



REVIEW ARTICLE

ALLOGRAFTS AND ITS SAFETY IN PERIODONTAL REGENERATIVE THERAPY.

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Abstract

Bone allografts are being widely used in field of periodontology, many of clinicians are not unfamiliar with their preparation and processing as well as their use as safe and effective graft materials. The major issues associated with these materials are antigenicity and risk of disease transmission from donor to recipient. To minimize the risk, the production of an allograft and its distribution and implantation requires strict attention to detail through a comprehensive process. With an increasing requirement in clinical practice for bone grafting procedures, there is an increase in patient's demands for assurance that bank bone will not be infected with pathogens. To ensure the patients, surgeons should be able to cite factual information and recommendations by responsible organizations regarding safety of allografts. Knowledge of human bone allograft procurement, processing, and tracking may allow dentists to better educate patients and address concerns about this valuable treatment option. The purpose of this review is to update about the procurement, safety and efficacy of allografts.

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Introduction:-

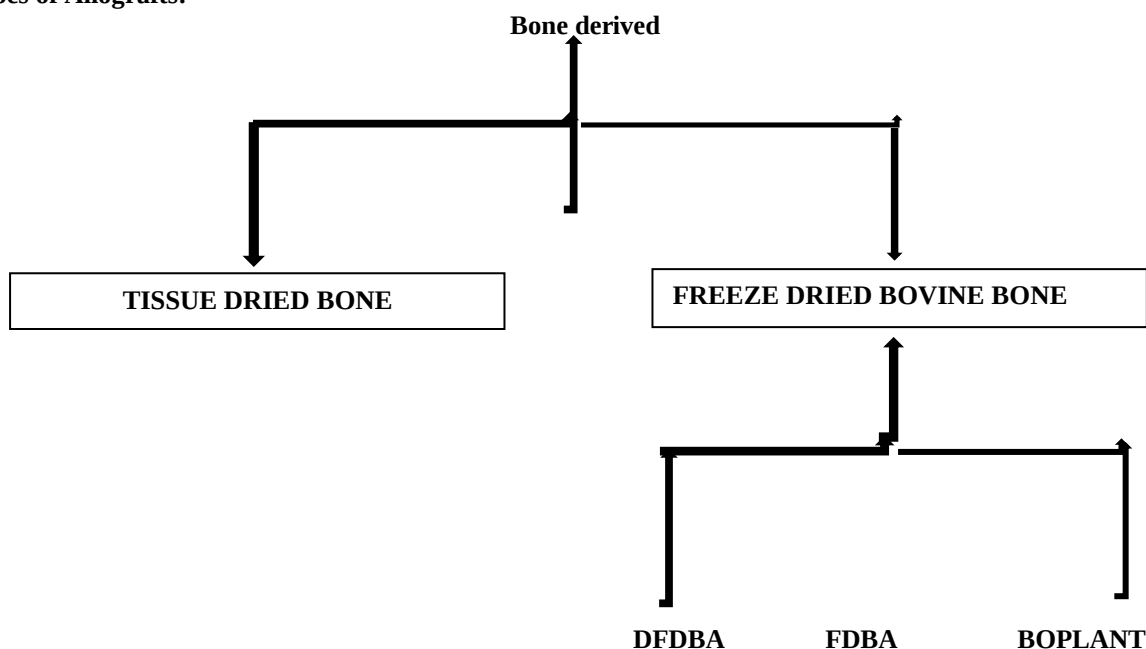
Several types of bone grafts have been studied over the years and search is still continued for an ideal bone replacement material. Bone allograft material has been used in dentistry for the past four decades. Allografts are bone grafts taken from one individual for transplantation to another. Bone allografts are being widely used in the field of dentistry¹⁻³, orthopedics⁴⁻⁶, and craniofacial surgery⁸⁻¹¹. They are generally used in two forms freeze dried bone allograft (FDBA) and demineralized freeze dried bone allograft (DFDBA). In reconstructive craniofacial surgery, autogenous bone was the material of optimal choice despite serious shortcomings, before the emergence of demineralized allogenic bone which was accepted as the most promising alternative to autogenous bone in 1900s.¹⁰

FDBA was first used in periodontal therapy in early 1970s although it has been used clinically in orthopedic therapy since 1950s³. FDBA provides an osteoconductive scaffold for bone growth and elicits resorption when implanted in mesenchymal tissues¹². DFDBA was first used in dentistry and medicine in 1965 but for the treatment of periodontal defects in humans it was utilized in 1975 for the first time⁵. DFDBA also provides osteoconductive surface, and in addition, it also acts as a source of osteoinductive factors. So, it elicits mesenchymal cell migration, attachment, and osteogenesis when implanted in well vascularized bone; it induces endochondral bone formation when implanted in tissues that would otherwise not form bone. DFDBA contains bone morphogenic proteins

(BMPs) such as BMP 2, 4 and 7, which help stimulate osteoinduction¹³. Thus, commercially prepared, allograft retained proteins have the capacity to influence cell behavior in vivo. BMPs produce multiple effects on bone by:

1. acting as mitogens on undifferentiated mesenchymal cells and osteoblast precursors;
2. inducing the expression of the osteoblast phenotype (e.g., increasing alkaline phosphatase activity in bone cells; and
3. acting as chemoattractants for mesenchymal cells and monocytes as well as binding to extracellular matrix type IV collagen. Studies have determined that the minimal effective amount of BMP necessary to affect bone growth is about 2 µg/40 mg wet weight of explants. The optimal amount is about 10 µg.

Types of Allografts:-



1. Demineralized
2. Bovine powder
3. Bovine derived
4. Hydroxyapatite
5. Anorganic bovine bon

Procurement of Allografts:-

Use of any substitute for autogenous tissue requires consideration of its biological and biomechanical potential as a graft material and the possibility of transfer of disease from donor to recipient as well as the presence and significance of immune responses to foreign antigens.¹⁴ Thus, bone banks accredited by responsible organizations exist for the purpose of supplying the surgeon with safe and effective bone tissue that is suitable for intended clinical application and are available whenever the need arises. The goals of bone banking are to preserve the physical integrity of the graft and the inductive protein, to reduce its immunogenicity, and to ensure sterility¹⁵. Bone banking has greatly increased the options for the periodontal therapist in the management of severe osseous defects. Bone graft procedures are no longer limited by available autogenous bone. The possibility of disease transfer with bone allografts is very unlikely if the material is procured and processed according to tissue banking protocols.

There are certain organizations regulating allograft acquisition, processing, and use:

FDA:-

FDA Centre for Biologics Evaluation and Research (CBER) regulates human cells, tissues, and cellular based products under federal law, title 21 of U.S. Code of Federal Regulations (CFR), parts 1270 and 1271. CFR Title 21 part 1271 requires HCT/P (Human Cellular and Tissue Based Products) manufacturers to register their companies and products with FDA CBER and comply with applicable FDA regulations.

Aatb:-

American Association of Tissue Banks is an independent non profitable organization dedicated to ensuring and maintaining the safety, consistency, and availability of allografts in the United States. To fulfill this mission, the AATB, publishes tissue banking industry standards and offers rigorous accreditation for institutional members as well as a certification program for people working in the field. By accepting AATB accreditation, tissue banks agree to comply with onsite inspections of processing facilities, annual audits, and other various AATB prescribed safety regulations. Additionally, by satisfying AATB accreditation, tissue banks help ensure their compliance with FDA HTC/P regulations.

Producing an allograft worthy of distribution and implantation requires strict attention to detail throughout a comprehensive process. This process begins with donor screening.

Donor Screening and Testing:-

The donor's medical/social history is screened for medical conditions or disease processes that would contraindicate the donation of tissues in accordance with current policies and procedures approved by bone banks meeting the standards established by FDA. A donor should be in good systemic health and free of infectious diseases having potential risk of transmission. The contraindications for bone tissue donation include:

1. Donor from high risk groups, as determined by medical testing and/or behavioral risk assessments.
2. Donors testing 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).

Procurement of Allografts:-

Various steps in the preprocurement of human bone allografts are as follows¹⁶:

1. **Notification of prospective donor's death** - Hospitals or morgues notify tissue recovery agencies of human deaths.
2. **Determination of initial donor eligibility** -The tissue recovery agency determines donor eligibility on the basis of readily available information (for example, age, cause of death, evidence of infection, history of systemic disease, evidence of drug use).
3. **Consent**- If a potential donor is deemed acceptable, the tissue recovery agency obtains and documents consent from relatives or caretaker of the donor according to U.S. Food and Drug Administration regulations and state anatomical gift laws.
4. **Dispatch of recovery team**- Most tissue recovery agencies use their own recovery teams to evaluate and procure potential donor tissues.
5. **Assignment of tracking number to prospective donor**-The dispatched tissue recovery team assigns a unique tracking number to the potential donor.
6. **Determination of additional donor eligibility**- The tissue recovery team confirms donor identity, reviews medical records, performs a full body physical assessment, reviews critical time limits and verifies the temperature of the cadaver's storage.
7. **Tissue procurement** -The tissue recovery team must procure the tissue within 12 hours of death for non - refrigerated cadavers or within 24 hours for refrigerated cadavers.
8. **Autopsy**- Some tissue procurement agencies perform autopsies on potential donors as an additional screening procedure.
9. **Transport**- The tissue recovery team transports harvested donor tissue, blood samples and relevant medical records to the tissue processing center.

Steps in manufacturing and processing of allografts^{1, 9, 17}:-

Long bones are the source for periodontal bone allografts. Cortical bone is the material of choice because it has been found to be less antigenic than cancellous bone. BMP is located in the bone matrix and since the mass of bone matrix is greater in cortical than cancellous bone, the increased amount of BMP is present in cortical bone. BMP concentration is greater in cortical than cancellous bone in quantities 1 mg/kg of wet weight of fresh bone.

1. First of all soft tissue stripping is done to remove residual muscle, tendon, ligament, and so forth.
2. The cortical bone is rough cut to particle size ranging from 500 µm to 5 mm. This fragmentation increases the efficiency of defatting of bone and subsequent decalcification.

3. The graft material is then immersed in 100% ethyl alcohol for 1 h to remove fat that may inhibit osteogenesis and to inactivate viruses. Viral infectivity is undetectable within 1 min of treatment with 70% ethyl alcohol.
4. The bone is frozen at -80°C for 1 to 2 weeks to interrupt the degradation process and the tissue water is removed by the process of lyophilization. This process is commonly referred to as freeze drying. During this time, the results from bacterial cultures, serologic tests, and antibody and direct antigen assays are analyzed. If contamination is found, the bone is discarded or sterilized by additional means.
5. Freeze drying removes more than 95% of water content from the bone. Although freeze drying kills all cells, it has the advantage of facilitating long term storage and reducing antigenicity.
6. The cortical bone is ground and sieved to a particle size of approximately 250 to 750 μm .
7. Particle sizes within this range have been shown to promote osteogenesis, whereas a particle size below 125 μm can induce a significant foreign body giant cell response.
8. The graft material is again immersed in 100% ethyl alcohol and washed repeatedly to remove chemicals used in processing.
9. Decalcification with 0.6 N hydrochloric acid removes the calcium from the bone matrix and exposes the bone inductive proteins. This step is not needed if unmineralized freeze dried bone is the desired end product, such as in orthopedic and oral surgery procedures in which structural stability is necessary.
10. The bone is washed in a sodium phosphate buffer to remove residual acid.
11. If the bone is demineralized, it is refreeze dried.
12. Vacuum sealing in glass containers protects against contamination and degradation of the material while permitting storage at room temperatures for an indefinite period of time.

As a result of allograft processing, there is an exponential reduction in the potential for graft contamination, disease transfer, or both. With proper processing, allografts for dental purposes routinely achieve sterility assurance level (SAL) of 10^{-6} . SAL is probability that an item will not be sterile after it has been subjected to a validated sterilization process⁸. With a SAL of 10^{-6} , the odds of an organism's surviving after allograft processing are less than one in 1 million⁶. There is no need of secondary sterilization after procuring the bone as usually most bone banks procure the bone under sterile conditions. But if bone allograft is contaminated at the time of procurement, it has to be sterilized using ionizing radiation or ethylene oxide.

After processing bone allograft has to undergo certain tests which include:-

1. **Visual inspection test**- Visual detection is done for problems such as gross graft contamination, packaging defects and product mislabeling.
2. **Residual moisture test** -Testing of freeze dried allografts is done to ensure residual moisture is 6 percent or less.
3. **Residual calcium test** - Testing of demineralized freeze dried bone allograft is done to ensure residual calcium content is 8% or less.

Demineralized Freeze- Dried Bone Allograft (Dfdba):-

Advantage of demineralized bone arises from the fact that the organic bone matrix (collagen fibers) has to be exposed in order to remove its mineral components, and therefore so-called matrix proteins (e.g. morphogenetic proteins) can easily diffuse into the implantation site and work osteoinductively (Barradas et al., 2011)¹⁶. Most common products of demineralized freeze dried bone allograft are (Bio-Oss®, Endobone®). Due to their osteoconductive properties they can serve as an inactive scaffold or platform for the maturation of bone cells present within the defect. They are used in orthopedics, dental and maxillofacial surgery, as well as in periodontology and implantology, etc. (Baldini et al., 2011¹⁹; Cao et al., 2009²⁰; Jian et al., 2008²¹; Merks et al., 2003²²; Precheur, 2007²³; von Wattenwyl et al., 2011)²⁴. An alternative solution, eliminating the potential complications associated with the application of materials of autogenous, allogenic or xenogenic origin, is the use of alloplastic implants for the purpose of bone replacement. Such implants can be synthesized from both natural and synthetic materials (Bohner, 2010; Giannoudis et al., 2005)²⁵.

Effectiveness of DFDBA:-

The effectiveness of demineralized bone matrices might differ depending on the age and gender of the donor, the residual mineral, the particle size, or the preparation method^{26,27}. According to Sayler et al the success and safety of demineralized bone implants as well as different characteristics of the product, including its osteoinductive potential, depend on the technological process used to produce them^{10,11}. 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. Commercial bone banks do not verify the specific amount of BMPs or any level of inductive capacity in any graft material they sell. Therefore, graft quality cannot be considered standardized. Delaying the procurement of donor bone after death, improper storage conditions or other processing factors may play a significant role in the bioactivity of allograft that makes the way to the clinician's office. In addition, age, gender and medical status of deceased donors may also affect osteogenic activity in grafts taken from them.

Another concern is what happens to DFDBA when placed in periodontal defect over time. If DFDBA particles remain in the site for longer than a year acting as bone matrix, they may weaken the host bone and delay normal bone formation possibly by interfering with the osteoclasts' ability to resorb the DFDBA particles. 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 1000 μm possess a higher osteogenic potential than do particles below 125 microns. Optimal particle size appears to be between 100 to 300 μm .²⁶

This may be due to a combined effect 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 μm is the most frequently available. Glowacki and Mulliken developed the technology of preparing demineralized bone implants in powder form. Powder provides the maximum surface area necessary for interaction with recipient target cells, which stimulates endochondral proliferation. Glowacki and colleagues demonstrated that the extent of bone induction is a function of the surface area of the implanted bone.²⁷

Safety of allografts:-

Two important concerns on use of allografts are antigenicity and disease risk transmission.

Antigenicity:-

Use of allografts whether medical or dental field have always been a major concern as these grafts are procured from healthy donors. The Proceedings from the State of Art Workshop 1 held in 1982 stated "a principal concern with allografts is the problem of graft rejection." In humans, chromosome 6 contains the major histocompatibility complex (MHC), which codes for the human lymphocyte antigens (HLA). These antigens are expressed on the cell surface of nearly every nucleated cell in the body and represent the primary stimulus for transplant tissue rejection when HLA mismatches occur between donor and recipient. Detection of donor-specific anti-HLA antibody formation in a patient receiving allografts is an important measure of the clinical immunogenicity of the respective graft material²⁸. With tissue processing, cell death occurs, whether this is performed after aseptic procurement or during terminal sterilization, the magnitude of a possible immune reaction is considerably diminished.

Risk of disease transmission with the use of allografts:-

Viral disease transmission has been more frequently encountered especially HIV which is associated with bone allografts. First case was reported with HIV and bone allografts in 1988²⁹. Donor medical records revealed history of intravenous drug abuse and also history of lymphadenopathy which was suggestive of HIV. Second issue about the disease transmission is bone tissue banks which is not accredited and follow the instructions according to AATB and when these grafts are taken from such banks results in transmission of diseases. Most common method used for sterility of grafts is irradiation which prevents the risk of infection of HIV. A study conducted by Smith et al have reported that doses in which tissue is compromised at 1.5-2.5Mrads was not effective for sterilization of graft and was ineffective against HIV. Delay in processing of FDBA and DFDBA should ensure testing and safety of these grafts. Deep freezing of allografts reduces the disease transmission to 1 in 8 million³⁰. Risk of HIV transmission following proper processing of DFDBA has been reported in 1 in 2.8 billion^{31,32}. Hence, DFDBA offers more reliability over FDBA in an environment where there is possibility of viral contaminants³³.

Human trafficking of allografts:-

Human bone allograft taken from tissue bank must be tracked according to FDA and informed to recipient by the clinician about the product recall. In 1997 FDA proposed an approach to regulate HCT/Ps under 21 CFR part 1271.290 which deals with tracking protocol for human allografts and also investigates the disease risk and contamination of bone allografts. According to this regulation HCT/P must label the processing facilities with a unique alphabetical code which does not contain donor identity. The significance of this code is the graft can be

tracked to its and recipient and also manufacture details are also provided. Most of the tissue banks issues tracking form of the graft, these forms consists of three copies: one to the patient, one form to the clinician and one form to the tissue bank. Clinicians who have used recalled allografts should be notified to the recipients and it is tested for any susceptible pathogen for minimum of 6 months after the graft has been implanted.

Conclusion:-

Human bone allografts are predominantly used in clinical practice for the treatment of periodontal defects, this reflects its safety and usefulness of these products. FDA and agencies like AATB allows tissue processing facilities to uphold their fiduciary responsibilities to the public. These products when used in clinical practice should be investigated thoroughly and be familiar with such agencies which patronize it. This gives a clear knowledge about the processing of the products and its safety measures. So while purchasing allografts, it should be ensured to choose the products accredited by responsible organizations to ensure the safety and quality of the product and better educate the patients regarding concerns about this valuable treatment option.

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