal per cage) under the same laboratory conditions [room temperature (22±1 °C) under a 12-h light/dark cycle] and were fed with standard laboratory diet and water ad libitum. Animals were randomly divided into 3 equal groups: CTBC (Kryptonite™), DBB (Gen-Os®), and control (without graft).

SURGICAL PROCEDURES

Full anesthesia was obtained with a combination of ketamine (75-95 mg/kg intra-muscular injection) and xylazine (5 mg/kg intra-muscular injection). The right manbibular inferior border of each rat was shaved and cleaned with an iodine solution before surgery. Surgical procedures followed the procedures reported in a previously published study.[12] Skin and periosteal incisions were done along the inferior border of the mandible and a muscle-periosteal flap was elevated at both sides.

Four holes, in 0.5 mm diameter were created as corners in a square (6 mm away from each other). According to the manufacturer's instructions, the three components of CTBC were mixed for one minute and then allowed to polymerize for 8 minutes. At the end of the 8 minute, it was ready to be filled into capsules. Custom-made teflon capsules with 6 mm inner diameter and 1 mm circumferential collar were filled with graft material (CTBC; DBB); or left empty (control) and placed to the lateral surface of the mandible (Figure 1).

The capsules were sutured with 4.0 vicryl sutures through the 4 holes in the bone for stabilization. After closing the periosteum with 4-0 vicryl sutures and the skin with 4-0 silk sutures, an antibiotic/analgesic combination was administered as intramuscular injection for 3 days postoperatively.

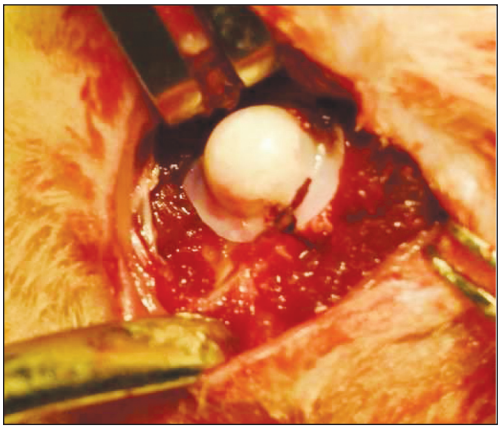

FIGURE 1: Capsules are filled with grafts, or left empty. Capsules are placed on the lateral surface of the mandibular ramus and fixed by means of silk sutures.

HISTOLOGICAL ANALYSIS

Animals were sacrificed 4 months postoperatively under high dose ketamine anesthesia. The right mandibles were removed, fixed in formalin for 10 days and then decalcified in a 10% formic acid solution. Mandibles were dried with increasing concentrations of ethanol and cleaned with xylene before embedding in paraffin. Bones were cut to make 5 µm thick sections by a microtome. For volumetric estimation, partitions were performed starting from a random partition and selecting one every 50th partition. A total of 2,000-3,000 sections were taken from each tissue block by random sampling and 40-45 sections were selected from each animal. Selected sections were stained with haematoxylin-eosin, evaluated at x4 and x10 magnification, and photographed under a light microscope. Sections were examined histologically to evaluate primary bone tissue, graft particles and connective tissue within the capsules in all groups. A histological scoring system modified from Lane and Sandhu was used to determine histopathological changes.[13,14]

STATISTICAL ANALYSIS

The sample size was calculated based on the data of a previous study with a similar design to estimate the sample size.[15,16] The parameter used for sample size calculation was morphometric analysis of lateral augmentation of the alveolar crest and Bio-Oss (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland) in rats. Taking into account a Type I error equal to 0.05, and a power of 80%, a minimum sample size of 13 rats per group was necessary. To compensate for possible losses, three rats were added to each group. SPSS 17.0 Software Package Program (IBM, USA) was used for the statistics. The Kruskal-Wallis test determined whether there was a statistical difference between the groups in terms of newly formed bone, graft particles and connective tissue. Comparisons of the groups were evaluated using Bonferroni corrected Mann-Whitney U test and a p value of <0.05 was considered statistically significant.

RESULTS

Surgical procedures were completed without any known complications in all animals and there was no loss until the end of the study. No failures occurred during histological possessing and thus all 48 samples were histologically analyzed. It was observed that a limited amount of mineralized bone was formed in the capsules filled with CTBC and DBB, and most of the capsule cavity was filled with residual bone graft substitute particles embedded in the connective tissue (Table 1, Figure 2 and Figure 3). The newly formed bone was in continuity with the host bone and was limited to the lower part of the capsule. There was no statistically significant difference between CTBC and DBB groups in terms of all evaluated parameters (Table 1). Abundant new bone formation was demonstrated histologically in control samples where the capsules were initially left empty (Figure 4). The differences in new bone formation between control and either of the grafted groups were statistically significant ($p<0.05$) (Table 1). Significantly less fibrous connective tissue formation was observed in the control group compared to the CTBC group ($p<0.05$) (Table 1).

DISCUSSION

This study revealed the limited new bone formation in capsules filled with CTBC and this material delayed bone formation when used in addition to GBR. GBR has become a standard treatment option to enhance bone volume in deficient sites prior to- or in conjunction with implant placement.[17] The concept of GBR is based on blocking the non-osteogenic connective tissue cells from entering the bone resorption site and repopulating the area by osteogenic cells.[18]

TABLE 1: Differences between the three groups in terms of newly formed bone, particles and connective tissue.

Group	N	Newly formed bone	Graft particles	Connective tissue
CTBC	16	1.00 (0-1)[a]	2.00 (1-3)[a]	2.00 (1-3)[a]
DBB	16	1.00 (0-2)[a]	2.00 (1-3)[a]	2.00 (1-2)[ab]
Control	16	3.00 (2-3)[b]	0.00 (0-0)[b]	1.00 (1-2)[b]
p value		0.000*	0.000*	0.002*

The results are presented as median (minimum-maximum). There is a significant difference between a and b. There is no difference between the groups with the same letters. *Kruskal-Wallis test was performed for non-paired observations and p<0.05 was considered to be statistically significant. Comparisonal analyzes between the three groups were analyzed by Bonferroni corrected Mann-Whitney U test and p<0.017 were considered as statistically significant. CTBC: Calcified triglyceride bone cement; DBB: Deproteinized bovine bone.

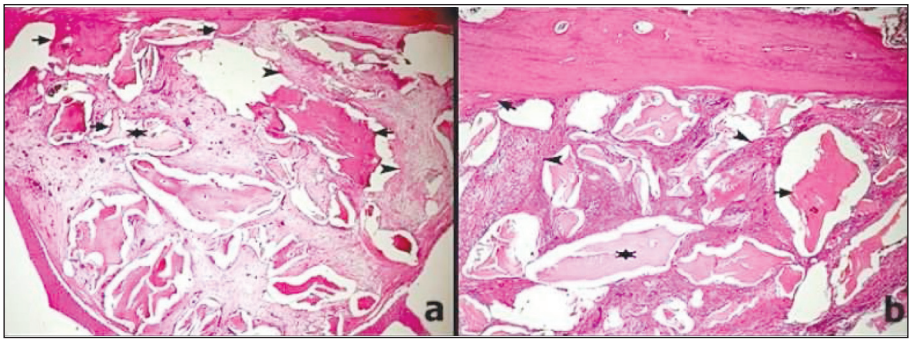

FIGURE 2: Calcified triglyceride bone cement-grafted specimens. New bone (arrows) formation is limited to the lower part of the capsule. The major portion of the tissue consisted of graft particles (stars) embedded in the connective tissue (arrowheads). **a)** x4 haematoxylin-eosin, **b)** x10 haematoxylin-eosin.

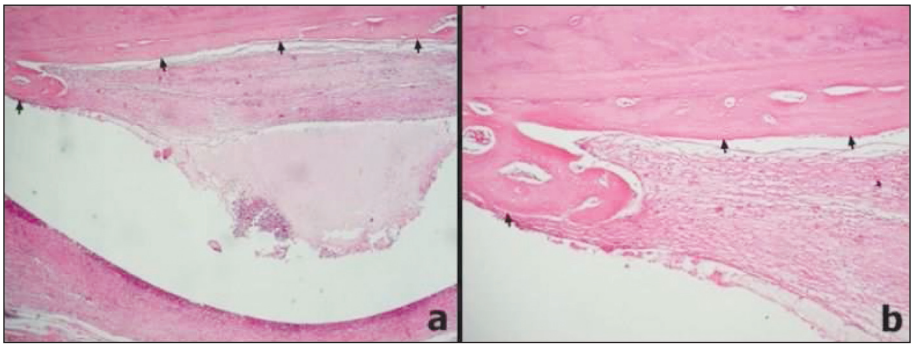

FIGURE 3: Deproteinized bovine bone-grafted specimens. New bone (arrows) formation is limited and confined to the lower part of the tissue formed under the capsule. Graft particles (stars) embedded close to the connective tissue. **a)** x4 haematoxylin-eosin, **b)** x10 haematoxylin-eosin.

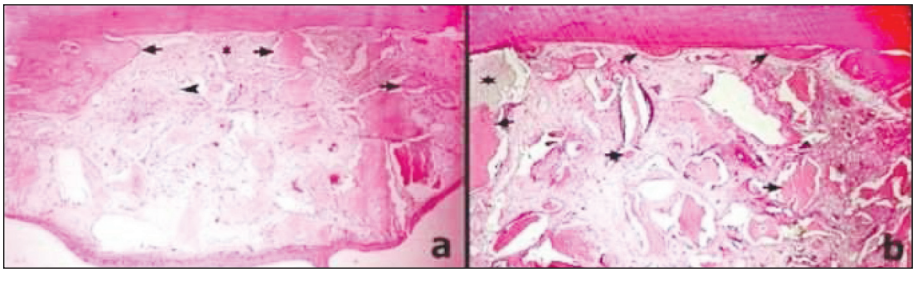

FIGURE 4: Control specimens. New bone (arrows) predominate the space created by the capsule. **a)** x4 haematoxylin-eosin, **b)** x10 haematoxylin-eosin.

Dahlin et al. reported that a non-absorbable membrane as a mechanical barrier resulted in healing of bone defects. Their work repopulated the interest in osteogenesis beneath a semipermeable membrane.[19] Regeneration that occurs within the space formed by the barrier membrane starts with the formation of well-vascularized granulation tissue, followed by angiogenesis and migration of osteogenic cells from existing bone. This is followed by woven bone deposition and lamellar bone formation.[20] In this context, a critical requirement for bone formation by GBR is provision of space for new tissue ingrowth. Thus, GBR is often combined with a bone graft and/or substitute, which can support the supple barrier material and assure space provision, clinically. Ideally the bone graft and/or substitute should have also the potential to enhance bone formation, either in terms of rate, amount or both.[19,20] Autogenous bone graft is still the best option for the augmentation of atrophic alveolar ridge due to its osteoinductive, osteoconductive and osteogenic properties.[21] However, their use is limited due to the risks of donor site morbidity and complications such as infection, resorption, and the need for bone removal from intraoral or extraoral sites. Allografts and xenografts have osteoconductive and limited osteoinductive characteristics but lack the osteogenic properties of autografts.[22,23] The necessity of using a graft material beneath the membrane is also a point of discussion in GBR.

CTBC is an Food and Drug Administration-approved bone cement for use in orthopedic surgery as well as in craniofacial reconstruction procedures and an another important advantage of the material is its stickiness, which ensures that it remains stable in the area where it is applied.[7-11,24] Bone formation within the CTBC (Kryptonite®) graft material in 2-4 months was shown in animal studies and its success in cranial reconstruction and in primer sternotomy when used as fixation were also reported.[7,11,25]

In the study, the amounts of new bone produced with CTBC were similar to those produced with DBB. DBB is amongst the most widely used bone substitute materials for oral indications and numerous pre-clinical and clinical studies have shown positive results after its use.[26] Nevertheless, several pre-clinical studies, using various animal platforms, have indicated that DBB may in fact not increase bone formation in GBR. For example, in a study employing the same "capsule model" and time-frame as herein, limited new bone formation were reported with a similar (but not identical) DBB substitute as DBB.[16] In another study, also using the same experimental set-up as herein, but a prolonged healing period (1 year), unremarkable bone formation was again observed in the grafted capsules; in contrast, originally empty capsules were consistently almost completely filled out with new bone.[12] Nevertheless, it has to be pointed out that simply the fact that a) the CTBC and DBB grafted capsules herein had similar amounts of bone after 4 months of healing and b) the amount of bone formed in DBB grafted capsules in the previous experiments did not increase dramatically after 1 year, does not necessarily imply that CTBC grafted capsules will never get filled with new bone. Obviously, the single observation period of 4 months is a limitation of the present study. In context, no assumptions can be made on the long-term performance of CTBC as adjunct to GBR.

Also, the fact that CTBC did not enhance bone formation in the present experiment, i.e. under optimal conditions for healing, i.e. space provision, wound stability, primary intention healing and no infection, does not necessary imply that this material may not facilitate bone formation, clinically. It may well be that in a clinical situation, where the dimensions and/or morphology of the defect is not supportive, CTBC could support a supple barrier and thereby could facilitate space provision for new bone tissue ingrowth.

Several studies have shown that bone substitutes used as an adjunct to GBR interfere with bone formation. Stavropoulos et al. demonstrated that the amount of newly formed bone under a teflon capsule was significantly larger when no graft material was used. They compared two graft materials, BioOss and Biogran (Biogran®, Orthovita, Malvern, PA, USA), and found no significant differences on behalf of bone formation in short and long terms.[12,16] In the present study, we used the same Teflon capsules to evaluate the efficiency of a newly developed graft material CTBC; Kryptonite™ when compared to DBB in GBR. Consistent with the previous studies

about the ineffectiveness of DBB, we found the best results in empty capsules. No significant difference was found between the 2 graft materials, CTBC and DBB on behalf of new bone formation.

Our literature research revealed two studies on the properties of Kryptonite bone cement when used on dental tissues and there is no way to compare our results about CTBC hence, there exists no available data about the effect of CTBC in GBR.[8,27] However, the results of the present study are in accordance with a latest study in which CTBC was used as an alternative agent in bone reconstruction and augmentation for sinus augmentation.[8] CTBC was not found to be suitable for maxillary sinus augmentation and was less successful to autogenous bone or bovine hydroxyapatite in this experimental study performed in New Zealand rabbits.[8] The other study showed that Kryptonite cement provided optimal apical seal in a manner similar to mineral trioxide aggregate, amalgam and intermediate restorative material when used as a retrograde filling cement.[27]

The migration of bone substitutes away from the grafted area is a commonly encountered complication when the membrane was not fixed tightly over the grafted area.[28] Our main hypothesis was to reveal the advantage of this material especially in horizontal and vertical bone augmentation procedures depending its excellent adhesive property that could be easily fixed on the bone surface. CTBC seems to support this main hypothesis and any kind of membran could also be fixed on the material without need of fixation pins.

CONCLUSION

The present study revealed that DBB or CTBC both arrested bone formation when used as an adjunct to GBR. Considering the current knowledge in the field, the present report is the first study using this cement for GBR and further studies should be performed to found the effect of CTBC on bone regeneration around the titanium dental implants.

Source of Finance

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Feyza Otan Özden, Esra Demir, Seda Koçyiğit;* ***Design:*** *Feyza Otan Özden, Seda Koçyiğit;* ***Control/Supervision:*** *Feyza Otan Özden, Seda Koçyiğit, Esra Demir, Mehmet Öndder Karayiğit;* ***Data Collection and/or Processing:*** *Seda Koçyiğit, Esra Demir;* ***Analysis and/or Interpretation:*** *Feyza Otan Özdden, Mehmet Önder Karayiğit;* ***Literature Review:*** *Seda Koçyiğit, Esra Demir;* ***Writing the Article:*** *Feyza Otan Özden, Esra Demir;* ***Critical Review:*** *Mehmet Önder Karayiğit;* ***References and Fundings:*** *Feyza Otan Özden, Seda Koçyiğit;* ***Materials:*** *Feyza Otan Özdden, Mehmet Önder Karayiğit.*

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