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Bone Grafts in Periodontics - An Overview

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***Abstract*: This paper presents an overview bone grafts in periodontics.**

***Keywords*: Bone Grafts, Periodontics.**

1. Introduction

Bone grafts are tissues taken from a patient's body or a natural substitute or synthetic biomaterial that is placed in bone defects to achieve re-establishment of the lost bone tissue. Ideally, a bone graft should undergo resorption after serving as a space maker over a definite period of time for its subsequent replacement by the host bone tissues. Bone tissue has the ability to regenerate completely if provided the space into which it can grow. As natural bone grows, it generally replaces the graft material completely, resulting in a fully integrated region of new bone.

2. Rationale

The rationale behind bone graft use originates from the osteogenic, osteoinductive, and/or osteoconductive properties possessed by the grafting materials. Osteogenic material contains bone-forming cells within the graft itself. The vital osteoblasts originating from the bone graft material contribute to new bone growth. Osteoinductive materials stimulate osteoprogenitor cells to differentiate into osteoblasts that then begin new bone formation in the surrounding tissue immediately adjacent to the graft. The most widely studied type of osteoinductive cell mediators are BMPs, PDGF and VEGF etc. Osteoconductive materials serve as scaffolds for bone growth within existing bony walls. Osteoblasts from the margin of the defect utilize the bone graft material as a framework upon which they spread and produce new bone [1].

3. Types of bone grafts

Bone grafts and bone substitutes can be classified into four groups, according to their origin: autografts, allografts, xenografts and alloplasts and their application modality encompasses several forms for application, such as block, granular, moldable, injectable or in-situ hardening materials.

A. Autogenous bone graft

Autogenous bone has been considered as the "gold standard" for regenerative therapy as it has osteogenic, osteoinductive, and osteoconductive properties. Autogenous bone provides mechanical support to the vessels and cellular elements that colonize the grafting site, and also stimulates bone formation at the graft site as it contains mature cellular elements that can directly create new bone. The inorganic component of bone (hydroxyapatite) contributes to the rigidity of the graft and the organic component (collagen) provides strength, durability, and stability. Autologous bone grafts may be the cortical, cancellous, or corticocancellous type.

1) Cortical Bone Grafts

Cortical bone grafts are blocks composed predominantly of cortical bone. They provide a very dense, compact bone that offers great structural support. A cortical bone graft is suitable for reconstruction of both horizontal and vertical defects and is usually placed as a block graft secured with screws to the underlying ridge. This type of graft takes longer to revascularize than a cancellous graft.

2) Cancellous (particulate) Bone Grafts

Cancellous bone grafts consist predominantly of trabecular bone tissue. Cancellous bone has higher osteogenic and osteoinductive properties than cortical bone and has a larger number of osteoblasts and progenitor cells. The structure of cancellous bone allows rapid revascularization of the graft. It

Graft material	Origin	Bone regeneration potential	Resorption time
Autograft	Patient's own tissue	Osteogenic Osteoinductive Osteoconductive	Weeks to months
Allograft	Tissue from individuals of the same species	Osteoconductive Osteoinductive (DFDB)	Months (4-12 months)
Xenograft	Tissue from other species	Osteoconductive	Months to a year
Alloplast	Synthetically produced material	Osteoconductive	Wide range (rapidly resorbable to non resorbable)

also reduces the number of cells that undergo necrosis, allowing rapid neoangiogenesis, with early incorporation of the graft. These grafts often exhibit greater resorption than block grafts because of their lower density. A limitation of cancellous bone grafts is their instability immediately after placement. This type of graft requires a rigid biologic scaffold provided by barriers or walls of bone. Cancellous bone grafts are suitable for covering periodontal fenestrations, peri-implant osseous defects, for obtaining small alveolar reconstructions in GBR, for filling the spaces between cortical bone grafts, and for sinus lift and split-crest procedures.

3) Corticocancellous Bone Grafts

Corticocancellous bone grafts are composed partly of compact (cortical) tissue and partly of spongy (trabecular) tissue. Ideally they have the best features as they have a large number of osteoblasts and osteoprogenitor cells and they also give good structural support [2].

4) Osseous coagulum

Osseous coagulum is a mixture of bone dust obtained from small particles ground from cortical bone and blood. Bone is obtained by using carbide bur, no.6 or no.8 at speeds between 5000 and 30000 rpm. The use of this material is based on the rationale that the small particle size is predictably resorbed and replaced by host bone. The advantage of the particle size is that it provides additional surface area for the interaction of cellular and vascular elements. The mineralized fragments are also thought to induce bone formation. Osseous coagulum procedures have disadvantages, which include aspiration problems, complications of salivary contamination and bleeding, an unknown quantity of collected bone fragments, and limitations concerning the quantity of bone that can be obtained.

5) Bone blend

The bone blend technique was designed to overcome some of the problems of osseous coagulum procedures. The bone blend technique uses an autoclaved plastic capsule and pestle. Bone is removed from a predetermined site (extraction socket, exostosis, edentulous area, or region of the defect) with chisels or rongeur forceps. The pestle and bone fragments are placed in the capsule, and a few drops of sterile saline are added. The capsue is closed, wrapped in sterile gauze, and placed in the triturator for 60 seconds (100 to 200 μm). Trituration reduces the bone fragments to a workable plastic like osseous mass, similar in consistency to slushy amalgam, that can be packed or molded into bony defects [3].

B. Donor site

The choice of the site for bone harvesting depends on the quantity and quality of bone needed to restore the proper morphology of the alveolar ridge. The choice also is influenced by the conditions at the recipient site, patient's expectations, and the dental practitioner's capabilities and preferences.

1) Intraoral Donor Sites

Intraoral bone sites are indicated for reconstruction of bone defects that affect the edentulous areas caused by one to three missing teeth or in maxillary sinus augmentation, because the amount of bone that can be harvested from intra oral sites is limited. The donor sites are maxillary tuberosity, tori, edentulous alveolar areas, including healing extraction sockets, symphysis, rami, mental and mandibular retromolar areas. A major advantage of an intraoral donor site is that the harvest site is close to the defect, which translates into reduced operating and anesthesia time and often accelerated healing because of the rapidity of mucosal healing in the oral cavity. There is also reduced postoperative morbidity compared with extraoral sites. Also, intraoral sites leave less scarring. Intraoral grafting procedure can be performed using local anesthesia or intravenous sedation, which reduces costs.

2) Extraoral Donor Sites

An advantage of extraoral donor sites is that a large amount of bone can be harvested for reconstruction of larger defects. A disadvantage of all extraoral sites is the need for a surgical site in addition to the intraoral site and the possibility of postoperative morbidity associated with the donor site. General anesthesia and hospitalization are often required for patient undergoing extraoral bone harvest [4].

4. Advantages of autogenous grafts

The use of autogenous bone graft has several advantages.

1. The osteogenic ability of autogenous bone results in more efficient release of osteoinductive growth factors {BMP 2, BMP 6 and BMP 9 (most osteoinductive), BMP 4, and BMP 7 (limited osteoinductivity), Insulin like growth factor I & II, Acidic and Basic fibroblast growth factors, Platelet derived growth factors, Interleukins, Granulocyte macrophage colony stimulating factors, etc.).
2. Has better osteoconductive surface for cell attachment and growth.
3. Acts as a space maker when used in combination with a barrier membrane.
4. Highly biocompatible with the recipient grafting site.
5. Low cost.

5. Disadvantages of autogenous grafts

Despite its advantages, autogenous bone has some disadvantages.

1. Unpredictable resorption time. Loss of graft volume in the magnitude of 50% has been reported during healing over a period of 6 months.
2. Risk of donor site morbidity and the need for two surgical sites.
3. Two surgical sites can increase both postoperative stress and the risk of infection.
4. Longer recovery time.

To overcome these disadvantages faced with autografts, alternative materials have been developed.

6. Allografts

Allografts are grafts transferred between different individuals of the same species. The graft tissues typically are processed from cadavers under sterile conditions to prevent an immune reaction in the recipient.

The steps in processing allografts for dental use usually include the following:

1. Cortical bone is obtained in a sterile manner within 12 hours of death of the donor. It is preferred over cancellous bone since it is less antigenic and contains more bone-inductive proteins.
2. Following procurement, the bone is cut into particle size of 0.5 to 5 mm and then immersed in 100% ethanol for 1 hour to remove fat that may inhibit osteogenesis.
3. Ethanol also has virucidal activity. Within 1 min of ethanol treatment, cortical bone is completely penetrated by ethanol and the viruses are inactivated.
4. Then, bone is frozen, further decreasing the risk of disease transfer.
5. Next, the frozen bone is ground to a particle size of 250 - 800 µm. This particle size range promotes osteogenesis, whereas particles smaller than 125 µm induce a macrophage response.
6. Then the bone particles are again immersed in ethanol.
7. Next, decalcification with 0.6 or 0.5 N hydrochloric acid is done, which removes calcium from the bone matrix and exposes the bone-inductive proteins.
8. This step is bypassed if mineralized freeze-dried bone is the desired end product
9. Bone is then washed in a sodium phosphate buffer to remove residual acid.
10. The cortical bone is frozen at – 80 C for 1 to 2 weeks to interrupt the degradation process.
11. During this time, the results from bacterial cultures, serologic tests, antibody and antigen assays are analyzed.
12. If contamination is found, the bone is discarded or sterilized by additional methods and so labeled.

Allografts may or may not be demineralized. The allograft is freeze-dried to permit long-term storage and reduce antigenicity.

A major concern with allografts in general is the potential for disease transfer, particularly viral transmission, and even more particularly HIV. Tissue banks have adopted rigorous exclusionary techniques, testing for HIV antigen and HIV antibody and lymph node biopsy in order to reduce this potential risk. Additionally, mere freezing of bone allografts reduces the risk of disease transfer to 1 in 8 million. Treatment of cadaveric bone spiked with viral particles and cortical bone procured from a donor who had died of AIDS with a viricidal agent and demineralization in HCL has been found to inactivate HIV. The probability of HIV transfer following appropriate demineralized freeze- dried bone allograft preparation has been calculated to be 1 in 2.8 billion. To date there is no reported case of disease transmission from freeze-dried bone allografts used for dental purposes in over 1 million cases over 25 years of use. A rare disease entity, Creutzfeldt-Jacobs disease, transmitted via preons (smallest proteinaceous infectious particles) has also never been reported from a bone-derived material. However, due to difficult exclusionary technicalities, Creutzfeldt-Jacobs disease may remain a remote potential risk.

The types of bone allografts used in periodontics are:

1. Frozen iliac cancellous bone (less frequently used).
2. Nondemineralized freeze-dried bone (FDB)
3. Demineralized freeze-dried bone (DFDB) (most often used)
4. Partially decalcified bone.

The methods of preparation of these allografts are distinct as are their biological properties.

A. Frozen iliac cancellous bone

They are osteoconductive and have proved successful in management of bony defects with various number of walls and even supracrestally to some extent. The possibility of disease transfer, antigenicity, and the need for extensive cross matching has precluded the use of frozen iliac allografts in modern periodontics.

B. Mineralized freeze-dried bone allografts

Freeze drying markedly reduces the health risks associated with fresh frozen bone and they are used extensively in the treatment of periodontal osseous defects. Cortical bone allografts preserved by freeze-drying were shown not to to elicit an immune response in non-human primates. Freeze-drying partially distorts the three-dimensional presentation of human leukocyte antigens, affecting immune recognition. The American Academy of Periodontology recommends use of cortical rather than cancellous bone allografts since cancellous bone is more antigenic. The freeze-dried bone allograft is osteoconductive. Mineralized freeze-dried bone allografts can be used alone or in combination with other graft materials. Experimental studies have shown that the addition of autogenous bone to freeze-dried bone allograft dramatically enhance its osteogenic potential.

C. Demineralized freeze-dried bone (DFDB)

DFDB is prepared by simply demineralizing bone in hydrochloric acid until the calcium content is reduced to less than 2%. Since DBM is prepared from particulate bone, its preparation entails freeze-drying the bone, grinding it, demineralizing it, and refreeze-drying it again. Thus in contradistinction to other freeze-dried bone allografts, DBM is freeze-dried twice.

The demineralized freeze-dried bone allograft has osteogenic potential. It is grossly amorphous, soft and does not provide structural support. Demineralization is said to increase the availability of bone matrix associated bone morphogenetic proteins [BMP], rendering these grafts highly osteoinductive. Demineralization of allografts is performed because the bone mineral blocks the effect of the factors stimulating bone growth

sequestered in bone matrix i.e. bone morphogenetic proteins. Bone morphogenetic proteins are a group of acidic polypeptides belonging to the transforming growth factor-β gene super-family. They stimulate bone formation through osteoinduction by inducing pleuripotential stem cells to differentiate into osteoblasts. Although demineralization releases BMP from bone, it also facilitates BMP elution and loss into the acid bath. Furthermore, other potentially antigenic proteins may be exposed during the demineralization process, eliciting an immune response that may result in inflammation and rapid graft resorption. The bioactivity of demineralized freeze-dried bone allograft appears to be age dependent and bone from younger animals has been shown to have an increased osteogenic potential compared with bone from older animals.

DFDB can be used alone or in combination with other graft materials. Experimental studies have indicated that the addition of autogenous bone to demineralized freeze-dried bone allograft does not dramatically enhance its osteogenic potential.

D. Partially demineralized bone

Partially demineralized, (partially decalcified or surface decalcified bone) is chemosterilized by Urist's technique and is an antigen extracted allogeneic bone. The calcium content of surface demineralized bone allograft is somewhat higher, usually around 20%. It has osteoconductive properties, but the preparation of this allograft is complex and time consuming.

E. Advantages of allografts

The use of allografts has many advantages,

1. Osteoconductive ability.
2. Physical structure similar to that of the recipient.
3. Availability of large amounts of donor bone.
4. Elimination of the risk of donor site morbidity.
5. Reduced surgical time.

F. Disadvantages

1. In rare cases it can cause an immune reaction.
2. It could transmit a viral infection from the donor to the recipient.
3. Although large amounts of bone are available, the clinician still must depend on a bone bank as a source [5].

7. Xenografts

They are heterologous materials derived from species other than the recipient. These materials are inert and resorb slowly (e.g, bovine, porcine derived, and natural coral HA). These sources, through different processing techniques, provide products which are biocompatible and structurally similar to human bone. Xenografts are osteoconductive, readily available, and free of disease transmission.

A. Bovine derived bone replacement graft

It is heterologous, anorganic, deproteinized bovine bone. Bovine bone is processed to yield natural bone mineral without the organic component. Anorganic bovine bone is the hydroxyapatite "skeleton" that retains the macroporous and microporous structure of cortical and cancellous bone remaining after chemical or low-heat extraction of the organic component.

Historically, bovine xenografts have failed due to rejection, probably because earlier materials used chemical detergent extraction, which left residual protein and therefore produced adverse reactions and clinically unacceptable results. Currently available bovine-derived hydroxyapatite is deproteinated, retaining its natural microporous structure, which supports cell-mediated resorption. This is important as the graft is to be replaced with new bone.

Bovine-derived hydroxypatite bone replacement grafts have increased available surface area that can act as an osteoconductive scaffold due to their porosity and have a mineral content comparable to that of human bone, allowing them to integrate with host bone. They have been used with success for the treatment of intrabony defects and in ridge augmentation.

B. Advantages

1. They provide a structure similar to that of human bone, with improved osteoconductive capability compared to synthetically derived materials.
2. These materials have micropores and macropores that promote both the stability of the clot and the apposition of new bone within the graft.

Coralline calcium carbonate: Biocoral (Inoteb, Saint Gonnery, France)

Calcium carbonate obtained from a natural coral (genus Porites) is composed primarily of aragonite (>98% $CaCO_3$,). It has a pore size of 100 to 200 μm, which is similar to the porosity of spongy bone. It has a calcium-to-phosphate ratio of 10:6 and is resorbable. It has a porosity of greater than 45%, which provides a large surface area for resorption and replacement by bone.

It does not require a surface transformation into a carbonate phase (which occurs with synthetic coral) to initiate bone formation. Hence, it more rapidly initiates bone formation and has a high osteoconductive potential with no fibrous encapsulation. Brittleness and difficulty in handling are its disadvantages. Coralline calcium carbonate produces comparable results to other bone replacement grafts with significant gain in clinical attachment, reduction of probing depth and defect fill.

Xenografts can be used alone or can be mixed with autologous bone to improve the osteoinductive capacity of the graft. They integrate well into the recipient site, histologically show direct contact with the parent bone, and undergo slow resorption.

C. Advantages

1. Osteoconductive
2. Relatively inexpensive

3. Elimination of need for a second surgical site
4. Less healing time

D. Disadvantages

1. They carry a rare risk of causing an immune reaction
2. Inability to gain adequate height and width for large defects.
3. Not always available in formulations that allow easy adaptation or modeling [6].

8. Alloplasts

Alloplastic bone substitutes represent a large group of chemically diverse synthetic biomaterials, including calcium phosphate (e.g. tricalcium phosphate, hydroxyapatite and calcium phosphate cements), calcium sulfate, bioactive glass and polymers. Depending on their construction, alloplasts may be resorbable or nonresorbable. These materials vary in structure and in chemical composition, as well as in mechanical and biological properties.

The 1996 World Workshop in Periodontics has concluded that "synthetic graft materials function primarily as defect fillers. If regeneration is the desired treatment outcome, other materials are recommended".

A. Bioceramics

They are biocompatible osteoconductive materials that offer a chemical environment and surface that are suitable for new bone formation. Bioceramics are being increasingly used as an alternative to autogenous cortico-cancellous bone graft. Calcium hydroxyapatite (HA) and β tricalcium phosphate (P-TCP) belongs to the calcium phosphate ceramic family.

B. Tricalcium phosphate (TCP)

Tricalcium phosphate is a porous form of calcium phosphate. The most commonly used form of which is β-tricalcium phosphate as the α form is less stable than β TCP.

β TCP has a rhombohedral structure, has high osteoconductivity with a calcium to phosphate ratio of 1.5, is mineralogically B- whitlockite. It is not as stable as HA and is resorbed at a faster rate and subsequently replaced with host tissue. They first, serve as a scaffold for bone formation, and then permits subsequent replacement with bone. Tricalcium phosphate as a bone substitute has gained clinical acceptance, but the results are not always predictable. The tricalcium phosphate particles generally become encapsulated by fibrous connective tissue and do not stimulate bone growth.

C. Commercial Types

1. Bioresorb: micropore size 0.5 to 10 µm, macropore size 50 to 700 µm.
2. Cersorb: pore size 150-500 µm.
3. Vitoss: pore size 1-1000 µm

D. Hydroxyapatite: $Ca_4(PO_4)6(OH)_2$

Hydroxyapatite is the primary mineral component of bone and is the least soluble of the naturally occurring calcium phosphate salts. It is therefore highly resistant to physiologic resorption and has good space maintainance property. HA alloplasts has a stoichiometry similar to that of bone mineral. They are manufactured by hydrothermal exchange reaction, they are sintered at 1100 to 1300 degree celcius, where the calcium carbonate skeleton is converted into calcium phosphate, while the trabecular bone structure of coral is unchanged. The calcium to phosphate ratio is 1.67, which is similar to that found in bone material.

Hydroxyapatite resorbability is determined by the temperature at which it is processed. Resorbability is desirable if the graft is eventually to be replaced by the host bone. Its compressive strength is enhanced by bone ingrowth but is only comparable to that of cancellous bone. They are osteophillic and osteoconductive rather than osteogenic or osteoinductive. It acts as a trellis for the ingrowth and subsequent deposition of new bone.

There are three available forms of hydroxyapatite:
Solid particulate, nonresorbable form. (Dense HA)

1. Porous non-resorbable form derived from the exoskeleton of coral.
2. Resorbable porous non-ceramic hydroxyapatite.

E. Solid particulate, nonresorbable form (Dense hydroxyapatite)

Dense hydroxyapatite grafts are prepared at high temperature (by sintering). They are nonresorbable, nonporous, dense, and have a larger crystal size. They are osteophillic, osteoconductive and act primarily as inert biocompatible fillers. Histologically, new attachment is not achieved. They yield similar defect fill as other bone replacement grafts and the clinical improvement is more stable than with debridement alone.

F. Porous hydroxyapatite

Porous hydroxyapatite (eg. Interpore 200, Irvine, CA) is obtained by the hydrothermal conversion of the calcium carbonate exoskeleton of the natural coral genus Porites into calcium phosphate hydroxyapatite. It has a pore size of 190 to 200 pm, which allows bone ingrowth into the pores and ultimately within the defect.

G. Resorbable hydroxyapatite

This synthetic hydroxyapatite is a resorbable, particulate material processed at a low temperature (eg. OsteoGen, Impladent, Holliswood, NY; OsteoGraf LD, CeraMed Dental, LLC, Lakewood, CO). This resorbable form is a non-sintered (non- ceramic) precipitate with particles measuring 300 to 400 µm. The non-sintered hydroxyapatite resorbs slowly acting as a mineral reservoir inducing bone formation via osteoconductive mechanisms. Its slow resorption rate has the advantage of allowing it to act as a mineral reservoir at the same time acting as a scaffold for bone replacement.

H. Commercial HA types

1. Polycrystalline ceramic form of pure densely sintered

HA: The particle size ranges from 250 to 420 µm (Osseo graf /N -300) and 420 to 1000 µm (Osseo graf/ N -700).
2. Coralline porous non resorbable hydroxyl apatite (Interpore 200 µm)
3. Resorbable non ceramic hydroxyl apatite: OsteoGen 300 to 400 µm.
4. Fluorohydroxyapatitic biomaterial: Pore diameter 10 µm. (FRIOS Algipore).

Combinations of the two primary forms of calcium phosphate have been studied to take advantage of the rapid resorption of β-tricalcium phosphate and the inert scaffold of dense hydroxyapatite.

I. Biphasic alloplastic materials

Biphasic compounds of hydroxyapatite and tricalcium phosphate have been developed to combine the features of space maintenance and bioresorption, allowing for bone ingrowth. The quantity of HA is higher than TCP, enhanced bone formation. Preclinical studies using different experimental models has provided histological evidence that particulate or moldable in-situ hardening hydroxyapatite/tricalcium phosphate shows osteoconductivity and resorption properties similar to those of deproteinized bovine-derived bone mineral.

Types

1. Tricos 60% HA + 40% TCP
2. Osteon TM 70% HA + 30% TCP
3. Bone Ceramic: 60% HA + 40% TCP
4. Ceraform: 65% HA + 35% TCP
5. MBCP: Micro Macroporous Resorbable Biphasic Calcium Phosphate bone substitute. 20% HA + 80% TCP.

J. Glasses

There are two forms of bioactive glass currently available. PerioGlas® and BiogranTM.

Bioactive glasses are composed of CaO, Na_2O, SiO_2, P_2O_5 and they bond to bone through the development of a surface layer of carbonated hydroxyapatite. When exposed to tissue fluids, bioactive glasses are covered by a double layer composed of silica gel and a calcium-phosphorous rich (apatite) layer. The calcium phosphate rich layer promotes adsorption and concentration of proteins, which is utilized by osteoblasts to form a mineralized extracellular matrix and osteogenesis, allowing rapid bone formation [7].

K. PerioGlas®

PerioGlas® has a particle size ranging from 90 to 710 µm, which facilitates manageability and packing into osseous defects. Compared to tricalcium phosphate, hydroxyapatite and unimplanted controls, Fetner et al. showed that PerioGlas® produced significantly greater bone repair. It is osteoconductive and also has hemostatic properties. It may also act as a barrier retarding epithelial downgrowth [8].

L. BiogranTM

BiogranTM has a narrower range of particle sizes - 300 to 355 µm size range which favours formation of hollow calcium phosphate growth chambers. Phagocytosing cells can penetrate the outer silica gel layer of BiogranTM by means of small cracks in the calcium phosphorous layer and partially resorb the gel. This resorption leads to the formation of protective pouches where osteoprogenitor cells can adhere, differentiate and proliferate. BiogranTM has a more uniform size compared to PerioGlas® and this has a clinical advantage over PerioGlas®, which has multiple particle sizes.

M. Polymers: htr polymer

- They are non resorbable, microporous, biocompatible composite of polymethylmethacrylate, poly hydroxylethylmethacrylate and calcium hydroxide. The acronym HTR™ stands for hard tissue replacement Histologically, new bone growth has been found deposited on HTR™ particles. Its hydrophilicity enhances clotting, and its negative particle surface charge allows adherence to bone. It serves as a scaffold for bone formation when in close contact with alveolar bone. Clinical defect fill and resolution can be achieved supporting its use as a biocompatible alloplastic bone substitute in the treatment of intrabony and furcation defects.

N. Advantages of alloplast

- Unlimited availability
- Unlimited durability
- No transfer of pathogens
- No immune reaction

O. Disadvantages

- No osteogenesis
- No definable absorption and transformation rates [9].

9. Conclusion

This paper presented an overview on bone grafts in periodontics.

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