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# VALIDATION OF BONE GRAFTS IN PERIODONTAL THERAPY-A REVIEW

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## ABSTRACT

**Background:** Bone grafts are widely used in the treatment of periodontal osseous defects. However, many are unfamiliar with their preparation, exact mechanism of action and processing as well as their use as safe and effective graft materials in periodontal therapy. Also, the clinical benefits of this therapeutic practice require further clarification through a proper review.

**Aim:** The purpose of this review is to access the efficacy of bone replacement grafts in proving demonstrable clinical improvements in periodontal osseous defects.

**Methods**: Data Sources: A literature search was conducted on several medical databases. For study inclusion, all studies that used bone graft in the treatment of periodontal osseous defects were included. Around 80 relevant articles were selected for this review.

**Results**: A large body of evidence clearly indicates that grafts consistently lead to better bone fill than non grafted controls. As more is learned about the biologic process of periodontal regeneration, new graft materials are expected to make the task of periodontal regeneration even more predictable.

**Conclusion**: Bone replacement grafts (BRG) are widely used in the treatment of periodontal osseous defects. Our review of literature strongly suggests better bone fill, gain in clinical attachment level and reduced probing depths with the use of various grafts as compared to non grafted sites. However, the clinical benefits of this therapeutic practice require further clarification through a systematic review of randomized controlled studies.

**Keywords:** bone grafts, periodontal regeneration, prognosis

## INTRODUCTION

Bone grafting is a surgical procedure that replaces missing bone. Bone generally has the ability to regenerate completely but requires a very small fracture space or some sort of scaffold to do so. Most bone grafts are expected to be reabsorbed and replaced as the natural bone heals over a few months' time.

Bone grafts can effect bone replacement through three different mechanisms: osteogenesis, osteoinduction and osteoconduction. [1]

Osteogenesis refers to organic material capable of forming bone directly from osteoblast.[1] An osteogenic graft is derived from or composed of tissues involved in the natural growth or repair of bone. It is for this reason that they can even encourage bone formation in soft tissues or

activate more rapid bone growth in bone sites. [2], [3]

Osteoinductive materials are capable of inducing the transformation of undifferentiated mesenchymal cells into osteoblasts or chondroblast and enhance bone growth or even grow bone where it is not expected. [1] This process, first described by Urist, does not necessitate the presence of living cells in the grafts. Some factors now known as Bone Morphogenetic Proteins (BMPs) located primarily in cortical bone are involved in this mechanism. [4]

Osteoconduction is characteristic of a material (often organic) which permits bone apposition from existing bone and requires the presence of bone or differentiated mesenchymal cells.[5],[6] Osteoconduction provides a physical matrix or scaffolding suitable for the deposition of new bone. Osteoconductive grafts do not produce bone formation themselves when placed within soft tissue.[2], [3] It permits Osteogenesis when cells already committed to bone formation are present in a closed environment. A material is said to be osteoconductive when its structure and its chemical composition facilitate new bone formation from existing bone. This means that this material has to be inserted in a bony site, an orthotopic site.

## CLASSIFICATION OF BONE GRAFTS

Human bone
Auto grafts
- Extra oral
- Intraoral

Allografts
- Fresh frozen bone
- Freeze-dried bone allografts
- Demineralized freeze-dried bone allografts

Bone substitutes
Xenografts
- Bovine-derived hydroxyapatite
- Coralline calcium carbonate

Alloplastic grafts
- Polymers
- Bioceramics
  - Tricalcium phosphate
  - Hydroxyapatite
- Bioactive glasses

Mechanism of action - autogenous bone grafts[2]

To understand the mechanism of action of bone grafts, biology of bone healing has to be clearly understood. The bone repair process begins with an inflammatory response that prompts granulation tissue to proliferate in the wound site. This granulation tissue brings in capillaries, fibroblasts, and osteoprogenitor cells.

Similarly, revascularization is initiated within the first few days following the grafting procedure. Blood vessels originating from the host bone invade the graft. If the graft material contains vital osteogenic precursor cells that survive the transplantation process, these cells may contribute to new bone formation. Osteoblasts, are produced by the osteoprogenitor cells in the granulation tissue, stimulate organic matrix of woven bone and to initiate mineralization. This healing mass of new tissue is called the callus, and it is an architecturally disorganized mass. Woven bone - replaced by lamellar bone as bone remodeling units invade the healing area. This entire process is called osteogenesis.

Autogenous bone grafts are composed of organic and inorganic structures. Resilience, toughness, and continuity are related to collagen, of the organic component. Stiffness, hardness and rigidity are characteristics of the inorganic aspect; a crystalline, ceramic-like material which is primarily hydroxyapatite (HA). This inorganic matrix contains organic components of osteocytes, osteoclasts, osteoblasts, osteogenic signaling proteins and various amount of mesenclymal tissue.

Grafted autogenous bone heals in three phases. During the first phase, the surviving cells are responsible for the formation of osteoid by osteogenesis. They are most active within the first four weeks after bone grafting. [7] The blood vessels from the host bone and the connecting

tissue invade the graft. Bone cells from the host tissue follow the blood vessels and remodel the graft by a coupled resorption and formation phenomenon.[8] The BMP derived from the mineral matrix of the grafted bone through the resorbing action of osteoclast, acts as a mediator for the second phase. [4], [9] The BMP and other proteins must be released prior to the osteoinduction cycle. Phase three occurs as the inorganic component of bone acts as a matrix and source of minerals during replacement of the matrix by the surrounding bone and resembles an osteoconductive mode of action. The three phases overlaps in the time sequence and are not separate phases of growing bone from the grafted autogenous material. Grafted autogenous bone can be trabecular (cancellous), cortico-cancellous or cortical. The cancellous portion of grafts provides the cells for osteogenesis and survives best when a blood supply from the host bone is readily available. Cortico-cancellous block grafts permit contouring and adaptation of the graft to the recipient bed anatomy.

The trabecular portion is placed on the host bone and the cortical aspect is positioned on the surface of the graft. The cancellous portion is primarily responsible for the living bone cells and osteogenesis and therefore placed closest to the new blood vessels which arrive from the host bone and enter the graft at a rate of 0.5mm/day. [10] The cortical graft supports osteogenesis only from the surviving cells fewer than trabecular bone and also provides more of the BMP compared with trabecular bone for the second osteoinductive phase [11] The cortical aspect also provides a more resistant scaffold for the third osteoconductive phase. In addition, it may act as a barrier to soft tissue invasion thus excluding the need for a GTR membrane and provide an extended period for blood vessels to enter the graft from the host bone [1]

Autogenous bone grafts:

Harvested from the patient's own body and is the gold standard among graft materials because they are superior at retaining cell viability. These grafts contain live osteoblasts and osteoprogenitor stem cells and heal by osteogenesis.

Avoid the potential problems of histocompatibility differences and the risk of disease transfer. Autogenous cancellous bone graft is the most effective bone graft material possessing all 3 characteristics.

Limitations include the increased operative time, limited availability and significant morbidity related to blood loss, wound complications, local sensory loss and, most importantly, chronic pain. [4] Donor site pain persisting for more than 3 months has been reported in up to 15% of patients having an iliac graft harvested. The amount of pain seems to be proportional to the extent of dissection required to obtain the graft. [5]

Mellonig JT, Bowers GM reported 3 to 3.5 mm of clinical bone fill is usually obtained in intraosseous defects when the site is grafted compared with less than 1 mm of fill in sites that are not grafted.[12]

Histologic evaluations suggest that at least partial periodontal regeneration occurs after autogenous grafting. In the early stages of autograft healing, new bone originates from the surviving osteoprogenitor cells, and in later stages, it originates from the osteoinduction response of the host bone. The area of new bone interdigitation and the quantity of donor bone that is resorbed is higher for cancellous bone grafts compared with cortical grafts. Autogenous bone should be used whenever possible in graft cases. [12]

Autogenous bone from intraoral sites :

In 1923, Hegedus attempted to use bone grafts for the reconstruction of bone defects produced by periodontal disease. Sources of bone include- bone from healing extraction wounds, bone from edentulous ridges, tori, the maxillary tuberosity, bone trephined from within the jaw without damaging the roots, newly formed bone in wounds especially created for the purpose, and bone removed during osteoplasty and ostectomy.

Types of autogenous bone grafts osseous coagulum, Bone blend of cortical and cancellous intraoral bone, cortical bone chips, Bone swaging & Intraoral Cancellous Bone Marrow Transplants

Autogenous bone from extraoral sites – In 1969 Cushing claimed that extra oral cancellous bone and marrow offer the greatest potential new bone growth. In 1968 Schallhorn obtained this material either from anterior or posterior iliac crest.

Mechanism of action of allograft: [7]

Bone allografts are procured usually within 12 hours of death of a suitable donor. Cortical bone is harvested in a sterile manner. Long bones are the source for periodontal bone allografts. The cortical bone is rough cut to a particle size ranging from 500 um to 5mm. The graft material is then immersed in 100% ethyl alcohol or a similar solvent for 1 hour to remove fat that may inhibit osteogenesis. The cortical bone is ground and sieved to a particle size range of approximately 250 to 750 um. 5. Decalcification with 0.6 or 0.5 N hydrochloric acid removes the calcium, leaves the bone matrix, and exposes the bone-inductive proteins. The bone is washed in a sodium phosphate buffer to remove residual acid. The cortical bone is frozen at -80°C for 1 to 2 weeks to interrupt the degradation process. During this time, the results from bacterial cultures, serologic tests, and antibody and antigen assays are analyzed. If contamination is found, the bone is discarded or sterilized by additional methods and so labeled. Freeze-drying removes more than 95% of the water content from the bone.

Allografts are obtained from cadavers or from patients' living relatives or non-relatives. Basically these bone grafts are of the same species but different genotypes. After processing, they are stored in bone banks. The advantages of allografts are availability, elimination of the donor site in the patient, decreased anesthetics and surgery time, decreased blood loss, and fewer complications.

However, it is associated with some disadvantages which relate to bone tissues coming from another individual. Consequently the medical history must be thoroughly checked to eliminate donors with history of infection, malignant neoplasm's, degenerative bone diseases, hepatitis B or C, sexually transmitted diseases, autoimmune disease and other problems which affects the quality of the bone and the health of the recipient. [13]

There are 3 main types of bone allografts:

1. Frozen, freeze-dried (lyophilized),
2. Demineralized freezed dried bone (DFDB),
3. Mineralized deproteinized and irradiated allograft.

Fresh allografts are the most antigenic. Freezing or freeze-drying the bone significantly reduces its antigenicity. Allografts are not osteogenic and so, bone formation takes longer and results in less volume than can be achieved with autogenous grafts [1] . Allograft is said to form bone by osteoinductive effect on surrounding undifferentiated mesenclymal cells in the soft tissue over the graft as the blood vessels grow into the graft. It may also form bone by the osteoconduction phenomenon when the host bone resorbs the material and grows into its scaffold.

Mineralized Freeze-Dried Bone Allograft (FDBA):

It was introduced to periodontal therapy in 1976. This material is osteoconductive. Although FDBA contains inductive proteins, the polypeptides are sequestered by calcium. This material is resorbed and replaced by host bone very slowly. Freeze-dried bone allograft is the only graft material that has undergone extensive field testing for the treatment of adult periodontitis.

FDBA is still used today, but a large-scale research review showed that FDBA mixed with autogenous bone is more effective at increasing bone fill than FDBA alone. [14]

Sanders et al in 1983 found that more than 50% bone fill was achieved in 80% of test cases grafted with FDBA plus autogenous bone but in only 63% of controls grafted with FDBA alone.

Mellonig and co-workers - reported bone fill exceeding 50% in 67% of the defects grafted with

FDBA and in 78% of the defects grafted with FDBA plus autogenous bone. [15]

FDBA, however, is considered an osteoconductive material, whereas decalcified FDBA (DFDBA) is considered an osteoinductive graft.

Decalcified freeze-dried bone allograft (DFDBA):

Also referred to as allogeneic, autolyzed, antigen-extracted (AAA) bone. Experiments by Urist have established the osteogenic potential of DFDBA. [4] Demineralization in cold, diluted hydrochloric acid exposes the components of bone matrix, closely associated with collagen fibrils, which have been termed bone morphogenetic protein.

Bowers et al in 1991 evaluated osteogenin combined with DFDBA, DFDBA alone, osteogenin combined with bovine collagen, and bovine collagen alone in human periodontal osseous defects. The ability of each material to regenerate a new attachment apparatus of bone, cementum, and periodontal ligament was evaluated in submerged and nonsubmerged environments using two patient populations. Mean results indicated that osteogenin combined with DFDBA significantly enhanced regeneration in a submerged environment.

Mellonig and associates tested DFDBA against autogenous materials in the calvaria of guinea pigs and showed it to have similar osteogenic potential. [16]

These studies provided strong evidence that DFDBA in periodontal defects results in significant probing depth reduction, attachment level gain, and osseous regeneration.

The combination of DFDBA and guided tissue regeneration has also proven very successful.

Human clinical studies have shown DFDBA grafts result in 2.5 to 3 mm of bone fill, which is somewhat less than autogenous bone.[16]

Delipidization with ether, alcohol, acetone, hexachlorophene, common detergents may even enhance bone induction. [4] The osteogenic ability of different grafting materials, autogenous osseous coagulum, autogenous bone blend, FDBA and DFDBA, packed in nylon chambers, and implanted in guinea pig calvaria defects, were compared in an investigation.[14] The newly formed bone was determined by the incorporation of a radionuclide, 85 Strontium, and evaluated histologically at different time intervals ranging from 3 to 42 days. The authors concluded that DFDBA is a material of high osteogenic potential, while osseous coagulum and bone blend show less potential, and FDBA is the least effective.

Future Directions with DFDBA

The enhanced osteogenic potential of DFDBA is the result of a variety of bone-inductive proteins located within the bone matrix. [4] These proteins have been termed bone morphogenetic proteins (BMPs).

At the very least, nine BMPs (BMP-1 through BMP-9) have been cloned and characterized, and some are available in human recombinant form.

Animal experiments have demonstrated that the BMPs have the ability to induce bone and repair bone defects at a variety of anatomic sites.[17] Therefore, more recent studies have attempted either to combine DFDBA with BMPs or to evaluate BMPs with a carrier.

Gendler demonstrated by experiments that perforated demineralized bone matrix was a new form of osteoinductive material. [18] It was demonstrated that subcutaneous implantation of perforated decalcified bone matrix (PDBM) induced multiple centers of endochondral osteogenesis with subsequent resorption of bone matrix and replacement by new bone.

**Mechanism of action of alloplast bioceramics**

Bioceramic alloplasts are primarily composed of calcium phosphate. Calcium phosphate biomaterials have excellent tissue compatibility and do not elicit any inflammation or foreign body response. These materials are osteoconductive.

Two types of calcium phosphate ceramics are Hydroxyapatite (HA) and Tricalcium phosphate (TCP)

Hydroxyapatite (HA)

Synthetic HA's have been marketed in a variety of forms, primarily as a porous or dense nonresorbable material or as resorbable, porous. HA ceramic's resorbability is determined by the temperature at which it forms. Dense HA grafts are osteoconductive, and act primarily as inert biocompatible fillers.

They have produced clinical defect fill greater than flap debridement alone in the treatment of intrabony defects. [19] Histologically, new attachment was not achieved. They yield similar defect fill as other graft materials, and the clinical improvement achieved is more stable than with debridement alone. Yukna et al in 1989 demonstrated that over a 5-year period open flap debridement was not stable and regressed three to five times faster than sites treated with HA.

Porous HA is obtained by the hydrothermal conversion of the calcium carbonate exoskeleton of the natural coral into HA.

It has a pore size of 190 to 200µm, which allows fibrovascular in growth and subsequent bone formation into the pores and ultimately within the lesion itself. Clinical defect fill, reduction of probing depth, and attachment level gain have been reported.

As with dense HA, any regeneration is limited to only the apical portion of the defect. Kenney et al provided histologic evidence suggesting that porous HA could stimulate osteogenesis, but because no evidence of new connective tissue attachment or cementum was noted.

Another form of synthetic HA is a resorbable, low-temperature-processed, particulate material. The resorbable form is nonsintered with particles measuring 300 to 400µm. It has been proposed that nonsintered HA resorbs acting as a mineral reservoir inducing new bone formation via osteoconductive mechanisms. Its reported advantage is its slow resorption rate allowing it to act as a mineral reservoir at the same time acting as a scaffold for bone replacement.

Tricalcium phosphate (TCP)

It is mineralogically B-whitlockite. TCP is partially bioresorbable. Tricalcium phosphate is a porous form of calcium phosphate. The proportion of calcium and phosphate is similar to bone.

It serves as biologic filler, which is partially resorbable and allows bone replacement of the implant material. Conversion of graft material is pivotal to periodontal regeneration. First, serving as a scaffold for bone formation, then permitting replacement with new bone.

Tricalcium phosphate as a bone substitute has gained clinical acceptance, but results are not always predictable. Tricalcium phosphate does not seem to initiate osteogenesis.[20] The particles generally become encapsulated by fibrous connective tissue and do not stimulate bone growth. Some bone fill, however, has been achieved with tricalcium phosphate grafts.

These ceramics form the new bone strictly by osteoconduction with the new bone formation taking place along their surface.[1] A chemical contact between the host bone and grafted material may be developed as well as possible stimulus for bone activity.[21]

**HTR Polymer**

The acronym stands for hard tissue replacement. HTR (Bioplant) is a nonresorbable biocompatible microporous composite of polymethylmethacrylate (PMMA), polyhydroxyl ethylmethacrylate (PHEMA), and calcium hydroxide.

Favorable clinical results have been achieved with HTR for the treatment of infrabony defects and furcation defects. Improved clinical results with this synthetic substitute have not always been achieved. Although Shahmiri et al in1992 demonstrated no clinical improvement in probing depth; most reports have supported the use of HTR as a bone substitute.

Histologically, new bone growth has been found deposited on HTR particles.[22] It appears to serve as a scaffold for new bone formation when in close contact to alveolar bone.

Its hydrophilicity enhances clotting, and its negative particle surface charge allows it to adhere to bone. In moderately severe intrabony human periodontal defects, a mean defect resolution of 75% has been reported with HTR.[23] They also reported significant clinical defect fill and resolution can be achieved, supporting its use as a biocompatible alloplastic bone substitute.

Furcation repair was better with HTR than that achieved with autogenous bone.[24] It is a clinically beneficial, biocompatible, osteophilic, and osteoconductive alloplastic bone substitute.

**Mechanism of action – Xenografts**

Two available sources of xenografts used as bone substitutes in clinical practice: bovine bone and natural coral.

Both sources, through different processing techniques, provide end products that are biocompatible and structurally similar to human bone. Xenografts are osteoconductive. Commercially available bovine bone is processed to yield natural bone mineral minus the organic component. Advantage of the product as a bone substitute is that it is natural in that it can provide structural components similar to that of human bone, improving its osteoconductive capability over synthetically derived mineral.

Boplant (Calf bone): treated by detergent extraction, sterilized, and freeze dried, has been used for the treatment of osseous defects.

Kiel bone: is calf or ox bone denatured with 20% hydrogen peroxide, dried with acetone, and sterilized with ethylene oxide.

Ospurum: Fosberg described the use of ospurum for treatment of periodontal defects.[25] This is Ox bone which is soaked in warm potassium hydroxide to remove connective tissue, in acetone to remove lipids, and in a soft solution to remove proteins.

Anorganic bone: is ox bone from which the organic material has been extracted by means of ethylenediamine; it is then sterilized by autoclaving. Anorganic bovine bone is the HA skeleton, which retains a highly porous structure similar to cancellous bone that remains after chemical or low heat extraction of the organic component.

Yukna has used a natural, anorganic, microporous, bovine-derived hydroxyapatite bone matrix, in combination with a cell-binding polypeptide that is a synthetic clone of the 15 amino acid sequence of type I collagen. The addition of the cell binding polypeptide was shown to enhance the bone regenerative results of the matrix alone in periodontal defects.

Historically, bovine xenografts have failed owing to rejection, probably because earlier materials used chemical detergent extraction that left residual protein and therefore produced adverse reactions and clinically unacceptable results.

Currently available bovine-derived HA is deproteinated, retaining its natural microporous structure that supports cell-mediated resorption. This becomes important if the product is to be replaced with new bone.

Two products are currently available: OsteoGraf and BioOss. Both have been reported to have good tissue acceptance with natural osteotrophic properties. Histologically, no fibrous tissue or space between the HA and newly formed bone is found. This is in contrast to histologic reports obtained with synthetic HA.

Bovine-derived HA bone substitutes increase the available surface area that can act as an osteoconductive scaffold because of their porosity. This HA mineral content is comparable to that of bone, allowing it to integrate with bone. They have been used with success for the treatment of intrabony defects and ridge augmentation.

**Coralline calcium carbonate**

Biocoral is calcium carbonate obtained from a natural coral, genus Ponies, and is composed primarily of aragonite (>98% calcium carbonate). It is biocompatible and resorbable with a pore size of 100 to 200μm, similar to the porosity of spongy bone. Its porosity, at greater than 45%, provides a

large surface area for resorption and replacement by bone.

In contrast to porous HA, derived from the same coral by heat conversion and nonresorbable, calcium carbonate is resorbable.

It does not require a surface transformation into a carbonate phase as do other bone substitutes to initiate bone formation; hence, it should more rapidly initiate bone formation.

Bicoral has a high osteoconductivity potential because no fibrous encapsulation has been reported. When compared with other bone substitutes, coralline calcium carbonate produces comparable results. Significant gain in clinical attachment, reduction of probing depth, and defect fill has consistently been reported.

**Mechanism of action of Bio active glass:**

There are two forms of bioactive glass currently available PerioGlass and bioGran. Bioactive glasses are composed of $SIO_2$, CaO, $Na_2O$, $P_3O_5$ and bond to bone through the development of a surface layer of carbonated HA. When exposed to tissue fluids in vivo, the bioactive glass is covered by a double layer composed of silica gel and a calcium phosphorus-rich (apatite) layer. The calcium phosphate-rich layer promotes adsorption and concentration of proteins used by osteoblasts to form a mineralized extracellular matrix. It is theorized that these bioactive properties guide and promote osteogenesis, allowing rapid and quick formation of new bone

PerioGlas is osteoconductive. Has particle size ranging from 90 to 710 µm. 68% defect repair was achieved when used on surgically created defects in monkeys. [26]

He also compared tricalcium phosphate, HA, and unimplanted controls, and showed PerioGlas to produce significantly greater osseous and cementum repair. It also appeared to retard epithelial downgrowth, which the authors contend may be responsible for its enhanced cementum and bone repair. [26]

Biogran has particle size of 300 to 355 µm size range. Formation of hollow calcium phosphate growth chambers occurs with this particle size because phagocytosing cells can penetrate the outer silica gel layer by means of small cracks in the calcium phosphorus layer and partially resorb the gel. This resorption leads to the formation of protective pouches where osteoprogenitor cells can adhere, differentiate, and proliferate.

According to the manufacturer, larger particles do not resorb in the same manner, which slows the healing process theoretically because bone healing must progress from the bony walls of the defect and smaller particles cause a transient inflammatory response, which retards the stimulation of osteoprogenitor cells.

Biogran has a clinical advantage over the PerioGlas preparation, which has multiple particle sizes. Clinically, no comparison has been made between the products, and no human periodontal studies are available.

A human study by Schepers et al in 1993 demonstrated that Biogran could be used successfully in the treatment of oral osseous defects.

## CONCLUSION

Although complete periodontal regeneration is unpredictable with any regenerative therapy currently used, periodontal bone grafts show strong potential. Requirements for a successful graft includes Patient Selection, material Selection, Proper Flap Reflection and Wound Stability, Revascularization, Root Debridement, Postsurgical care .A large body of clinical evidence clearly indicates that grafts consistently lead to better bone fill than nongrafted controls. As more is learned about the biologic process of periodontal regeneration, new graft materials are expected to make the task of periodontal regeneration even more predictable.

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