Position Paper

Tissue Banking of Bone Allografts Used in Periodontal Regeneration*

This paper was prepared by the Committee on Research, Science and Therapy of the American Academy of Periodontology for the information of the dental profession. However, it may also be of interest to the lay public. It represents the position of the American Academy of Periodontology regarding tissue banking and the use of bone allografts in periodontal therapy. *J Periodontol 2001;72:834-838.*

The aim of this review paper is to evaluate current knowledge on bone allograft material supplied by different bone banks to the clinician, including knowledge about the safety and effectiveness of such material. Allograft material has been used in periodontal therapy for the last three decades.[1] It is generally used in one of two forms: freeze-dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFDBA). Both FDBA[2-5] and DFDBA[6-12] have been used successfully to regenerate the attachment apparatus during periodontal treatment, when compared to treatment without allograft.

The two types of graft materials work by different mechanisms. FDBA provides an osteoconductive scaffold and elicits resorption when implanted in mesenchymal tissues.[13] DFDBA also provides an osteoconductive surface. In addition, it provides a source of osteoinductive factors.[14] Therefore, it elicits mesenchymal cell migration, attachment, and osteogenesis when implanted in well-vascularized bone, and it induces endochondral bone formation when implanted in tissues that would otherwise not form bone.

The decision about which form of allograft to use should be based on the clinical condition of the site to be grafted. Because it is still mineralized, FDBA may have better physical characteristics. However, FDBA is not osteoinductive. Although no significant differences have been found clinically between FDBA and DFDBA in primarily intraosseous defects,[4,5,12] in sites where regeneration may be more problematic, DFDBA may be a more appropriate choice.

Some studies suggest that guided tissue regeneration (GTR) combined with the use of DFDBA is more predictable than membranes alone in periodontal treatment of infrabony pockets and furcations.[8,9,15-18] The clinical results using DFDBA, however, have been variable. Some investigators have reported success, while others have failed to demonstrate clinical improvement that could be attributed to DFDBA.[19,20] This variability also has been observed when DFDBA was used in combination with GTR.[21-23] A meta-analysis of the treatment of infrabony defects with DFDBA has questioned the benefit of using DFDBA in these treatments.[24] Variability also has been reported in the ability of DFDBA to induce new bone formation in animals.[25,26]

Several possible explanations could account for the wide variation in reported clinical results with the use of DFDBA. One potential cause might be that bone induction proteins are not present in sufficient quantity to produce detectable bone formation. Another possibility is that the bone-inductive components of DFDBA are present but in an inactive form.[27,28] Alternatively, it is possible that the natural variability in human donors is reflected in the bone-induction ability of the preparations, and some DFDBA batches are simply more active than others, even when identical procedures have been used to prepare them. The issue is further complicated by the fact that tissue banks do not use identical methods of DFDBA preparation.

Most bone banks adhere to the guidelines of the American Association of Tissue Banks (AATB) with respect to procurement, processing, and sterilization of bone grafts. AATB's guidelines apply to quality control and compliance, ensuring safety. The U.S. Food and Drug Administration does have guidelines for implants manufactured from biomaterials, indicating upper limits on residuals and contaminants introduced during processing, including ethylene oxide (ETO) sterilization (discussed below), as well as the acceptable bioburden for an implantable device. At least with respect to ETO, these guide-

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lines could and should be applied to banked bone. The AATB[29] advocates excluding collection of bone under the following circumstances:

1. Donors from high-risk groups, as determined by medical testing and/or behavioral risk assessments.
2. Donors test positive for HIV antibody by ELISA.
3. Autopsy of donor reveals occult disease.
4. Donor bone tests positive for bacterial contamination.
5. Donor and bone test positive for hepatitis B surface antigen (HBsAG) or hepatitis C virus (HCV).
6. Donor tests positive for syphilis.

There have been no reports of virus contamination or acquired pathology from DFDBA, although this material is in wide use clinically. This may be a consequence of the processing involved.[30] Thus, DFDBA appears to be safe from disease transmission based on current knowledge. It should be emphasized that even though many bone banks do not sterilize bone allografts, they do collect and process bone under sterile conditions, and no reports of contaminated DFDBA have been noted. However, those tissue banks that sterilize their samples by radiation or ETO may not report this fact on the package insert or labeling. Studies examining the effect of ETO on the ability of DFDBA to induce bone[31-33] have shown that it can decrease effectiveness and resorption of the allograft. Some of this may be due to inadequate removal of residuals formed during the sterilization process,[33] or to exposure of the bone graft to temperatures that cause protein denaturation. Thus, sterilization processing may be an important contributor to the variability in DFDBA's osteoinductive properties.

The effect of radiation is more controversial. Irradiated bone has been shown to support normal healing of bony defects.[33] However, irradiation of DFDBA reduced bone induction ability by 40%.[34] A study to examine the use of irradiation to sterilize HIV-contaminated bone graft found that the dose used exceeds current practice for sterilization of medical products. The authors concluded that gamma irradiation should not be used for this purpose.[35]

Recent studies have examined the ability of commercial DFDBA to induce new bone formation in vivo in order to assess if the broad variation in clinical response was due to differences in the preparations or to variations in host response. It was found that wide variations in commercial bone bank preparations of DFDBA do exist, including the ability to induce new bone formation, even within the same bank.[36,37] These results may explain the variability of the clinical response when using DFDBA in periodontal therapy. Because the methods used by bone banks to process donor bone are proprietary, these studies did not evaluate which procedures are best for preserving bone-induction ability while maintaining sterility of the DFDBA.

Differences in processing methods do not explain the variability in bone-induction ability among DFDBA batches from the same bone bank, however. A recent study examining the effects of donor age and gender on the variability in bone-induction ability indicated that donor age but not donor gender may play a role.[37,38] Because of the publication of these studies, some bone banks limit the age of the donor for bone harvesting.

Today, the use of DFDBA from an AATB-accredited bone bank is generally safe and may be considered as a bone graft substitute during regeneration procedures. The clinician needs to be aware that donor age may be important. Because DFDBA bone-induction ability may not have been examined, some of the allograft will be effective only as a space maintainer or bone void filler. Even though preparations of DFDBA may not be osteoinductive, they may still have potential as a carrier for bioactive components of known activity, like bone morphogenetic protein (BMP). A study examining this option of using DFDBA as a carrier for BMP has proven the strategy to be successful in an animal model.[39]

In vitro assays used to assess bone-induction ability must be relied on with caution. To date, the only definitive assay of osteoinduction remains implantation of the DFDBA in a tissue that otherwise would not form bone, such as in immunodeficient rat or mouse muscle. A quantitative histologic analysis must be performed of the amount of new bone that is formed in association with the implanted DFDBA. Moreover, it is essential that evidence be provided that the assay has been validated using DFDBA of known inductive ability. In vitro assays should be validated against in vivo determinations of osteoinduction and should examine relevant markers of osteogenesis.

When DFDBA is used in particulate form, particle size also appears to be an important variable in the success of DFDBA as a bone-inductive material. Particles in the range of 125 to 1,000 microns possess a higher osteogenic potential than do particles below 125 microns.[40] Optimal particle size appears to be between 100 to 300 microns.[40] This may be due to a combination of surface area and packing density. Very small DFDBA particles elicit a macrophage

response and are rapidly resorbed with little or no new bone formation. Tissue banks providing DFDBA for dental use will usually have this graft material in various particle sizes, and the range from 250 to 750 microns is the most frequently available.[32]

FDBA is also a useful material clinically. There have been no reports of virus contamination or acquired pathology from FDBA, although this material is in wide use clinically. It can be combined with antimicrobial therapy, and has been used with tetracycline to regenerate experimental defects in baboons,[41] during treatment of localized juvenile periodontitis,[42,43] and during treatment of periapical lesions.[44] FDBA does not appear to be antigenic.[45] Sterilization with ETO is problematic in that some batches have exhibited residuals which were toxic to human gingival fibroblasts.[46] Although four cases of HIV have been reported following procedures using frozen bone allografts,[47,48] it should be emphasized that frozen and fresh allografts typically are not used in periodontal therapy. The delay required to process DFDBA and FDBA ensures that there is adequate time for testing for potential pathogens, helping to assure the safety of these implant materials.

With the increase in use of more complex periodontal procedures like sinus lifts, surgical placement of implants, and ridge augmentation, DFDBA is now being provided in sheets of various thicknesses from 20 to 100 microns to 100 to 300 microns (lamellar bone or laminar bone) and as blocks of ilium. The results of using these materials in the clinic have been primarily published as case reports.[49] Freeze-dried skin, fascia, and cartilage are also available from tissue banks. FDBA and DFDBA continue to be important bone substitutes for use in a variety of periodontal regenerative procedures around teeth as well as regeneration of bone for dental implants.

SUMMARY

Freeze-dried bone allografts and demineralized freeze-dried bone allografts have been widely used in periodontal therapy in the past and continue to be used in contemporary clinical practice. They have been demonstrated to be safe and capable of supporting new bone formation and, in the case of DFDBA, have been shown to induce new bone formation and periodontal regeneration. Numerous reports indicate that wide variability exists in the ability of commercial preparations of DFDBA to induce new bone. Recently, it has been shown that this variability is related to the age of the donor as well as to the content of bone-inductive factors in the donor bone. Residuals due to inadequate evacuation following ETO or radiation sterilization may also contribute to the variability in response. It is likely that more consistent and reliable results could be achieved with DFDBA if bone banks evaluated the potency of their preparations and reported this information to the clinician.

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