Eight-Year Results of Site Retention of Anorganic Bovine Bone and Anorganic Bovine Matrix

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The long-term fate of some biomaterials is still unknown, and the reports present in the literature are not conclusive as to whether these biomaterials are resorbed over time or not. Different reports can be found with regard to the resorption behavior of anorganic bovine bone (ABB). The aim of the present study was to provide a comparative histological and histomorphometrical evaluation, in the same patient, of 2 specimens retrieved from a sinus augmented with ABB and with anorganic bovine matrix added to a cell-binding peptide (PepGen P-15), respectively, after a healing period of 6 months and after 8 years of implant loading, to evaluate the resorption of both biomaterials. A unilateral sinus augmentation procedure with ABB (50%) and with PepGen P-15 (50%) was performed in a 54-year-old male patient. Two titanium dental implants with a sandblasted and acid-etched surface were inserted after 6 months. During this procedure, 2 tissue cores were retrieved from the sinus with a trephine, before implant insertion. After an additional 6 months, a fixed prosthetic restoration was fabricated. One of these implants, after a loading period of 8 years, fractured in the coronal portion and was removed. Both specimens, one retrieved after a 6-month healing period and the other after an 8-year loading period, were treated to obtain thin ground sections. In the 6-month specimen, the histomorphometry showed that the percentage of newly formed bone was 27.2\% ± 3.6\%, marrow spaces 35.6\% ± 2.3\%, residual ABB particles 25.1\% ± 1.2\%, and residual PepGen P-15 particles 12.1\% ± 2.2\%. In the 8-year specimen, the histomorphometry showed that the percentage of newly formed bone was 51.4\% ± 4.8\%, marrow spaces 40\% ± 7.1\%, residual ABB particles 6.2\% ± 0.7\%, and residual PepGen P-15 particles 2.4\% ± 0.5\%. Both biomaterials underwent significant resorption over the course of this study.

Key Words: anorganic bovine bone, biomaterials resorption, cell-binding peptide, sinus augmentation

INTRODUCTION

The long-term fate of some biomaterials is still unknown, and the reports present in the literature are not conclusive as to whether these biomaterials are resorbed over time or not.\textsuperscript{1-11} Different reports can be found about the resorption behavior of anorganic bovine bone (ABB), and this topic is still a subject of controversy.\textsuperscript{2} A group of researchers found that ABB resorbs over time.\textsuperscript{5} Wallace et al\textsuperscript{3} reported that the amount of ABB gradually decreased over time and was completely absent in a 20-month sample. The resorption rate of ABB in vivo has been reported to be 2–3 years,\textsuperscript{4} and Tadjoedin et al\textsuperscript{5} reported a decrease of about 10% per year. Sartori et al\textsuperscript{6} found that the potential metabolization of ABB by osteoclasts could be confirmed by the progressive increase in relative bone volume in a 10-year period (29.8\% at 8 months vs 86.7\% at 10 years). Valentini et al\textsuperscript{7} reported that the density of ABB decreased by a little more than 10% between 6 and 12 months, suggesting a slow but active resorption. Berglundh and Lindhe\textsuperscript{8} found that ABB with time became integrated and subsequently replaced by newly formed bone. Zaffe et al\textsuperscript{9} found that in most patients, there was a scarcity or absence of ABB granules in the biopsies. Artzi et
al\textsuperscript{10} evaluated morphometrically the rate of resorb-
ability of an inorganic bovine bone material in a
standardized intrabony defect in dogs at 3, 6, 12,
and 24 months. They observed a resorptive phase
of ABB up to 6 months but did not notice a
continued resorption at up to 24 months examina-
tion. According to Yildirim et al,\textsuperscript{11} the inward
growth of bone indicated a slow resorption of
ABB. In in vitro studies from our laboratory, it was
found that it was possible to generate cells with the
characteristics of osteoclasts on the surface of
different xenografts and that these cells were able
to resorb these different biomaterials.\textsuperscript{12–14} On the
other hand, no evident signs of any major ABB
resorption was found by other researchers\textsuperscript{1,15,16};
Hallman et al\textsuperscript{1} reported that the mean size of the
particles was similar in the 6-month and 3-year
specimens. Mordenfeld et al\textsuperscript{17} did not find, after 11
years, any obvious signs of resorption or decrease in
size of ABB particles over time.

The aim of the present study was a comparative
histological and histomorphometrical evaluation, in
the same patient, of 2 specimens retrieved from a
sinus augmented with ABB and with anorganic
bovine matrix added to a cell-binding peptide
(PepGen P-15), respectively, after a healing period
of 6 months and after an 8-year implant loading, to
evaluate the resorption of both biomaterials. The
peri-implant bone response to the implant surface
and the bone-implant contact percentage have
already been reported in another study.\textsuperscript{18}

\textbf{Materials and Methods}

A unilateral sinus augmentation procedure, by a
lateral wall approach, with ABB (50%; Geistlich Bio-
Oss, Geistlich, Wohlhusen, Switzerland) and Pep-
Gen P-15 (50%; DENTSPLY, Tulsa Dental Specialties,
Tulsa, OK) was performed in a 54-year-old male
patient. The patient had a noncontributory past
medical history. At the initial visit, the patient
underwent a clinical and occlusal examination, and
radiographs were performed (Figures 1 and 2). Two
titanium dental implants with a sandblasted and
acid-etched surface (DPS implants, DENTSPLY-
Friadent, Mannheim, Germany) were inserted after
6 months (Figure 3). During this procedure, 2 tissue
cores were retrieved from the sinus with a
trephine, before implant insertion. After an addi-
tional 6 months, a fixed prosthetic restoration was
fabricated. One of these implants, after an 8-year
loading period, fractured in the coronal portion
(Figure 4) and was removed with a 5-mm trephine
bur. Both specimens, one retrieved after a 6-month
healing period and the other after an 8-year
loading period, were treated to obtain thin ground
sections.

\textbf{Specimen processing}

Both specimens were washed in saline solution and
immediately fixed in 4% paraformaldehyde and
0.1% glutaraldehyde in 0.15 M cacodylate buffer at
4°C and pH 7.4, to be processed for histology. The
specimens were treated to obtain thin ground
sections with the Precise 1 Automated System
(Assing, Rome, Italy).\textsuperscript{19} The specimens were dehy-
drated in an ascending series of alcohol rinses and
embedded in a glycolmethacrylate resin (Technovit
7200 VLC, Kulzer, Wehrheim, Germany). After
polymerization, the specimens were sectioned,
along their longitudinal axis, with a high-precision
diamond disk at about 150 \(\mu\)m and ground down to
about 30 \(\mu\)m with a specially designed grinding
machine (Exakt, Norderstedt, Germany). A total of 2
slides were obtained for each specimen. The slides
were stained with acid fuchs in and toluidine blue.
The slides were observed in normal transmitted
light under a Leitz Laborlux microscope (Leitz,
Wetzlar, Germany) and polarized-light microscopy
(Leitz, Wetzlar, Germany).

Percentages of newly formed bone, residual
grafted materials, and marrow spaces were mea-
sured by histomorphometric analysis, which were
carried out using a light microscope (Laborlux S,
Leitz, Wetzlar, Germany) connected to a high-
resolution video camera (3CCD, JVC KY-F55B, JVC,
Yokohama, Japan) and interfaced to a monitor and
PC (Intel Pentium III 1200 MMX, Intel, Santa Clara,
CA). This optical system was associated with a
digitizing pad (Matrix Vision GmbH, Oppenweiler,
Germany) and a histometry software package with
image-capturing capabilities (Image-Pro Plus 4.5,
Media Cybernetics Inc, Immagini & Computer Snc,
Milano, Italy).

\textbf{Results}

\textbf{Six-month specimen}

It was possible to observe residual particles of both
biomaterials surrounded by newly formed bone.
The presence of newly formed bone tissue was indicated by the high staining affinity for acid fuchsin. Some particles of ABB were surrounded by mineralized tissues, and the biomaterial particles had served as an osteoconductive scaffold (Figure 5). Some ABB particles were bridged by newly formed bone. Only a few particles of PepGen p-15 were in contact with newly formed bone, while other particles were encapsulated by fibrous soft tissues. No inflammatory cells or foreign-body reaction cells were present around the biomaterial particles. No gaps were present at the bone-particle interface, and the bone was always in close contact with the particles. Osteoblasts could be seen depositing osteoid matrix directly on the ABB particles (Figure 6). No osteoclasts were observed. Marrow spaces contained marrow stromal cells, adipocytes, and small blood vessels.

Histomorphometry showed that the percentage of newly formed bone was 27.2% ± 3.6%, marrow spaces 35.6% ± 2.3%, residual ABB particles 25.1% ± 1.2%, and residual PepGen P-15 particles 12.1% ± 2.2%.

**Eight-year specimen**

Biomaterial particles appeared to be embedded in compact, mature bone (Figure 7). Most of the ABB particles and some of the PepGen P-15 particles were completely surrounded by bone. No inflammatory cell infiltrate and no foreign-body reaction were present (Figure 8). No gaps were present at the bone-biomaterial interface. Osteons and cement lines, indicating areas of remodeling, were present.

Histomorphometry showed that the percentage of newly formed bone was 51.4% ± 4.8%, marrow spaces 40% ± 7.1%, residual ABB particles 6.2% ± 0.7%, and residual PepGen P-15 particles 2.4% ± 0.5%.

**DISCUSSION**

The resorption times and the ultimate replacement of some xenografts by newly formed vital bone are
still not fully understood. Most reports concern the resorption capability of ABB, while, according to the author’s knowledge, no study is present in the literature about the metabolization rate of PepGen P-15. An ideal material should provide a framework for continuous bone resorption and bone deposition. The osteoclasts’ resorption behavior plays a pivotal role in the integration of different bone substitute biomaterials into the bone tissue. The ability to form new bone must be balanced by the resorption rate of the biomaterial. Opposing views have been reported about the continued long-term presence of the grafted biomaterials particles, and the controversy continues. On one hand, the presence of graft particles in about 25% to 30% of the treated site volume may interfere with normal healing, by disrupting vascularization and depriving cells of nourishment. This could create problems for
the osseointegration of implants. From a theoretical standpoint, the lack of resorption of a xenograft could produce a negative consequence on the biomechanical properties of the augmented sites and their implant-supporting capabilities, because the augmented area is composed of a composite rather than a homogenous bone structure. It is still unknown if the long-term success of implants could be compromised by their insertion into sites where there is still the presence of residual graft particles. Some clinicians prefer implant insertion in areas of only vital bone and not in bone areas regenerated by bone substitute materials. However, the structure constituted by bone and grafted particles could, on the other hand, be advantageous and behave as a cancellous bone network around an implant. In some clinical indications, a low substitution rate may be beneficial, during which the physical support from the graft material maintains the initial dimensions of the augmented volume area and prevents soft-tissue collapse, providing a stress shield against pressure exercised by the overlying gingival or mucosa of the sinus. Moreover, the almost complete incorporation of the ABB particles in bone creates a dense, hard tissue network. It seems likely that this network provides mechanical support to loaded dental implants, which is comparable to or even exceeds that of native bone. ABB resorption does not seem to be absolutely necessary to provide predictable osseointegration. In the present specimens, a significant decrease in the percentage of both biomaterials over the long term was found. This is in contrast with the studies by Hallman et al., Artzi et al. and Mordenfeld et al., who did not find any sign of resorption of the grafted biomaterials over time. Moreover, the progressive increase in the percentage of the newly formed bone over time could point, as already stated by Sartori et al., to a significant resorption of both grafted biomaterials.

**CONCLUSION**

Both biomaterials underwent a significant resorption over the period of this study. Both biomaterials were osteoconductive, and their continued presence in the peri-implant bone did not produce any untoward effect. Both biomaterials appeared to be histologically biocompatible in that no inflammatory cell reactions and no foreign-body reactions were observed.

**ABBREVIATION**

ABB: anorganic bovine bone

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**REFERENCES**


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