In Vitro Osteoclast Resorption of Bone Substitute Biomaterials Used for Implant Site Augmentation: A Pilot Study

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Purpose: *This observational study examined the resorptive behavior of normal neonatal rabbit osteoclasts grown on slices of bovine cortical bone as compared to samples of commercially available bone substitute biomaterials. It also examined the surface characteristics of these materials.* ***Materials and Methods:*** *The 11 materials tested fell into 3 groups: (1) bone-derived, including freeze-dried human rib block, human demineralized freeze-dried bone, and deproteinated bovine bone; (2) synthetic hydroxyapatites (HA); and (3) synthetic non-HA, including coated methacrylates and coated silica glass. After 4 days in culture, 1 group of samples of each material underwent scanning electron microscopy (SEM) to evaluate resorptive pitting versus controls, while another group underwent tartrate-resistant acid phosphatase staining and light microscopy to examine osteoclast numbers and morphology. The 2 bovine-derived HA materials also underwent immunohistochemical staining and surface chemistry analysis.* ***Results:*** *While most of these materials supported osteoclast attachment, some spreading, and survival in culture, only the bone-derived materials, with the exception of sintered deproteinated bovine bone, showed large scalloped-edged resorption pits with trails and exposed collagen when examined by SEM, although not to the same extent as unprocessed natural bone material. The HA materials and the sintered deproteinated bovine bone showed evidence of etching with smaller pits but no evidence of resorptive trail formation. The non-HA materials showed no evidence of pit formation or trails. Under immunohistochemical staining, Bio-Oss appeared to be positive for type I collagen after osteoclast activity on its surface, while Osteograf/N showed no positive staining. Surface chemistry analysis revealed nitrogen present in Bio-Oss specimens (0.17% to 0.47%), while there was no nitrogen detected in the Osteograf/N (0.00%); the percent nitrogen observed in normal bovine bone controls was 6.01% to 9.25%.* ***Discussion:*** *The bone-derived materials supported osteoclast activity on the material surface in a way that facilitated formation of the more complex resorption pits in vitro. Assuming the rate of pit formation observed in vitro mimics that observed in vivo, the quantity and type of osteoclastic remodeling seen on non–bone-derived materials—and perhaps sintered bone-derived materials—would be extremely slow to negligible. Physiologic removal of non–bone-derived bone substitutes in vivo may occur by methods other than osteoclast resorption.* ***Conclusions:*** *Allogenous and xenogenous bone-derived materials that undergo delayed physiologic resorption may be more appropriately used with a staged surgical approach when used in sites intended to support osseointegrated dental implants. The combination of collagen staining and the presence of nitrogen suggest that there may be residual protein in Bio-Oss.* (Int J Oral Maxillofac Implants 2002;17:321–330)

Key words: *alveolar ridge, alveolar ridge augmentation, biocompatibility, bone remodeling, bone resorption, bone substitutes, cell culture, collagen, hydroxyapatites, immunohistochemistry, osteoclasts, scanning electron microscopy, x-ray emission spectrometry*

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Since the first reports of the use of autogenous bone grafts to support endosseous dental implants,[1–3] bone augmentation procedures have been employed frequently in the practice of implant dentistry for implant site augmentation. Currently available bone substitute biomaterials fall into the categories of autogenous (transplanted host bone), allogenous (transplanted bone from the same species), xenogenous (transplanted bone from a different species), and alloplastic (synthetic materials). Although autogenous bone continues to be recognized as the "gold standard,"[4] largely because of its osteogenicity, autograft harvest has the disadvantages of increased cost and morbidity. These considerations have led researchers and clinicians to explore the clinical application of allogenous, xenogenous, and alloplastic materials.[5]

The establishment and long-term maintenance of the dynamic bone-implant interface requires initial and continuous remodeling.[6] Some manufacturers suggest that their bone substitute biomaterials are remodeled in the same manner as the host bone, while others suggest that theirs are nonresorbable. Bone substitute materials must, of course, be biocompatible, noninfectious, and nonantigenic. Although most are not considered to be osteogenic or osteoinductive, they should at least be osteoconductive and capable of undergoing normal physiologic remodeling to yield functional bone capable of supporting functional osseointegration of dental implants.

There is still debate as to the extent to which hydroxyapatite (HA) materials truly undergo osteoclastic remodeling in vivo.[7] Increasingly, it appears that osteoclast-resorptive behavior on alloplastic HAs will depend on factors such as solubility of the material and surface rugosity, as well as the physicochemical features of the HA formed, which depend partly on the sintering temperature.[8–12]

The common source of xenogenous HA bone substitutes is bovine bone. Two established products in this category are Osteograf/N (Dentsply CeraMed Dental, Lakewood, CO) and Bio-Oss (Osteohealth, Shirley, NY), which differ principally in their methods of processing of the source bovine bone. Osteograf/N is produced by a high-temperature, nonchemical process involving sintering, while Bio-Oss is produced by a low-temperature, chemical extraction process. Both materials are described as "anorganic" in their respective product literature.

The present study compared the cell attachment, morphology, and resorptive behavior of osteoclasts in vitro on various types of bone substitutes commonly used for implant site augmentation, as well as the surface characteristics of these materials.

MATERIALS AND METHODS

Materials Examined

The following materials were investigated:

1. Bovine cortical bone slices (as positive control) (prepared in-house from fresh-frozen bovine radii)
2. Freeze-dried human rib block (University of Miami Tissue Bank, Miami, FL)
3. Demineralized freeze-dried human cancellous bone particulate (LifeNet Tissue Services, Virginia Beach, VA)
4. Deproteinated bovine cancellous and cortical bone block and particulate ("B") (Bio-Oss, Osteohealth)
5. Deproteinated bovine bone particulate ("N") (Osteograf/N-700, Dentsply CeraMed Dental)
6. Synthetic HA particulate ("C") (Calcitek, SulzerMedica, Houston, TX)
7. Synthetic HA particulate ("P") (Osteograf/P, Dentsply CeraMed Dental)
8. HA cement (BoneSource, Howmedica Leibinger, Dallas, TX)
9. Coated silica glass particulate (Perioglas, Block Drug, Jersey City, NJ)
10. Coated acrylic particulate (Bioplant HTR, Septodont, New Castle, DE)

Four groups (n = 5) of each material were used for each repeat, 2 groups for scanning electron microscopy (SEM), and 2 for light microscopy (LM) following tartrate-resistant acid phosphatase (TRAP) staining.

Harvesting and Plating of Osteoclasts

This was performed ad modum Chambers and coworkers.[13] Osteoclasts were isolated from the endosteal surfaces of the long bones of normal neonatal male New Zealand white rabbits and resuspended in α-minimum essential medium (MEM) with 15% fetal calf serum, vitamin C (50 μL/mL), Vitamin D_3 (10^{-8} mol/L) (Sigma, Sigma-Aldrich, St. Louis, MO), and antibiotics (100 μg/mL of penicillin, 0.3 μg/mL of fungizone, and 50 μg/mL of gentamicin). All tissue culture chemicals were obtained from Gibco BRL Products (Canadian Life Technologies, Burlington, Ontario, Canada) unless otherwise stated.

The suspension was plated onto 2 groups of samples (n = 5) of each material at equivalent densities; another group (n = 5) each of unprocessed bovine bone and both deproteinated bovine bone materials was also plated for eventual immunohistologic investigation. Two further groups of samples

(n = 5) of each material were plated with medium only as control. The cultures were maintained in a humidified incubator at 37°C/5% CO_2 with medium changed every 48 hours. This protocol was repeated twice.

All animal procedures were performed according to an approved protocol from the Dalhousie University Animal Care and Use Committee in compliance with the regulations of the Canadian Council on Animal Care.

TRAP Staining

This was performed ad modum Minkin.[14] After 4 days in culture, the medium was removed and samples were washed twice with phosphate-buffered saline (PBS). Adherent cells were fixed with 10% neutral buffered formalin for 30 minutes and washed twice with distilled water. Specimens were then incubated with ASBI phosphate as substrate in Michaelis-Veronal acetate buffer at pH 5.0 in the presence of 50 mmol/L L-tartaric acid (ICN Pharmaceuticals, Montreal, Quebec, Canada). Hexazonium pararosanilin was used as a coupling agent. TRAP+ cells stained ruby red after 30 minutes of exposure. The reaction was stopped by repeated washing with distilled water. All staining chemicals were obtained from Sigma (Sigma-Aldrich) unless otherwise stated. Because of the inherent difficulties of quantitation of cells per unit area as a result of the complex 3-dimensional surfaces of these diverse materials, a subjective ordinal scoring system was used.

Type I Collagen Immunohistochemistry

Following 4 days of incubation, 5 samples each of unprocessed bovine bone and both deproteinated bovine bone groups were removed from cell culture and sonicated in PBS. Adherent cells were removed from samples using 0.25 mmol/L ammonium hydroxide and then samples were treated with 3% hydrogen peroxide in methanol for 30 minutes to inhibit endogenous peroxidases. Specimens were then washed with PBS, and blocking buffer (10:1 ratio of 0.1 mol/L Tris-HCl and 0.1 mol/L Tween to PBS and 1% bovine serum albumin) was added for another 30 minutes. The materials were subjected to mouse monoclonal antibovine type I collagen antibody (1:2,000 dilution) (#C2456, Sigma) for 30 minutes and then washed twice with PBS. The primary antibody was then conjugated with a secondary antibody (F-Ab anti-mouse IgG labeled with horseradish peroxidase at 10 mL per 8 µL distilled water) for 30 minutes followed by another PBS washing. Finally, a 1:10 dilution of 3,3' diaminobenzidine tetrachloride (DAB) solution was added and left to react with the secondary antibody for 30 minutes before a final rinsing with PBS. The bovine type I collagen can then be identified by the dark brown to black staining.

The Sigma anti-bovine type I collagen antibody was listed as also potentially recognizing human, lapine, cervine, and porcine type I collagen as well as that from rat, but not mouse, type I collagen. Thus, there was a very slight possibility that the rabbit stromal cells in the osteoclast preparation could synthesize some type I collagen, producing a false-positive reaction. Hence, to control for the possibility of cross-reactivity of the mouse anti-bovine type I collagen antibody with rabbit collagen produced by the rabbit stromal cell from the osteoclast preparation, a second series of assays was undertaken using mouse marrow to generate murine osteoclasts on these specimens. For these experiments the mouse marrow was harvested from 6-week-old female CD1 mice and cultured for 8 days in the presence of Vitamin D_3, antibiotics, and 15% fetal calf serum supplemented by α-MEM as above.

Light Microscopy

All TRAP-stained and DAB-stained samples were examined and photographed using the 4× or 10× objective on a Zeiss Tessovar microscope (Carl Zeiss, Jena, Germany).

Scanning Electron Microscopy

Specimens were dehydrated by the critical point–drying method, whereby they were placed in ethanol and subjected to a series of liquid CO_2 treatments in a high-pressure chamber (Polaron, Watford, United Kingdom). The dried samples were then sputter-coated with Au-40 Pd (gold palladium) (Tousimis Sputter Coater, Tousimis Research, Rockville, MD) and underwent SEM (Nanolab 2000 ARL, Bausch & Lomb, Ottawa, Ontario, Canada). All 5 specimens of each material group were prepared and scanned to assess resorption pit formation.

Surface Chemistry Analysis

Specimens of unprocessed bovine bone and both types of deproteinated bovine bone underwent x-ray photoelectron spectroscopic (XPS) analysis. The spectra were obtained using a Leybold MAX 200 XPS (Leybold Inficon, East Syracuse, NY) system utilizing an unmonochromatized magnesium Kalpha x-ray source operated at 12 kV and 35 mA. The atomic ratios of nitrogen were derived from the spectrum run in low-energy mode (pass energy = 192 eV), which were normalized to unit transmission of the electron spectrometer. Binding energies and peak areas were obtained using the curve-fitting

Figs 1a to 1c Attachment of TRAP+ cells on normal bovine bone as compared to bone-derived and synthetic samples, following plating with rabbit osteoclasts, fixing, and TRAP staining.

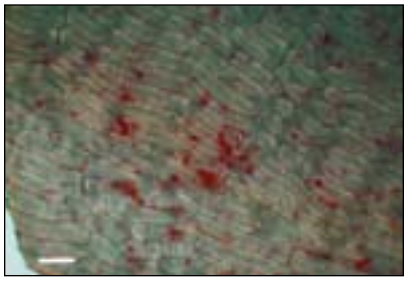

Fig 1a Bovine cortical bone slice. Note the relatively even distribution of both mono- and multinucleated TRAP+ cells throughout the surface (bar = 300 μm).

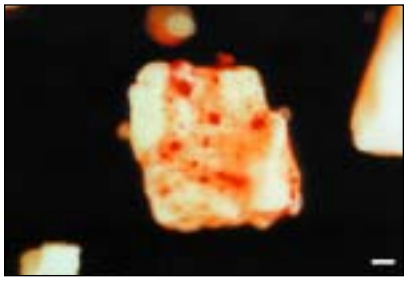

Fig 1b Chemically deproteinated bovine trabecular bone. Note the moderate numbers of both larger and smaller TRAP+ cells (bar = 100 μm).

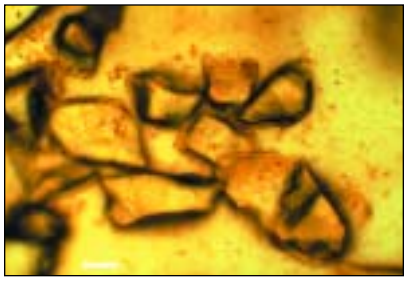

Fig 1c Coated silica particulate. Note the diminished numbers and size of TRAP+ cells (bar = 300 μm).

Figs 2a to 2c SEMs of normal bovine bone as compared to deproteinated bovine bone samples following rabbit osteoclast culture.

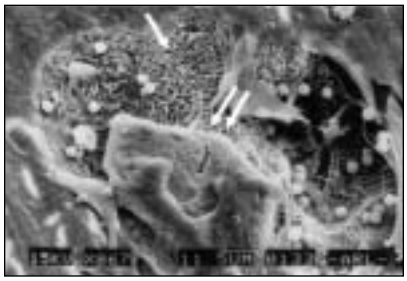

Fig 2a Normal bovine bone. Note the large resorption pit with exposed collagen fibrils *(single arrow)* seen beneath the retracted osteoclast cell body *(double arrow)* (original magnification ×867).

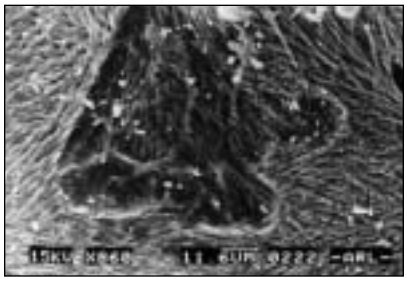

Fig 2b Chemically deproteinated bovine bone. Note the mesh-like mineral matrix that remains following chemical processing. Resorption pits, such as the one depicted, were present on specimens cultured in the presence of osteoclasts but not on those control specimens cultured in the absence of osteoclasts (original magnification ×860).

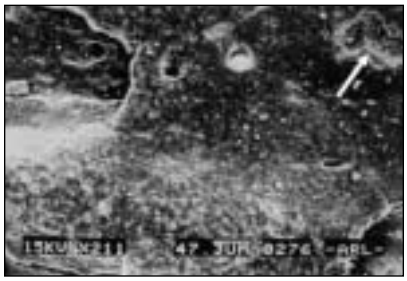

Fig 2c Thermally deproteinated bovine bone. Note the relatively amorphous mineral surface which remains following sintering. There was no evidence of fully developed resorption pits on these specimens. There were, however, occasional areas of etching *(arrow)* observed in specimens cultured in the presence of osteoclasts that were not present on those control specimens cultured in the absence of osteoclasts (original magnification ×211).

routines provided with the spectrometer.[15] This method allows characterization of the elements present and their chemical bonding state in the surface layer (ie, the superficial 2 to 10 atomic layers of the material being examined).

RESULTS

TRAP Staining and SEM

Results of osteoclast cultures and subsequent TRAP staining and LM or SEM examination are presented in Figs 1 and 2 and in Table 1. Under LM, bone-derived materials exhibited TRAP+ multinucleated cells with greater than 3 nuclei (osteoclasts)[16–18] in greater numbers and of more characteristic size and shape than was evident on the synthetic HA or synthetic non-HA materials. Deproteinated and demineralized sample groups, however, demonstrated cells in lower numbers and of less typical morphology than was evident on the freeze-dried and control bone groups. Under SEM, bone-derived materials exhibited scalloped trails and pits consistent with osteoclast-resorptive activity. Modified bone-derived materials exhibited evidence of osteoclast-resorptive activity less frequently per area observed than the control bovine bone slices, with the sintered deproteinated bone material displaying the least.

Under LM, synthetic HA materials exhibited moderate numbers of TRAP+ multinucleated cells with normal morphology, but fewer than the bone-derived materials. Under SEM, synthetic HA materials exhibited surface etching, at times with tiny

Table 1 Observations on Cell and Pit Numbers Subsequent to Osteoclast Culture Followed by TRAP Staining and LM Examination, or SEM Evaluation, of Resorption Pit Formation

Materials examined	LM appearance	SEM appearance
Bone-derived		
Bovine cortical bone slices (control)	+++	+++
Freeze-dried human rib block	+++	++
Demineralized freeze-dried human bone	++	++
Deproteinated bovine bone (B) particulate and block	++	++
Deproteinated bovine bone (N) particulate	++	+
Synthetic HA		
HA particulate (C)	++	+/–
HA particulate (P)	++	+
HA cement slices	++	+
Synthetic non-HA		
Coated acrylic particulate	+	–
Coated silica particulate	+	–

B = Bio-Oss; N= Osteograf/N-700; C = Calcitek; P = Osteograf/P.
LM appearance: +++ = significant numbers of osteoclasts, large well-spread cells abundant; ++ = moderate osteoclast numbers, slight decrease in cell size; + = diminished osteoclast numbers and/or size.
SEM appearance: +++ = Large well-defined, scalloped, trail-like resorptive pits apparent, pits numerous; ++ = Resorptive pits exhibiting less than typical size and shape and/or number; + = Surface etching and/or tiny pit-like surface irregularities; +/– = Surface etching and/or tiny pit-like surface irregularities/No pit formation apparent; – = No pit formation apparent.

(~ 10 μm) pit-like surface irregularities, consistent with limited osteoclastic activity but without the scalloped pits and trails observed in the bone-derived materials.

Under LM, synthetic non-HA materials exhibited TRAP+ multinucleated cells present in lower numbers and with less well spread morphology than the cells on the bone-derived materials. Under SEM, synthetic non-HA material surfaces exhibited no evidence of osteoclastic activity.

Collagen Immunohistochemistry Staining

The bovine bone slices showed extensive evidence of osteoclast-resorptive activity, as demonstrated by the positive staining of the exposed bovine type I collagen remaining in the pits. The pits seen were circular in shape as well as exhibiting the typical scalloped edges with evidence of continuous trail formation (Fig 3a).

Bio-Oss specimens revealed discrete areas positive for type I collagen staining in both the rabbit and mouse experiments. There were smaller continuous trails seen using this method on all the Bio-Oss specimens examined (Fig 3b). Repeated examinations revealed small, dark brown or black areas resistant to removal by further sonication after staining. These positive areas were similar in shape to the scalloped trails seen on normal bone, but much less frequent and much smaller in length than those seen on normal bone. There was no evidence of any collagen staining associated seen with this method on any of the Osteograf/N specimens (Fig 3c).

Surface Chemistry Analysis

Pure HA should exhibit calcium, oxygen, hydrogen, and phosphorus using this method, but no nitrogen. Specimens were therefore examined for nitrogen as a marker for the possible presence of protein within the surface layers of these materials. Analysis of the bovine bone surface composition showed between 6% and 9% nitrogen. In contrast, the Bio-Oss surface revealed between 0.17% and 0.47% nitrogen. Osteograf/N specimens did not reveal any surface nitrogen.

DISCUSSION

The classical spreading and resorptive pit formation of osteoclasts that is normally seen on bovine bone

Figs 3a to 3c Immunostaining for type I collagen on normal bovine bone as compared to deproteinated bovine bone samples following mouse osteoclast culture.

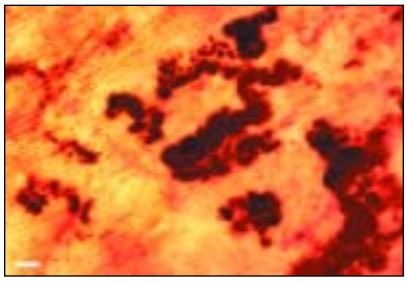

Fig 3a Normal bovine bone. Large, scalloped-edged resorption pits and trails, as revealed by the brown staining of exposed type I collagen, were present throughout the surface of specimens. The linear striations are specimen preparation artifacts left by the Beuhler saw blade (bar = 100 μm).

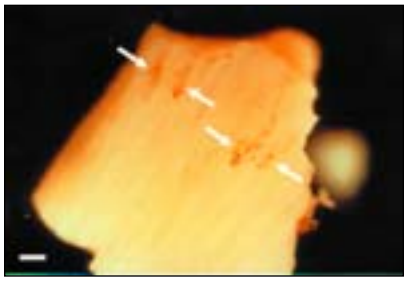

Fig 3b Chemically deproteinated bovine bone. Although substantially less than seen on normal bovine bone controls, limited staining *(arrows)* was consistently observed on these specimens (bar = 100 μm).

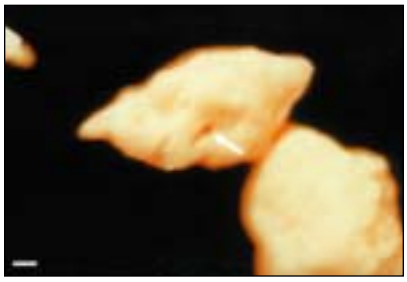

Fig 3c Thermally deproteinated bovine bone. There was no evidence of staining on any of these specimens. The small dark structure in this specimen *(arrow)* is an anatomic void (bar = 100 μm).

slices in tissue culture was most closely mimicked on the non-sintered bone-derived materials, with only areas of "etching" of the synthetic HA materials and sintered bone-derived material seen when cultured with osteoclasts. Osteoclast attachment and resorptive activity involves the formation of cellular attachments to proteins, within either normal bone matrix or proteins adsorbed onto biomaterial surfaces.[19] These cell-attachment zones involve binding of cell membrane components to the extracellular matrix proteins (eg, osteopontin or bone sialoprotein). On modified biomaterials, the osteoclast relies on adsorbed surface proteins, eg, vitronectin (a protein found in serum), to gain attachment.[20–23] The formation of the very specialized subcellular resorptive lysosome that allows normal osteoclast-resorptive activity appears not to occur normally under these conditions.[24] When most, or all, of the noncollagenous proteins were absent from bone matrix, as with Bio-Oss and Osteograf/N, the quality of the osteoclast cell attachment was compromised, as reflected by the lower osteoclast numbers and abnormal osteoclastic pit formation on these materials as compared with controls. The differences seen in the resorption pit formation between the Bio-Oss and Osteograf/N on SEM possibly reflect the extent to which these materials have been altered relative to intact bone matrix. There were relatively more normal pits on Bio-Oss, which appeared to have some residual proteins, whereas there was very little resorption of the Osteograf/N material, which appeared to be completely anorganic.

The presence of nitrogen in the surface layers of the Bio-Oss, in combination with the evidence of positive staining for bovine type I collagen after the material had been exposed to osteoclastic activity, strongly suggests that there is residual protein in this product. However, the Bio-Oss material does appear to undergo more normal osteoclast resorption in vitro when compared with the apparently anorganic sintered product, Osteograf/N, which exhibited only minimal "etched" areas of osteoclastic activity. Of related interest is the recent work of Schwartz and coworkers,[25] which revealed the presence of the proteins transforming growth factor-beta and bone morphogenetic protein-2 in Bio-Oss following solvent extraction, gel electrophoresis, and silver staining. They then demonstrated these same extracts to be osteoinductive in mice. The implications of protein residues with respect to immunologic reactions to a material or infectious considerations from materials derived from bovine sources remain unclear.

It would appear that the synthetic HA materials do not undergo osteoclast-mediated "dissolution" to the same extent as materials derived from bone. No evidence of osteoclast-mediated dissolution or etching of the synthetic non-HA materials was observed under SEM. Although these findings are not entirely consistent with those made by de Bruijn and associates[8] on experimental sintered HA samples in osteoclastic cell culture (where "no resorption or surface alteration" was observed), these observations may be attributable to differences in the chemical and surface properties of the different HAs being used, or to their use of a rat-derived osteoclastic cell culture versus the present rabbit-derived osteoclastic cell culture. However, the findings of the present study may be consistent with observations made by Gomi and colleagues[9] on

experimental sintered HA samples in osteoclastic cell culture. In that study, TRAP+ cells were reported to have created 15- to 25-μm (small) surface pitting, while multinucleated cells caused erosion of the ceramic surface without pit formation. Further, Doi and coworkers[26] reported that in rabbit osteoclast culture, bone and sintered carbonate apatite were absorbed whereas sintered HA was not.

The lack of non-collagenous cell-attachment proteins (eg, osteopontin and bone sialoprotein) in the synthetic HA and synthetic non-HA materials may be largely responsible for this difference in osteoclast behavior. The addition of RGD(Argo-Gly-Asp)-containing peptides that block normal osteoclast cell attachment will also markedly decrease their capacity to form resorption pits on bone.[21–23]

At the surface of HA-containing biomaterials, there will be local high concentrations of calcium related to ion exchange. These local higher-than-physiologic calcium concentrations near the surfaces of these materials may also play a role in inhibiting normal osteoclast differentiation[27] and/or function.[28–30] HA materials vary in their composition with respect to true HA versus calcium phosphate and/or carbonate in various forms, as well as variation in crystallinity versus amorphous content; there is also great variation in surface rugosity or texture. All these factors may alter cell behavior on these materials.

The success of the phenomenon of osseointegration has been correlated with the histologic appearance of the bone-implant interface,[31] and the degree of bone-implant contact has been positively correlated with removal force.[32] Histomorphometric analysis of this interface of the percentage of vital bone apposition to the implant surface has become accepted as a method of quantifying osseointegration,[33] with values over 60% suggested as an indicator of success in functioning implants in humans[34] and values of 56% to 85% demonstrated.[35] Biomechanically, the degree of bone apposition to an implant surface has been described as a key determinant of functional stress transfer,[36] and a correlation of osseointegrated contact area with the biomechanical behavior of an implant has been demonstrated.[37]

Although the requirements for a conventional pontic site augmentation generally involve only the biocompatibility and space-filling capability of the material used, the requirements for an implant site augmentation material are significantly more complex. Because of their mere presence and lack of osteogenicity (and putative lack of osteoinductivity[38–44]), nonautogenous materials used in conjunction with implants at stage I surgery may interfere with the complex initial stages of human osseointegration.[45] The use of these materials may result in diminished apposition of vital bone at the implant surface, as has been suggested by several canine studies.[46–49] Following the initial development of a stable bone-implant interface, it has been demonstrated that the immediate peri-implant region undergoes a comparatively higher turnover than the surrounding bone[50] and that this rapidly remodeling region provides the biomechanical tissue compliance at the bone-implant interface necessary to limit microdamage in function.[51] If that peri-implant milieu is disrupted by diminishing the amount of vital bone contact by substituting biomaterials that are not subject to normal physiologic remodeling, the risk of failure at the bone-implant interface could arguably be significantly increased. Even allogenous and xenogenous materials that undergo delayed physiologic resorption may be more appropriately used with a staged approach (ie, delayed implant placement), given that numerous animal studies[43,52–70] and human studies and reports[71–99] have demonstrated that various nonautogenous materials linger in grafting sites.

Despite the findings of a major retrospective consensus conference,[100] which reported favorable 3- and 5-year survival rates for implants associated with human maxillary sinuses augmented with a variety of grafting materials, and retrospective human studies that concluded that neither the use of nonautogenous materials[101,102] nor the use of a staged approach[101,103] affects implant success rates, it is suggested that the protocols for usage of nonautogenous bone substitute biomaterials be more carefully considered in clinical implant dentistry.

CONCLUSIONS

Under the conditions of this investigation the following were found:

1. Bone-derived materials, unless sintered, demonstrated osteoclast attachment, spreading, and resorptive activity involving the formation of large complex resorption pits, albeit in reduced numbers relative to unmodified bone controls.
2. Synthetic HA materials and the sintered bone-derived material allowed osteoclast attachment but exhibited limited surface etching, which is consistent with limited osteoclast-resorptive activity.
3. Synthetic non-HA materials, while they supported osteoclast attachment, demonstrated no evidence of resorption.
4. Bio-Oss showed evidence of the presence both of type I collagen and surface nitrogen, while Osteograf/N showed neither.

ACKNOWLEDGMENTS

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