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Title: How Cadaver Bone Mineralizes and Sclerotic Bone Fails

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Abstract

Cadaver bone is the most common bone grafting material used in dentistry. It was theorized that this material mineralizes by way of osteoinduction and subsequent osteogenesis. This theory has been proven false, and with that knowledge, cadaver bone grafts are now thought to heal by osteoconduction. However, there is no scientific support that osteoconduction occurs during the mineralization of cadaver bone grafts. In addition, many in dentistry believe that cadaver bone grafts are resorbed and become normal functioning bone. However, there are no studies that support this belief.

Objectives: The goal of this study was to evaluate the how cadaver bone grafts produce mineralization and what type of bone is produced by these bone grafts.

Materials and Methods: This study was a histological analysis of cadaver bone graft healing sites ranging from the incipient stages of mineralization and various time points through to bone failure years after grafting.

Results: The mineralization of cadaver bone grafts was produced via an inflammatory response produced by a foreign body reaction. The histologic findings of the mineralized bone produced by this process was sclerotic bone. Over time the sclerotic bone was found to fail via an accumulation of microfractures producing bone particulate in the granulation tissue.

Conclusions: Cadaver bone grafts produce sclerotic bone that is not capable of self-repair which ultimately leads to bone failure.

Clinical Relevance: These findings describe a new mechanism by which dental implants can be lost.

Introduction

The popularity of cadaver bone for use in the treatment of maxillofacial skeletal defects has coincided with the findings of Urist et al. (1), which identified the presence and function of bone morphogenic proteins (BMPs). With the discovery that cadaver bone contained proteins that have the potential for osteoinduction and stimulating osteogenesis, it was assumed that the growth factors in cadaver bone also stimulated bone growth when grafted into humans. However, beginning in the 1990's, research showed that autografts and allografts are not osteoinductive in humans (2-7). Likewise, studies proved that cadaver bone does not stimulate osteogenesis in humans (8-12). Most current publications now claim that allografts are osteoconductive, presuming that this is the only other way bone can mineralize. However, again, there is no scientific support for the assumption that allografts are osteoconductive.

Since cadaver bone is not osteoinductive, does not stimulate osteogenesis, and there is no scientific support showing that cadaver bone is osteoconductive, the question remains as to the process by which these materials produce mineralization. Irrespective of the extensive use of cadaver bone for bone grafting over decades and the hundreds of histologic studies, there is no published histologic research that can be found on the available databases on how these materials produce mineralization. This article will present the early histology of cadaver bone mineralization and discuss the type of tissue that these materials produce.

Materials and Methods

Human histologic samples were taken at various time points. The early histologic samples were taken from healing extraction sites during the mineralization period. The samples were acquired from patients who requested that the cadaver bone be removed from their jaw and replaced with synthetic resorbable graft materials. The histologic samples taken of allografts in the mineralizing phase were from patients who were asymptomatic and had no signs of pathology. Histologic samples of mineralized cadaver bone were taken via trephine at the time of implant placement. The sites had no clinical signs of pathology. Histology and clinical cases of sclerotic bone failure were obtained from patients who were treated for implant failure after grafting with cadaver bone. None of these patients were enrolled in a controlled clinical trial. All cases were retrospective. All patients gave written permission consenting that any tissue removed during routine therapy could be used for scientific evaluation.

Results

Early cadaver bone mineralization

In the following case, teeth #7 and 8 were grafted with freeze-dried bone allograft and covered with a collagen membrane. At seven weeks, the patient requested that the allografts be removed, and the sockets re-grafted with a synthetic biocompatible, resorbable graft material. The patient was a health professional who came to understand that unmatched cadaver tissue posed health risks she was not informed of prior to the grafting. The extraction socket for tooth #8 appeared to be normal both radiographically and clinically, but histologically found to be infected with actinomycetes with no bone formation (data not shown).

In Figure 1A, the radiograph of extraction socket #7 at seven weeks shows a normal appearance. However, there is a clear distinction between the extraction socket wall and the bone graft in the socket. There is no evidence that bone is growing from the socket wall into the extraction socket. In Figure 1B, the cadaver bone particles are identified and the black arrow points to newly formed mineralized tissue on the surface of an allograft particle. New mineralization is only present on the surface of the allograft particles. There is no growth of mineralized tissue from the periphery. Figure 1C is a high-magnification view of an allograft particle with new mineralization forming on the surface as identified by the black arrow. What is also evident in image 1C is intense inflammation with the presence of cytotoxic T cells. The identification of the cells as cytotoxic T cells was make by a medical pathologist. This finding makes sense because these cells are involved in rejection during organ transplantation (13).

While some clinicians contend that inflammation is a component of bone regeneration that is only present when trauma is involved to remove damaged necrotic tissue prior to bone regeneration. In medicine and dentistry, it is known that inflammation results in bone resorption; not bone regeneration. The mineralizing cells in this histology lack the anatomy of normal mineralizing osteoblasts. In addition, osteoblasts mineralize in a very controlled environment and it is doubtful that osteoblasts would function correctly in this degree of intense inflammation. As a comparison to the mineralization process produced by cadaver bone in Figure 1B and 1C, Figure 1D shows stimulated osteogenesis in an extraction socket 6 weeks after grafting with a biocompatible synthetic bone graft (Socket Graft Putty). The osteoblasts are plump and tightly packed and in the process of producing osteoid, ultimately forming woven bone. No inflammation is present, and the tissue shows normal anatomy.

Figure 1

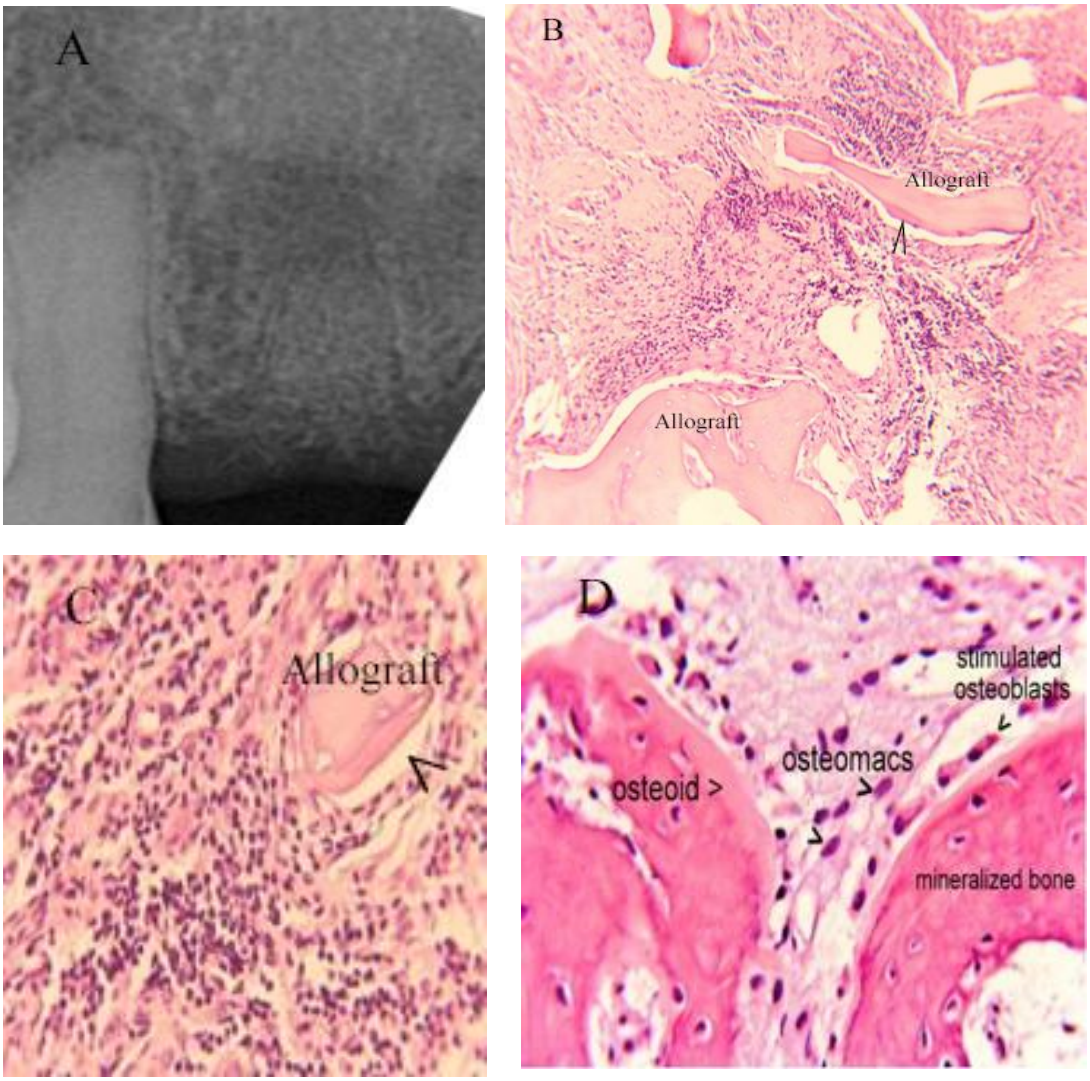

Figure 1. Mineralized freeze dried bone allograft. (A) Radiograph of extraction socket. (B and C) Hematoxylin and Eosin staining of mineralized allograft site containing intense inflammatory infiltrate. The black arrows indicate incipient mineralization. (D) Hematoxylin and Eosin staining of biocompatible synthetic bone graft showing robust osteogenesis and no inflammation.

Figure 2 is of a molar extraction site 8 weeks after extraction and grafting with mineralized allograft and covered with a collagen membrane. The patient requested that the cadaver bone be removed, and the socket grafted with a synthetic biocompatible resorbable bone graft. Figure 2A is a photograph of the molar extraction site after the cadaver bone had been removed. Note that no bone formation has occurred on the extraction socket wall at eight weeks. Figure 2B is a low-

magnification photomicrograph of the allograft removed from the extraction socket. While there is inflammation present, it is significantly less than the amount of inflammation found in the previous case presented at 7 weeks. Figure 2C is a high-magnification image of Figure 2B. The allograft shows most of the particles covered with new mineralization and a decrease in inflammation. There is no evidence of mineralization proceeding from the periphery, which again rules out osteoconduction.

In the histology of the early mineralization process of allograft particles, the amount of mineralization is inversely related to the amount of inflammation present in the tissue. As the allograft particles become covered by mineralized tissue this isolates the foreign antigenic graft material from the host immune system and inflammation subsides.

Figure 2

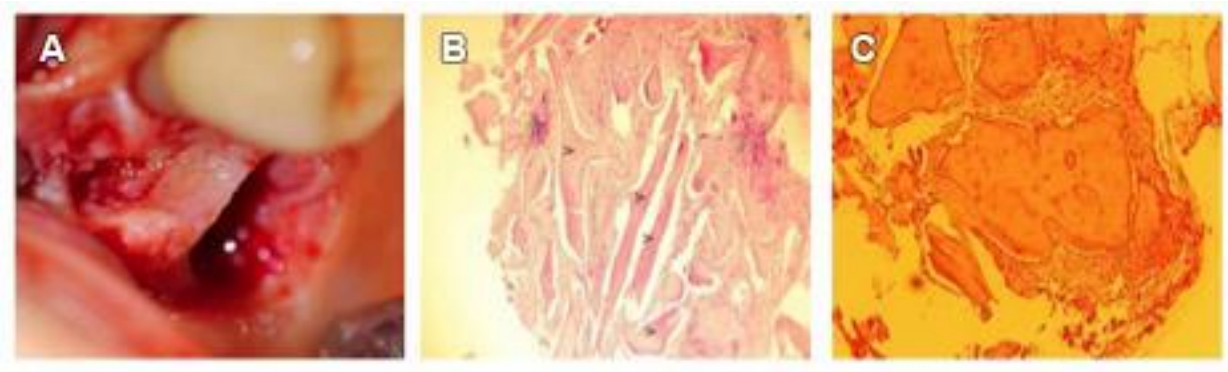

Figure 2. Mineralized freeze dried bone allograft. (A) Photograph of molar extraction site after allograft was removed. (B and C) Hematoxylin and Eosin staining of mineralized freeze dried allograft.

Figure 3A is a radiograph of an extraction site 6 months after being grafted with mineralized freeze-dried bone allograft. The white arrows outline the radiolucent border around the grafted site, which is common when a non-biocompatible, non-resorbable graft material is placed in bone. Figure 3B shows the ridge 6 months after grafting. The pebble-like surface is composed of allograft particles covered in a thin layer of mineralization. When a non-biocompatible bone graft material such as mineralized freeze-dried bone allograft is used, the particles on the surface are completely covered in mineralized tissue. However, when a biocompatible bone graft material is used, the surface graft particles are not covered in mineralized tissue. This finding supports the concept that transplanted tissue produces mineralization for the purpose of encapsulating the antigenic graft material and isolating it from the host. Figure 3C is a core sample taken from the second molar site at the time of implant placement. The histology shows remaining allograft particles encased in sclerotic bone. Sclerotic bone is found in other bony lesions such as arthritic joints and calcified arteries and is formed in response to inflammation (17,18). Figure 3D is a site that was grafted with mineralized allograft prior to implant placement. The Implant was in function for a number of years before it failed abruptly with the diagnosis of sclerotic bone failure.

Figure 3

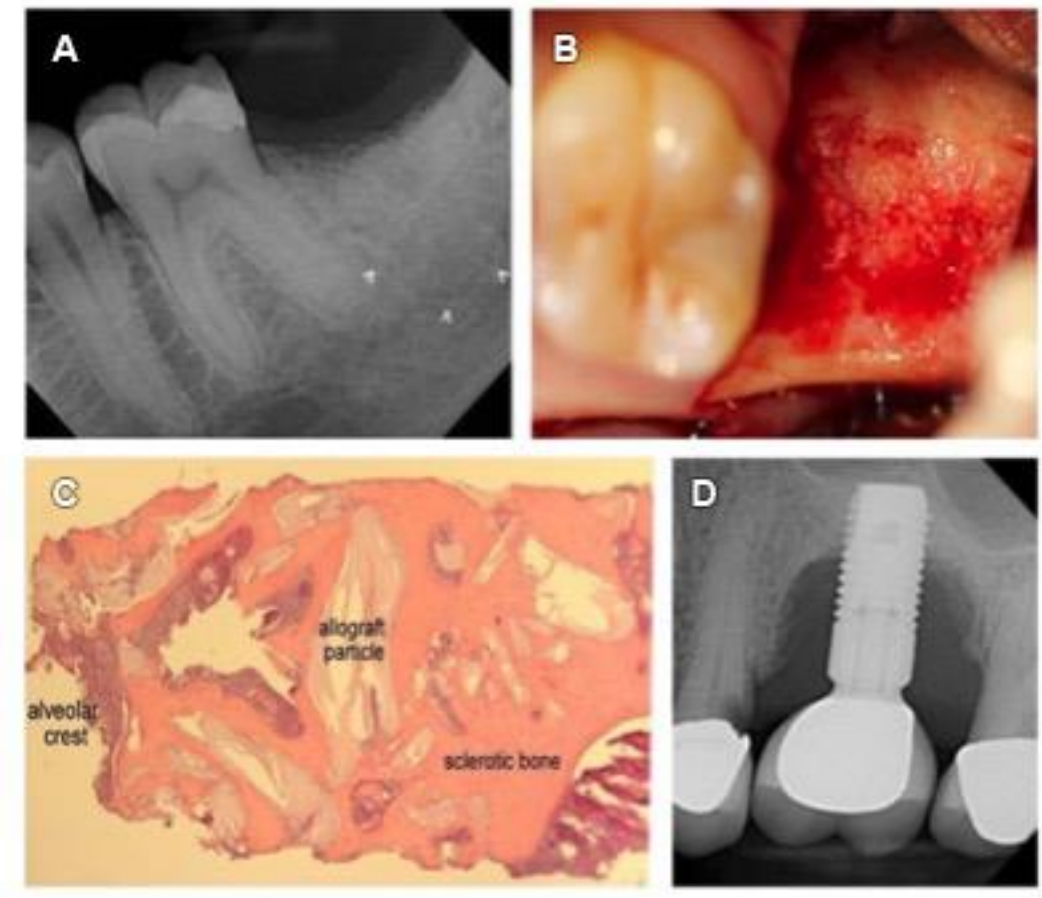

Figure 3. Sclerotic bone from mineralized freeze-dried bone allograft. (A) Radiograph of extraction site 6 month after grafting with mineralized freeze dried bone allograft. White arrows indicate radiolucent boarder around graft site. (B) Photograph of the ridge 6 months after grafting. (C) Hematoxylin and Eosin staining showing sclerotic bone. (D) Radiograph of site that was grafted with allograft prior to implant placement showing bone failure of grafted bone.

Figure 4 shows a site grafted with Bio-Oss. The bone graft was in place for 10 years. The radiograph in figure 4A shows a very dense mineralized area mesial to the molar outlined by black arrows. The image in figure 4B is the trephine immediately after taking a core sample from the densely mineralized tissue. Particles of Bio-Oss graft material are obvious but what is most striking is the lack a vascularity with essentially no bleeding. Image 4C is the demineralized histology stained with H and E. With the mineralization removed from the Bio-Oss particles the histology shows the collagen matrix that was inside the mineralized bovine bone. The bone in the histologic sample is amorphous with no cement lines and no laminar bone. No resorption is occurring in association with either the Bio-Oss particles or the mineralization produced by the patient. No blood supply is evident. This histology is pathognomonic for sclerotic bone.

Figure 4

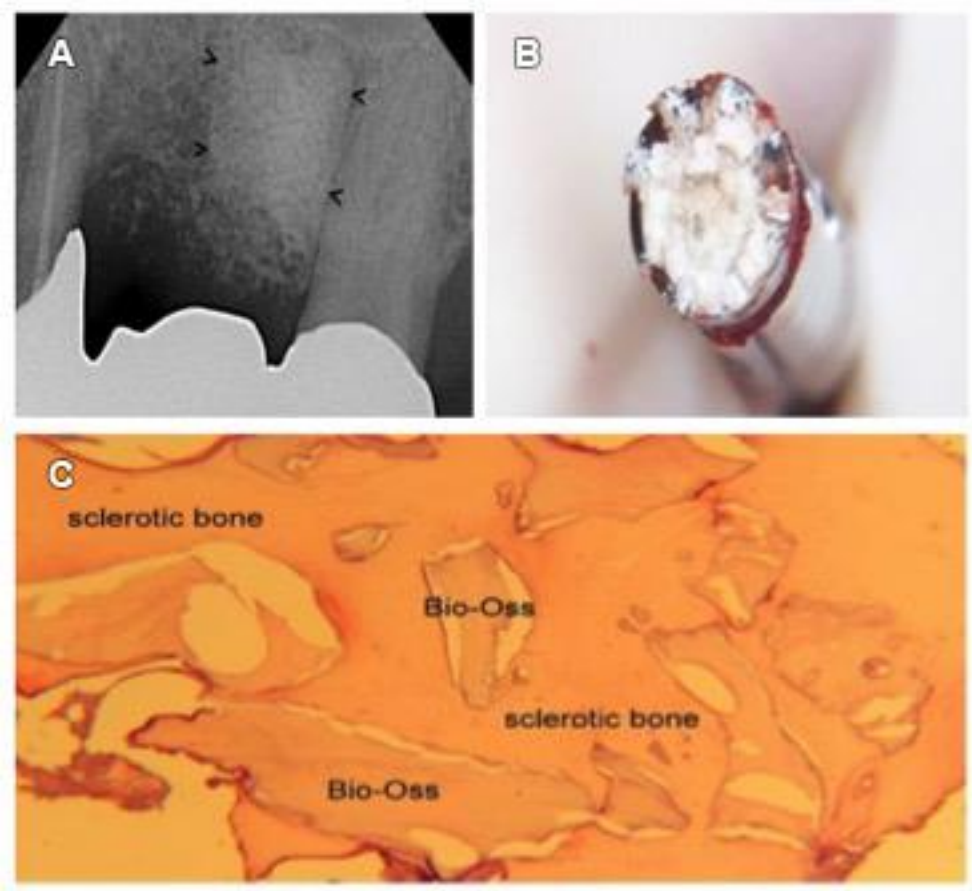

Figure 4. Sclerotic bone produced by Bio-Oss. (A) Radiograph of Bio-Oss. (B) Photograph of trephine sample. (C) Hematoxylin and Eosin staining of Bio-Oss showing sclerotic bone.

Sclerotic bone failure

Mineralized cadaver bone that contains proteins, irrespective of whether it is of animal or human origin, produces sclerotic bone (20). While sclerotic bone is pathologic, the question remains about how this affects the ability of this tissue to support a dental implant. A study published in the Journal of Periodontology found that the most significant factor associated with marginal bone loss and implant failure was implants placed in sites grafted with cadaver bone (21). Most dentists think of implant failure as a result of infection, referring to the process of bone loss as periimplantitis. However, sclerotic bone in other parts of the human body fails by way of microfracture; not infection. This process was well documented by studying the formation of sclerotic bone in the joints of horses (22)

Joint inflammation resulting from trauma or disease converts normal bone into sclerotic bone (22). Once sclerotic bone is formed it is unable to be reversed and with continual loading

microfractures develop in the sclerotic bone. Microfractures are a common occurrence in normal bone, as the microfracture allows bone to absorb load without resulting in a complete fracture of the bone (23). However, normal bone microfractures can be repaired because normal bone contains basic multicellular units that are comprised of both osteoblasts and osteoclasts that remodel the microfracture to repair the damaged bone. In sclerotic bone produced by cadaver bone grafts, there are no functioning basic multicellular units, and therefore this bone lacks the ability to repair microfractures. As shown in the horse sclerotic bone model, the microfractures are unable to be repaired and the microfractures accumulate until the bone collapses (22). The same process occurs in sites that are grafted with cadaver bone and have received a dental implant. While the dental profession assumes that an implant placed in sclerotic bone integrates, there is no scientific support for this assumption. It is probable that implants placed in the sclerotic bone produced by cadaver bone grafts are retained by mechanical support alone.

In Figure 5, a bicuspid was extracted and grafted with freeze-dried bone allograft. The implant was placed and was functional for several years before the implant was lost. The radiograph in Figure 5A shows radiopaque material in the lesion, which reflects bone chips contained in the granulation tissue. Upon flapping the site to remove the failed implant, the bone chips are readily visible in the granulation tissue as noted by the arrows in image 5B. Figure 5C is a biopsy of the granulation tissue removed from the site of the failed allograft, which shows the inclusion of multiple bone particles. The presence of bone particles in granulation tissue around a failed implant is pathognomonic of sclerotic bone failure and this has only been seen in sites grafted with cadaver bone. Figure 5D shows an implant placed in Bio-Oss with the diagnosis of sclerotic bone failure.

Figure 5

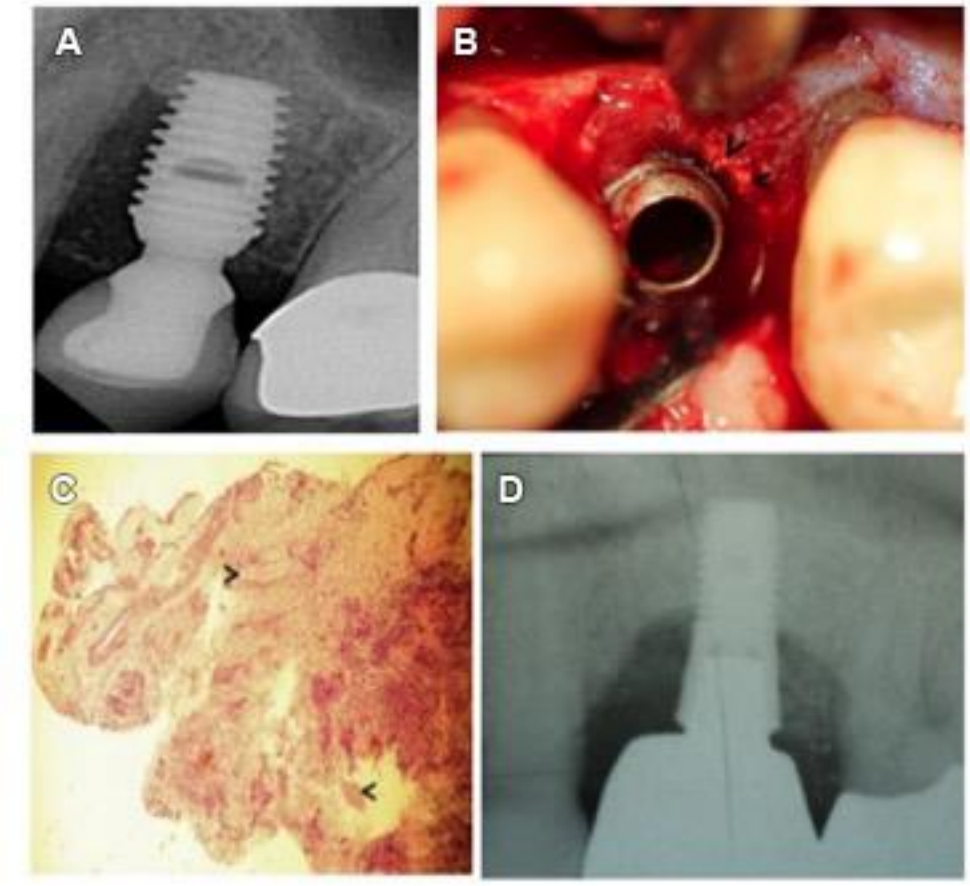

Figure 5. Bone chips in site grafted with mineralized freeze dried bone allograft. (A) Radiograph of radiopaque bone chips in the granulation tissue. (B) Photograph of visible bone chips (black arrows). (C) Hematoxylin and Eosin staining of biopsy granulation tissue from the implant site showing sclerotic bone chips. (D) radiograph of bone graft failure.

The radiograph in Figure 6A is of a mobile implant that was placed approximately 4 years prior to implant loss. Upon communication with the surgeon that removed the molar, the site was grafted with a combination of Bio-Oss and mineralized freeze-dried bone allograft in 1998. Figure 6B shows removal of the implant, which contained a portion of the bone that was prepared for histology. Figure 6C shows the histology of the grafted site, which shows allograft particles and Bio-Oss particles encased in sclerotic bone. The differing size of the osteocyte lacuna allows the determination of what particles are from the allograft and what particles are bovine. After approximately 18 years there was no resorption of the cadaver bone and the implant was lost due to bone graft failure after being functional for approximately 4 years. Figure 6 shows a failed combination allograft/Bio-Oss bone graft after 1.5 years in function. The images of these two cases of bone failure are very similar.

Figure 6

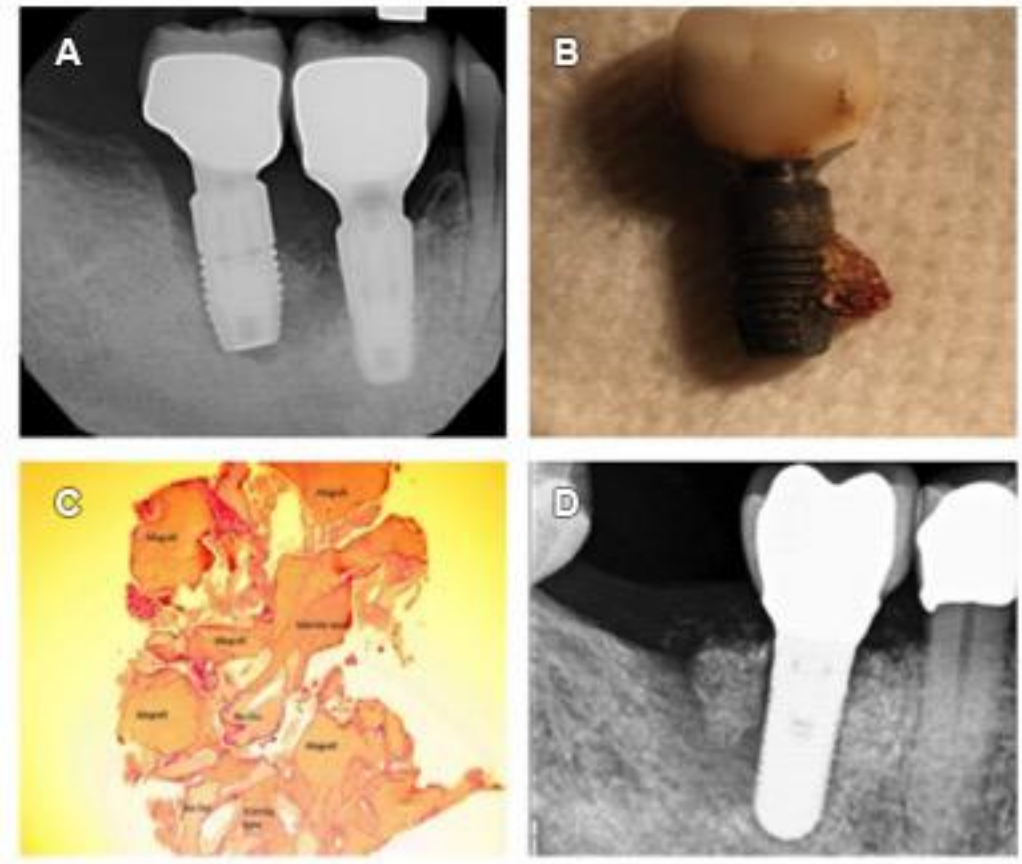

Figure 6. Failure of combination Allograft/Bio-Oss. (A) Radiograph of implant placement approximately 4 years prior to implant loss. (B) Photograph of the removed implant with attached bone used for histology. (C) Hematoxylin and Eosin staining showing sclerotic bone. (D) Radiograph of a failed combination allograft/Bio-Oss bone graft.

Discussion

The presence of inflammation can induce cells other than osteoblasts to become mineralizing cells. In cardiovascular disease, it is known that the cells that form the mineralized tissue in our arteries are reprogramed smooth muscle cells (14). The mineralized tissue that forms in our arteries is bone but is not produced by cells of osteogenic origin. The inflammation caused by cardiovascular disease prompts smooth muscle cells to be deprogramed into stem cells and these cells are then reprogramed to produce mineralized tissue. In the presence of inflammation, endothelial cells can also become reprogramed into mineralizing cells that produces the calcified tissue found in atherosclerosis (15).

Bone that forms in soft tissues outside the skeleton is known as heterotopic ossification. In the presence of inflammation, bone forms in muscle through an inflammatory process that stimulates endothelial cells to be reprogramed into mineralizing cells. The process of endothelial-mesenchymal transition in the presence of inflammation is postulated as the process that produces ectopic mineralization (16). The conversion of smooth muscle cells in arteries into mineralizing cells is considered a protective mechanism. The bone produced in arteries surrounds the soft plaques and prevents them from breaking loose and producing an infarct. The mineralization process on cadaver bone particles occurs on the surface of the graft particles and it is proposed that this process is also protective. Allografts are not resorbed and the mineralization of allograft particles isolates the foreign inflammatory tissue from the host. Therefore, it is postulated that cells that produce mineralization in sockets grafted with cadaver bone may be reprogramed endothelial cells.

The term sclerotic is defined as the inability of a tissue to respond or adapt to change. There are over 200 histologic studies on allograft cadaver bone in the literature. The studies of mineralized freeze-dried bone allografts report that an approximate average of 30% of the area is comprised of retained graft particles. Studies have shown that the percentage of retained graft particles does not change over time (19). No mineralized freeze-dried bone allograft particles have ever been reported to be in the process of resorption and there is no histology in the literature showing an osteoclast in a resorption lacuna on the surface of an allograft particle. There are no osteoclasts in Figure 3C and no remodeling has occurred. Basic multicellular units are required for bone remodeling and no basic multicellular units found in this histology. Therefore, the bone is termed sclerotic.

Different types of cadaver bone heal through the same process, but with varying mineral density related to the degree of inflammation produced. The antigenic response would be expected to be greater to bovine proteins than human proteins. A common low-temperature xenograft made from cow bone is an example of this process. Low-temperature bovine bone xenografts contain animal proteins and produce significant inflammation when grafted into humans. The difference in immune response to animal and human transplanted proteins can be seen in the degree of mineralization produced by the bone graft. The more antigenic the material, the higher the level inflammation and mineralization. The presence of antigenic material that produces inflammation resulting in mineralization is termed by the author as antigenic ossification.

Sclerotic bone produced by cadaver bone grafts lack a number of features found in normal bone. Because sclerotic bone never remodels into lamellar bone, sclerotic bone has no cement lines, which are exclusive to lamellar bone. The cement lines found in lamellar bone contain an elevated amount of elastic fibers that function to stop propagation of microfractures by absorbing the shock so the fracture is stopped at the cement line (23). Sclerotic bone has no cement lines and no osteoclasts to repair the microfractures, so the microfractures around the implant increase until the bone collapses and the implant is lost. Since sclerotic bone is a homogeneous material without the ability to repair, the point at which sclerotic bone fails is simply a function of the amount of load and the frequency of loading.

In periodontal disease and periimplantitis, bacterial invasion produces inflammation and the bone is resorbed ahead of the infection. There is no bone found in the site of the lesion because it is fully resorbed ahead of the infection. However, sclerotic bone fails by a breaking up of the bone and the granulation tissue contains fragments of bone.

The granulation tissue in sites of failed sclerotic bone is commonly infected, however the infection is secondary to the breakup of the sclerotic bone. Sclerotic bone failure is commonly misdiagnosed as periimplantitis because the clinical picture of sclerotic bone failure and periimplantitis can appear to be very similar radiographically. Because the dental profession does not understand the concept of sclerotic bone failure, it is common for different clinicians to have varying opinions about why an implant is lost. In actuality, in the United States where cadaver bone grafts are common it appears that approximately 50% of lost implants are a result of sclerotic bone failure, and with the continued use of cadaver bone grafts this percentage is likely to increase.

It is proposed that freeze-dried allografts and xenografts are non-biocompatible and heal by way of an inflammatory process called antigenic ossification. The mineralization process produces sclerotic bone, which never resorbs and fails by the accumulation of microfractures.

Compliance with Ethical Standards All research studies have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Conflict of Interest: The author declares no conflict of interest.

Funding: The study was privately funded by the author.

Ethical Approval: There was no research done to acquire the tissue samples. The tissue samples were acquired during routine dental treatment where the tissue needed to be removed in order to treat the patient. Therefore, no ethical approval was needed.

Informed Consent: Informed consent was obtained from all individual participants to use discarded tissue obtained during routine surgery for scientific evaluation.

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