Continuity of bone after tooth extraction must be preserved to keep the structural and mechanical strength of the hard tissue support, even in cases where implantation is not necessary later on.1 After mandibular third molar removal, postoperative pain and swelling are well-known and frequent complications, especially when surgical techniques have to be applied, such as in cases of impacted or recessed teeth.2 In these cases, excess bone loss is caused when drilling is applied in order to facilitate tooth removal compared with a simple extraction. The most pronounced changes of the alveolar bone loss occur in the first 6 months.3 The decreased amount of hard tissue makes it difficult to carry out both conventional and implantation treatments.4 Therefore, filling the iatrogenic bone loss plus the extraction socket is preferred to keep the integrity of the bone surface and to expedite healing of the bone void. Mandibular third molar sockets are considered to be useful for in vivo testing of alveolar ridge preservation techniques and graft materials.

Purpose: The goal of this study was to compare bone graft materials in mandibular third molar extraction sockets and to monitor bone remodeling and complications. Materials and Methods: Patients with bilateral, impacted mandibular third molars were involved. Twenty-four patients were planned to be randomly assigned to three possible treatments: (1) the control sockets were left empty; (2) the socket was filled with bovine xenograft (Bio-Oss); or (3) the socket was filled with albumin-impregnated bone allograft (BoneAlbumin). Postoperative pain during the first week was determined with the visual analog scale. Cone beam computed tomography (CBCT) images were taken at 6 and 12 weeks and 1 year postoperatively for micromorphologic analysis and measurement of pocket depth at the second molar. Patients and image analyses were blinded toward the treatment group (randomized double-blind split-mouth design). Results: Postoperative pain was lowest in the allograft group (control: 5.06 ± 0.53; xenograft: 5.85 ± 0.42; allograft: 3.94 ± 0.52; P < .05). At weeks 6 and 12, early signs of remodeling were observed in the allograft group and the controls, while bone xenograft was still demarcated from the host bone. The 1-year CBCT images showed complete remodeling and integration of allograft with natural trabecular structure, while the xenograft particles were still visible. Support for the second molar was significantly better, as evidenced by less deep and prevalent pockets in the allograft-filled group compared with the controls (P = .017). Conclusion: Filling an extraction socket with albumin-integrated allografts provides superior bone regeneration compared to either native bone build-up or xenograft application or socket regeneration without bone grafting. INT J ORAL MAXILLOFAC IMPLANTS 2020;35:297–304. doi: 10.11607/jomi.7554

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An ideal bone graft should have osteoconductive, osteoinductive, and osteogenic features. Osteoconductivity is provided by the natural structure of the bone, osteoinductivity is granted by structural proteins such as growth factors and bone morphogenetic proteins, and osteogenicity stems from the presence of live mesenchymal stem cells and osteoblasts. Only autograft meets all of these criteria,6,7 although limited availability and donor site morbidity limit its use.7,8 Several bone grafting materials are used as alternatives, each with compromised healing potential compared with autograft.9–12 The most common issues with synthetic and xenogeneic grafts are imperfect integration, graft resorption, and lack of osteoinductive capacity.13–15

Conservation of human bones and using them as allografts overcomes most of these challenges, and the perceived risks of infection and graft resorption are insignificant with modern preparation techniques.16 However, this is achieved at the cost of losing osteogenicity and much-reduced osteoinductivity since the cells and most of the proteins are washed out during the cleaning steps of processing.17 Previous studies have shown that albumin impregnation can restore osteoinductivity by providing the lost albumin content of the tissue.18 Albumin is a well-known protein commonly used in cell culture to increase cell proliferation.19,20 In an in vitro study, albumin increased mesenchymal stem cell adhesion and proliferation on the surface of human bone allografts.21 Animal experiments in bone healing showed that more regenerative bone tissue was formed when the implanted allograft was previously impregnated and freeze-dried with serum albumin than with native grafts.22,23 Similar results were achieved in human studies. In hip and knee implant revisions, the albumin-impregnated graft incorporated perfectly, and active remodeling was observed even in very challenging cases.24 In another study on donor site filling in knee surgery, less postoperative pain and better bone quality were seen with albumin-impregnated allograft than with native grafts.22,23 Similar results were achieved in human studies. In hip and knee implant revisions, the albumin-impregnated graft incorporated perfectly, and active remodeling was observed even in very challenging cases.24

The materials and methods

Study Protocol

The study was a prospective, randomized, double-blind observation with a self-controlled, split-mouth design. After the removal of mandibular third molars, the socket was left empty in the control group, while in the alveolar preservation groups, it was filled in with either bovine xenograft (Bio-Oss, Geistlich) or serum albumin-impregnated human allograft (BoneAlbumin, OrthoSera Dental Zrt). Sockets were randomly assigned to each treatment group with the same incidence rate, so it was planned to perform 16 operations in each group.

Patients

A total of 28 patients (18 men and 10 women) with a mean age of 22.57 ± 1.93 years (range: 30 to 72 years) participated in the study. Panoramic radiographs were taken preoperatively to observe the position and the depth of impaction of the third molar. The inclusion criteria were impacted third molars in both sides of the mandible, covered by intact mucosa. The indication of removal was always preventive and orthodontic due to lack of space or abnormal position of the third molar. All patients needed to be healthy without regular medicament consumption. Exclusion criteria were standard surgical contraindications: acute oral inflammation, previous or present application of bisphosphonates, therapeutic radiologic exposure of the jaws, anticoagulant therapy without International Normalized Ratio (INR) stabilization, and compromised medical status. Patients who met the inclusion and exclusion criteria were required to read and sign an informed consent. To avoid any allocation bias, surgical sites were randomly assigned to the three groups. The randomization codes were enclosed in numbered sealed envelopes.

The study was conducted in accordance with Helsinki guidelines and was reviewed and approved by the Regional and Institutional Committee of Science and Research Ethics of Semmelweis University, Budapest, Hungary (IRB ID: 7786-9/2014/EKU).

Albumin-Coated Allograft

The bone graft was harvested during hip prosthesis revision surgery from the femoral heads, which would have been discarded otherwise. After defatting, extensive washing, and chemical antigen removal, the grafts were ground to a particle size of 0.5 to 1 mm and sterilized with ethylene oxide.27 Then, the grafts were preserved by freeze-drying, followed by submerging the bone granules in a 10% human serum albumin solution under aseptic conditions followed by a second freeze-drying step. Albumin
impregnation does not influence the physical properties of the bone graft.\textsuperscript{21}

**Surgical Method**

Two dentoalveolar surgeons performed the operations. A three-corner mucoperiosteal flap extending from the retromolar area to the level of the first molar was prepared through a vertical release incision in the anterior corner of the flap. Then, the bone covering the impacted tooth was removed as far as the level of the cervical line by using a fissure bur in a surgical handpiece. Heat damage to the bone was prevented by water cooling. When the position of the tooth made it necessary, the molar was separated, and then it was delivered from the socket.\textsuperscript{28} After the removal of the tooth, the socket was debrided mechanically and irrigated with saline. At this time, an assistant opened the allocated envelope for identifying the assignment before applying the graft materials, and the corresponding process was carried out. In the control group, the alveolar socket was left empty; only the blood clot filled it. In two groups, alveolar preservation was applied: the sockets were filled in with either xenograft or allograft. The flap was repositioned to its original place, and it was carefully attached with sutures so that tension in the mucosa was avoided. All patients went through the same rehabilitation protocol. Antibiotics (875 mg amoxicillin + 125 mg clavulanic acid 2 times per day or 300 mg clindamycin 4 times per day) were prescribed for a week, and the patients were advised to take NSAID analgesics (275 mg naproxen maximum 3 times per day) when needed.\textsuperscript{29} The sutures were removed 1 week after surgery. After the removal of the mandibular third molar on one side, the second surgery on the contralateral side was performed 6 weeks later.

**Follow-up Evaluation**

One week after each operation, postoperative pain was evaluated by visual analog scale (VAS) from 0 to 10.\textsuperscript{25,26} The number and duration of analgesics taken were also determined. Ultra-low-dose cone beam computed tomography (CBCT) images were taken at 12 weeks and 12 months following the first surgery, as shown in Fig 1. Since 6 weeks passed during the operation of the two sites of a patient, two types of results were obtained with a single CT: a 12-week image of the earlier operated side and a 6-week image of the contralateral side. The CBCT examinations were performed with 75- or 100-μm voxel sizes (Promax 3D Mid, Planmeca Oy). Localized field of view (FOV) CBCT images were taken at 1 year. Two investigators (F.M., C.D.-N.) experienced in dental radiology, both blinded toward the treatment groups, analyzed the records individually, and then the results were averaged. During the qualitative analyses for trabecular bone formation, the presence or lack of a cortical layer above the socket, graft remnants, and demarcation of the graft from the host bone were observed. Quantitative analysis included density measurements of the newly formed bone and the graft remnants if visible. At the distal site of the second molar, the distance was measured between the cementoenamel junction (CEJ) and the deepest point of the surrounding bone edge defining both the initial and postoperative pocket depth values with DataViewer software (Bruker). One-year CBCT images were converted to DICOM format and imported into CTAn software (Bruker) to carry out micromorphometric analysis. Percent bone volume (BV/TV), bone surface/volume ratio (BS/BV), trabecular thickness (TbTh), trabecular separation (TbSp), trabecular number (TbN), total porosity (Po(tot)), and connectivity (Conn) were measured and compared. For statistical analysis, data were subjected to Kruskal-Wallis analysis of variance (ANOVA) and Tukey’s post hoc test.

**RESULTS**

Three patients did not appear at the 1-year follow-up, which resulted in a final unequal distribution of patients in the three groups (control, n = 13; xenograft, n = 13; allograft, n = 16; with each patient having a different treatment at the two sides). There was no significant difference in age, sex, and morbidity among the groups. Angulation and depth impaction of third molars were also analyzed (Fig 2).

**One-week Results**

The 1-week results showed a similar trend with the number and duration of analgesics taken, with BoneAlbumin getting the lowest scores. Postoperative pain was the lowest when BoneAlbumin was placed into the socket, and the most pain was caused by xenograft.
application (VAS: control, 5.06 ± 0.53; xenograft, 5.85 ± 0.42; allograft, 3.94 ± 0.52). A statistically significant difference was found between the xenograft and the allograft groups ($P = .0122$) (Fig 3).

**Six-week Results**

During the analysis of the CBCT images at 6 weeks, initial regeneration was observed in the control group. A small amount of radiopaque tissue was seen at the bottom of the socket, indicating the beginning of bone formation, although the alveolar socket was mostly filled with connective tissue. In the case of xenograft, radiopaque particles filled the whole alveolar socket, demonstrating the difference in the structure of the bovine bone compared and contrasted with the human tissue. When the socket was filled with allograft, the graft particles were similar to the surrounding original bone tissue in their density. Graft integration was not yet observed at the 6-week time point (Fig 4).

**Twelve-week Results**

At 12 weeks, the control group images showed more opacity in the sockets representing the ongoing regeneration of the bone defect. Where xenograft was applied, the graft granules started to get absorbed at the bottom of the alveolar socket, and significant demarcation was seen at the graft-host interface (Fig 4). When allograft was used for socket preservation, significant bone regeneration was observed. Graft granules seemed more similar to the surrounding bone than they had in the images taken at week 6; in some cases, the graft-host interface was barely noticeable. Less demarcation was observed and more interruptions occurred in the alveolar cortical tissue than in the case of xenograft, indicating the active resorption and rebuilding of the bone (Fig 4).

**One-year Results: Pocket Depth at the Distal Site of Second Molar**

In CBCT images at 1 year, bone development and maturation were observed in each group (Fig 5a). The initial pocket depth at the distal site of the second molar increased by an average of 0.49 mm in five cases of the control group. The initial pocket depth increased in four cases of the xenograft group by an average of 0.39 mm and in two cases of the allograft group by an average of 0.23 mm. In five of seven, one of five, and two of nine cases, the initial pocket remained deeper than 3 mm postoperatively in the control, xenograft, and allograft groups, respectively. The postoperative reduction of the initial pocket depth and the postoperative pocket depth in the different groups are presented in Figs 5b and 5c.

**One-year Results: Visible Signs of Bone Formation**

The alveolar socket was filled with trabecular bone in controls covered by a cortical bone, which formed a continuous layer in 69.23% and was fenestrated in 30.77% of cases. The edge of the alveolar socket remained visible only in 26.89% of cases; the rest exhibited remodeling. The mean density of the newly formed bone was highly variable (162 ± 107 HU).

In the xenograft group, homogenous bone formation was not found; the graft remained visible in each case and became demarcated in 42.31% of cases. The preserved area was never covered by intact cortical bone, and in 65.39% of cases, bone layer could not be detected at all. A fenestrated cortical layer was
Fig 4  6- and 12-week CBCT images. (a) 6-week control: connective tissue fills the socket; (b) 6-week xenograft: high-density graft particles; (c) 6-week allograft: graft particles similar to surrounding bone; (d) 12-week control: connective tissue and initial bone regeneration; (e) 12-week xenograft: demarcation and resorption of graft particles; (f) 12-week allograft: regenerative bone tissue. * = signs of new bone formation.

Fig 5  1-year follow-up CBCT images. (a) White * indicates residual xenograft graft. Note the marginal bone loss at the distal site of the second molar at 1 year. (b) Postoperative reduction of the initial pocket depth. (c) Comparison of postoperative pocket depth. Data are presented as mean ± SE; black * indicates $P < .05$. 

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observed in 34.62% of cases. The mean density of the regenerated bone was 841 ± 350 HU, which is close to the mean density of the graft, 1,074 ± 187 HU.

The most homogenous trabecular bone formation was observed in the allograft group in 43.75% of the cases. The allograft graft particles were detected in only 34.38% and became demarcated in 21.88% of cases. In the vast majority of cases, the regenerated bone was covered by a cortical layer, which was continuous in 15.63% and fenestrated in 68.75% of cases. The mean density of the newly formed bone and the persisting graft were 308 ± 156 HU and 674 ± 278 HU, respectively.

One-year Results: Bone Micromorphology Analysis
The CBCT bone morphology analysis showed significant differences between each group. Bone volume per tissue volume (BV/TV) and trabecular number (TbN) were significantly higher in both groups when alveolar preservation was applied. Bone surface per tissue volume (BS/TV) ratio was the highest in the control group with thin trabeculae and lots of resorption lacunae; it was the lowest in the xenograft group, which had a very dense structure. Allograft had values in between the two. Trabecular thickness (TbTh) showed the same structural properties; it was the highest in the control group and lowest in the xenograft group, with the allograft group having intermediate values. Total porosity (Po(tot)) and connectivity (Conn) refers to remodeling; porosity was the highest at the control group, indicating numerous resorption lacunae, and connectivity was significantly lower in the xenograft group, probably due to the persistent graft particles (Fig 6).

DISCUSSION
Swelling and pain are well-known consequences of third molar removal, and indeed, these cause postoperative discomfort to patients in this type of elective surgery.2

![Fig 6](image_url)

1-year micromorphometry analysis. (a) Bone volume per tissue volume (BV/TV). (b) Bone surface/volume ratio (BS/BV). (c) Trabecular thickness (TbTh). (d) Trabecular number (TbN). (e) Total porosity (Po(tot)). (f) Connectivity (Conn). All data are presented as mean ± SE; * P < .05; ** P < .01.
The lowest pain score was achieved in patients where allograft was applied, meaning its application for alveolar preservation resulted in the least uncomfortable post-operative recovery. Xenograft showed similar results to those of the unfilled alveolar sockets. The underlying mechanism can be accelerated wound healing induced by albumin. It is already known in plastic and reconstructive surgery that circulating albumin levels correlate with healing potential. The high local albumin content may cause fewer foreign body reactions and provides an optimal milieu for soft tissue closure, ultimately resulting in less pain for the patients. The observation that this discomfort of patients can be alleviated by allograft implantation is a significant improvement of the procedure.

Previous microcomputed tomography (micro-CT) analysis of the bone core biopsy samples collected from either the alveolar process or from sinus analysis of the bone core biopsy samples collected at 8 months after the procedure. Xenograft showed similar results to those of the unfilled alveolar sockets. The underlying mechanism can be accelerated wound healing induced by albumin. It is already known in plastic and reconstructive surgery that circulating albumin levels correlate with healing potential. The high local albumin content may cause fewer foreign body reactions and provides an optimal milieu for soft tissue closure, ultimately resulting in less pain for the patients. The observation that this discomfort of patients can be alleviated by allograft implantation is a significant improvement of the procedure.

An interesting observation of the present study is bone regeneration has been extensively studied using various filling materials. One way of investigating regeneration is the observation of persistent bone filler materials: at the 12-month follow-up, these can be considered sequestrated foreign bodies since new bone formation is already completed at this time point. The new bone/residual graft ratio at 1 year was dramatically reduced in both grafted groups. In the case of xenograft, the percentage of residual graft material is reported to be approximately 20% at 6 months or 12% at 21 weeks. A longitudinal study reported that this ratio is dramatically reduced by the end of the first year, which is in line with the results of the present study. Hypercalcified islands of xenograft were found but not allograft at the 1-year follow-up. The aforementioned studies also reported the presence of secluded islands of xenograft residual graft incorporated in the new bone, indicating that the highly mineralized, dense bovine xenograft cannot be fully integrated into the human body and partially persists as a sequestrated dead ceramic particle. Histology further supports this observation: similar xenograft particles connected with newly formed bone were found in bone core biopsy samples collected at 8 months after ridge augmentation. Therefore, a key difference between an advanced human allograft and a mineralized xenograft is that the former fully morphs into the new bone and becomes indistinguishable from the host, while the latter persists as ceramic particles embedded in the tissue.

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An interesting observation of the present study is the high prevalence of initial pocket behind the second molar. The initial pocket is caused by the angulation and the deep impaction of the third molar. Without bone grafting, the postoperative pocket is caused by the inadequate height of the newly formed bone in place of the former third molar, leaving behind a hard-to-clean recess. This recess might be a constant threat for infection, causing inflammation of the extraction socket, as a later complication of third molar removal. Grafting of the third molar socket can significantly alleviate this problem. Similar results were reported in a dog histomorphometry study; however, this phenomenon has not been adequately researched and may require further studies to come to a conclusion on the importance of sulcus depth after third molar extraction. It was concluded that alveolar preservation with a serum-albumin–enhanced allograft is beneficial in regard to the amount and quality of the newly formed bone tissue and also decreases the marginal bone loss on the side of the second molar. The improvement of the advanced allograft over xenograft is also evident in the significantly less pain after surgery, which makes life easier for both the patients and the medical staff.

CONCLUSIONS

Within the limitations of this study, it was concluded that: (1) filling an extraction socket with albumin-integrated allografts provides superior bone regeneration compared to either xenograft or healing without bone grafting; (2) allograft application for alveolar preservation in the extraction socket resulted in the least uncomfortable postoperative recovery; and (3)
alveolar preservation after mandibular third molar removal can significantly prevent the high prevalence of initial pocket formation behind the second molar.

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