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The issue of bioresorption of the Bio-Oss xenogeneic bone substitute in bone defects

Bone grafts and bone substitute biomaterial implemented in guided tissue regeneration should undergo the process of biological decomposition in the recipient's system. Essential in the process of bioresorption are, among others, the mechanism and the duration of biological decomposition, the escalation and type of inflammatory reaction as well as the quality of the final bone structure obtained. Vital is also the issue of safety in bone substitute implementation.

Bio-Oss (Geistlich Pharma AG, Switzerland) is a bovine xenogeneic bone substitute, deproteinised, containing mineral structure close to that of a human bone, which undergoes conversion into bone tissue in the recipient's system. It is thought that the osteo-conduction of Bio-Oss is based on supporting the organisation of bone growth and a closed integration with the bone that is regenerating (4). The newly--created bone, after the procedures of augmentation with the implementation of Bio-Oss, has been described as mature and compact (12) or as a mix of dense and spongy bone (4, 22). With the implementation of histomorphometric methods, tests on biopsies from areas augmented with Bio-Oss have demonstrated a content percentage of regenerated bone tissue amounting to 14.7% (healing 6–8 months, sinus lifting) (6/4) or 48.3% – 63.9% (healing 9 months, postextraction defects) (4). The process of creation of new bone by osteoblasts is connected with simultaneous resorption of the bone substitute by osteoclasts and multinuclear cells. Histological tests of 8 month-old specimens showed the presence of secretorily active osteoblasts (10), whilst in samples taken within a period from 6 months to 4 years from the procedure in some Havers' canals small capillary vessels, mesenchymal cells and osteoblasts have been detected (12). *In vitro* tests showed that human osteoblasts isolated from the marrow and cultured in the presence of Bio-Oss as the matrix after 3 months displayed morphological and functional features typical of those cells (5). Similar osteoblasts were cultured in the three-dimensional model – porous mineral of Bio-Oss bone. After 6 weeks, an accumulation of mature collagen fibres in intra- and extracellular areas was noticed with the arrangement of osteoblasts resembling a natural, three-dimensional structure, whilst the mature collagen from the tested osteoblasts cultured on the porous bone mineral displayed identical formula in tests with the implementation of electrophoresis in comparison with human bone collagen (1). Apart from osteoblasts, histological tests have also shown the presence of lacunae containing osteocytes (10) as well as the presence of osteoclasts resorbing biomaterial (12).

MATERIAL AND METHODS

A 56-year-old female patient underwent the procedure of maxillary sinus lifting with the implementation of Bio-Oss biomaterial. The implantation of regenerated bone took place 30 months

after the procedure by introducing one stomatological implant. During implantation trepanobiopsy was carried out, thereby obtaining a bone cylinder 1.2 cm long and 0.2 cm wide. The tissue was preserved in 10% buffered formalin, and consequently decalcified in a solution of soda diversenate. Following decalcification, the bone was sunk into a paraffin-embedded tissue block and cut into 3.5 μm thick scraps of which some were made into stained preparation with the implementation of the routine hematoxylin-eosin method in the automatic stainer. On the remaining paraffin scraps immnunohistochemical determination was carried out with the implementation of monoclonal antibodies (Dako and Novocastra) in compliance with procedures recommended by the producer. Each staining was carried out with positive and negative control. Prior to the commencement of the test, the preparations were subjected to the process of antigen unblocking. The choice of method: trypsin etching boiling in a water bath or citrate buffer depended on the recommendations of the antibody producer. In order to demonstrate: macrophages, T and B lymphocytes, vascular endotheliums as well as mast cells, a reaction with the following antibodies was performed: CD68, CD3, CD 79α, CD34 and Mast Cell Tryptase. All preparations, both routine and those determined immunohistochemically, were evaluated under a light microscope.

RESULTS

The bone cylinder evaluated in preparations routine stained with hematoxylin-eosin contained correct, packed bone smoothly changing into spongy bone, with osteocytes in bone lacunae (Fig. 1). The presence of correct, round and elongated osteoblasts was noticed on the surface of bone trabeculae, focally creating clusters of several cells (Fig. 2). Osteoblasts were accompanied by multinuclear osteoclasts.

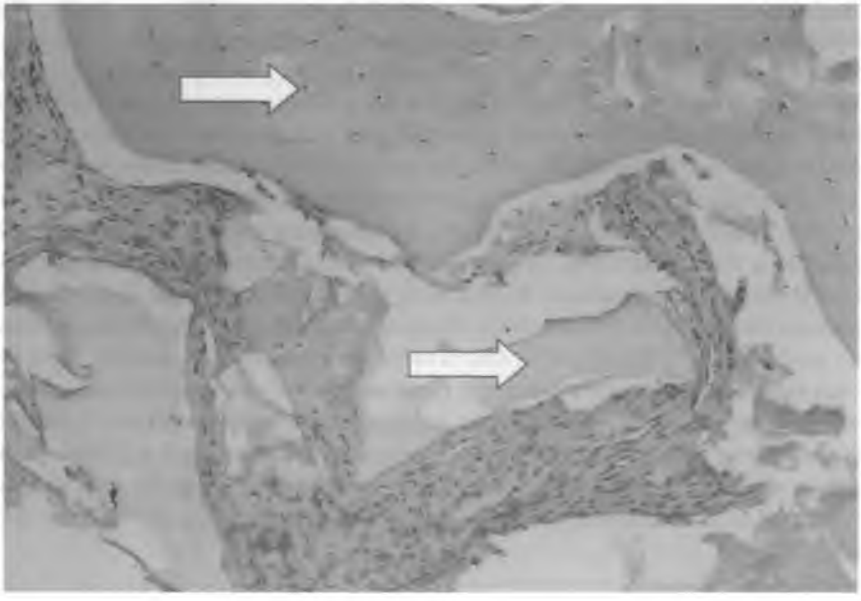

Fig. 1. HE staining, magn. 100 x. Normal compact bone smoothly changes into spongy bone; with osteocytes in the osseous lacunae. Remnants of the Bio-Oss biomaterial in the form of fine particles between the osseous trabeculae

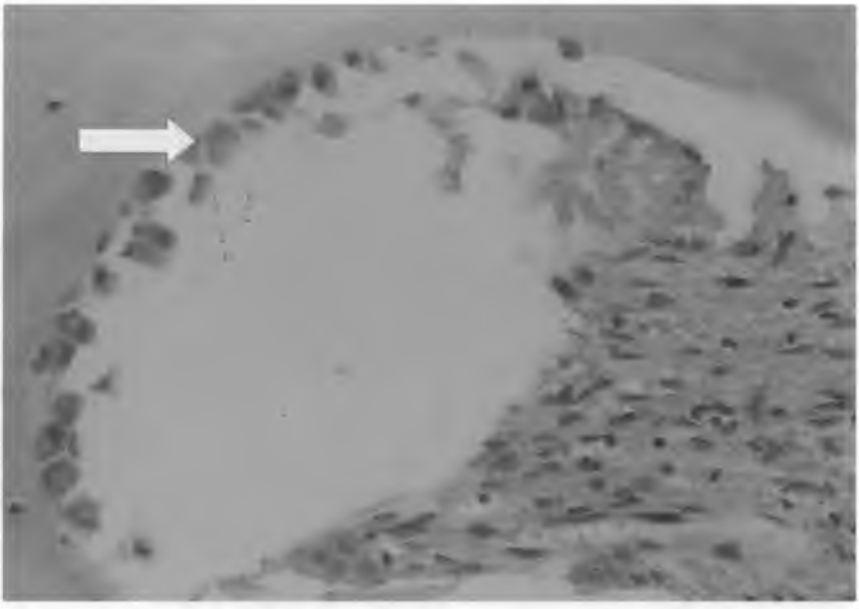

Fig. 2. HE staining, magn. 250 x. Normal, round and elongated osteoblasts on the surface of the osseous trabeculae, focally creating clusters of several cells

Most of the intratrabecular space was filled with fibrous connective tissue with numerous capillary vessels (Fig. 3). Among the bone trabeculae, there were also the remnants of Bio-Oss biomaterial in the form of minute particles (Fig. 1). The presence of red marrow, ostoid focuses in the connective tissue, inflammatory infiltration with granulocytes as well as active resorptive changes in the bone tissue and around the particles of biomaterial was not recorded.

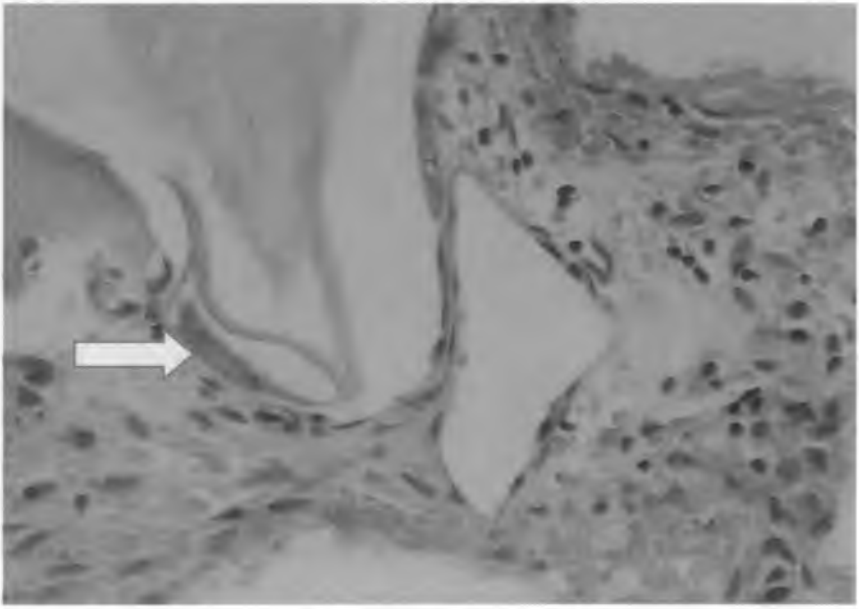

Fig. 3. HE staining, magn. 320 x. Multinuclear osteoclasts accompany osteoblasts

Immunohistochemical staining disclosed the presence in the connective tissue in the intratrabecular areas of single macrophages (Fig. 4, 5), few reactive fine lymphocytes T (Fig. 6) and B (Fig. 7). No increase in the number of mast cells was noticed (Fig. 8).

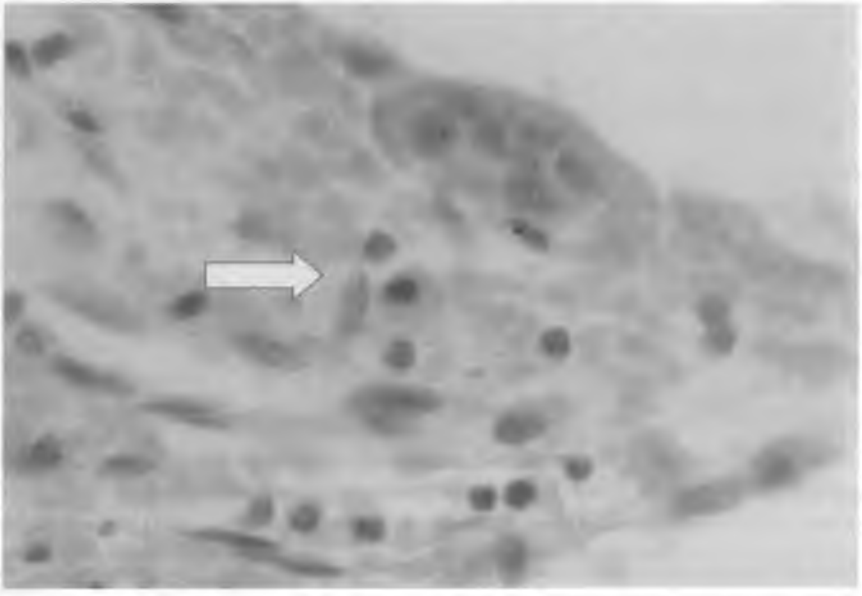

Fig. 4. HE staining, magn. 750 x. The intratrabecular space is filled with fibrous tissue with numerous capillary vessels

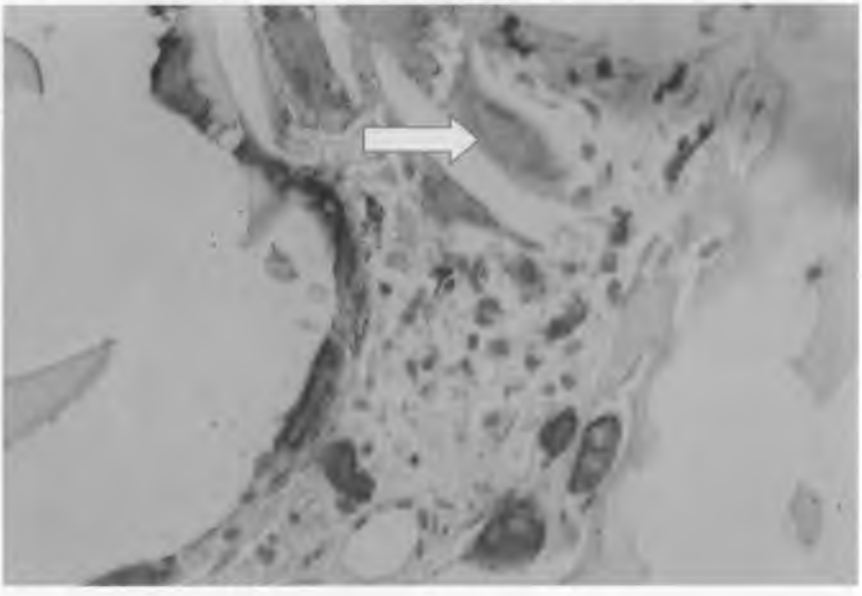

Fig. 5. CD 68 staining, magn. 320 x. Single macrophages are present in the intratrabecular spaces

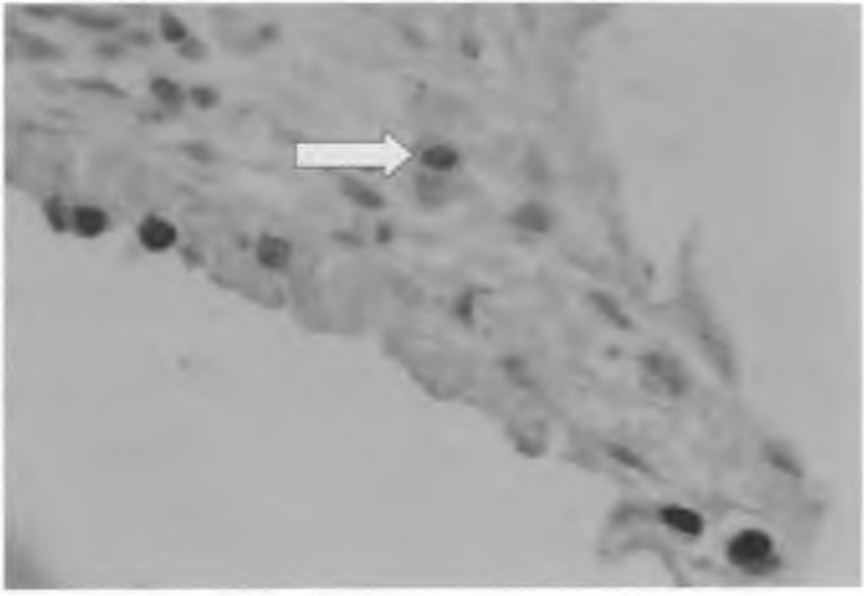

Fig. 6. CD 3 staining, magn. 640 x. Rare, reactive, fine T lymphocytes in the intratrabecular spaces

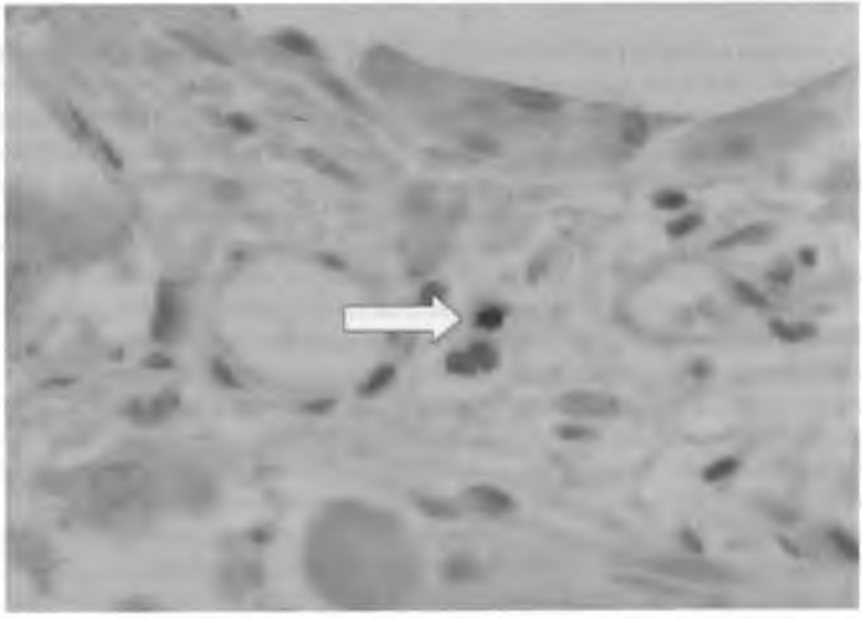

Fig. 7. CD 79 staining, magn. 640 x. Rare, reactive, fine B lymphocytes in the intrabecular spaces

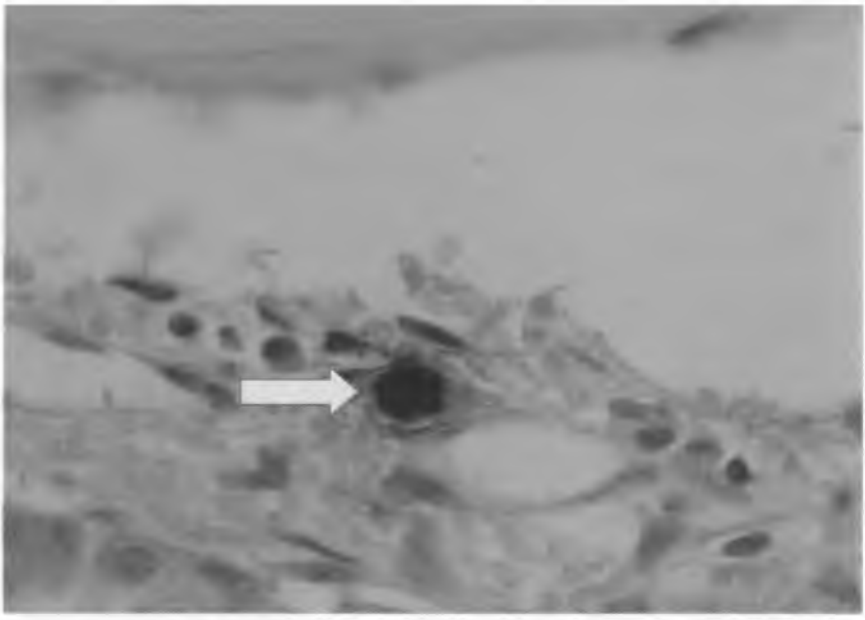

Fig. 8. Mast cells staining, magn. 640 x. Normal number of mast cells

DISCUSSION

The main issue concerning Bio-Oss biomaterial is connected with its biodegradation and replacement by the recipient's bone. Some researchers observed its fast restructuring into the recipient's own bone, whilst other authors noticed slow resorption of the biomaterial or its total lack (12). In our research we have noticed the presence of remnant particles of Bio-Oss in the vicinity of the correct bone 30 months following the implantation of bone substitute, which testifies to slow bioresorption. Similarly, in biopsy preparations obtained by S k o g l u n d et al. from patients in 9 to 44 months following the implantation of Bio-Oss, the presence of biomaterial particles was observed. This is why the fact of Bio-Oss as being an absorbable material has been questioned (16). Yet, histological tests conducted by S c h l e g e l and D o n a t h demonstrated the presence of non-resorbed Bio-Oss particles 6 years and more following implantation. Therefore, the authors described that material as non-resorbable (15).

Our observations have shown that after 30 months, the regenerated bone has a mature character, as we have not detected the presence of osteoid which would indicate an active process of new bone creation. The occurrence of osteoid has, however, been described in biopsies obtained 9 months after Bio-Oss augmentation (4). So far, we have not come across research on regenerated bone with the implementation of Bio-Oss concerning the presence of immunologically competent cells. In our preparations we have determined a small number of T and B lymphocytes as well as the absence of granulocytes, whilst the mast cells appeared in a number consistent with the norm.

Tests results testify to the absence of an active inflammatory process and antigen stimulation as well as to a descending character of the inflammatory infiltration, in the given case 30 months from the augmentation procedure which, however, seems to require further research on a greater number of cases.

P i a t e l l i et al. describe the newly-created bone in maxillary sinuses following the procedure of sinus lifting with the implementation of Bio-Oss as mature and compact (12); however, on the basis of histopathological tests, the regenerated bone tissue after the augmentation of postextraction dental alveoluses has been described as a mix of packed and spongy bone (4, 22). Our observations have

shown the presence of correct bone appearing to be on the verge of packed and spongy bone. A vital issue connected with the implementation of bone substitutes is the question of safety.

Practical tests concerning the production process as well as theoretical calculations indicate that the implementation of Bio-Oss does not carry risks of infecting with prions and Creutzfeld Jacob disease (20). It has been established in detailed biochemical, histochemical and biophysical tests that Bio-Oss does not contain any proteins in measurable quantities (6); nevertheless, according to other reports it may contain residual proteins (17).

Independent of the fact that apart from the Bio-Oss biomaterial resorption and the new creation of bone tissue also enduring the presence of unabsorbed Bio-Oss particles around the restored bone after 44 months (16), 4 years (12) and 6 years (15) from the moment of implantation has been noted, it seems that the quality of regenerated bone filling defects or participating in the process of implant osteointegration is satisfactory for the clinician.

Bio-Oss is successfully utilised in a number of dental surgery procedures: in augmentation of the alveolar process following tooth extraction, in order to avoid alveolar atrophy (3, 4), in the height and width restoration of the alveolar process (8), in implantation to fill bone defects around the implants (9, 19), in regenerating of the process bone defects around the exposed implants (2, 21), in the procedure of sinus lifting (3, 18), in filling bone defects following tooth resection (14), in filling bone cavities after cyst removal (11, 14) and in treating bone defects in parodontium diseases (7, 13).

CONCLUSIONS

Performed observations have shown that after 30 months from implementing Bio-Oss, the regenerated bone in the above-described case had a mature character, which is consistent with previous reports.

It seems that although the fact of creation of new bone structure is indisputable, nevertheless the process of Bio-Oss biological decomposition should be described as slow bioresorption since unresorbed particles of the biomaterial were noticed 30 months after implantation.

The small number of T and B lymphocytes observed, as well as the absence of granulocytes and mast cells in a number consistent with the norm suggest that despite the presence of residual particles of Bio-Oss in the researched bone tissue, no active inflammatory processes and antigen stimulation take place. Nevertheless, the above-described observations require confirmation by a larger number of cases.

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SUMMARY

Bone grafts and bone substitute biomaterial implemented in guided tissue regeneration should undergo the process of biological decomposition in the recipient's system. The aim of this work is the presentation of current views concerning the issue of Bio-Oss bovine bone bioresorption and their

juxtaposition with the results of the author's own research. The work presents histopathological and immunohistochemical tests of the xenogeneic Bio-Oss preparation from biopsies carried out 30 months after implantation. It was observed that the preparations contained correct bone neighbouring remnant particles of Bio-Oss, intratrabecular fibromatosis around the implant, abundant vascularisation, absence of osteoid and of active inflammatory process. A small number of T and B lymphocytes was detected. The results obtained in the above-described case testify to the descending character of the inflammatory infiltration 30 months after the implementation of Bio-Oss and efficient restoration of the bone. The prevalent view in literature is that Bio-Oss is resorbable biomaterial. However, there are also reports questioning this view as remnants of Bio-Oss have been detected even 44 months after implantation into the bone defect. In the author's own cases, Bio-Oss remnants could be observed 30 months after implanting. It seems that although the creation of new bone structure is indisputable, the process of biological decomposition of Bio-Oss should be described as slow bioresorption.

Zagadnienie bioresorpcji ksenogenicznego substytutu kostnego Bio-Oss w ubytkach kostnych

Przeszczepy kostne i biomateriały kościozastępcze stosowane w sterowanej regeneracji tkanek powinny ulegać procesowi biologicznego rozkładu w organizmie biorcy. Celem pracy jest przedstawienie aktualnych poglądów dotyczących zagadnienia bioresorpcji minerału kości bydlęcej Bio-Oss oraz zestawienie ich z wynikami badań własnych. W pracy zaprezentowano badania histopatologiczne i immunohistochemiczne ksenogennego preparatu Bio-Oss z biopsji pobranych w 30 miesięcy po wszczepieniu. W preparatach stwierdzono obecność kości prawidłowej sąsiadującej z resztkowymi cząstkami Bio-Oss, włóknienie międzybeleczkowe koło implantu, bogate unaczynienie, brak osteoidu oraz brak aktywnego procesu zapalnego. Zaobserwowano niewielką liczbę limfocytów T i limfocytów B oraz komórek tucznych. Uzyskane wyniki w opisanym przypadku świadczą o zejściowym charakterze nacieku zapalnego w 30 miesięcy po zastosowaniu Bio-Oss i skutecznej odbudowie kości. W piśmiennictwie przeważa opinia, że Bio-Oss jest biomateriałem resorbowalnym, aczkolwiek spotyka się doniesienia poddające w wątpliwość ten pogląd, gdyż resztki Bio-Oss spotykano nawet w 44 miesiące po wszczepieniu w ubytku kostnym. W badaniach własnych cząstki tego materiału obserwowano 30 miesięcy po wszczepieniu. Chociaż nie podlega dyskusji fakt powstawania nowej struktury kostnej, to proces rozkładu biologicznego Bio-Oss należałoby określić jako powolną bioresorpcję.