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Current Concepts in Periodontal Regeneration

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Abstract : Periodontal regeneration is a field of dentistry focused on restoring and regenerating the tissues that support the teeth, including the gums, periodontal ligament, and alveolar bone. Recent advancements in periodontal regeneration have aimed to improve treatment outcomes, enhance tissue healing, and provide more effective and predictable results for patients with periodontal diseases. The restoration of missing tooth-supporting tissues, such as the periodontal ligament, alveolar bone, and cementum, has been the focus of numerous regenerative periodontal therapies over the past 20 years, including guided tissue regeneration (GTR), enamel matrix derivative, bone grafts, growth factor delivery, and the combination of cells and growth factors with matrix-based scaffolds.

IndexTerms **-Periodontal regeneration, Bone grafts, Guided tissue regeneration, Emd, Growth factors, Stem cells**

INTRODUCTION

Regeneration is defined as the formation of new alveolar bone, new cementum, and new functionally oriented periodontal ligament. Periodontal regeneration is a field of dentistry focused on restoring and regenerating the tissues that support the teeth, including the gums, periodontal ligament, and alveolar bone. Recent advancements in periodontal regeneration have aimed to improve treatment outcomes, enhance tissue healing, and provide more effective and predictable results for patients with periodontal diseases.

The restoration of missing tooth-supporting tissues, such as the periodontal ligament, alveolar bone, and cementum, has been the focus of numerous regenerative periodontal therapies over the past 20 years, including guided tissue regeneration (GTR), enamel matrix derivative, bone grafts, growth factor delivery, and the combination of cells and growth factors with matrix-based scaffolds.[1]

Periodontal tissue is destroyed by chronic periodontitis, which can lead to tooth loss Various biomaterials have been used to help treat gum diseased teeth. It started as a contact inhibitory membrane in guided tissue regeneration (GTR), which is now the gold standard in dental clinics. The objectives of periodontal therapy include not just stopping the spread of periodontal disease but, where necessary, regenerating diseased structure losses. Traditional surgical techniques, including as flap debridement, still provide tried-and-true ways to reach root surfaces, minimize periodontal pockets, and achieve improved periodontal form/architecture. These methods, however, have little potential for replacing tissues that were lost earlier in the course of the disease. Even though the causes or aggravating elements of the various types of periodontal diseases vary, they all result in clinical attachment loss.

The loss of periodontal structures is still present after the initial phase of periodontal therapy is finished, even when the etiological and contributory factors have been managed and the periodontal inflammation has subsided. If plaque and calculus are removed, a periodontal pocket may heal with the formation of a long junctional epithelium . The practitioner is now faced with the difficult choice of whether to treat the deformities brought on by the disease's long-term progression. Horizontal bone defects, periodontal intrabony defects, furcation defects, and/or gingival recession are a few examples of the abnormalities.[2]

Numerous bone grafting materials are available for the clinician today and have been used to achieve periodontal regeneration or alveolar ridge reconstructions. Autogenous bone, allogeneic bone replacements including freeze-dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFDBA), xenogeneic, and alloplastic are the four kinds of hard tissue replacement materials for periodontal regeneration.

To provide an Extracellular matrix -mimicking microenvironment, biomimetic nanofibrous and multilayer scaffolds have been developed for periodontal tissue regeneration in recent years. A few studies attempted to regenerate periodontal tissues with the proper structures, such as the oriented PDL fibers, but achieved limited success. The apical migration of gingival epithelium between the gingival connective tissue and the root surface. This healing process does not fully restore either the form or the function of the lost structures and hence does not constitute regeneration. Current regenerative techniques are aimed at the treatment of intrabony and furcation defects .

Background Of Periodontal Regeneration Therapy :

Periodontitis is caused by bacterial infection and includes enhanced neutrophil and macrophage infiltration, osteoclast activation via RANKL signaling, and bone resorption as a result. By creating a space in the periodontium, dental plaque and calculus frequently increase susceptibility to bacterial infection and periodontitis. Current therapeutic techniques include a conservative, non-surgical approach to treating periodontitis' causes, such as dental plaque and calculus, as well as ressective surgery to shallower the periodontal pocket. But these methods typically result in the attachment of lengthy junctional epithelium to root surfaces as a sort of repair. Hemidesmosomes, which join long junctional epithelium to root surfaces, have a lower ability to preserve the periodontium than do connective tissue fibers embedded in the CM.[3]

Therefore, if patients ignore plaque control or if the host immune response is compromised, periodontitis is likely to return. Regenerative periodontal therapy is now required in order to solve this issue. Bone grafting techniques were once often carried out as regenerative therapy, however they do not stop the downgrowth of long junctional epithelium on their own. Therefore, over the past 30 years, GTR has been developed and employed. It used the concept of contact inhibition to regenerate periodontal tissue.

Karring and Nyman developed the GTR concept in the 1980s while looking for potential regenerative components in periodontium. In their dog experiment, Karring et al. discovered that osteoblasts may cause root resorption and ankylosis when periodontitis-affected roots were implanted into Alveolar bone In the same year, Nyman et al. reported that in their dog and monkey experiment, root resorption took place when a periodontitis-affected root was placed into gingival connective tissue. In a monkey experiment, Nyman et al. proposed in 1982 that PDL cells have the potential to regenerate. Additionally, it was revealed that in clinical studies, GTR employing Millipore filters to treat a tooth damaged by periodontitis resulted in a new attachment by the periodontal ligament without the development of lengthy junctional epithelium or ankylosis.

There have been sporadic reports that root resorption and ankylosis can still occur despite a successful GTR operation. Moreover, when the regeneration ability of PDL and Cementum significantly reduced as chronic periodontitis

persists over an extended period of time, it is often difficult to orchestrate harmonious regeneration of multiple types of periodontal tissues. Moreover, persistent periodontitis can significantly reduce regeneration of PDL and Cementum and thus regeneration of the multiple periodontal tissues may be uncertain . As a new solution to overcome such obstacles, tissue engineering Recently, methods for periodontal regeneration have been researched. Along with GTR, various biomaterial scaffolds that are supplied with cells and/or bioactive materials can be used. More recent scaffolding systems have been created to direct integrated periodontium regeneration. These scaffolds are made to give bioactive signals for periodontium regeneration and to degrade so that new tissues can take their place.[4]

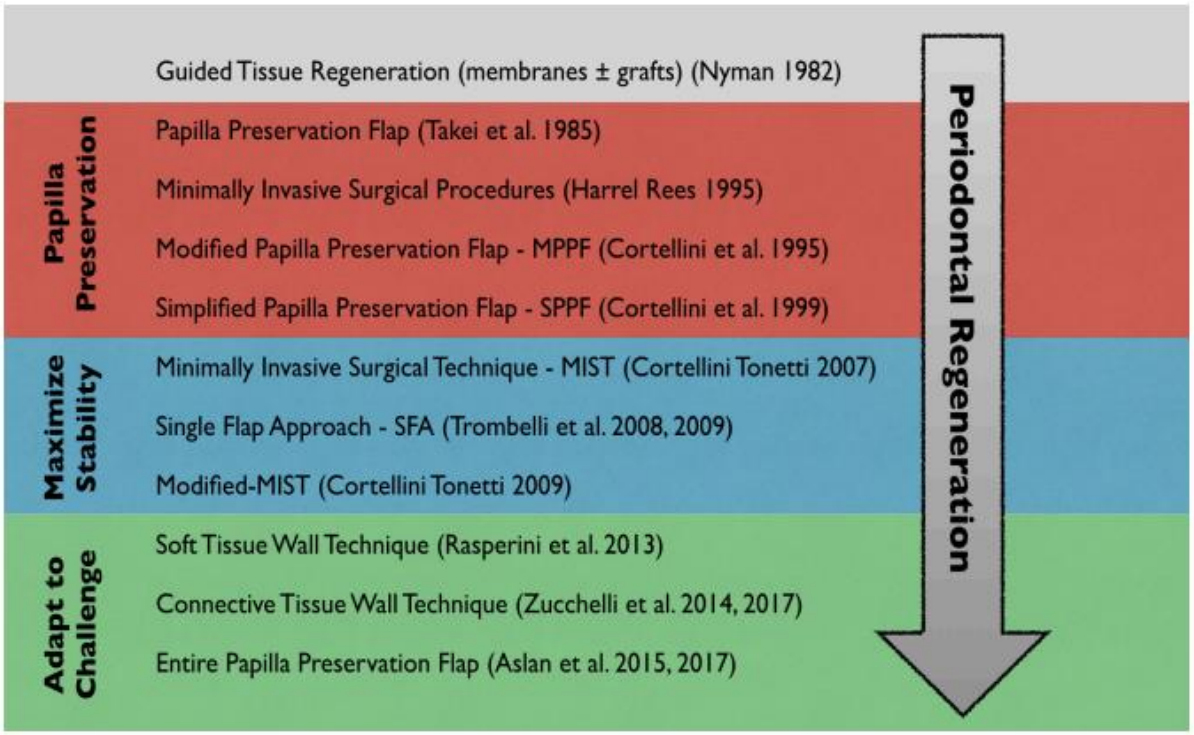


Figure 1. Evolution of flap designs for periodontal regeneration in relation to biological and clinical concepts.

The Regenerative Properties Of Bone Grafts: A Comparison Between Autografts, Allografts, Xenografts, And Alloplasts

BONE GRAFT :

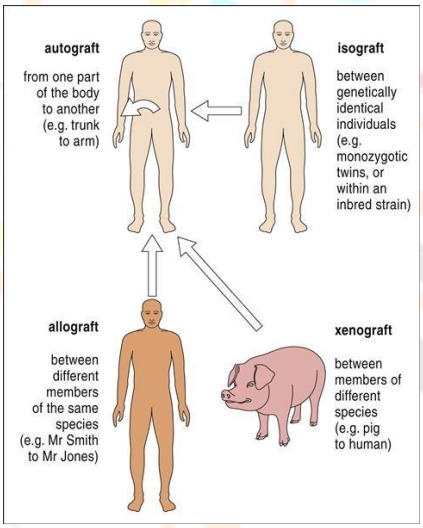
A bone graft is defined as a living tissue capable of promoting bone healing, transplanted into a bony defect, either alone or in combination with other materials,

A bone substitute is a natural or artificial substance that can serve the same function but typically only contains a mineralized bone matrix and no living cells. Since the first reported use of bone grafts in 1682, when a cranial lesion was successfully repaired with a cranial bone transplant from a dead dog, bone grafts and substitutes have been utilized in the medical field. Bone grafts are categorized by the US Food and Drug Administration as Class II devices (filling bony voids and defects) and Class III devices (bone graft containing).

Due to improvements in dental implantology and the increasing demand for the correction of cranial bony abnormalities, the use of bone grafts and substitutes in dentistry has significantly expanded in recent years. Trauma, periodontal disease, surgical excision, cranioplasty, infection, congenital deformities, and oral cancer are some of the conditions that can cause these bony or skeletal anomalies . Following tooth loss, where rapid resorption of alveolar bone occurs due to a lack of intraosseous stimulation that would ordinarily occur via the periodontal ligament fibers, inadequate bone is most frequently observed in dentistry.

Comparison Of Allografts, Xenografts, And Autografts

Grafting is a treatment used in medicine to replace damaged tissue. Depending on where it comes from, there are three different types of graft.They consist of allografts, autografts, and xenografts. The definition of a xenograft is the transplantation of tissue from a single donor into an entirely different species (from an animal to a human). The pericardium, fetal skin, swine small intestine, bovine dermis, and horse dermis are the most often used harvest sources for xenografts. However, xenografts from nations with a history of spongiform encephalopathy are prohibited in the United States. They are different from allografts, when the donor and the recipient are both humans (human to human). When a tissue is transferred from one part to another by an autograft, the receiver is regarded as the source.



Common uses of bone graft

1. Socket Preservation

The graft fills in the hole left by an extracted or missing tooth. It prevents the teeth on both sides of the socket from collapsing into it and the jawbone from receding.

2. Creation of a Healthy Base

It is used in ridge augmentations to increase the width of the jawbone. A wider jawbone helps provide a solid foundation for implants.

3. Sinus Lift

Sometimes the sinuses move down and occupy the space left behind by a missing upper back tooth. Bone graft can lift the sinuses back to their proper place. In addition, a dental bone graft will keep the sinuses from coming back down.[5]

4. Supporting Loose Teeth

Gum disease causes infections that erode the jawbone and loosen teeth. Dentistwill perform bone grafts to reduce tooth movement and provide support.

5. Nerve Removal

Sometimes dentists must remove nerves to make room for a dental implant. After removing the nerve, they fill the space with a bone graft.

Qualities of the Perfect Bone Grafting Material:

In order to replace missing bone, bone grafts' primary roles are to offer mechanical support and promote osteo-regeneration . Effective performance of this job depends heavily on the four biological characteristics of osseointegration: osteogenesis, osteoconduction, and osteoinduction . Osteointegration is the capacity of a grafting material to chemically bind to the surface of the bone without the presence of a fibrous tissue layer in between.[6]

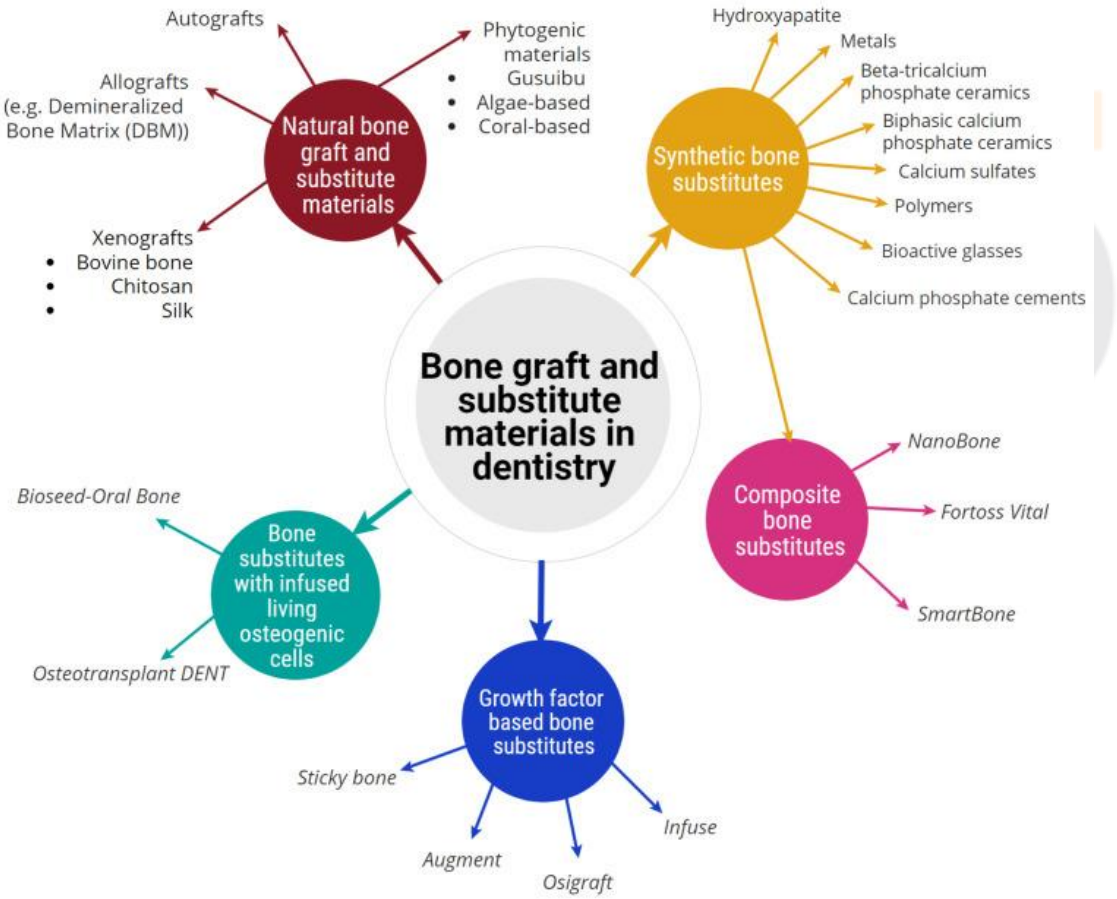
Osteoinduction is the recruitment of host stem cells to the grafting site, where they stimulate the development of stem cells into osteoblasts by the action of regional proteins and other factors . Platelet-derived growth factors (PDGFs), fibroblast growth factors

(FGFs), and transforming growth factors (TGFs) are a few of the growth factors that have an impact on this process. Direct osseous linkage and fresh bone production are made possible by these four key characteristics.

BONE GRAFT CLASSIFICATION BY MATERIAL SOURCE

TYPE	SOURCE	PROS	CONS
AUTOGRAFT	**PATIENT**	TRUE OSTEOGENIC LIVING CELLS GROWTH FACTORS NO DISEASE TRANSMISSION GOOD WITH CORTICAL BONE	PAIN INFECTION COMPLEX SURGERY LIMITED SUPPLY
ALLOGRAFT	**OTHER HUMAN**	OSTEOINDUCTIVE OSTEOCONDUCTIVE EFFECTIVE AS "SHELLS"	RISK OF DISEASE TRANSMISSION
XENOGRAFT	**OTHER SPECIES** (mostly bovine)	HA: SIMILAR TO HUMAN VOLUME STABILITY **COLLAGEN**: ACCELERATES BONE FORMATION	OSTEOCONDUCTIVE ONLY
ALLOPLAST	**SYNTHETIC**	NO RISK OF DISEASE TRANSMISSION	OSTEOCONDUCTIVE ONLY
	HYDROXYAPATITE	RESORBED SLOWLY →PRESERVES VOLUME GOOD GROWTH FACTOR CARRIER	
	TCP	RESORBED QUICKLY →REPLACED BY NEW BONE	
	BIOGLASS	BIOACTIVE →ACCELERATES BONE FORMATION RESORBED QUICKLY →REPLACED BY NEW BONE	

Dental Bone Graft and Replacement Material Classification:
Based on the tissue source or the material group, there are two main ways to categorize bone transplant and substitution materials . Currently employed bone graft and substitute materials in the dentistry industry can be broadly divided into five groups . In order to replace bony voids and supplement or reconstruct periodontal and alveolar bone abnormalities, this section examines various dental bone transplant and substitute materials.



Classification of bone graft and substitute materials used in dentistry, broadly classified into five categories and showing their associated sub-categories.

Clinical comparison :

As it is well known, fibroblast penetration, acellularization, and remodeling enable tissue repair. In order to enhance acellurization, fibroblast penetration at the graft site is crucial for tissue repair. Compared to xenograft, autograft and allograft encourage higher fibroblast infiltration. This is a result of the scaffold's extensive cross links. A large number of fibroblast is present at the site of grafts in comparison to bovine, where a substantial amount of fibroblast was discovered afterwards, according to an experiment performed on human acellularized dermal matrix and pig dermal matrix.[7] The image below displays the level of fibroblast infiltration in the human and bovine scaffold.Compared to allograft and autograft, xenograft also has a slower rate of wound healing. Various research indicate that the cross-linking of xenograft scaffolds' permeability to fibroblasts is reduced, which slows the chain of events necessary for effective wound healing. After fibroblast infiltration and acellularization, remodeling takes place. The recently implanted graft will attempt to adapt to the new surroundings during this stage. Failure to rebuild adequately could lead to tissue deterioration and rejection.

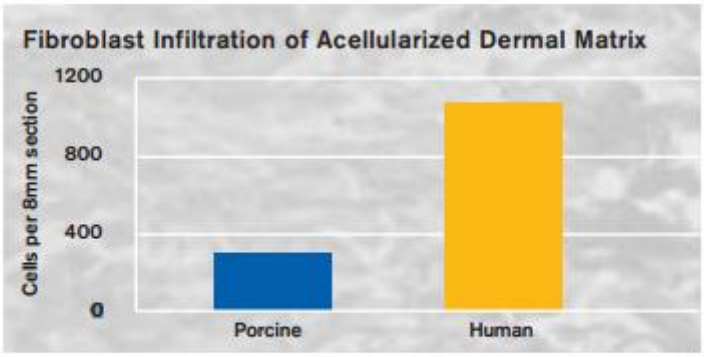


Figure 1. *P < .05. Wilcoxin test. Fibroblast infiltration of porcine and human acellularized dermal matrix at four weeks, determined by automated cell counting.*[8]

Type	Available Sources	Advantages	Disadvantages
Autogenous	Extra-oral sites: Crest of the iliac bone, tibia, parietal bone, ribs, sternum. Intra-oral sites: Mandibular symphysis, ramus, maxillary tuberosity, zygomatic buttress, extraction socket, coronoid process, autogenous tooth.	Potential for osteogenesis, osteoinduction, osteoconduction, and osteopromotion. No allergenic and immune-mediated reaction and no possibility of graft rejection. Low cost.	Donor site surgery and morbidity. Increased surgical time which may require general anesthesia. Very large quantities cannot be harvested without significant donor site deficit.
Allogenic	FDBA DFDBA DBM	Acts as a scaffold and allows osteoconduction. No donor site surgery/morbidity. Can be combined with other materials such as BMP, GFs, PRF to enhance its healing potential.	Processing required to remove allergenic component Rejection by the host is possible.
Xenogenic (different species)	Porcine source Bovine source Corals Algae	Acts as a scaffold and allows osteoconduction. No donor site surgery. Can be combined with other materials such as BMP, GFs, PRF to enhance its healing potential. Low cost. Significant quantities can be acquired.	Processing required to remove allergenic components but still can transmit disease. Possibility of rejection.
Alloplastic (synthetically produced)	TCP β-TCP Bioactive Glass Bio-ceramics Hydroxyapatite	Acts as a scaffold and allows osteoconduction. No donor site surgery. Can be combined with other materials such as BMP, GFs, PRF to enhance its healing potential. No allergenic potential.	Can be costly. Can act as foreign body.

Type	Available Sources	Advantages	Disadvantages
Engineered personalized bone grafts	Bioactive acellular scaffolds: Biodegradable synthetic materials with osteoinductive factors such as BMPs, PDGF, IGF. Cell seeded scaffolds: Autologous BMSCs in a customized scaffold mixed with PRP. Customized autologous bone grafts: Pluripotent stem cells induced to form bone.	Autologous stem cells with decreased chances of rejection. Can be molded into the desired anatomical shape using 3D modeling. Inclusion of bioactive molecules provides a better healing potential.	Still in infancy and further research needed to bring into clinical use. Requires facilities to harvest and culture stem cells. May have ethical issues.

Autogenous Bone: The Gold Standard for Bone Regeneration

Brief Historical context:

Hegedus used autogenous tibial grafts in 1923 to rebuild the alveolar ridges that had become inadequate as a result of "pyorrhea alveolaris." In "Dental Cosmos," he claimed that effective osteogenesis had occurred in the fourth week. Many publications up until the 1960s recommended using autogenous bone, although they came from different sources. Linghorne shown in his extensive histological analysis that autogenous grafts are superior than open flap debridement (OFD) in bone repair since histological research up to this point had been ambiguous. Burwell investigated the causes and circumstances of osteoblastic differentiation and found that a portion of the material needed to degrade in order for the RNA released to trigger the bone cell to form.[8]

The bone chips employed by Nabers, O'Leary, and Robinson required to totally resorb before new bone could develop, in their opinion. In 1971, Rivault et al. outlined the conditions for a successful graft. The size of the graft particles should be less than 100 microns, it should be kept in contact with the host bone, and the irritation factors of the grafted area should be kept under control, according to their recommendations. They also recommended that the bone graft be completely covered by soft tissue flaps for adequate blood supply.

Clinical experience and histology research over time revealed that autogenous cancellous bone containing hematopoietic marrow has the greatest potential for osteogenesis. In addition, autogenous bone has the lowest risk of host rejection, while cancellous bone's porous structure boosts the likelihood of fast revascularization and subsequent graft survival.Schallhorn has treated periodontal osseous lesions with autogenous cancellous bone combined with hematopoietic marrow from the iliac crest. A significant frequency of root resorption and consequent ankylosis were seen in beagle dogs, according to Sullivan et al. Dragoo and Sullivan reported external root resorption in a histopathological analysis of four individuals. The histological sections revealed a connection between inflammation and root resorption, suggesting that resorption may be more of a consequence than a cause of inflammation.

he resorption of sequestered bone, fresh marrow, which contains several undifferentiated cells, or the proteolytic activity of polymorphonuclear

leukocytes, macrophages, and other chronic inflammatory cells can all start the resorption of the root. When using frozen iliac autografts or allografts as well as fresh intraoral donor material, Schallhorn et al. reported no ankylosis or root resorption. In two cases treated without iliac autografts and in 16 of the 275 locations treated with it, root resorption was seen. Hiatt and Schallhorn reported an average fill of 3.44 mm in 166 transplants from tuberosity, which compares favorably with iliac autografts.

Linghorne 1951[5]	3 (dogs) 6	N/A	12-130 days Bone chips	Regeneration
Nabers 1965[4]	8 (Human) posteriors	Vertical defects 7-10mm	6 months Bone chips	Bone fill
Schallhorn 1968[9]	1 (Human) 2	Crater 4-5mm	5 months Cancellous bone & Iliac Marrow	Bone fill
Robinson 1969[20]	6 (Human) Posteriors	Vertical defects 7mm	3-24 months Osseous coagulum	Bone fill
Rivault 1971[7]	4 (Rhesus monkey) 32	Vertical defects N/A	180 days Osseous coagulum	Repair
Schallhorn 1971[12]	52 (Human) 182	Vertical defects N/A	5-6 months Iliac Transplant	Bone fill
Nabers 1972[4]	1 1	3 osseous defects	57 months	Regeneration
Hiatt 1973[13]	40 (Human) 78		Marrow & cancellous bone	Bone fill
Dragoo 1973[11]	4 12	1-2 osseous walls 1.4mm	2-8 months Iliac crest cancellous	Regeneration
Froum 1975[14]	19 32	Vertical defects 4mm	Bone blend & Iliac Marrow	Bone fill
Froum 1976[15]	28 75	Vertical defects >2mm	7-13 weeks Osseous coagulum	Bone fill
Moskow 1979[16]	1 1	1-3 osseous walls	28 weeks Mandible Alveolar crest	Long junctional epithelium Osseous repair

Sources Of Autogenous Grafts :

Autografts are commonly obtained from intraoral and extraoral sites from the same individual, such as the mandibular symphysis, mandibular ramus, external oblique ridge, iliac crest, proximal ulna, or distal radius, due to being good sources of cortical and cancellous bone . Autograft bone harvested from the mandibular ramus is associated with more minor downstream [9]complications compared with other intraoral sites although this presents a risk of damage to the inferior alveolar nerve. Mandibular ramus grafts are appropriate for use when the sites requiring augmentation are less than 4 mm in thickness and span a maximum of four teeth . There are no histocompatibility and immunogenicity issues associated with autografts, therefore they represent the highest degree of biological safety. However, there are several downsides associated with autografts, such as the

requirement for a secondary surgical visit, donor site injury and the potential for scarring. Additionally, autografts have been associated with higher surgical costs, more significant surgical risks, e.g., excessive bleeding, infection, inflammation and pain, limiting their application to relatively smaller bone defects. Thus, in large craniofacial defects, autografts may not represent a viable option .

Cancellous bone is most commonly used for autografts and contains osteoblasts and progenitor cells with considerable osteogenic potential. They possess relatively large trabecular surfaces, which facilitate establishment of an osteoinductive environment by encouraging revascularization and incorporation into the recipient site. In contrast, cortical bone lacks osteoblasts and osteogenic cells; instead, it provides structural-mechanical integrity and promotes bone healing through osteoconduction. Cortical grafts are slower to integrate relative to cancellous grafts due to their limited revascularization potential. Therefore, to maximize bone remodeling performance and healing potential, a combination of cancellous and cortical bone is used . Despite the development of numerous bone substitutes over recent years, autografts remain the gold standard for grafting materials as they are still the only graft material that possesses all of the four fundamental biological properties required . In dental applications, even though other bone substitutes are routinely used for management of localized alveolar bony defects and maxillary sinus bone grafting, autografts in block forms are still routinely used in alveolar ridge augmentation procedures. As very few bone substitutes can produce a volume of newly formed bone comparable to that produced by autograft materials therefore, autografts remain the material of choice for more complex augmentation procedures, such as posterior mandibular edentulous reconstruction . This is because autogenous block grafts can increase the bone quality and quantity in a predictable manner, allowing for placement of implants with maximal diameters which facilitate strength distribution for long-term survival.[9]

TYPES OF AUTOGENOUS BONE GRAFT :

Numerous varieties of autogenous bone transplants have been or are currently being used in therapeutic settings. They consist of extraoral cancellous bone and marrow, bone mix, intraoral bone chips, and osseous coagulum.

Cortical bone graft :

Autogenous cortical bone graft, which provides an osteoconductive medium with minimal osteoinductive and osteogenic properties, is best suited for structural defects for which immediate mechanical stability is required for healing.

Due to limited perfusion and donor osteocytes, the dense cortical matrix causes relatively sluggish revascularization and integration since resorption must occur before the deposition of new bone

For periodontal defects , Nabers and O'Leary (1965) observed that cortical bone chips removed with hand chisels during osteoplasty and ostectomy resulted in a coronal rise in bone height. Autogenous osseous coagulum and bone mix were used in place of cortical bone chips because of their comparatively large particle size (1,559.6 183 um) and propensity for sequestration. When assessing the impact of particle size for autologous bone grafts, the literature showed a wide range. It was claimed that values between 125 m and 2 mm were preferred. Particles smaller than 75-125 m are quickly resorbed and do not engage in successful osteogenesis, according to a key minimum value that was observed.

Cancellous bone graft:

The most popular type of autogenous graft is cancelled bone graft. The porous trabeculae are lined with functioning osteoblasts, creating an osteogenic graft that provides an osteoinductive, osteoconductive, and osteogenic substrate.A portion of the donor osteocytes that survive after implantation work in conjunction with the graft's porosity and local cytokines to encourage angiogenesis and host mesenchymal stem cell recruitment.[9]

Corticocancellous bone graft:

Corticocancellous bone grafts intuitively offer the advantages of both cortical bone and cancellous bone: An osteoconductive medium and immediate structural stability from cortical bone, and the osteoinductive and osteogenic capabilities of cancellous bone.

Intraoral cancellous bone and marrow : Intraoral cancellous bone and marrow can be obtained from healing extraction sockets, mandibular

retromolar areas, and maxillary tuberosity areas. A mean bone fill of 3.65 mm and >50% bone fill has been obtained on a predictable basis.

Extraoral cancellous bone and marrow : This material is obtained from either the anterior or the posterior iliac crest predictable bone growth ranging 3.53-4.36 mm and even complete eradication of furcation involvement and interdental craters have been reported by various authors.[7]

Osseous coagulum and bone blend :

Intraoral bone, when obtained with high or low speed round burs and mixed with blood becomes a coagulum. It was subsequently demonstrated in monkeys that small bone particles of 100 um could provide an earlier and greater osteogenic activity than particles 10 times as large. The bone blend technique was designed to overcome some of the disadvantages of osseous coagulum including inability to aspirate during the collection process and unknown quality and fluidity of the material. Bone blend is cortical or cancellous bone that is procured with a trephine or rongeurs, placed in an amalgam capsule and triturated to the consistency of a slushy osseous mass. The resultant particle size is in the range of 210 × 105 um. +Froum et al. reported that the osseous coagulum

bone blend type of grafts provided 2.98 mm coronal growth of the alveolar bone, compared with 0.66 mm obtained when open flap debridement alone was used .

Physical Properties :

Kim et al. compared the surface characteristics of AUTO-BG using a scanning electron microscope and found that the physical surface characteristics were quite similar to autogenous cortical bone (obtained from mandibular buccal cortical bone). Under high magnification, the root portion of the AUTO-BG showed a rough pattern while the crown portion of AUTO-BG was relatively smooth. The compactness of the cortical bone graft was wave-like due to its cortical nature while the surface of allogenic bone was fairly smooth as it contained cancellous bone. A smaller degree of compactness was demonstrated by the xenogenic graft. In an X-ray diffraction analysis (XRD), which is a method employed to study the crystalline nature of solids, AUTO-BG showed a crystalline structure similar to that of autogenous cortical bones. The calcium and phosphorus content from the Ca/P ion dissolution test was also found to be similar to that of autogenous cortical bone. This dissolution is an

indicator of biodegradability which is directly related to the release of calcium and phosphorus which is required for reprecipitation of apatite on the bone [7]surface .

Clinical Outcomes :

Long-term clinical studies conducted by Lee and Kim et al. revealed excellent biocompatibility of AUTO-BG . It was successfully depicted that AUTO-BG is resistant to infection and heals satisfactorily even with mild wound dehiscence. In another study, Kim et al. demonstrated that AUTO-BG undergoes a gradual resorption process and is ultimately replaced by good-quality bone, employing both the processes of osteoinduction and osteoconduction . They demonstrated through histological samples that after 4 months of grafting, the graft material directly fuses with the recipient bone and shows excellent vascularity and according to them, the graft is completely replaced by normal bone in 12–15 months. In their study, Jun et al. showed a mean bone density of 981 HU (D2 type) in healed auto-tooth bone graft versus 968 HU for Bio-Oss. Similarly, they depicted an almost 60% proportion of new bone volume to total bone volume with AUTO-BG . These studies show that the results of AUTO-BG in the maxillofacial region are comparable to other bone grafting sources.

Method of Preparation of AUTO-BG

Sound teeth that require removal are extracted using a minimally harmful approach. This protects the buccal and lingual cortical plates and thus allows a better adaptation of the graft. For producing the powder-type AUTO-BG, the extracted teeth are first thoroughly cleaned and made free from debris or any attached tissue remnants. Crown portions are separated from the root. The root portion is placed in a Smart Dentine Grinder (Kometa Bio, Fort Lee, NJ, USA) and is ground for approximately 30 s in order to produce a 300–1200 micron dentine powder. This dentin powder is then placed in a dentine cleanser for about 7 to 10 min. This dentin cleanser is a solution containing high pH (very basic) sodium hydroxide in 20% ethanol and is used to cleanse the particulate in an attempt to eliminate bacteria and any remaining organic material.[9] Once the cleansing process is complete, the excess cleanser is removed using sterile absorbent gauzes . Next, a dentine wash using a Smart Dentin Grinder (Kometa Bio, Fort Lee, NJ, USA) consisting of phosphate-

buffered saline, is poured onto the particulate material for 3 min. Once the soaking is complete, the excess liquid is removed by pouring out the excess, and the rest is absorbed with gauze. After this process, the AUTO-BG is now ready for use and can be easily transferred to the recipient site like any other graft material . In order to prepare the block-type AUTO-BG, the tooth is not subject to grinding while the rest of the process is essentially the same. Small holes may be drilled in the block-type graft in order to improve the ingrowth of vasculature in the grafted material from the recipient site. After this, it can be placed in the extraction socket for socket preservation. The root-on type resembles cortical plates and is used for vertical/horizontal augmentation of the bone .

Powdered form or tooth ash is prepared through a high-temperature sintering process. The tooth is soaked in hydrogen peroxide to remove debris of soft tissue and then disinfected by dipping in ethanol. The tooth powder is heated for one hour at 1200 °C to remove all impurities and any remaining infected material. In order to improve its handling and placing in bone defects, it can be mixed with gypsum or platelet-rich plasma .

Flow chart showing method of preparation of AUTO-BG.

Clinical applications of AUTO-BG:

Bone Augmentation

Dental implants are placed at an increasing number in clinical practice due to patients' awareness and evidence supporting long-term success with almost a million implants being placed every year . With this increasing number, implant surgeons are becoming increasingly confident and attempting to insert implants where the bone is inadequate in size for implants placement and in these cases, implant sites have to be grafted. Powder-type AUTO-BG is reported to be a successful bargain in such cases where osteogenic, osteoinductive, and osteoconductive properties are warranted . Ramanauskaite et al. showed that a mean gain in alveolar ridge width was around 5 mm with an annual resorption of approximately 0.1 mm. In their study, they were able to place implants in all the grafted cases within 26 weeks of grafting with adequate primary stability .[9]

In cases where the deficient bone needs to be corrected due to any reason other than implant placement, powder-type or block-type grafting, using AUTO-BG, can be employed successfully .

Sinus Augmentation

Bone resorption and sinus pneumatization in the posterior maxilla often precludes implant placement without performing sinus lift surgery . Sinus lifting can be carried out either via the crestal approach or through the lateral window approach . People have reported satisfactory results using various autogenous, allogenic, and/or alloplastic materials for sinus augmentation . In general, any material with a slow resorption rate would work in sinus lift surgery. AUTO-BG can be regarded as a possible alternative when the autogenous bone is needed for sinus augmentation without the need for donor site morbidity. It produces a positive effect to increase the quantity and quality of bone and minimizes re-pneumatization of the sinus . Kim et al showed an average increase in bone height of around 5 mm after sinus floor augmentation with AUTO-BG with an average of 0.76 mm/year bone loss after implant

loading with an overall reported implant survival of around 96% in AUTO-BG sinus augmentations as reported by Shavit et al.

Periodontal Defects

Research is being carried out in the field of bone regeneration for periodontal defects. Guided tissue regeneration (GTR) procedures , the use of enamel matrix derivatives (EMD) , growth factors (GF) , BMPs , platelet-rich plasma/fibrin (PRP/PRF) , and various bone grafting procedures have been extensively described in the literature. Autogenous material obtained from the same individual is always considered the gold standard because of its high osteogenic, osteoinductive, and osteoconductive potential . Keeping this under consideration, AUTO-BG-derived DDM and demineralized bone matrix (DBM) can provide the same beneficial effects as autogenous bone minus the need for donor site surgery and can promote bone formation in these intraosseous periodontal resorptive defects . Upadhyay et al. used AUTO-BG material for the treatment of class II furcation defects and followed the cases for one year. The results of their study showed that horizontal probing depths decreased in the range of 1–2 mm and approximately 3–4 mm of bone was gained in the linear dimension .

A study by Indurkur et al. (2018) showed that individuals treated with AUTO-BG combined with a chorion membrane demonstrated insignificant outcomes to DFDBA with a chorion membrane in intrabony defects in all clinical outcomes, suggesting that AUTO-BG can be used as a useful alternative to DFDBA in periodontal regenerative therapy for intrabony defects. Furthermore, a case series found that AUTO-BG material with osteoinductive and osteoconductive capacities can be utilized for the treatment of intrabony defects .[9]

Guided Bone Regeneration:

Guided bone regeneration (GBR) is a process where new bone formation is guided by the use of a resorbable or non-resorbable membrane . In most cases, GBR is performed with or prior to implant placement . Powder-type AUTO-BG material can be placed along with an implant if the osseous defect is larger than 2 mm vertically or horizontally around the implant . The use of a

resorbable or non-resorbable membrane is the discretion of the surgeon. In case the operator feels that the amount of grafted material is less, it can be combined with allogenic material or PRF can be added to increase its bone-forming potential . A study was conducted by Lee et al. where they used AUTO-BG for the purpose of GBR with and without the use of membranes. The results of their study showed that there was a net gain of bone of around 87% with no statistical difference whether the membrane was used or not .[9]

Alveolar Bone Grafting

Alveolar bone grafting (ABG) is an important and commonly performed procedure in complete cleft lip and palate patients . Various autogenous, allogenic, and alloplastic techniques for grafting have been described in the literature . The use of AUTO-BG has also been reported. Authors have used both powder-type and block-type grafts in ABG. The advantage of AUTO-BG is that it can be combined with other graft materials and PRF can be placed alongside this graft in order to enhance the quality of the forming bone . Authors have combined AUTO-BG along with distraction osteogenesis in alveolar cleft patients and have reported satisfactory results . In cleft patients, the AUTO-BG can be acquired from non-functional third molars or supernumerary teeth which are quite common in cleft afflicted patients. In addition, any other tooth which must be extracted as per the orthodontic plan can also be used for this purpose [10].

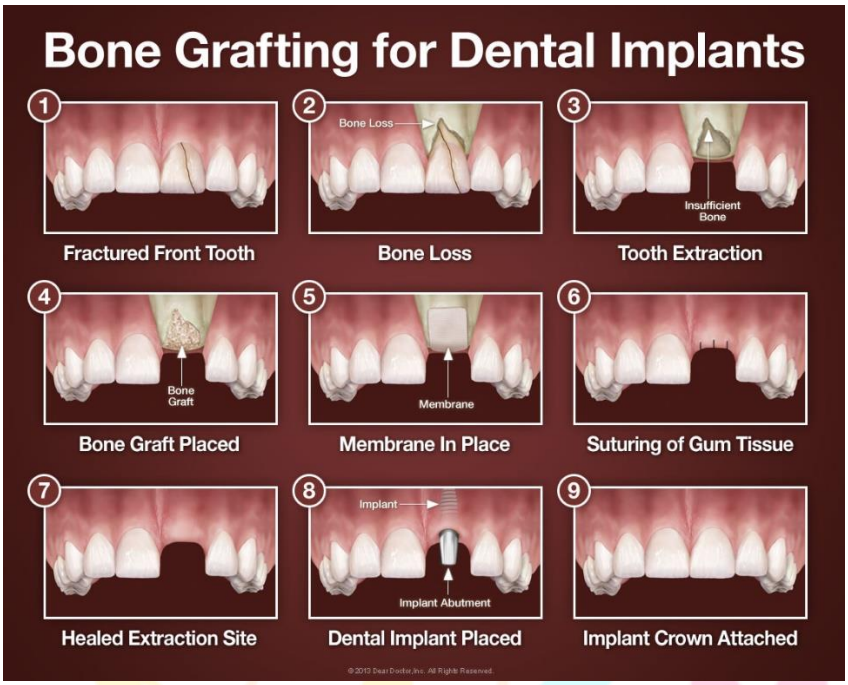
In addition, pilot research intended to assess the effectiveness of an AUTO-BG in avoiding periodontal abnormalities after surgical extraction of impacted or semi-impacted lower third molars. Radiographic and periodontal evaluations of post-extractive sockets were done for this aim. The study included 10 patients, and 20 lower third molar extraction sockets were treated with a split-mouth technique. The experimental sites were filled with AUTO-BG derived from the removed lower third molars, whereas the control sites were filled with blood clot alone. Flaps were closed with the purpose of ensuring the wound's stability. In all cases, the healing was unaffected by any problems connected with the use of the AUTO-BG. The probing pocket depth distal to the second lower molar was reduced at both surgery sites after 6 months, with a higher decrease reported at the experimental locations. Radiographic assessment also revealed that the transplanted sites had more bone gain than the control sites. The findings of this exploratory investigation show that AUTO-BG may be effective in reducing the establishment of periodontal abnormalities distal to the second lower molar after surgical extraction of the lower third molars .

Ridge Augmentation

When ridge resorption precludes the placement of the implant(s), augmentation procedures are warranted . The deficiency can be in horizontal, vertical, or both dimensions . In such situations, AUTO-BG can be used successfully, especially the root-on type block graft. Various clinicians have used block-type AUTO-BG material for ridge augmentations when the defects were equal to or more than 3 mm . The placement of graft material in these cases helps to increase bone mass and follow-up studies have shown successful implant placement in ridges where AUTO-BG material was used . Kim et al. showed through long-term follow-up studies that marginal bone resorption of around 2.5 mm occurs over a 5-year period following ridge augmentation and, in most cases, implants placed in the ridges augmented with AUTO-BG could be functionally loaded within 5–7 months .

Socket Preservation and Reconstruction

After tooth extraction, a sequence of biological cascades is set in motion which ultimately heals the extraction socket with secondary intention and in turn, leads to some degree of bone loss which is directly proportional to the trauma induced to the bone during tooth extraction . Socket preservation techniques attempt to minimize this bone loss and tend to improve the quality and quantity of the bone in the healed socket. Grafting with or without a membrane has been described in the literature . AUTO-BG can be used in such cases in two different ways: one being the extraction socket packed with powder-type tooth graft material and which may or may not be covered with a membrane; secondly, the root-type AUTO-BG can be used to preserve healing sockets . They resemble a tooth root and can be placed in the socket and again may or may not be covered with a membrane according to the choice of the operator. Optimal healing of the sockets has been described in both conditions . Radoczy-Drajko et al. demonstrated that AUTO-BG when used as a graft material for socket preservation leads to a mean bone loss of 15% in the horizontal dimension (maximum at the coronal portion and minimum at the apical) with negligible loss in the vertical dimension. These findings show that successful results can be obtained with AUTO-BG with functional restoration using implants later on .[11]



Allografts for Bone and Periodontal Regeneration

Brief history :

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 dentistry , orthopedics, and craniofacial surgery. 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 the 1900s [10]. FDBA was first used in periodontal therapy in the early 1970s although it has been used clinically in orthopedic therapy since 1950s.[12]

FDBA provides an osteoconductive scaffold for bone growth and elicits resorption when implanted in mesenchymal tissues. 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 time. 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 not form bone otherwise. DFDBA contains bone morphogenic proteins (BMPs) such as BMP 2, 4 and 7, which help stimulate osteoinduction. Thus, commercially prepared, allograft-retained proteins have the capacity to influence cell behavior in vivo. BMPs produce multiple effects on bone by 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 µm

The bone allogeneic grafts are usually procured within twelve hours of the donor's death and placed in tissue banks. Four types of allogeneic grafts have been used in periodontal reconstructive therapy:

LAMINAR BONE ALLOGRAFT MEMBRANES :

The use of this barrier membrane in combination with particulate demineralized freeze-dried bone allograft (DFDBA) may Deep persisting periodontal pocket mesial of the LR6;[12] b. Radiographic signs of angular bone loss mesial of the LR6 and furcation involvement; c. Alveolar bone defects as revealed following the elevation of a buccal flap; d. Placement of xenograft and collagen membrane in the defects (Bio-Oss and Bio-Gide respectively); e. Radiographic signs of bone fill mesial and at the furcation area of LR6. An overview of periodontal regenerative procedures for the general dental practitioner also be of promise as demonstrated in a randomized clinical trial (RCT) in patients with twelve pairs of Class II mandibular molar furcation lesions (Scott et al., 1997). However, further studies with much higher power are necessary for conclusive evidence on the use of this material as membrane barrier in GTR procedures

FROZEN ILIAC ALLOGRAFT:

it has demonstrated favourable results. However, the need for extensive cross-matching to decrease the possibility of disease transmission and graft rejection has limited the widespread use of it in periodontal treatment (Rosen et al., 2000).

FREEZE-DRIED BONE ALLOGRAFT (FDBA):

This type of graft has been reported to be effective as a scaffold over which new bone may form (Goldgerg and Stevenson, 1987). [12]

DEMINERALIZED FREEZE-DRIED BONE ALLOGRAFT (DFDBA):

It has been reported that hydrochloric acid and freeze-drying of cortical bone graft may expose the morphogenetic proteins in the bone matrix, and therefore enhance its osteogenic potential (Urist and Mikulski, 1979). DFDBA has been considered to be one of the ‘‘gold standard” grafts in periodontal regeneration, with favourable outcomes (Libin et al., 1975; Pearson et al., 1981; Rosen et al., 2000). The risk of disease transmission has always been a concern with the use of allografts. However, this may be minimal if the graft is harvested and processed according to the standards and guidelines of established bodies (e.g.,

American Association of Tissue Banking) (Mellonig, 1995). In addition, human studies have shown no immune reaction (antigenicity) following treatment with both FDBA and DFDBA (Quattlebaum et al., 1988

SYNTHETIC VARIANTS :

Flexible hydrogel-hydroxyapatite (HA) composite which has a mineral to organic matrix ratio, approximating that of human bone.[12]

Artificial bone can be created from ceramics such as calcium phosphates (e.g., HA and tricalcium phosphate), bioglass, and calcium sulphate are biologically active depending on solubility in physiological environment. These materials combine with growth factors, ions such as strontium or mixed with bone marrow aspirate to increase biological activity. The presence of elements such as strontium can result in higher bone mineral density (BMD) and enhanced osteoblast proliferation.

STEPS IN MANUFACTURING AND PROCESSING OF ALLOGRAFTS :

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.

• First of all soft tissue stripping is done to remove residual muscle, tendon, ligament, and so forth. • 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. [13]

• 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.

• 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. [13]

• 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.

• The cortical bone is ground and sieved to a particle size of approximately 250 to 750 µm.

• 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.

• The graft material is again immersed in 100% ethyl alcohol and washed repeatedly to remove chemicals used in processing.

• 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. • The bone is washed in a sodium phosphate buffer to acid remove residual. • If the bone is demineralized, it is refreeze dried.

• 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 . SAL is probability that an item will not be sterile after it has been subjected to a validated sterilization process .

With a SAL of 10-6, the odds of an organism’s surviving after allograft processing are less than one in 1 million . 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:

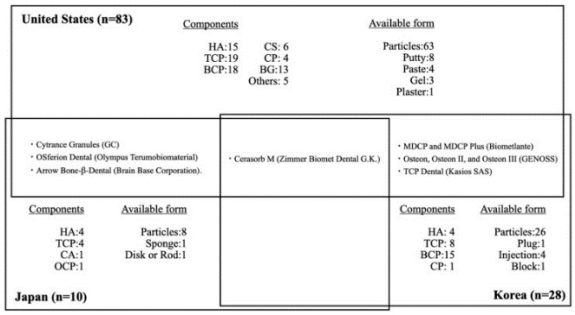
• Visual inspection test - Visual detection is done for problems such as gross graft contamination, packaging defects and product mislabeling.

• Residual moisture test - Testing of freeze dried allografts is done to ensure residual moisture is 6 percent or less.

• Residual calcium test - Testing of demineralized freeze dried bone allograft is done to ensure residual calcium content is 8% or less

Puros (Zimmer Dental, Carlsbad, California) is a new allograft of cancellous bone in the market. It is human bone that undergoes a patented tutoplast process. The patented Tutoplast process gently removes unwanted material such as fats, cells, antigens, and inactivates pathogens, [14]while preserving the valuable minerals and collagen matrix, leading to complete and rapid bone regeneration. This process involves delipidization with acetone and ultrasound, osmotic treatment, oxidation with hydrogen peroxide to destroy unwanted proteins, solvent dehydration with acetone to preserve the collagenous fiber structure, and low-dose gamma irradiation.

Manufacturers believe that this new solvent preservation method preserves the trabecular pattern and mineral structure better than the freeze-drying process, thus providing a more osteoconductive material. Grafton DBM 21 (BioHorizons, Birmingham, Alabama) is another allograft processed from cadaver long bones by aseptically processing the bone to remove lipid, blood, and cellular components before it is frozen. Cortical bone is milled into elongated fibers of 0.5 mm in diameter or pulverized into particles of 100 to 500 mm. It is combined with a glycerol carrier to stabilize the proteins and improve the graft handling.



Distribution of dental alloplastic bone substitute products commercially available in the United States, Japan, and Korea. HA = hydroxyapatite, TCP = tricalcium phosphate, BCP = biphasic calcium phosphate, CS = calcium sulfate, CP = calcium phosphate, BG = bioglass, CA = carbonate apatite, OCP = octacalcium phosphate.

SAFETY OF BONE ALLOGRAFT :

There are two major concerns regarding the use of bone allografts, antigenicity, and the risk of disease transmission.

Antigenicity:

A concern about the antigenicity of the donor material arises with any dental/medical procedure using tissues derived from human 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."[15] 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 material.

Risk of Disease Transmission Associated with use of Allografts :

The potential for disease transfer particularly viral transmission and even more particularly HIV is a crucial factor associated with use of bone allografts. The first case of HIV transmission through allogenic bone was reported in 1988. [29] A femoral head specimen from a 52-year-old man was resected as a part of hip arthroplasty and implanted in recipient 24 days after its procurement in November 1984. The recipient developed lymphadenopathy, diarrhea, nausea and vomiting, and night sweats within 21 days of surgery.[16] In February 1988, she was tested and found positive for HIV antibody. This case of HIV transmission represents the violation of basic principles in handling allogeneic tissues.

Bone Allografts for Clinical Transplantation and regeneration :

GUIDED TISSUE REGENERATION WITH ALLOGRAFT:

A physical barrier or membrane inserted between the mucogingival flap and the root surface may retard apical migration of epithelium prevents gingival connective tissue from contacting the root surface permitting cells originating from the periodontal ligament space to occupy the created space and undergo amplifying cell division. This procedure has been termed guided tissue regeneration. Wound healing studies in animals indicate that periodontal

regeneration is the result. However, histologic observations in humans suggest that wound healing is by new cementum with inserting new connective fibers with little or no bone formation. Clinical studies indicate that healing of the osseous defects is mainly by soft tissue. This correlates with histologic observations. A number of case reports indicate that the combination of decalcified freeze-dried bone allograft and the physical barrier enhances bone fill. Antleregg et a 12 compared 15 pairs of periodontal osseous defects treated by guided tissue regeneration with decalcified freeze-dried bone allograft or with the physical barrier alone.[17] They found bone fill to be significantly more favorable with the use of the bone graft and the barrier . A subsequent study did not find significant improvement in any of the clinical measurements between guided tissue regeneration alone and decalcified freeze-dried bone allograft plus guided tissue regeneration.83 Decalcified freeze-dried bone allograft plus guided tissue regeneration also was compared with decalcified freeze-dried bone allograft alone. The results of this study likewise suggest a beneficial effect with the use of either technique, but no differences between groups. However, long-term (5 year) results of guided tissue regeneration procedures used alone or in combination with a bone graft indicate that the success of guided tissue regeneratron is enhanced significantly by the addition of a bone graft

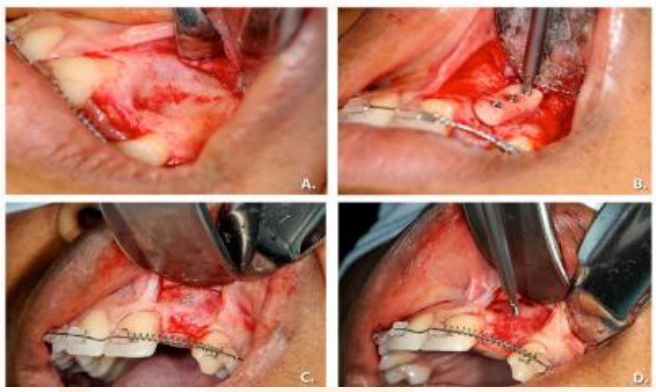
GUIDED BONE REGENERATION WITH ALLOGRAFT :

Guided bone regeneration is a new technique that evolved following the guided tissue regeneration procedure for reconstruction of lost periodontium. The procedure consists of the use of a physical barrier to exclude connective tissue from the defect. The objective of guided bone regeneration is to promote bone formation in osseous deformities either before, or in conjunction with, endosseous dental implant placement. Such deformities may be localized alveolar ridge defects associated with loss of bone before, or at the time of, implant removal, fenestrations/ dehiscences of alveolar bone at the implant interface, or defects associated with immediate implant placement in fresh extraction sockets. Small natural spacemaking defects that provide support for the physical barrier do not require bone grafts to prevent collapse of the barrier into the defect whereas large nonspacemaking defects do. Both free-dried bone allograft and decalcified freeze-dried bone allograft are used as support for the physical barrier and to provide either a lattice network for osteoconduction, or bone inductive proteins for osteoinduction.[18]

Demineralized bone products are used in dentistry primarily in periodontal regeneration and in reconstruction of the jaws for dental implant placement. Periodontal regeneration is challenging because grafting is performed in an infected site, often complicated by patients who smoke or are diabetic. Furthermore, the grafting attempts to reconstruct not only bone, but also cementum, and periodontal ligament. The classical approach to periodontal regeneration has been to use bone grafts. However, demineralized products began to attract attention for periodontal regeneration in the early 1980s. Bowers and colleagues showed that the average new attachment formation was only 1.21 mm compared to no attachment in periodontal defects that were only debrided .

There are many varieties of grafting materials available. Freezedried allografts are the most common. When bone allografts which retain bone morphogenic proteins [BMP's] are placed in contact with vascularized host bone, they will unite with it and their calcified matrix will be replaced by new bone. The individual peculiarities of the human skeleton are such that each bone has its own requirements for healing, immobilization and bone grafting. Therefore, there is no universal, all purpose bone allograft, and there is no single way of preparing all bone allografts. To date, in reconstructive dental surgery, the most successful bone allografts have been aseptically excised and processed freeze-dried cortical, cancellous and corticocancellous grafts which have not been subjected to extensive manipulations, such as exposure to chemical agents, heating, irradiation, ethylene oxide etc.

Bone allografts commonly used in reconstructive dental procedures can be broadly divided into particulate and structural grafts. The former are used most frequently. Particulate grafts can be crushed cancellous or cortical bone [bone chips], ground bone, morselized bone or microparticulate grafts. [18] .These are used for filling defects with largely intact walls (closed intraosseous defects). The structural grafts are bone plates [bone struts], sections of mandibles and cortical and cancellous bone blocks. The former are used primarily to reconstruct large defects in bone



Reconstruction of mandibular and maxillary defects with freeze-dried structural allografts. (A.) Bone defect in area of tooth #10. (B.) Bone allograft shaped to obliterate a defect and secured in place with screws. (C.) Grafted site uncovered after 5 months shows good healthy bone regeneration. (D.) Fixation screws removed for preparation of implant placement

Xenografts For Bone And Periodontal Regeneration

INTRODUCTION :

Xenografts are bone grafts from other species (typically bovine and porcine) and transplanted in humans. It is osteoconductive, biocompatible and structurally similar to human bone. Plenty of donor sources can found for bone grafting. Bovine, Equine Porcine bones and natural coral are used for xenografting. Among them bovine bones are commonly used for grafting procedures due to structural similarity with human cancellous bone. Anorganic bovine bone graft (ABM) is a naturally derived porous and deproteinized bovine bone mineral with comparable mineral composition and microporous structure similar to native human bone. Anorganic bovine bone has been shown significant improvement in clinical attachment level and hard tissue fill in human intrabony defects. The bovine derived xenografts BIO-OSS and OSTEOGRAF/N-300 currently are in widespread clinical use. Bio-Oss exhibits osteoconductive properties with a crystalline structure similar to human bone and is said to resorb within 12 to 24 months based on human histological sections of sinus core samples.[19]

Bovine derived bone is available in different particle sizes, ranging from 240 to 2,000 µm. A relatively small particle size, 240 to 1000 µm, provides a correspondingly larger surface area, which will enhance angiogenesis and osteoconduction, serving as a scaffold for the formation of new bone . Human and animal studies also have shown that ABB is osteoconductive and facilitates new bone formation . ABB contains growth factors that might facilitate the induction of newbone .On the otherhand, a variety of animal and human studies have suggested that the resorption of ABB and its replacement with new bone appears to be a relatively slow compared to allografts.

However, despite the positive results obtained from studies conducted in xenograft materials, the tissue /bone regeneration with this graft material might be unpredictable. In one study, where defects were treated with bovine derived bone grafts, at one year follow up, 78% of defect healed successfully. Furthermore, in another study, eight intrabony defects were filled with xenografts and the results showed that seven defects went through successful healing, but one defect healed by repair. Great advantage of these types of graft is that only one surgical procedure is necessary.

Commercially available xenograft materials :

Brand	Manufacturer	Available as
Bio-Oss cortical and cancellous	Osteohealth Co.	Granular 25- 1 -0 or 1 .O-2.0mm block
Osteograft / N-300 /N-700	CeraMed	Granular 250-420mm Granular 420-1000mm

Bovine derived graft material not used in dentistry: Endobon@, Laddecm

XENOGRAFTS IN PERIODONTAL RREGENERATION:

Sources :

BOVINE SUBSTITUTES:

Bovine bone (BB) derived xenografts were widely used for alveolar bone re Deproteinized bovine bone, often known as BioOssTM, is the most widely used source of xenograft materials in the dental industry.

It is claimed that they come from young herds of carefully bred, highly selected young animals—typically young calves—who are clear of all known ailments. A chemical low heat processing method preserves mineral structure with a calcium phosphate ratio of 2.1:1 and porosity of 75 to 80%, i.e. similar to natural hydroxyapatite, while eliminating all organic components.

The porous structure exhibits a vast surface area, and promotes the growth of new blood vessels via angiogenesis which enhances bone growth .[19]

Due to their better stability and minimal immunogenicity, bovine bone substitutes have been employed widely in maxillary sinus lifting and implant treatments. According to 24 studies, 39% of new bone formed at maxillary sinus defect sites treated with BioOssTM after 6 months, which was comparable to 40% of new bone formed at the same site after treatment with autograft bone. Additionally, they discovered that 31% of the BioOssTM that was grafted stayed at the graft site as opposed to just 18% of the autograft bone. With osteoconductive qualities and a crystalline structure resembling that of real bone, Bio-Oss and is said to resorb within 12 to 24 months.[21]

In a study where Bio-Oss was combined with pig collagen fibers and grafted into canine periodontal lesions, Clergeau et al. (1996) explored the periodontal application of Bio-Oss. After the regenerative procedure, the animals were slaughtered 6, 18, and 36 weeks later. The findings showed that collagen-Bio-Oss material-implanted sites had higher bone repair than control sites.

There are further commercially available goods made from bovine bone, including OsteoGrafTM and CeraboneTM. These products, including BioOssTM, have structural and biochemical characteristics that are strikingly comparable to those of human bone and can be used as efficient osteoconductive grafting materials.

Bovine bone xenografts have a number of benefits but also some drawbacks. A substance utilized therapeutically in periodontology or alveolar bone regeneration must be uncontaminated and safe for the patient's long-term health. Multiple forms of sinusitis, a maxillary fungal ball, material displacement, persistent inflammation and other inflammatory reactions, and a reaction to a foreign body are among the serious consequences that have been recorded. Therefore, the efficacy and legitimacy of grafts made from bovine bone should be questioned, and novel procedures utilizing various materials must be tried.[19]

PORCINE SUBSTITUTES:

Recently created alternatives generated from porcine are thought to resemble human bone in terms of structure and development. The porous anorganic bone graft material porcine bone graft tissue is primarily composed of calcium phosphate. These are made by removing the organic materials from swine bone and are offered in granular form with particle sizes of 0.25-1 mm and 1-2 mm (Gen-Os®).

They have osteoconductive traits and a minimal risk of spreading illness. Excellent osteoconductivity, cell survival, and osteoblast-like cell development are all provided by porcine collagen in vitro. The biocompatible anorganic bone mineral matrix stimulates the production and ingrowth of new bone at the implantation site thanks to its interconnected macro- and microscopic porosity structure.[20]

EQUINE SUBSTITUTES :

Equine derived bone substitutes have the ability to induce osteoblastic differentiation and angiogenesis In addition, the presence of neoplastic bone associated with remodeling effects was observed around the graft material 6 months postoperatively in case of successful sinus lift .

MARINE SUBSTITUTES :

Because of their distinctive structural networks, marine skeletons can serve as models for the development of human tissues. Corals, sponges, mollusk shells, cuttlefish, and fish bones are frequently employed. Scaffolds made of coral skeletons and calcium phosphates from converted coralline are good.

Calcium carbonates and phosphates such as hydroxyapatite have similarities with the mineral constituents of bones. The coral skeletal carbonate also possess unique architectural properties like porosity, pore size and pore interconnectivity which are important in periodontal regeneration.[19] Significant gain in clinical attachment level, reduction of probing depth and defect fill have been reported.Chitosan is available in a variety of forms, including beads, films, hydrogels, and more complex structures, such as porous scaffolds. Due to the poor mechanical properties exhibited by chitosan, it is often combined with other materials such as gelatin, calcium phosphates and bioglass to provide more desirable properties.[19]

Fishbone-derived and fish scale-derived scaffolds (FSS) are another de-mineralized bone matrix (DBM) alternative against the bovine bone grafts. Demineralized bone matrix has successfully been practiced in various studies to fill defects, reconstruct cranio-maxillofacial fractures, bridge large bone and high risk defects, and induce bone formation. Hence these marine based biomaterials offer excellent osteoconductivity and supports cell adhesion, proliferation and differentiation making them an attractive option in regenerative scenario.

IMMUNOGENICITY :

Histologic studies performed in animals have shown that xenografts such as ABB are biocompatible materials that evoke minimal inflammation, without the induction of foreign body reactions, and their use generally results in normal uneventful healing.

CLINICAL APPLICATIONS :

TISSUE ENGINEERING IN DENTISTRY :

Collagen, alginates, and other marine biomaterials have been used for a range of applications. Tilapia collagen helps in pulp and dentin regeneration. Additionally, they increase the survival of human periodontal ligament stem cells and up-regulate the expression of osteogenic markers, which supports the regeneration of alveolar bone. In dentistry, collagen extracted from fish can be employed as membranes, local delivery systems, and hemostatic agents. Due to its outstanding biocompatibility, bioactivity, and antibacterial qualities, chitosan is another important agent that can be used in a wide range of dental applications. Chitosan hydrogels are used to treat chronic periodontitis by filling up bone defects. Scaffolds made of chitosan help in pulp and dentin regeneration.[19]

FURCATION DEFECTS :

Clinical attachment gain, pocket depth reduction, and gingival margin position changes have been used as measures of improved clinical outcomes following the application of anorganic bovine bone with or without GTR in the treatment of class III furcation abnormalities. Furcation flaws were better resolved when GTR with bio-absorbable collagen membrane and anorganic bovine bone/collagen were combined.

Combination therapy seems to work best for more severe osseous defects including class II furcation defects and intrabony defects with one or two walls.

Additionally, long-term clinical results, notably in furcation abnormalities, appear to be stable when GTR and xenograft graft material are used in[20] combination therapy.

INTRABONY DEFECTS:

The number of bony walls (1, 2, or 3 walls) and depth of the defect (measured from the crestal height of bone to the depth of the defect) are frequently used to describe intrabony flaws.

the root of the defect. In comparison to surgical debridement alone, bone transplants offer better clinical results in the treatment of periodontal bone

abnormalities. When compared to open flap debridement techniques, bone grafts improve intrabony defects by raising bone level, decreasing crestal bone loss, raising clinical attachment level, and shallowening probing pocket depths. When compared to presurgical levels, deproteinized bovine bone has the capacity to enhance the effects of enamel matrix protein in reducing probing pocket depth, enhancing clinical attachment levels, and accelerating defect fill. [21]

In various human clinical investigations, deproteinized bovine bone has been investigated for periodontal defects alone or in combination with autogenous bone, collagen membranes, enamel matrix derivatives, or collagen matrix. Recent research has shown that periodontal repair achieved using GTR therapy, with or without the addition of deproteinized bovine bone, appears to hold up over time.

Fig : xenograft

Sinus lifting procedures :

The most common bone substitutes used in maxillary sinus floor elevation surgery are xenografts made from inorganic bovine bone. There are also other sources, including horse and porcine bone. A bone substitute's biocompatibility, osteoconductivity, integration, and resorption may all be affected by changes in its physicochemical qualities. Numerous publications have demonstrated successful regeneration operations in individuals who underwent bovine bone augmentation of the maxillary sinus. Osteoplant Osteoxenon® derived from equine bone is composed of flexible cortical and cancellous bone tissue, and is resorbable by osteoclast activation, promoting new bone formation as a scaffold.[22]

Socket preservation:

Recently, some investigators studied a xenogenic bone substitute that consists of corticocancellous porcine bone in the form of particles with a high porosity and a diameter ranging from 600 to 1,000 microm. The ridge preservation procedures using

corticocancellous porcine bone and collagen membrane limited the reabsorption of hard tissue ridge after tooth extraction compared to extraction alone and allowed a more favorable implant position. The histologic analysis after 7 months of tooth removal showed higher percentages of trabecular bone and total mineralized tissue in ridge-preservation sites compared to extraction-alone .

Wound healing:
Collagen from marine sources had stimulating effects on fibroblasts proliferation, collagen synthesis and reepithelialization there by assisting in wound contraction and dermal reconstitution. Hence they are valuable agents for scaffold fabrication. Chitosan is also gaining attention due their desirable qualities of non toxicity, biocompatibility, biodegradability and thus helps in wound healing .[23]

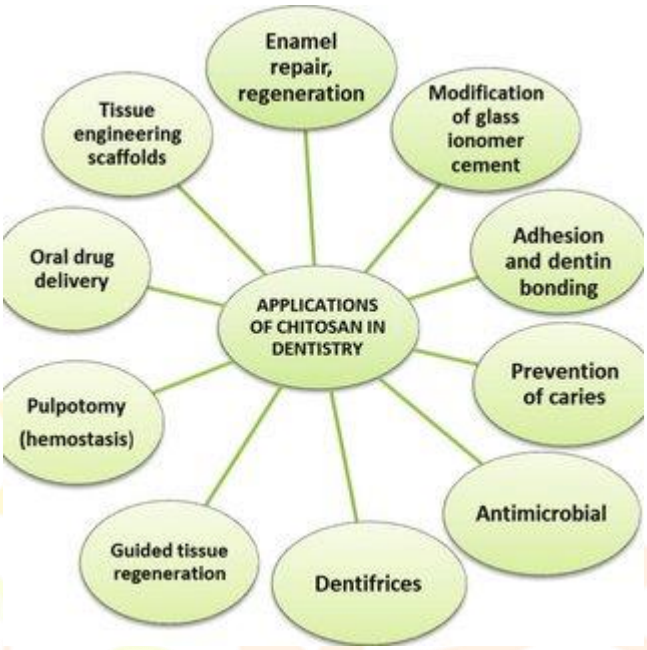


Fig : chitosan

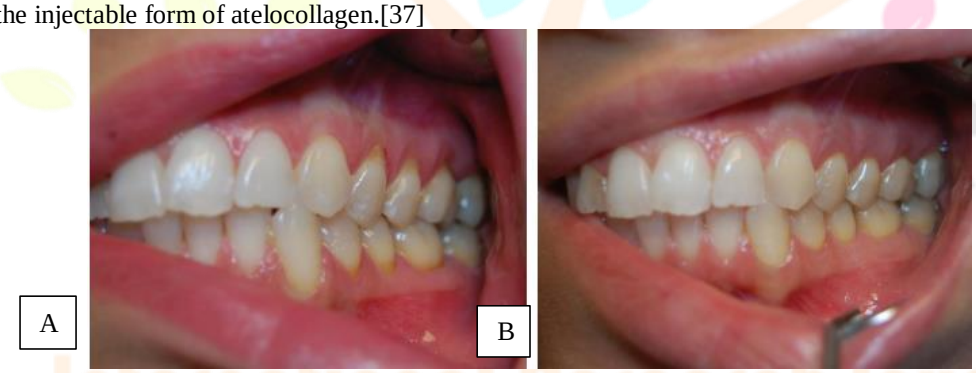
Novel Natural Bovine Bone Mineral Grafting Material with Integrated Atelocollagen Type 1
Introduction :
Atelocollagen was the first naturally occurring biomaterial with potential application as gene delivery vector and is prepared by pepsin treatment from type I collagen of calf dermis. Atelocollagen/DNA complexes can be fabricated into beads, sponge, membrane, minipellet, etc. without use of heat or without using any organic solvent. Further, high concentration of atelocollagen allows the complex to be stable for prolonged times, which is advantageous for a sustained release carrier.[40] On the contrary, low concentration of atelocollagen results in formation of complex particles with diameter in size 100–300 nm, which is considered adequate for systemic applications. Furthermore, treatment with atelocollagen did not alter expression level of toxicity related genes, suggesting it to be practically non-toxic and potential candidate for gene vector. Atelocollagen has been successfully employed in several *in vitro* and *in vivo* gene delivery studies.
Atelocollagen is a low-immunogenic derivative of collagen obtained by removal of N- and C-terminal telopeptide components (Ochiya et al., 1999), which are known to induce antigenicity in humans. Telopeptides are removed by treatment of collagen with type I pepsin. The resulting atelocollagen (300 kDa) has a stick-like structure, 300 nm in length and 1.5 nm in diameter. One advantage of atelocollagen compared to other experimental carriers is that it is already used clinically in a variety of applications, including wound healing, and as a bone cartilage substitute and haemostatic agent (Ochiya et al., 2001).

Atelocollagen Type 1 in periodontal regeneration :
Gingival recessions, as the result of dental plaque accumulation, aggressive tooth brushing, periodontal diseases and orthodontic treatment, are large-scale for the population's problem . Gingival connective tissue consists predominantly of fibroblasts (5%), as well as other cells, such as mast cells, macrophages, neutrophilic granulocytes, lymphocytes and plasma cells, present in the lamina propria of oral mucosa. The extracellular matrix contains glycosaminoglicans, such as hyaluronic acid, dermatan sulfate and heparan sulfate, proteoglycans, glycoproteins and fibers, such as reticulin fibers, collagen fibers, oxytalan and elastic fibers. Collagen fibers (65% of volume) play

a key role in the gingival architecture of healthy gingiva, as well as in periodontal disease progression . The level of collagen loss is highlighted as the main marker of this process . The early signs of collagen loss in the perivascular area are observed in the first stage of gingivitis (initial lesion). This is due to the fact that almost 70% of collagen is destroyed around the cellular infiltrate at that stage. The main fiber groups affected during this process are circular and dentinogingival fibers. In the established lesion, there is an adverse relation between number of inflammatory cells and the amount of intact collagen .[39] The process starts apicaly to the junctional epithelium and spreads into the periodontal ligament. The activities of various enzymes, such as collagenase and the phagocytosis, are mechanisms associated with collagen degradation. Collagen is an essential protein of the connective tissue matrix, which influences the migration of keratinocytes to damaged sites of epidermis or epithelium, and it is an important component of

accelerating wound healing and tissue regeneration. Over the embryonic period, the predominant type of collagen in gingiva, called the "fetal collagen", is collagen type III.
After birth, collagen type III is gradually replaced by collagen type I, which becomes the dominant type. However, type III is detected in the reticulum, type V in the pericell area and type IV in blood vessel basement membranes . Type I collagen fibers are situated in the deep layer of gingival connective tissues and type III in more superficial layers. There are two α1 and one α2 chains, but three identical α1 chains are the difference in the structure of the triple helix of collagens type I and III. Both collagenous and noncollagenous molecules are combined to create fibrils . Collagen for biomaterial production may be extracted from animals and humans (cadavers, placenta, amniotic sac) [6] or may be synthetized using recombinant techniques . For extraction from animal tissue, two different methods are used: pepsin digestion or acid solubilization, obtaining two different collagen forms: atelocollagen and tropocollagen. [37]
Atelocollagen is preferred in commercial use due to the associated cross-species antigenicity of the p-determinant located in the telopeptides [29]. For this reason, both allogenic and xenogenic collagen are widely recognized as safe biomaterials. Collagen is seen as one of the most valuable biomaterials, due to its excellent biocompatibility, weak antigenicity and its biodegradability. Collagen products may have various forms, such as liquid, gel, membrane and granules.

This makes it very useful in medical applications, especially for tissue engineering: oral mucosa, gingival and bone tissue, skin regeneration and stimulation, as well as a scaffold for artificial blood vessels and valves. Currently, injectable collagen is available, not only as a tissue filler, but also as a connective tissue biostimulator. This form of collagen is traditionally used for skin applications; however, similar results may be expected after collagen stimulation in oral mucosa, as it also contains connective tissue. The injectable form of atelocollagen is a promising material for gingival soft tissue regeneration and stimulation and allows for reduction in the number of procedures and support in a variety of surgical scenarios according to recent study condudcted by Marzena Wyganowska-Swiatkowska 1 in 2020 in Department of Dental Surgery and Periodontology, Poznan University of Medical Sciences, Poland i.e In this study 18 patients, 97 gingival class I Miller recessions were divided according to recession height, gingival papillae loss and thickness of gingivae. Atelocollagen (Linerase, 100 mg) was injected into keratinized gingivae twice or thrice, at two-week intervals. Results were statistically significant changes in gingival recession, amount of gingival papillae loss and thickness of gingiva were observed, after both two and three collagen injections. Although the degree (height) of recession decreased and gingival tissue thickness increased with every injection; there was no difference in gingival papillae loss between second and third collagen injections . This was the first clinical examination of gingival tissue outcome done after gingival recession treatment with the injectable form of atelocollagen.[37]



Patient with class I Miller recessions in upper and lower arch before (A) and after 3 Atelocollagen injection (B)

In 2018 a study was conducted in vivo study using an animal model (sheep). Four hollow titanium cylinders 4.0-mm internal diameter and 8-mm length, termed "bone growing chambers" (BGC), were inserted in two sheep mandibles. In the right side of each animal, the BGC was filled by the novel natural bovine bone graft with integrated atelocollagen Type I (test group), while the left BGC was left empty to be filled only by blood clot (control group). After 2 months of healing, the animals were sacrifced, and both histological and morphometric analysis were performed in which they found All titanium chambers were well osseointegrated after 2 months of healing. In the test group, newly formed bone mixed with residual granules appeared incorporated in the new trabeculae was found .

Synthetic Bone Substitute Materials
INTRODUCTION :
An autologous bone graft is still the ideal material for the repair of craniofacial defects, but its availability is limited and harvesting can be associated with complications. Bone replacement materials as an alternative have a long history of success. With increasing technological advances the spectrum of grafting materials has broadened to allografts, xenografts, and synthetic materials, providing material specific advantages. A large number of bone-graft substitutes are available including allograft bone preparations such as demineralized bone matrix and calcium-based materials. More and more replacement materials consist of one or more components: an osteoconductive matrix, which supports the ingrowth of new bone; and osteoinductive proteins, which sustain mitogenesis of undifferentiated cells; and osteogenic cells (osteoblasts or osteoblast precursors), which are capable of forming bone in the proper environment. All substitutes can either replace autologous bone or expand an existing amount of autologous bone graft.
Because an understanding of the properties of each material enables individual treatment concepts this review presents an overview of the principles of bone replacement, the types of graft materials available, and considers future perspectives. Bone substitutes are undergoing a change from a simple replacement material to an individually created composite biomaterial with osteoinductive properties to enable enhanced defect bridging.
HISTORICAL BACKGROUND :
The breakthrough in the present-day development of bone substitute materials (BSM) was initially achieved by Barth and Ollier who carried out animal experiments in order to study different bone replacement materials for the first time (Barth, 1895).

Historically, autogenous bone grafts, allografts, and a variety of biomaterials have been used for the repair of osseous defects and the augmentation of compromised bone. The ideal bone-graft substitute is biocompatible, bioresorbable, osteoconductive, osteoinductive, structurally similar to bone, easy to use, and cost-effective.

Problems related to the availability of graft material, donor-site morbidity, immunogenicity and biomechanical integrity have limited its success. An increasing number of bone graft materials with completely different origins are commercially available for many applications throughout the human body. They are variable in their composition, their mechanism of action and, therefore, their indications.

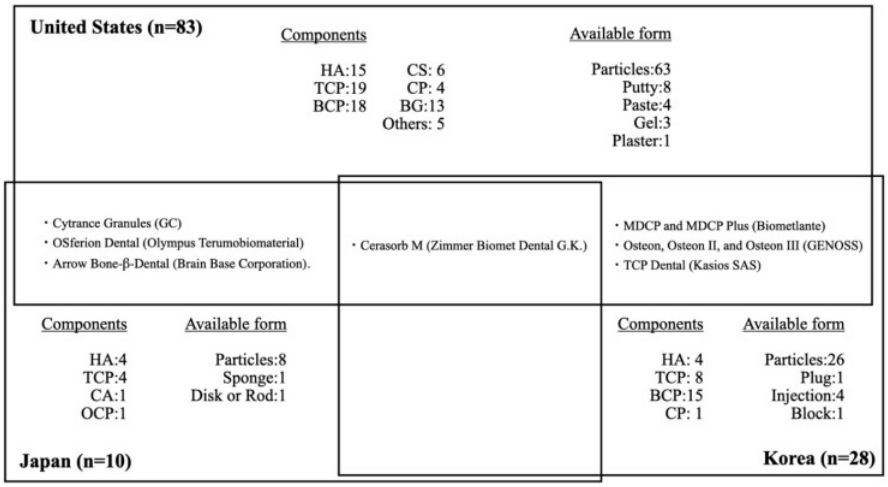
BSM are generally considered to be a highly important alternative to bone grafting in dental surgery, implantology and periodontology.[41] Donor site morbidity is diminished while simultaneously guaranteeing a nearly unlimited level of material disposition. In this way, a large variety of osseous defects can be repaired using BSM. Due to current developments innovative BSMs with new chemical, structural and subsequent biological properties will embrace a lot of requirements in order to imitate the characteristics of the bone defect. Crucial for the clinical success of BSMs are their interactions with the adjacent tissue structures and cells due to a macroporous interconnecting structure of >100 micron diameter promoting cell infiltration, bone growth and vascularisation. In the context of large osseous augmentations, autogenous bone is still used as the preferred gold standard material. However, in certain clinical settings and appropriate indications a combination of BSM with living tissue/cells or BSM alone may be suitable.

THE FUNCTIONS OF BSM ARE AS FOLLOWS :

- space maintenance for bone regeneration
- pre-setting of the desired anatomical form
- supporting functions for the periosteum and associated membranes
- acceleration of bone remodelling
- osteoconductive structural guidance for the regeneration of osseous tissue
- carrier substance for antibiotics, growth factors or approaches by gene therapy (Rupprecht et al., 2007, Fischer et al., 2011, Maus et al., 2008a, Smeets et al., 2009, Kolk et al., 2011)
- scaffolds for tissue engineering approaches

THE REQUIREMENTS FOR AN IDEAL BSM ARE :

- biocompatibility
- osteoinduction and osteopromotion/osteoconduction
- porosity
- stability under stress
- resorbability/degradability
- plasticity
- sterility
- stable and long-term integration of implants.



dental alloplastic bone substitute products commercially available in the United States, Japan, and Korea. HA = hydroxyapatite, TCP = tricalcium phosphate, BCP = biphasic calcium phosphate, CS = calcium sulfate, CP = calcium phosphate, BG = bioglass, CA = carbonate apatite, OCP = octacalcium phosphate.

VARIOUS BSM FORMS :

BSMS OF NATURAL ORIGIN :

- Materials are subdivided into harvested bone grafts and graft substitutes: autogen (from same individual) , allogen (from same species) , xenogen (other species) .

SYNTHETIC (ALLOPLASTIC) MATERIALS :

- ceramics: biological glasses, TCP, HA and glass ionomer cements

- metal: titanium
- polymers: polymethylmethacrylate, polylactides/polyglycolides and copolymers

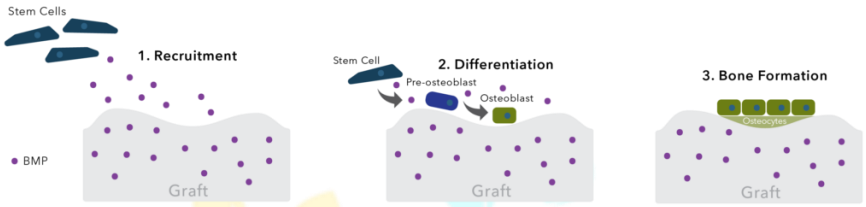
- cements: calcium phosphate (CP) cements

COMPOSITE MATERIALS

- In ideal cases composite biosynthetic materials have osteoconductive osteogenic and osteoinductive activities .

BSM COMBINED WITH GROWTH FACTORS :

- Among the bone growth factors tested in heterotopic and orthotopic locations, bone morphogenic proteins (BMPs), either in native (BMP) or recombinant forms (rhBMPs), appear to be the most effective and therefore the most promising.



Growth factor based bone grafts play an active role in bone healing through protein-based cell stimulation.

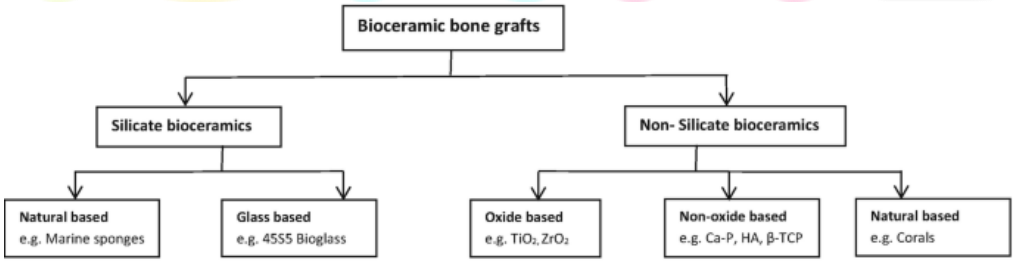
BSM WITH LIVING CELLS :

- Composite biosynthetic transplants consist of a carrier as an osteoconductive scaffold combined with osteogenic cells and/or growth factors (Lane et al., 1999, Vaccaro et al., 2002). A ‘‘combined graft’’ contains osteogenic cells and cytokines along with a BSM as a synthetic osteoconductive matrix[42]

Ceramic and ceramic composites:
Ceramic bone substitutes are typical calcium-based synthetic bone substitutes that are already approved in terms of stability and effect. Given the problems with autogenous bone and allogeneic bone, osteoconductive ceramic with biodegradation draws considerable attention these days. For synthesized graft to exert its biological effects, several conditions are required: compatibility with

surrounding tissues, chemical stability in body fluid, biomechanical and physical compatibility, durability in sterilization process, reasonable price, and consistency of reliable quality . Today, various types of ceramic products are composed of calcium phosphate, including hydroxyapatite (HA) and tricalciumphosphate (TCP), or (calcium sulfate), or their compounds .

Ceramic features no limitations of quantity, no risk of morbidity and infection of the donor site, and easy sterilization and storage. However, primary application of ceramics is mainly focused on bone defects, such as fracture with joint depression, because ceramics are fragile and have poor mechanical strength. Since the amount of resorption of ceramic varies depending on material, if resorption does not occur properly, it could possibly impede bone remodeling. As a result, the speed of bony union and the process of remodeling to obtain a proper strength are delayed. In addition, due to its fragility, it is difficult to mold ceramic into a desired shape during operation. The remodeling process relies mainly on ceramic biodegradability.[43] At this time, material that is not absorbed biologically impedes the remodeling process and becomes a region of mechanical stress concentration . Too slow absorption impedes bone remodeling, and too fast absorption reduces mechanical stability and causes fibrous tissue formation instead of osteogenesis .



Classification of bioceramic bone grafts

Hydroxyapatite (HA)
HA is bioactive ceramic and a main mineral of bone. Given its density, HA with a porous structure is easily bio-absorbable and exhibits good osteoconductivity. Therefore, when it is introduced in vivo, surrounding bone tissues grows and gradually progresses through the bone substitution. Regarding the material features of HA, it can be

inserted in line with a shape of a defective region. In addition, it is easily absorbed, does not generate metabolite impeding osteogenesis, and causes almost no foreign body reaction due to its excellent biocompatibility . HA has very high compression and tensile strength compared with TCP. Since HA is slowly degraded and retained in vivo for a long period of time, it impedes bone remodeling extends the mechanical vulnerability of new bone, and remains as permanent stressor.

Tri-calcium phosphate (TCP)

Tri-calcium phosphate is osteoconductive calcium phosphate and has the most similar chemical composition to human bone. It has better absorption than hydroxyapatite (HA) . It is more porous than HA and features weak mechanical strength and fast absorption. More porous TCP undergoes biodegradation within 6 weeks after its introduction into the bone defect. Since its compression and tensile strength is very similar to that of cancellous bone, it is used in regions with no mechanical load . Moreover, TCP has better osteoconductivity and biocompatibility than conventional bone cement with PMMA, and it is possible to inject TCP with a syringe into a bone defect or the screw insertion site in case of fracture fixation . As another main component, polyphosphate is highly concentrated in osteoblasts and is involved in mineralization of bone metabolism . In contrast of HA, ceramic TCP is biodegraded fast in vivo. It is biodegraded within 4–8 weeks after graft, and it is difficult to obtain proper bone formation during the early period . In consideration of these properties, biphasic ceramic with a mixture of HA and TCP is manufactured. Depending on the mixture ratio of these two components, it is possible to adjust the speed and degree of absorption and mechanical strength

Calcium phosphate cement (CPC)

The discovery of the first CPC occurred coincidentally via the observation of calcium phosphate solubility in 1986 . CPC consists of calcium phosphate. Calcium phosphate cements (CPCs) are frequently used to repair bone defects. Currently, CPC are defined as a combination of one or more calcium phosphate powders which, upon mixing with a liquid phase, form a paste able to self-set and harden in situ in the bone defect site to form a scaffold . A body-temperature dissolution-precipitation reaction is one of the most important

characteristics of CPC, which facilitates is ability to mold and fill the bone defect . Injectability, one of the advantages of CPC, allows

application of CPC in minimally invasive surgery . Therefore, it is clinically used to fill metaphyseal or subchondral cortical defects caused by articular fracture. Since CPC has the material property of ceramic, bioabsorbable-enhancing additives, such as chitosan or Vicryl meshes, can be used to improve mechanical strength .[49] CPC has osteoconductivity; it is gradually absorbed in the bone remodeling process and is replaced by a new bone . Currently, the paradigm has moved toward enhancement of biological interactions of CPC, such as bone tissue engineering, in addition to improvement in the mechanical strength of CPC and the addition of cells and growth factors in cement . In addition, 3D printing for fabricating CPC scaffolds is rapidly developing with a high degree of accuracy. Here, 3D printed CPC offers specific benefits for clinical applications, including easy adaptation and fixation, reduced surgical time, and good esthetic results . Furthermore, with recent advances in tissue engineering, “tissue regeneration by natural tissues” instead of “tissue replacement by biomaterials” has been proposed and emphasized. This new emphasis on tissue engineering is enhanced by CPC’s excellent biological interaction such as osteoconductivity, osteoinductivity, biodegradability and bioactivity .

Calcium sulfate

Calcium sulfate is clinically used to fill defects, such as bone cavities, and segmental bone defect, and moreover expansion use for spinal fusion and even for filling of harvest site of autogenous bone. Through recrystallization, it becomes a solid material and gives mechanical stability to its inserted region. Calcium sulfate normally undergoes biodegradation within 6–8 weeks after its insertion into the bone defect. Given its lack of porosity, calcium sulfate has limited osteoconductivity. Given its mechanical disadvantage and rapid resorption. Compared with calcium phosphate, calcium sulfate is not often used .[50]

PROPERTIES AND SYNTHETIC ROUTES OF EACH COMPOSITION OF ALLOPLASTIC BONE SUBSTITUTES :

The properties of alloplastic bone substitutes are known to vary according to their compositions, as follows.

CP is a generic term that loosely describes various compositions. LeGeros has described the following types of commercially available CP compounds: (1) calcium HA: $Ca_{10}(PO_4)_6(OH)_2$, either naturally derived (coralline or bovine) or synthetic; (2) β-TCP: $Ca_3(PO_4)_2$; (3) BCP, consisting of a mixture of β-TCP and HA; and (4) unsintered CPs .

Pure HA ($Ca_{10}(PO_4)_6(OH)_2$) is among the least soluble of the CP compounds and is not found in biologic systems . Synthetic HA is prepared by numerous techniques, broadly divided into (1) solid-state chemical reactions or (2) wet reactions. These preparations have distinct sintering temperatures.

β-TCP (β-$Ca_3[PO_4]_2$) is one of the two polymorphs of TCP. Typically, β-TCP is prepared by sintering calcium-deficient HA to high temperatures . It can be also be prepared at lower temperatures in water-free mediums or by solid-state acid–base chemical interactions.[51]

Bioactive glasses (BGs) are amorphous materials, based on acid oxides (e.g., phosphorus pentoxide), silica (or alumina oxide), and alkaline oxides (e.g., calcium oxide, magnesium oxide, and zinc oxide). BGs possess an interconnective pore system and are available in both compact and porous forms . The bioactivity of the BG surface enables the growth of osseous tissue .

CS is the oldest ceramic bone substitute material, first described by Dressman in 1892 for the filling of osseous defects in human patients . Recent studies continue to demonstrate the bone healing properties of CS [40,41]. CS hemihydrate (CaSO4·1/2H2O) powder is hydrated to form CS dihydrate (CaSO4·2H2O), undergoing a slight exothermic reaction to set to a solid form.
The resorption rate of bone grafts is a feature that clinicians consider very important; there is substantial variability among alloplastic materials. HA is known to require a long interval for replacement by native bone due to its low substitution rate . If socket grafting and early re-entry for implant placement is planned, there may be insufficient time for bone formation. Conversely, if the objective is correction of a contour defect (e.g., a buccal defect at a missing tooth site) and the majority of the implant is inserted into native bone for osseointegration, a slowly replaced material will presumably provide long-term space maintenance.[53]
β-TCP is probably best known for its rapid resorption . Lambert et al. compared the healing of rabbit sinuses augmented with xenograft, BCP, and pure β-TCP . Each material supported the formation of new bone, but the bone architecture differed among materials. At 2 months after augmentation, the xenograft had formed an intimate bone bridge between the particles, while the β-TCP graft showed no bone formation. At 6 months after augmentation, there was nothing left in the β-TCP graft. These findings implied more rapid resorption of pure phase β-TCP compared to xenograft and BCP. In another study, Jensen et al. created defects in the mandibles of mini-pigs and grafted them with either autograft, xenograft, or β-TCP; they then harvested bone sections after 1, 2, 4, or 8 weeks . Consistent with the results of other studies, they found that autografts and β-TCP produced slightly more new bone during initial healing (after 4 weeks).
BCP is a combination of two alloplastic materials, generally β-TCP and HA, with ratios adjusted to potentially manipulate their biomedical properties. Cordaro et al. carried out a randomized controlled trial comparing bone healing in grafted human sinuses with either BCP or xenograft at 6 to 8 months after engraftment . The materials differed during later healing, such that less residual synthetic material remained, compared with xenograft material (26.6%). Mahesh et al. grafted human sockets with BGs, then compared bone formation with that achieved using xenografts. Significantly more new bone formed from the BG putty (36–57%) between 4 and 6 months after engraftment. Furthermore, the BG resorbed at approximately 20% per month . Unlike the slower resorbing CP compounds, CS compounds resorb relatively quickly, generally within 8 weeks and certainly by 6 months after engraftment .[54]

Alloplastic Bone Graft Products For Periodontal And Bone Regeneration :
In the context of periodontal regeneration, bone graft materials are required to increase space in patients with non-contained defects such as one-wall defects and class II furcation involvement . Preferably, alloplastic bone substitutes will be completely resorbed. A previous study showed that non-resorbable products such as HA sintered at high temperatures tended not to be used for periodontal regeneration because of concerns that residual bone graft materials may cause long-term inhibition of periodontal tissue formation and weak resistance due to re-infection . For complete bone substitute resorption, 3–6 months is an appropriate interval considering the speed of bone remodeling and creation of space . In contrast, materials with slow resorption rates are required in situations involving GBR and sinus lift where robust space creation and primary implant stability are needed [59]. Although autologous bone is generally considered the gold standard, single-use autologous bone is not appropriate for GBR because of its high resorption rate . Selection of a product with a suitable resorption rate is necessary for each clinical situation. We also emphasize that an appropriate surgical procedure should be considered in clinical situations. This procedure may include the concomitant use of alloplastic bone substitutes with growth factors, or the use of alternative surgical techniques such as onlay block grafting and distraction osteogenesis .[55]

Effective for bone-to-bone as well as bone-to-metal wet field applications, the revolutionary biomaterial Bone Adhesives: Tetranite

Introduction
Bone adhesives can be either synthetic or biologically derived and/or inspired. Poly(methyl methacrylate) (PMMA) bone cement is the best-known example of a synthetic adhesive considered for bone. PMMA bone cements are commonly used in bone surgeries for the fixation of implants such as hip and knee replacements into bone.However, they have little or no intrinsic adhesion to bone (the adhesion has to be enhanced using various pretreatments or chemical modifications),they lack chemical interaction, cause significant heat generation and shrinkage, are not biodegradable, and the methyl methacrylate monomer is toxic. Other synthetic adhesives include cyanoacrylates, polyurethanes, lactide-methacrylate-based systems,glass ionomer cements, and others. These systems tend to have higher adhesion strength than biological adhesives, but they often show relatively poor biocompatibility, and most are not biodegradable over clinically useful time frames. Biological or bioinspired adhesives, including fibrin adhesives,mussel adhesive proteins,and mimetic polymers, possess good biocompatibility and biodegradability but often have poor mechanical properties, which result in lower adhesion to bone[56].
PHRAGMATOPOMA CALIFORNICA WORM :
The capacity to make solid bindings in damp conditions is one of the main design hurdles for a bone adhesive, yet marine creatures have already found a solution to this issue.
The marine life that motivates bioadhesive research includes
is the Phragmatopoma californica worm, often known as the "sandcastle worm," which assembles sand grains and shell pieces into a protective tube shell around its body using a proteinaceous adhesive. The very acidic and basic proteins, as well as a significant amount of Mg2+ and Ca2+ ions, make up the adhesive. Lysine, histidine, and arginine make up about equal amounts of the basic residues, whereas phosphoserine dominates the acidic residues in natural glue.

TETRANITE AS BONE ADHESIVE :

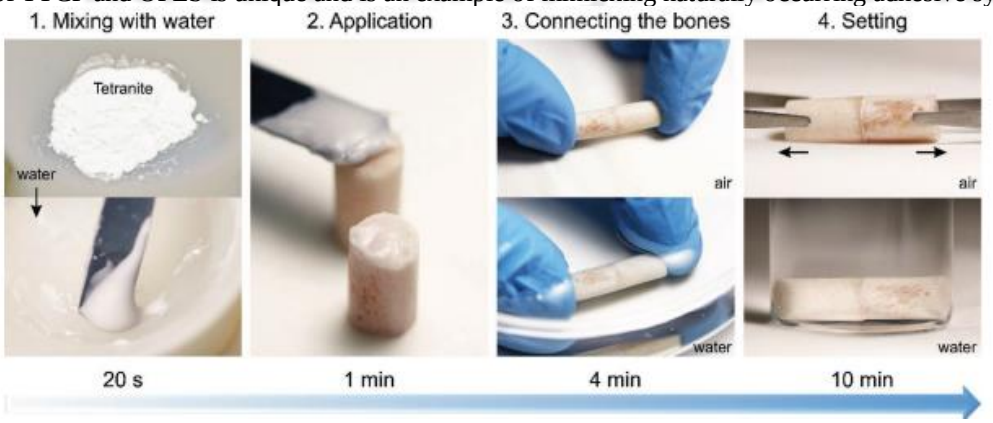
Tetranite bone adhesive and a fundamental understanding of its properties in uniaxial compression and shear. In addition, we use shear adhesive testing to explore the capacity of the material to create a fundamental adhesive bond between cortical and cancellous bone, smooth and porous 3D printed titanium, smooth and porous polyetheretherketone (PEEK), and smooth and porous 3D printed polylactic acid (PLA). By considering smooth and porous version of the same materials we are able to determine the capacity of the material to form both adhesive and cohesive bonds. In addition, investigation of the degradation and new bone formation of Tetranite placed in a critical size defect within the distal femoral condyle of a rabbit were found. Ultimately, this new bone adhesive may inspire innovative, cutting-edge clinical treatments of bone fractures and fusions with and without implants. [110]

Figure 4. Adhesive shear testing samples. a) Cylinders of varied materials and porosity before adhesive shear testing; b) samples adhered together using the Tetranite bone adhesive, HydroSet, or Palacos (lower rows) before and after shear testing showing different adhesive failure modes: adhesive, mixed, and cohesive.

Tetranite is a synthetic, injectable, cohesive, self-setting mineral–organic biomaterial that can be used as a wet-field bioresorbable bone adhesive, partially inspired by the concept of how marine species utilize their adhesive proteins to bind the objects underwater. An overview of the use of Tetranite as a bone adhesive is illustrated in Figure .

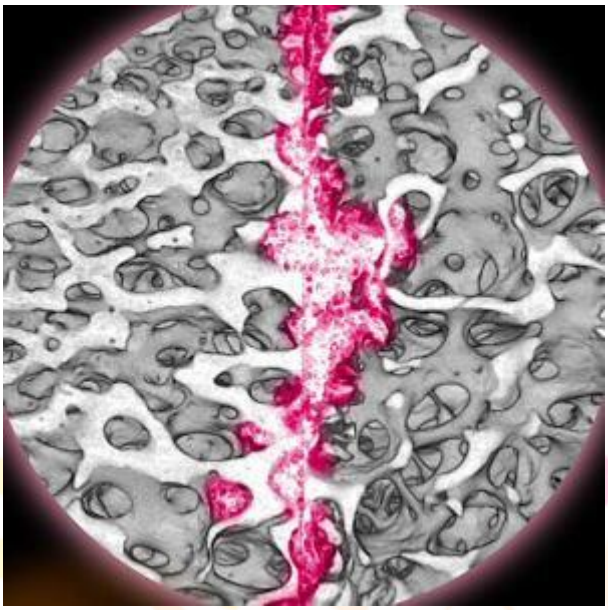
First, Tetranite powder is mixed with water in a liquid-to-powder ratio of 0.21 mL g–1 for 20 s. Upon mixing with water, a cohesive, viscous liquid is formed, which maintains its tacky character until set (step 1). The adhesive is applied onto the bone surface (step 2), and the two bone pieces are connected. One of the primary advantages of the present biomaterial is its inherent ability to set and maintain its adhesive character even in aqueous environments (step 3), similarly to the bioadhesive produced by the sandcastle worm, which results in the possibility to adhere the bones or other materials together even when immersed in water (or other biological medium, such as blood). Final setting of the bone adhesive occurs within 10 min from the start of mixing (step 4). Once set, significant force is required to break the formed bond between the adhesive and the bone surface. Samples are then incubated in phosphate-buffered saline (PBS) at 37 °C, where they continue to cure a small amount prior to final mechanical analysis. Tetranite stems from a class of CPCs. Its composition comprises TTCP and OPLS powders, which are mixed with water to produce the mineral–organic bioresorbable bone adhesive. TTCP is a key ingredient in commercial self-setting CPCs that have excellent biocompatibility and osseointegration, while OPLS is involved in the sandcastle worm glue as its key component, resulting in underwater adhesion, and has been shown to positively influence the bone remodeling process.[112]

The combination of TTCP and OPLS is unique and is an example of mimicking naturally occurring adhesive systems.



Bone adhesive application in both dry and wet environment. 1) Tetranite powder is mixed with water; 2) the adhesive paste is applied onto the bone surface; 3) bones are connected either in air or in an aqueous environment; 4)

the bone adhesive sets in 10 min either in air or in an aqueous environment, and significant force is required to pull the bones apart.
Mechanism of Action **:**



Tetranite bone adhesive micro mechanism

The components in Tetranite react within minutes to yield a uniquely adhesive composite system. After hardening, Tetranite is bioengineered to withstand tensile and shear bond stresses as high as 3 MPa, similar to the strength of human cancellous bone. The Tetranite scaffold is osteoconductive and bioactive, leading to the eventual replacement of Tetranite with new bone. Over time, load bearing responsibility is transferred to the new tissue such that mechanical integrity is maintained.[114]
HOW TETRANITE WORKS **:**
Tetranite uniquely combines two naturally occurring compounds–Tetracalcium Phosphate (TTCP) and the amino acid O-Phospho-L-Serine (OPLS) within an aqueous medium. Upon curing in situ, the biomatrial forms a multiphasic solid that is predominantly amorphous; however, several crystalline phases in lower proportion include (a) calcium-phosphoserine-monohydrate, (b) hydroxyapatite, (c) unreacted tetracalcium phosphate, and (d) unreacted α-tricalcium phosphate. Once on the market, it will become the first synthetic bone adhesive. As a synthetic product, Tetranite does not rely on large molecules and therefore can be produced in large quantities at a low cost. [110]
Tetranite supports the body's natural healing and bone regeneration process by providing a structure into which bone can infiltrate. Given the highly amorphous structure, which is dominated by ionic and coordination bonds, loss of ions from the cured substance is mediated by gradual dissolution as interstitial fluid contacts and penetrates the surface of Tetranite, leaving channels for bony ingrowth. New bone deposition (a reparative process naturally programmed into the connective tissue cells) is supported by neovascularization that develops in the Tetranite porosities and zones where the amorphous phase of the material has dissolved away. This is a continuous process that lasts for months until most of the Tetranite material is progressively resorbed and replaced by new bone during the healing process. The final removal of the residual crystallites of the most stable phase, hydroxyapatite, is carried out by multinucleated giant cells, the normal agents involved in the turnover of mineralized tissue.[111]

Scaffolding Design For Periodontal Regeneration
Introduction :
In tissue engineering, scaffolds mainly serve as a substrate for cell attachment, tissue ingrowth, as well as an initial structural support . In GTR, non-degradable or degradable membranes serve a contact inhibition of epithelium growth that in turn allow a relatively slow (4–6 weeks) healing of periodontal connective tissue and PDL . However, a prolonged period of periodontitis may deteriorate the outcome of GTR by deteriorating healing capacity of PDL cells, hindering host immune response or severely denaturalizing CM . Scaffolds used for the regeneration of periodontal tissues can provide a contact guidance that enables timely migration of cells into periodontal defects, followed by promoted regeneration . To further facilitate cell migration and tissue ingrowth, various bioactive cues including growth factors (GFs) and cytokines have also been delivered with the scaffolds . Evidence is still premature to suggest that scaffold-mediated periodontal healing is comparable to that of GTR. Most previous studies with biodegradable scaffolds have focused on the guided regeneration of CM and PDL . Cho et al. reported that CM-like tissue structure was formed on the surface of human dentin when incubated with human PDL stem/progenitor cells (PDLSCs) seeded in 3D-printed poly(ε-caprolactone) PCL scaffolds spatially delivered with connective tissue growth factor (CTGF), bone morphogenic protein 2 and 7 (BMP-2 and BMP-7) . Chen et al. fabricated an electrospun multiphasic scaffold which consisted of PCL, collagen type I (COL-I), and rhCEMP1/ACP that promoted formation of CM-like structure when implanted in rat calvaria with PDLSCs for 8 weeks . Park et al. performed a subcutaneous implantation of micro/macro-porous biphasic calcium (MBCP) blocks seeded with BMP-2 pre-treated PDLSCs into immunocompromised mice . After 4 weeks, BMP-2 pre-treatment group

showed formation of mineralized tissue integrated with fibrous tissues . [98] Several previous works also implemented tri-phasic scaffolds to guide an integrated regeneration of CM, PDL and AB . In 2014, Lee et al. reported the reconstruction of periodontium complex using a 3D printed tri-phasic scaffold. They spatiotemporally delivered amelogenin, CTGF and BMP-2 for regeneration of CM, PDL and AB, respectively, by single type of multipotent dental stem/progenitor cells . When implanted in dorsum of immunodeficient mice for 6 weeks, the tri-phasic scaffolds with spatiotemporal delivery of three different bioactive cues and dental stem/progenitor cells successfully promoted integrated formation of periodontium-like multi-tissue construct . Park et al. fabricated tri-phase, 3D-printed scaffolds consisted of regionally different micro-architectures that showed potential in promoting integrated healing of multi-tissue periodontium by PDLSCs .

As above-described, various scaffold systems have shown great potential for integrated periodontal regeneration. The recent technical development in micro-precise regional control in design of scaffolds has made important milestone toward integrated regeneration of multitissue periodontium. The existing approaches to regenerate integrated periodontium via various scaffolds and delivery system are discussed more in-depth below.[98]

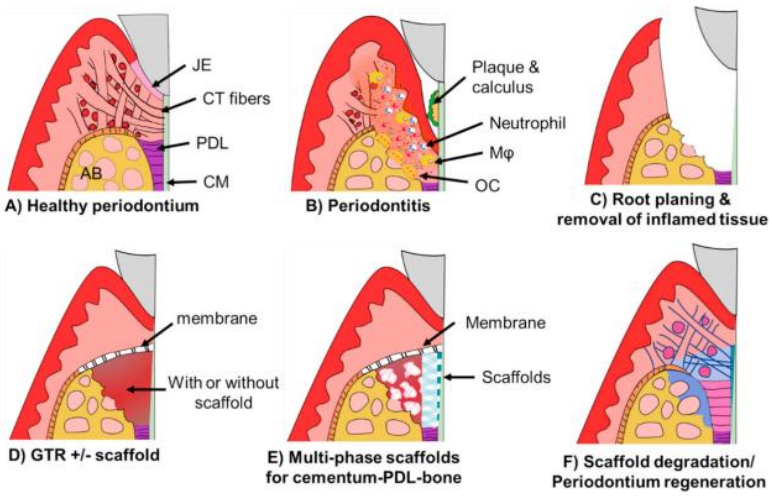


Fig. 1. Illustration of healthy periodontium, periodontitis, and scaffold-based regenerative approaches. A) Healthy periodontium is consisted of junctional epithelium (JE) (0.71–1.35 mm), connective tissue (CT) fibers (1.06–1.08 mm) [23], cementum (CM), periodontal ligament (PDL) and alveolar bone (AB). B) Periodontitis results in bone resorption by activated osteoclasts (OC), formation dental plaque & calculus, and epithelial down growth, as associated with inflammatory responses with increased number of neutrophil and macrophages (Mϕ). C) Surgical procedure involves root planing to remove calculus, necrotic CM and inflammatory granulation tissue. D) As a default treatment option, GTR with contact inhibition membrane is frequently performed that can be combined with filling the periodontal tissue gap with various scaffolds. E) Multi-phase scaffolds with delivery of bioactive cues can be implanted to induce integrative regeneration of multiple periodontal tissues. F) The implanted scaffolds are expected to undergo degradation as new tissues are forming.

PROPERTIES OF 3D SCAFFOLDS FOR APPLICATIONS IN ALVEOLAR BONE AND PERIODONTAL TISSUE REGENERATION :

Although conventional bone grafting materials serve the role of a supporting matrix, they have several disadvantages: allografts, xenografts, and alloplasts are brittle, poorly processable into porous forms, and are unable to generate structures tailored to the specific needs of patients. Likewise, they are unable to maintain the desired generated tissue volume under mechanical forces, hindering their ability to provide a proper template for effective cell interaction . Although autografts may have the ability to withstand mechanical forces, they are difficult to shape and conform to a bony defect , which is of a significant concern in the craniofacial region. BTE has opened new doors for regeneration through the introduction of scaffolds which possess three-dimensional (3D) architecture that closely mimics native extracellular matrix (ECM). Such arrangements eventually enhance cell adhesion, proliferation, differentiation, and overall tissue regeneration . As a matter of fact, scaffold properties are influenced by the used biomaterials and must be specific for the application while in harmony with the native environment to ensure that the defect area is replaced with a healthy, functional tissue matching the original one, without reparative scar formation .[64]

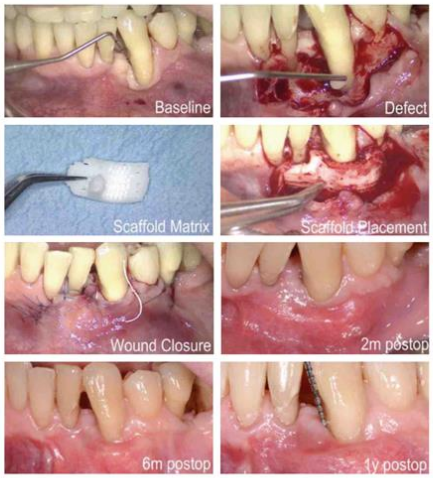


FIGURE 4: Custom-made 3D-printed PCL/HAp scaffold based on images from computed tomography (CT) scans and combined with CAD/CAM technologies for periodontal tissue regeneration. Courtesy of Rasperini et al., 2015 [136].

In general, scaffolds must exhibit an adequate degree of hydrophilicity , roughness , and specific surface topography; a topographic landscape on micro- and submicrometer scales must be developed to replicate the natural process of bone regeneration .

Nanotopography increases the overall surface area, surface-to-volume ratio, and surface roughness , which enhance the adhesion between osteoblasts and the underlying scaffold surfaces . As for microscale features, they facilitate cell penetration, vascularization, and diffusion of nutrients and offer better spatial organization for cell growth and ECM production . Development of a multiscale scaffold has been emphasized in periodontal tissue regeneration . Other important design characteristics are overall porosity, pore size, and interconnectivity. As human cancellous bone demonstrates a total porosity between 30% and 90%, any construct enclosing voids within this range is considered suitable for bone regeneration . However, extremely high porosity can jeopardize the overall mechanical stability of a scaffold by reducing its overall compressive strength . For alveolar bone regeneration applications, an overall porosity of 70% has been applied in preclinical and clinical studies . Regarding pore diameter, a range between 150 μm and 500 μm facilitates vascularization and penetration of new tissues without compromising the mechanical strength of the scaffold or cell infiltration into inner surface areas . These consequential events are also dictated by the presence of an interconnected pore network, which is essential for cell growth into the interior of the scaffold to prevent core necrosis.[69]

ANTI-MICROBIAL EFFECT OF SCAFFOLDS FOR PERIODONTAL REGENERATION:

As a simple and straightforward approach, material components with innate antimicrobial effect have been added to scaffold materials. An example of such component is chitosan, a natural sea shell-derived polymer with antibacterial, antifungal, bio-adhesive and hemostatic effects . Several previous studies showed the promising effects of chitosan in bacteria causing periodontitis. For example, Arancibia et al. reported that chitosan particles with a concentration of 5 mg/mL inhibited periodontal pathogens such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans . Similarly, Lee et al. reported that chitosan membrane with grafted epigallocatechin-3-gallate and lovastatin showed a bactericidal effect on periodontopathic bacteria such as Aggregatibacter actinomycetemcomitans, Prevotella nigrescens, and Porphyromonas gingivalis . Chitosan also showed its anti-microbial effects when added in scaffolds. Zhou et al. demonstrated that the scaffold composed of fish collagen/bioactive glass/chitosan composite nanofibers had antibacterial effects on Streptococcus mutans, leading to promoted regeneration of furcation defects in a dog model . Silver and magnesium (Mg) are another example of anti-microbial components'[70]

Summary of scaffolds for periodontal regeneration incorporated with anti-microbial component.

Antimicrobial component	Scaffold construct	Bioactive cue	Research design	Experimental duration	Outcomes
Chitosan	Chitosan (CS)/poly(vinyl alcohol) (PVA)/ hydroxyapatite (HA) electro-spun composite nanofibrous mats	Piroxicam (PX)	Initial material characteristics, release profile, and *in vitro* cytocompatibility	72 h	Sustained release of PX; appropriate mechanical behavior and minimum cytotoxicity; no direct observation for anti-microbial effect.
	Chitosan membrane with grafted epigallocatechin-3-gallate (EGCG) and lovastatin	Lovastatin	Initial material characteristics, antimicrobial activity test, and *in vivo* periodontal healing evaluation	21 days *in vitro*; 8 weeks *in vivo*	Lovastatin sustained release promoted osteogenesis; EGCG14-CS exhibited the promising bactericidal activity; improved periodontal healing in dog model.
	Chitosan/alginate/PLGA hybrid scaffolds	IGF-1, BMP-6	Initial material characteristics, release profile, gene expression, and mineralization assay	30 days	Alginate/PLGA released IGF-1 & BMP-6; hybrid scaffold activated proliferation and osteoblastic differentiation of cementoblasts; no direct observation for antimicrobial effect.
	Fish collagen/bioactive glass/ chitosan composite nanofibers	N/A	Initial material characteristics, antimicrobial activity assay, and *in vivo* periodontal healing evaluation	60 days	The composite nanofibers had antibacterial effect on *S. mutans*; improved periodontal healing in dog model
	Chitosan/β-glycerol phosphate (β-GP) hydrogel	TGF-β1, PDGF-BB, IGF-1	Initial material characteristics, release profile, and *in vitro* vitality assessment	2 weeks	Constantly released TGF-β1, PDGF-BB, IGF-1; no direct observation for antimicrobial effect.
	Injectable chitosan/ β-glycerophosphate hydrogels	BMP-7, ornidazole (ORN)	Initial material characteristics, release profile, antimicrobial assay, and *in vivo* periodontal healing evaluation	21 days *in vitro*; 8 weeks *in vivo*	Constantly released BMP-7 and ORN; the hydrogels loaded with chitosan and ORN showed clearly antimicrobial effect against *P. gingivalis*; improved periodontal regeneration in dog model
	Mesoporous HA/chitosan scaffolds	rhAmelogenin	Initial material characteristics, release profile, antimicrobial assay, and *in vivo* periodontal healing evaluation	7 days *in vivo*; 8 weeks *in vivo*	HA/chitosan scaffold showed antibacterial activity against *F. nucleatum* and *P. gingivalis*; enhanced formation of CM-like tissue in mouse model
	Sandwich-like chitosan/ polycaprolactone/gelatin scaffolds	N/A	Initial material characteristics, and *in vivo* subcutaneous implant to evaluate barrier effect	3 months *in vitro*, 4 weeks *in vivo*	Favorable stability and degradation rate; no direct observation for antimicrobial effect; cell occlusive effect in rat model
Tetracycline	PLGA/gum tragacanth nanofibers	N/A	Initial material characteristics, release profile, and antimicrobial properties	75 days	Nanofibers had a smooth and bead-less structure; tetracycline constantly released for 75 days after burst release during the first 2 h; Bacterial inhibition against G(+)
Metronidazole	Infection-responsive electrospun nanofiber mat	N/A	Initial material characteristics, release profile, *in vitro* cytocompatibility, and antimicrobial activity	72 h	Good cytocompatibility; the nanofiber mat released metronidazole and showed antibacterial effect.
	Dual drug loaded coaxial electrospun PLGA/PVP fiber	Naringin	Initial material characteristics, release profile, *in vitro* cytocompatibility, and antimicrobial activity	21 days	Fabricated fiber had adequate properties; metronidazole and naringin loaded fiber inhibited anaerobic bacteria.
Silver nanoparticles	Electrospinning nanofibrous with HA & silver nanoparticles	N/A	Initial material characteristics, release profile, and antimicrobial activity	32 days	Improved bone regeneration activity; silver nanoparticles enhanced antibacterial effect.
Doxycycline & simvastatin	Core-Shell poly-(D,L-Lactide-co-Glycolide)-chitosan Nanospheres	IL-1β, MMP-8, VEGF	Initial material characteristics, release profile, antibacterial examination, gene expression analysis, and *in vivo* periodontal healing evaluation	28 days	Scaffold constantly released simvastatin and doxycycline and significantly inhibited *P. gingivalis* and *S. sanguinis*; down-regulated IL-1β & MMP-8, up-regulated VEGF, and decreased bone loss in rat model.
nMgO	Biodegradable multifunctional nanofibrous membrane	N/A	Initial material characteristics, antibacterial effects, osteogenesis evaluation, and *in vivo* periodontal healing evaluation	14 days *in vitro*; 6 weeks *in vivo*	nMgO incorporated membranes enhanced osteogenic property & the antibacterial effect against *E. coli* and *S. aureus*; enhanced periodontal regeneration in rat model.
Mg doped HA nanoparticles	3D nano bilayered spatially and functionally graded scaffold	Bromelain	Initial material characteristics, release profile, antibacterial effects, and *in vivo* periodontal healing evaluation	12 days	Increased mechanochemical properties; improved antibacterial potential & sustained release; enhanced periodontal regeneration in rat model

ANTI-INFLAMMATORY EFFECTS OF SCAFFOLDS FOR PERIODONTAL REGENERATION :

Summary of scaffolds for periodontal regeneration incorporated with anti-inflammatory component.

Type of scaffold	Anti-inflammatory component	Research design	Experimental duration	Induction of inflammation	Outcomes
CS/PVA/HA electrospun fibers and films	Meloxicam (NSAIDs)	*In vitro* material characterization; cytocompatibility test (VERO cell culture)	72 h	N/A	Meloxicam, a selective COX-2 inhibitor, showed a sustained drug release over extended periods of time from CS/HA/PVA composite fibrous membranes and films; no direct observation in ant-inflammatory effect
Electrospun polycaprolactone (PCL) scaffold	Ibuprofen (IBU)	*In vitro* material characterization; cell vitality & wound closure assay, release profile, and *in vivo* periodontal regeneration evaluation	22 days	LPS	The anti-inflammatory effects of IBU on gingival cells were actively intensified; IBU-PCL scaffold significantly enhanced the clinical attachment and reduced bone destruction in mouse model.
3D BMP-2-Delivering Tannylated polycaprolactone (PCL) Scaffold	Tannic acid (TA)	*In vitro* material characterization; release profile; antioxidant assay; ROS measurement; anti-inflammatory effect test; ALPase activity assay	28 days	LPS	The BMP-2/TA/PCL scaffold significantly inhibited the mRNA levels of MMP-3, COX-2, IL-6, and TNF-α in LPS; increased osteogenic effect
Polycaprolactone - (Polyvinyl Alcohol/ Collagen) Hybrid Nanofiber	Ibuprofen (IBU)	*In vitro* material characterization; release profile	N/A	N/A	Both PCL and PVA/COL loaded membranes consistently released IBU; no direct observation in ant-inflammatory effect
Collagen membrane	Progranulin (PGRN)	*In vitro* coimmunoprecipitation assay; *in vivo* periodontal regeneration evaluation by microCT analysis, histomorphometric analysis & immunohistochemical staining	6 weeks	TNF-α	Collagen membrane containing PGRN had the effects of anti-inflammation, osteoclastogenic inhibition, and osteogenic promotion; PGRN enhanced periodontal regeneration in rat model.
Chitosan (CS)/b-sodium glycerophosphate/ gelatin hydrogels	Aspirin/ erythropoietin (EPO)	*In vitro* material characterization; release profile; toxicity assay & degradation evaluation; *in vivo* evaluation of anti-inflammatory effect & periodontal regeneration	2 weeks	Ligature wire (& LPS) with methods in Bhattarai 2016	No toxicity; hydrogel scaffold constantly released aspirin & EPO; CS/b-sodium glycerophosphate/ gelatin hydrogel aborted the inflammation and accomplished AB regeneration in rat model.

SCAFFOLD BIOMATERIALS FOR PERIODONTAL REGENERATION :

Natural materials :

Natural biomaterials generally have excellent cell affinity and biocompatibility. They are less toxic and rarely cause inflammatory responses or immune reactions. Therefore, natural biomaterials have been widely used as scaffolds for the regeneration of periodontal tissues . Collagen and chitosan are two most commonly investigated natural biomaterials for regeneration of periodontal tissues.

Bioceramics :

Bioceramics based materials such as hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and bioactive glass (BG) have been widely used to support healing of AB in periodontium. Bioceramic scaffolds typically provide high mechanical stability and biodegrability suitable for periodontal regeneration . The key advantages of bioceramic based scaffolds over other natural and synthetic materials are their outstanding osteoconductive and osteoinductive properties . Moreover, bioceramics can be delivered into periodontal defect in various forms such as granule, paste, and injectable format . On the other hand, the slow degradation rate of ceramics can be disadvantageous for periodontal regeneration as remaining ceramic particles can result in mechanical irritation or inflammation.[70]

Synthetic polymers:

Synthetic polymers have been predominantly used as materials for the second-generation degradable membrane to replace the nonresorbable membrane-PTFE. Such polymers have also been applied for scaffold materials. Polyester-based polymers such as polylactic acid (PLA), polyglycolic acid (PGA), polylactic-co-glycolic acid (PLGA) and polycaprolactone (PCL) have been frequently utilized for periodontal scaffold materials . Polyester's degradation byproduct may be toxic but it has been considered safe given the insignificant amount of residual particles that are released at a very slow rate . Synthetic polymers have a number of unique advantages including highly adjustable physio-chemical properties, controllable biodegradation rate, and simple and straightforward fabrication process allowing mass production.

Hydrogel :

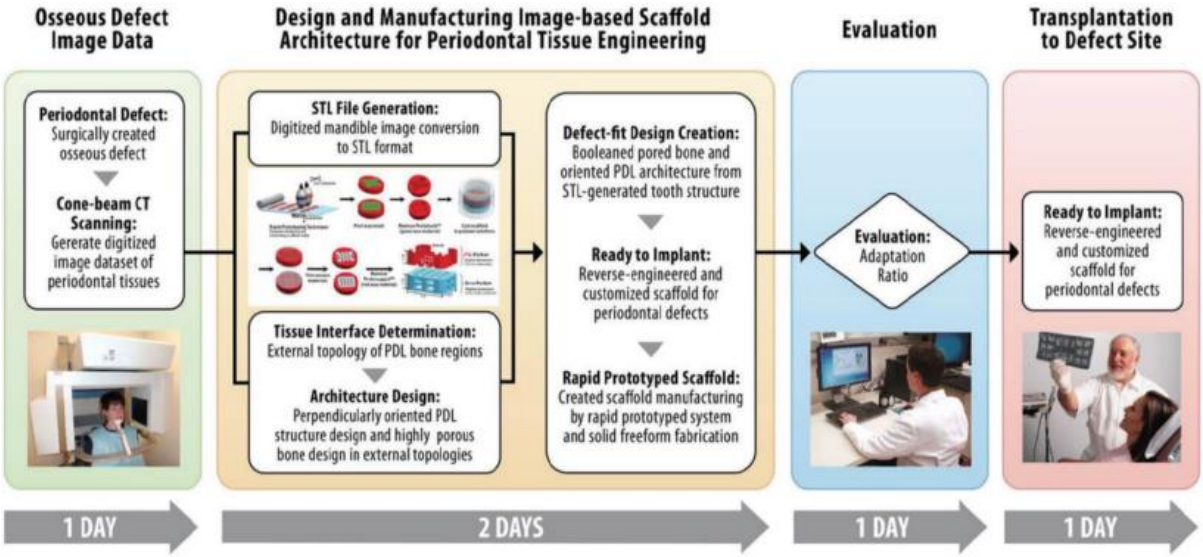
Hydrogel is a network of crosslinked macromolecular polymers with absorption characteristics and hydrophilic properties . Various types of biomaterials can be

formed as hydrogel . The advantages of a hydrogel form include high-water content, biocompatibility and the flexibility in structural design and formation. Various types of hydrogels have been applied for periodontal tissue regeneration. When collagen membrane with biphasic calcium phosphate (BCP) was compared with hydroxypropylmethyl cellulose (HPMC) hydrogel membrane with BCP HPMC with BCP showed superior outcome including inhibition of soft tissue invasion into periodontal defect as well as significant bone regeneration after 12 weeks in a canine model . Choi et al. demonstrated that proanthocyanidins (PAC)-treated collagen gel showed higher surface roughness and enhanced attachment of PDL cells . Collagen hydrogel scaffold loaded with FGF-2 showed CM-like tissue and PDL-like Sharpey's fibers formation without ankyloses and root resorption when treated a class II furcation defects in a canine model . HydroMatrix, an injectable peptide nanofiber hydrogel, also showed enhanced adherence, migration and proliferation of PDLSCs in vitro . Polyethylene glycol (PEG) hydrogel mixed with calcium phosphate (CaPs) and recombinant human cementum protein 1 (rhCEMP1) also improved periodontal regeneration in a rat model.

3D-printed scaffolds:

As an emerging technology, 3D printing allows us to better control macro- and micro-structure of tissue engineering scaffolds . Periodontium is a complex structure consisted of multiple tissue types as soft and hard tissues are integrated. To recapitulate such multi-phase tissue compositions, 3D printing technique has recently been adopted to fabricate scaffolds with regionally variant internal microstructures suitable for CM, PDL and/or AB . In addition, different growth factors can also be combined with 3D printed scaffolds to help the regeneration of each tissue in periodontium. Beside the internal microstructure, 3D printing with layer-

by-layer deposition enables to create custom-designed scaffold in a specific shape and dimension fitting to the anatomic shape of each periodontal defect.[71]



APPROACHES FOR INTEGRATED PERIODONTAL REGENERATION :

Multi-phasic scaffolds can be categorized into two groups: bi- and triphases . Each layer/phase is designed to guide a specific target tissue regeneration. In the integrated periodontium regeneration, of which the target tissues are PDL, AB, and CM, bi- or tri-phasic scaffolds are suitable for the purpose of true periodontium regeneration . Bi-phasic scaffolds have two different phases that can simultaneously target two different tissues: PDL-AB, AB-CM, or PDL-CM. Whereas tri-phasic scaffolds have three phases that simultaneously target three different tissues: PDL-AM-CM

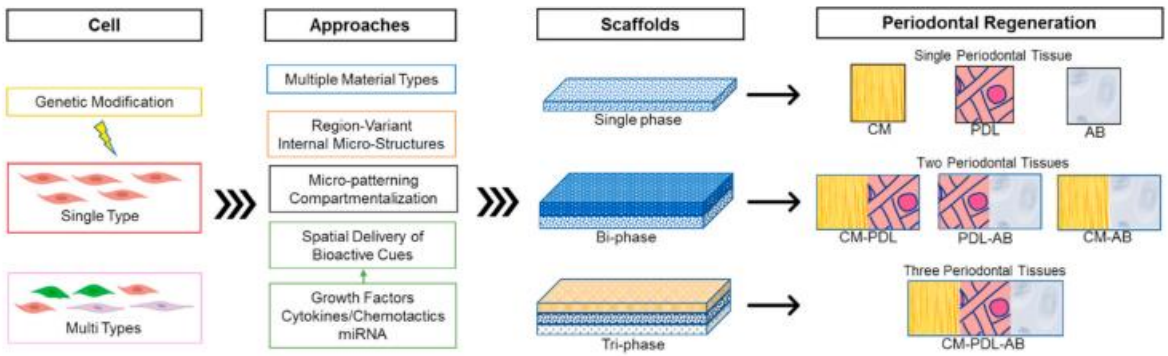


Fig. 3. Approaches for scaffold-based periodontal tissue regeneration in recent published studies from 2015 to 2020 (CM: cementum, PDL: periodontal ligament, AB: alveolar bone).

Scaffolds seeded with multiple types of cells:

Single or multiple cells seeded directly into the multi-phasic scaffold is a viable option to increase success of integrated periodontal regeneration. Cells that are most widely applied in integrated periodontal tissue regeneration are periodontal ligament stem cells (PDLSC) and bone marrow mesenchymal stem cells (BMMSC) as they are directly involved with target tissues Recent studies showed adipose-derived mesenchymal stem cells (AMSC) and gingival mesenchymal stem cells (GMSCs) also have the potential for periodontal tissue regeneration Since periodontium or periodontal tissue regeneration depends on four key factors (i) cells, (ii) scaffold, (iii) blood supply, and (iv) signaling molecules, advancement of tissue engineering strategies is an important

step forward to the successful periodontal tissue regeneration. [72]

Clinical applications of scaffolds for periodontal tissue regeneration.

Type of scaffold	Bioactive agents	Research design	Follow-up duration	Number of patients	Outcomes
3D printed PCL scaffold	rhPDGF-BB	Clinical case report, scaffold implantation after scaling and root planning	14 months	1	The implanted 3D scaffold served to fill the human periodontal osseous defect without signs of chronic inflammation or dehiscence. However, the implanted scaffold became exposed at 13 months, followed by a graft exposure 3 mm below the gingival margin. After removal of the exposed part of the graft, the site showed a larger dehiscence and wound failure, necessitating entire scaffold removal.
3D woven-fabric PLLA scaffold	MSC and PRP	Monocenter clinical trial; Implantation of scaffolds with MSC and PRP after phase I/II therapy	36 months	10	Clinical attachment level, pocket depth, and linear bone growth (LBG) were improved during the entire follow-up period. No clinical safety problems attributable to the investigational MSCs were identified.
β-TCP	rhFGF-2	Multicenter randomized controlled clinical trial; double-blinded; randomized to 1 of 4 treatment groups—β-TCP alone (control) and 0.1% recombinant human FGF-2 (rh-FGF-2), 0.3% rh-FGF-2, and 0.4% rh-FGF-2 with β-TCP—following scaling and root planing with EDTA	6 months	88	0.3% and 0.4% rh-FGF2/β-TCP groups showed significant improvements and 71% success rate at 6 months. Percentage bone fills of control, 0.1%, 0.3% and 0.4% group were 61%, 63%, 73% and 71%, respectively. No serious adverse events related to the products were reported.
Zn-substituted monetite-based scaffold	None	Randomized controlled clinical trial (split-mouth, double-blind); test group - open flap debridement (OFD) with Sil-Oss®, and control group - OFD with hydroxyapatite (HA) bone graft.	9 months	30	Zn-substituted monetite-based scaffold group (Sil-Oss®) exhibited a significant bone fill and the percentage of tissue mineralization compared to HA at 3 and 6 months. However, there were no significant differences in clinical attachment level and probing depth at 6 months
β-TCP, Autologous PDL-derived cell sheets	None	A single-arm and single-institute clinical study; bony defects were filled with β-TCP granules & 3-layered PDL-derived cell sheets following standard flap surgeries	6 months	10	Mean reduction of periodontal probing depth was 3.2 ± 1.9 mm. Mean clinical attachment gain was 2.5 ± 2.6 mm, and average increase of radiographic bone height was 2.3 ± 1.8 mm. Clinical improvements were maintained during a mean follow-up period. No serious adverse events were observed.
PCL scaffold, human umbilical cord mesenchymal stem cells	None	Randomized control clinical study; A patient of multiple gingival recession (Miller's Class II) was selected	6 months	N/A	Root recession was significantly reduced (over 80% root coverage).
β-TCP	None	Randomized clinical and biochemical trial; group I: β-calcium triphosphate (β-TCP) with collagen membrane, group II: cultured gingival fibroblasts (GF) on the β-TCP scaffold with collagen membrane.	6 months	20	Group II showed significantly higher reduction in vertical pocket depth (VPD), clinical attachment level (CAL) gain and radiographic bone gain than group I. The concentration of PDGF-BB in group II was significantly higher on 1, 3, 7 days than group I.
Demineralized porcine bone matrix (DPBM)	Enamel matrix derivatives (EMD)	Randomized clinical trial; group1: DPBM with EMD, group 2: DPBM only	24 months	42	Although both groups showed clinically and radiographically significant improvement, there were no statistically significant differences between 2 groups.

GUIDED TISSUE REGENERATION (GTR)

Introduction :

Gottlow coined the phrase "guided tissue regeneration (GTR)" in 1986. GTR is described as "procedures attempting to regenerate lost periodontal structures through differential tissue responses" by the 1996 World Workshop in Periodontics. In the belief that they hinder regeneration, barriers are used in an effort to keep gingival corium and epithelium away from the root surface. GTR membranes are used with the intention of excluding gingival connective tissue and epithelium and maintaining space (DHR-IJMS) . Stabilize the clot between the flaw and the tooth. When given the chance to populate the periodontium, specific cell populations have the capacity to produce new cementum, alveolar bone, and periodontal ligament, according to Melcher hypothesis .

Karring et al empirically established and histologically confirmed the Melcher theory. They have demonstrated that these situations develop when periodontal ligament cells are permitted to migrate and colonize the wound site but gingival epithelial cells or fibroblasts are kept out. The creation of periodontal devices known as barriers or membranes for directed tissue regeneration was prompted by the requirement to keep gingival epithelium and connective tissue cells from wounds. In 1982, Nyman et al. employed cellulose acetate laboratory filter paper as the first GTR membrane in periodontal surgery. This barrier lacks a number of qualities required for directed tissue regeneration.[73]

Guided tissue regeneration (GTR) techniques have been effectively used to treat periodontal problems and have supported the possibility of bone regeneration. GTR is a distinctive healing approach for periodontal infections (Caballé-Serrano et al., 2019). This technique uses the membranes as mechanical barriers to produce a gap around the flaws, allowing the formation of a new bone without the struggle for space by the nearby connective tissues. Membranes for GTR treatment must be biocompatible, have the appropriate degradation summary, have good physical and mechanical properties, and have enough continuous power (Shi et al., 2014; He et al., 2017; Khorshidi et al., 2018). GTR membranes should be permeable for cellular adaptation and adequate nutrient approval. On the other hand, bio-resorbable membranes involving collagen-based membranes are not essential to be removed since they reduce by period and do not need surgical removal (Caballé-Serrano et al., 2019; Ahmadi et al., 2020; Sadaf Ul et al., 2021).

Characteristics of GTR membranes :

Biocompatibility, cell exclusion, space maintenance, tissue integration, and ease of usage have been recommended by Scantlebury as characteristics or design requirements for GTR membranes. Future regeneration technologies should take biological activity into account as an additional attribute. Biomaterial is a nonviable material that is utilized in medical devices and is meant to interact

with biological processes, according to Black . The two main criteria for any technology that is put within the body to answer a need are safety and efficacy. Numerous in vitro and in vivo experiments that are intended to evaluate particular aspects of biocompatibility are used to address safety.[76]

According to Williams , biocompatibility is the capacity of a material to function with an appropriate host response in a given situation. This implies that neither the material nor the physiological tissue environment will negatively and significantly affect the material. Cell culture cytotoxicity, skin irritancy, subcutaneous implantation, blood compatibility, hemolysis, carcinogenesis, mutagenicity, pyrogenicity, sensitization, and short- and long-term histological tissue reaction are ten assays used to assess biocompatibility. The membrane must keep the growing fibrin clot in the wound space and the gingival flap apart in order to achieve cell exclusion. There have been no studies that directly examine this feature of the GTR membrane. Mechanical and/or structural elements that enable the membrane to tolerate the force of the flap (tissue tension) or occlusion are necessary for space maintenance during regeneration. Tissue integration dictates the incorporation of structural elements in the membrane to promote tissue ingrowth which concurrently achieve cell exclusion. Easy to use means membrane should be clinically manageable i.e. competent clinician can use membrane without undue difficulty.

GTR ADVANTAGE AND DISADVANTAGE :

Multiple advantages come across with using GTR systems for the treatment of periodontitis. GTR prevents connective tissue from entering the bone reformation site and interference in osteogenesis. It also creates a space under the surgical flap that acts as a scaffold for the growth of cells and blood vessels. GTR is capable of separating regenerative space from undesirable tissues, ensuring the mechanical stability of the healing complex, and preventing bacterial invasion which leads to the prevention of inflammatory response by the host immune system. GTR membrane must have a number of properties in order to function well as a barrier membrane. These properties include

biocompatibility, safety, non-allergic, non-toxic, mechanical stability, space maintaining between teeth, clinically manageable, cell occlusive, and tissue integrating Drug-loaded GTR systems have additional benefits which include; delivering the required therapeutic dose of the drug directly to the target site, prolongation of drug delivery, minimizing side effects compared to the systemic dosage forms, preventing the requirement for frequent administration of the drug, and inhibiting the occurrence of bacterial resistance by maintaining a continuous and high antibiotic concentration at the site of action .[79]

THE MEMBRANES ARE DISTRIBUTED INTO TWO CLASSES:

1) non-resorbable and 2) bio-resorbable membranes. Non-resorbable membranes like expanded polytetrafluoroethylene (*e*-PTFE) should be detached after implantation through the surgical process.

Absorbable barriers: Absorbable barriers are biodegradable, hence do not require their removal which reduce patient discomfort and eliminate surgery related complications. Absorbable membrane's disintegration process starts immediately after placement in the surgical site and their rate of disintegration vary from individual to individual, hence there is no control over length of application. Due to their biodegradable nature absorbable barriers elicit tissue reactions which influence wound healing and regeneration. Absorbable barriers can be natural or synthetic:

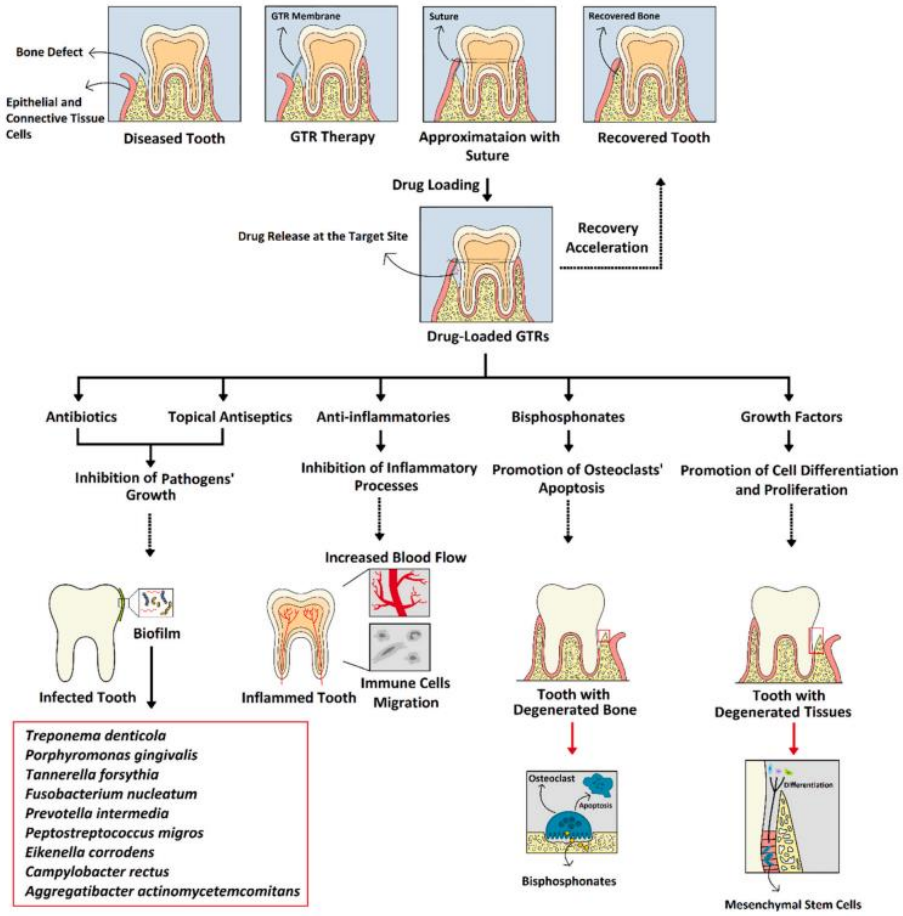
Natural absorbable barriers: COLLAGEN shows hemostatic activity, attracts and activates neutrophils, fibroblasts, interacts with various cells during tissue remodelling and wound healing, and low immunogenicity which make collagen an attractive biomaterials. Collagen is obtained from animal sources as bovine skin, tendon, intestine, ox cecum or rat tail. Isolation and purification are done by two ways, either enzymatic preparation of soluble collagen or chemical extraction of fibrillar collagen. After isolation and purification, collagen is processed by several means to make gels, sponges, filaments, membranes etc. for specific uses. Porphyromonas gingivalis produces collagenase, if membrane exposed during healing, uncontrolled degradation take place, resulting in unfavourable outcome. A histological evaluation of Avitene, a microfibrillar collagen hemostatic barrier produced from bovine corium, in humans revealed no more efficacy than the control group. Avitene was challenging to use both before and after being moistened with blood or saliva. Another collagen hemostatic is called Collistat. [79]After implantation, these two materials were mostly resorbed in 3 days and totally resorbed in 7 days .Bilayer porcine collage n membrane serves as the bio-guide.

Biomend is a semiocclusive (pore size 0.004 m) membrane that resorbs in 4 to 8 weeks from a bovin. There was a persistent inflammatory infiltration. Oxycel is a oxidised cellulose mesh hemostatic dressing material has been used as a GTR membrane which resorbed in 4 weeks of implantation. Histological study showed that it is well tolerated in soft tissue but delayed healing in bone tissue due to acidic nature. Paroguide is a collagen membrane enriched with chondroitin sulfate, showed no signs of inflammation and regeneration of PDL, cementum and alveolar bone, verified by histologically. DURA MATER, consists of irregular network of collagen fibers, obtained from cadavers can be used as GTR membrane. It was resorbed in 6 weeks, bone formation observed along material but risk to acquire Creutz-feldt-Jakob disease not only for recipient but also for operator may present . CONNECTIVE TISSUE GRAFT may consider as collagen based barrier has been used as natural barrier for GTR in mandibular class II furcation . AUTOGENOUS PERIOSTEUM can be used as a periosteal graft barrier for treatment of class II furcation involvements in lower molars . Type I collagen membrane derived from calf PERICARDIUM and cross-linked by diphenylphosphorylazide has been evaluated for GTR which showed significant inflammatory reaction, resorbed in 2 weeks and has week regenerative potential

Synthetic absorbable barriers: Synthetic absorbable barriers are manufactured from organic aliphatic thermoplastic polymers, most commonly used material are poly α-hydroxy acids which include POLYGLYCOLIC ACID (-O- CH2-(O)C-)n and POLYLACTIC ACID (-O-CH(CH3)-(O)C-)n and their copolymer, POLY GLYCOLIDE-LACTIDE. Poly α-hydroxy acid is degraded by hydrolysis into products that are metabolized to CO2 & H2O through citric/Kreb's cycle.[80]

DRUG-LOADED GTRS :

drug-loaded GTR systems showed that the drugs which were loaded in these systems included antibiotics, steroidal and non-steroidal anti-inflammatory drugs, bisphosphonates, calcium phosphate derivatives, growth factors, morphogenetic factors, and topical antiseptics.



Schematic summary of the mechanism of action in plain GTR systems and the classification of drug-loaded systems.

GTRS LOADED WITH STEROIDAL AND NON-STEROIDAL ANTI-INFLAMMATORIES:

Inflammation is one of the main symptoms of periodontitis which is commonly managed by systemic administration of anti-inflammatories especially non-steroidal agents. In fact, chronic periodontitis is considered an inflammatory disease . It was reported in previous studies that Non-steroidal anti-Inflammatory drugs (NSAID) had the potential to slow down the rate of bone loss associated with periodontitis due to inhibiting the inflammatory response . Oral administration of these drugs is related to multiple side effects which can be reduced using a topical preparation .[84]

A summary of the studies using GTR as the carrier of steroidal and non-steroidal anti-inflammatories.

Author and Year of Study	Main Matrix Component	Method of Fabrication	API	Solvent System	Type of Study	Release Pattern	Other Significant Results
Kharaziha et al. [64] 2015	PCL/forsterite nano-powder	Electrospinning	Dexamethasone	Chloroform/ Ethanol (9:1 v/ v)	*In vitro*	-No forsterite: Releasing 22% of drug during 12 h, followed by sustained release of 45% of drug during 21 days. –5% forsterite: Releasing 23% of drug during 12 h, followed by sustained release of 55% of drug during 21 days. –10% forsterite: Releasing 25% of drug during 12 h, followed by sustained release of 68% of drug during 21 days.	Increasing the forsterite concentration significantly improved the drug release while almost eliminating the burst-release phase.
Limoee et al. [65] 2019	PCL PVA/collagen	Electrospinning Glutaraldehyde crosslinking	Ibuprofen	Chloroform, Distilled water	*In vitro*	-PCL: released more than 80% of drug content during 48 h. - PVA/collagen: a burst release of 20% id drug in 5 h followed by sustained release of up to 80% during 48 h.	-Nanofibers remained intact without any tearing or fracture after 60 days.

DCM: Dichloromethane, **DMF:** Dimethylformamide, **PCL:** Polycaprolactone, **PVA:** Polyvinyl alcohol.

GTRS LOADED WITH TOPICAL ANTISEPTICS:

Topical antiseptics can have beneficial effects of symptom relief for periodontitis by inhibiting the growth of pathogens. These agents also have the advantage of lower side effects compared to the antibiotic, hence can be used in conjunction to other treatments such as SRP . Chlorhexidine is one of the most popular topical antiseptics indicated in a wide variety of dental conditions. It is commonly used for the prevention of plaque accumulation, gingivitis, periodontitis and other oral cavity diseases.

A summary of the studies using GTR as the carrier of topical antiseptics.

Author and Year of Study	Main Matrix Component	Method of Fabrication	API	Solvent System	Type of Study	Release Pattern	Other Significant Results
Tabary et al. [68] 2007	-PVDF/ Maltodextrin -PVDF/ β-cyclodextrin	Membrane impregnation	Chlorhexidine digluconate	–	*In vitro*	- PVDF/Maltodextrin: Prolonged release of 130 mg in 100 days - PVDF/β-cyclodextrin: Prolonged release of 40 mg in 100 days	- Inhibited growth of *Fusobacterium nucleatum* for 3 days.
Thomas et al. [69] 2012	-PLA	-Double emulsion solvent evaporation for particles -Film casting for membrane	Chlorhexidine digluconate	Ethanol	*In vitro*	Not reported	- Inhibited growth of *Methicillin-resistant Staphylococcus aureus*.
Zhou et al. [71] 2017	-Collagen -Bioactive glass -CS	-Electrospinning -Glutaraldehyde crosslinking	CS	-HFIP for collagen -Ethanol for bioactive glass -TFA for CS	*In vitro* *In vivo*	Not reported	-Enhanced expression of genes related to osteogenesis. - Inhibited growth of *Streptococcus mutans*. - Enhanced osteogenesis compared to control: 20% higher after 30 days. 25% higher after 60 days. -Decreased inflammation of the gingiva.

CA: Citric acid, **CS:** Chitosan, **HFIP:** Hexafluoro-2-propanol, **PLA:** Polylactic acid, **PVDF:** Polyvinylidene fluoride, **TFA:** Trifluoroacetic acid.

GTRS LOADED WITH BISPHOSPHONATES:

Bisphosphonates are bone-strengthening agents with advantageous effects in conditions with bone degeneration like periodontitis. Previous studies suggested an enhanced bone density in animal models after administration of bisphosphonates adjacent to other methods for the management of periodontitis-related bone loss . [89] The bisphosphonates main mechanism of action is inhibiting osteoclasts which are one of the main mediators in periodontitis-related bone loss . Topical administration of these drugs is more recommended due to the reduced systemic side effects.

A summary of the studies using GTR as the carrier of bisphosphonates.

Author and Year of Study	Main Matrix Component	Method of Fabrication	API	Solvent System	Type of Study	Release Pattern	Other Significant Results
Chakraborti et al. [60] 2011	PLGA/ MePEG/LDH clay	Film casting	-Tetracycline Hydrochloride -Alendronate	-DCM for PLGA/MePEG -DMSO for Tetracycline -Polysorbate 20 and deionized water for LDH clay and Alendronate	*In vitro*	-Film loaded with free Alendronate: releasing 35% of drug in burst phase followed by release 80% of drug until 10 days. -Film loaded with Alendronate ± free LDH clay: releasing 6% of drug in burst phase followed by release 80% of drug until 3 days. -Film loaded with Tetracycline ± LDH clay complexed with Alendronate: releasing 15% of alendronate in 10 days without any burst phase.	–

DMSO: Dimethyl sulfoxide, **LDH:** Layered double hydroxides, **MePEG:** Methoxy polyethylene glycol, **PLGA:** Poly (lactic-co-glycolic acid).

GTRS LOADED WITH CALCIUM PHOSPHATE DERIVATIVES, MORPHOGENIC FACTORS, AND GROWTH FACTORS :

The use of growth factors in the management of periodontitis has been a field of interest for researchers through the last decade. Growth factors demonstrated beneficial effects on accelerating tissue regeneration including PDL, alveolar bone, and root cementum . Generally, these factors affect the proliferation and differentiation of different tissues . Platelet-derived growth factor (PDGF) is a subtype of growth factors aiding periodontal tissue regeneration . Bone morphogenetic factors are another family that directly initiate the osteogenesis utilized in the management of periodontitis.

A summary of the studies using GTR as the carrier of calcium phosphate derivatives, morphogenic factors, and growth factors.

Author and Year of Study	Main Matrix Component	Method of Fabrication	API	Solvent System	Type of Study	Release Pattern	Other Significant Results
Zhanga et al. [79] 2020	PLGA/DEX/ Wool keratin	-Emulsion electrospinning	-bFGF	-PBS for DEX/ bFGF - TCM: DMF (7:3 v/v) for PLGA/Wool keratin	*In vitro*	-Releasing 25% of the drug content on the first day, 60% in 7 days and 90% in 28 days	−10 μg of bFGF led to higher cell growth
Chen et al. [80] 2019	PLA95	Electrospinning	β-TCP	DCM/DMF (7:3 v/v)	-*In vivo* -Clinical trial	–	-Hydrophilic nature. -Non-cytotoxicity. - *In vivo* study showed Higher bone and cementum height compared to control and Epi-Guide membrane. -Clinical trial showed a significant decrease in probing depth and attachment gain.
Gümüşderelioğlu et al. [81] 2019	-CS -Silica particles -HA -PCL	-Film casting for CS porous membrane -Microwave-assisted biomimetic method for HA coating -Electrospinning for PCL coating	-BMP-6 -HA	-AcOH (1% v/v) aqueous solution for CS/Silica -HFIP for PCL coating	*In vitro*	–	-Enhanced expression of OCN, COL I, RunX2, and OPN genes in MC3T3-E1cells. - Although PCL coating increased gene expression, no significant difference between the gene expression by PCL-coated and non-coated CS/HA membrane.
Park et al. [82] 1997	PLLA	-In-air drying phase inversion (Coating on PGA mesh)	PDGF-BB	-MeCl/EAc (2:1 v/v) for PLLA -BSA/Span 80 aqueous solution for PDGF-BB	*In vitro* *In vivo*	-By increasing the BSA from 0 to 10%, the PDGF-BB release, enhanced by 30 ng/cm^3 in 28 days. - By increasing the PDGF-BB content from 100 to 400 ng, the PDGF-BB release, enhanced by 45 ng/cm^3.	-Blank formulation: 10% weight loss after 4 weeks. -Loaded formulation: 40% weight loss after 4 weeks.
Ravi et al. [83] 2017	Collagen	Not reported	PRGF	–	Clinical trial	–	-Generally, no significant difference was observed between the efficacy of GTR alone or GTR + PRGF.

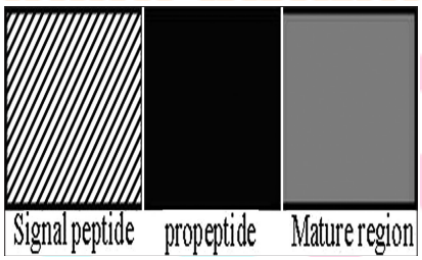
AcOH: Acetic acid, **β-TCP:** β-tricalcium phosphate, **bFGF:** basic fibroblast growth factor, **BMP-6:** Bone morphogenetic factor 6, **BSA:** Bovine serum albumin, **DEX:** Dextran, **DMF:** Dimethylformamide, **EAc:** Ethyl acetate, **HA:** hydroxyapatite, **HFIP:** Hexafluoroisopropanol, **MeCl:** Methylene Chloride, **PBS:** Phosphate-buffered saline, **PCL:** Polycaprolactone, **PDGF-BB:** Platelet-derived growth factor-BB, **PGA:** polyglycolic acid, **PLA95:** Poly-5D/95L-lactide, **PLGA:** Poly (lactic-co-glycolic acid), **PRDF:** Plasma rich in growth factors, **TCM:** Trichloromethane.

BONE REGENERATION WITH BONE MORPHOGENETIC PROTEIN :

Introduction :

Several decades ago, Dr. Marshal Urist, an orthopedic surgeon, discovered a group of proteins sequestered in bone and aptly named them bone morphogenetic proteins (BMPs). He observed that bone matrix preparations contained BMPs that induced cartilage, bone, and marrow formation when implanted intramuscularly in rodent models. Following purification and subsequent molecular cloning, the responsible proteins were identified. Most BMPs comprise three portions: signal peptide, propeptide, and mature region. The propeptide and mature region contains seven conserved cysteine residues characteristic of the transforming growth factor-β (TGF-β) superfamily.[90]

BMPs belong to a group of proteins called TGF-β gene superfamily that share common structural features. Currently, there are 43 members of this gene family. BMPs are synthesized as large precursors consisting of a prodomain and carboxy-terminal region of 100–125 amino acids. Most of the BMPs as well as TGF-β share a conserved pattern of seven cysteine residues in the mature domain. Each mature active BMP consists of dimers whose chains are connected by disulfide bonds, and the dimerization is a prerequisite for bone induction. BMPs are active both as homodimers (two identical chains) and heterodimers (two different chains) molecules.



Structure of the bone morphogenetic protein

The BMP family can be divided into four distinct subfamilies:

1. BMP-2 and BMP-4
2. BMP-3 and BMP-3B, the latter also known as growth/differentiation factor 10 (GDF10)
3. BMPs 5, 6, 7, and 8
4. GDFs 5, 6, and 7, also known as cartilage-derived morphogenetic
5. proteins 1, 2, and 3.

The influence of BMP may begin early and continue throughout postfetal life in embryonic bone formation.[12] BMPs act as growth and differentiation factors and chemotactic agents. They stimulate angiogenesis and migration, proliferation and differentiation of mesenchymal stem cells into cartilage and bone forming cells. More than 20 BMP-related proteins have been identified, several of which induce bone formation.

Table 1: Classification of bone morphogenetic proteins

BMP-1	Not part of TGF-β family
BMP-2	Osteoinductive, osteoblast differentiation, apoptosis
BMP-3 (osteogenin)	Most abundant BMP in bone, inhibits osteogenesis
BMP-4	Osteoinductive, lung and eye development
BMP-5	Chondrogenesis
BMP-6	Osteoblast differentiation, chondrogenesis
BMP-7 (osteogenic protein-1)	Osteoinductive, development of kidney and eye
BMP-8 (osteogenic protein-1)	Osteoinductive
BMP-9	Nervous system, hepatic reticuloendothelial system
BMP-10	Cardiac development
BMP-11 (growth/differentiation factor-8)	Neuronal tissues
BMP-12 (growth/differentiation factor-7)	Tendon-iliac tissue formation
BMP-13 (growth/differentiation factor-6)	Tendon and ligament-like tissue formation
BMP-14 (growth/differentiation factor-5)	Enhances tendon healing and bone formation
BMP-15	Follicle-stimulating hormone activity

BMP: Bone morphogenetic protein, TGF: Transforming growth factor

BONE MORPHOGENETIC PROTEIN CARRIERS:

As BMP is soluble in extracellular solution, it must have a carrier, without which it is phagocytized within 10 days. One of the major obstacles to the clinical use of BMPs is the challenge to define the optimal delivery system. Although a matrix carrier is not essential to promote bone formation, there are a number of advantages to an appropriate carrier including localization and

retention of BMP to the site, providing a 3D extracellular matrix scaffold for mesenchymal cell infiltration, a shape that may help and define the resulting new bone, and providing a substrate for cell growth and differentiation. The carrier material can be in the form of blocks, granules, paste, and solution or as self-setting cement.[96]

CLASSIFICATION OF CARRIERS:

BMP – DELIVERY SYSTEMS :

Several matrices and delivery systems have been used and evaluated for their efficacy and biocompatibility as carrier for BMPs. Three major strategies for growth factor delivery: gene therapy, cell therapy, and protein therapy. Gene therapy and stem cell-based therapy represent the major advance, however, presently are still in their infancy regarding safety and efficacy in human.Protein therapy, on the other hand, has demonstrated the most practical promise, mainly incorporating osteoinductive morphogens (BMPs) even so with some limitations

Most cell-seeding scaffolds are fabricated from two classes of biomaterials, derived from either synthetic or natural products. In addition, they may be constructed from either resorbable or nonresorbable materials. Examples of cell delivery devices and scaffolds in periodontics are:

Nonresorbable: Expanded polytetrafluoroethylene, ceramic, and titanium mesh

Resorbable: Alpha-hydroxy acids, polyglycolic acid, polylactic acid, copolymers of poly (lactic, glycolic acid), amino acid-based polymers, collagen-like proteins, and elastin-like proteins

Natural products: Collagen, hyaluronan, chitosan, gelatin, fibrin, and alginate

Synthetic hydrogels: Polyethylene glycol, polyethylene oxide, matrix extracts, and Matrigel.[120]

PRODUCTION OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN-2:

Recombinant proteins are produced from one of several cellular expression systems of bacteria, insect cells, or mammalian cells.

Recombinant human (rhBMP-2) is produced using a mammalian cell expression system. To produce an rhBMP-2 expressing cell line, the BMP-2 coding sequence or c-c DNA is linked to a strong promoter and a selectable marker. This construct is transfected

into the host cell, and the cells that contain the coding sequence are chosen using the selectable marker. A series of cell lines are created and one is chosen that expresses high levels of protein. The cell line goes through a series of validation steps, including a check of the fidelity of the BMP-2 coding sequence. For pharmaceutical production of recombinant proteins, the rhBMP-2 cell line is expanded and frozen in multiple aliquots so that the identical starting cells can be used for decades to come. Medium is harvested, the cells are removed by filtration, and rhBMP-2 is purified from the medium by a series of column chromatography steps to >98% purity. Final liquid rhBMP-2 is sterilized by ultrafiltration before placement in vials. A third method of obtaining bone GDFs entails gene therapy and direct delivery of a genetic growth factor to the site of interest to encode for certain desired factors.

BMPS IN BONE INDUCTION:

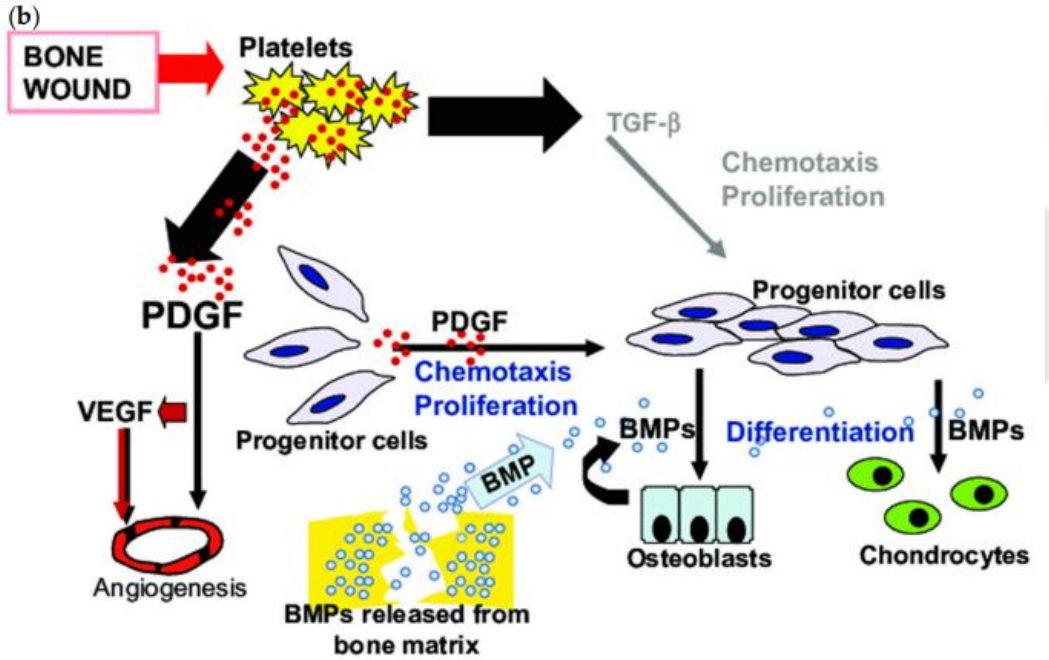
Bone formation can take place by an intramembraneous (direct) or endochondrol(indirect) process. Endochondral bone formation involves the formation of an intermediate cartilage that eventually becomes ossified that contains all the cellular components of mature bone. In both mechanisms, the induction of bone and cartilage occurs through an epithelial-mesenchymal interaction that initiates specific cell differentiation Depending on the concentration gradient BMPs can attract various types of cells and can act as chemotactic, mitogenic/or differentiating agent.BMPs can induce differentiation of mesenchymal progenitor cells into various cell types including chondroblasts and osteoblasts. This suggests that BMPs may be able to influence both direct and indirect bone formation.

BMPS – RECOMBINANT TECHNOLOGIES :

Identification of osteogenic proteins in bone matrix has been difficult to obtain due to small quantities of proteins tightly bound to organic and inorganic components of the extracellular matrix of bone; hence, recombinant technologies have been used to produce BMP for therapeutic evaluation. Because the structures of several human BMPs have been identified, it is possible to use DNA probes to obtain human complimentary DNA sequence. The human cDNA is cloned and spliced into a viral expression vector. Chinese hamster ovary cells and E. coli transfected to become carriers have been used to produce BMPs in large quantities for preclinical and clinical evaluation. Therefore rh-BMP (recombinant human – rh) produced provides optimum capability for clinical applications. In 2002, The US Food and Drug Administration (FDA) approved BMP-2 and BMP-7 for use in bone regeneration.

BMPS IN BONE TISSUE HEALING:

To develop a strategy for improving the amount of new bone formation, understanding wound healing processes at the cellular level is important because growth factors are dynamically orchestrated to recruit the appropriate cells into the defects and stimulate bone formation . In inflammatory phases, platelets secrete platelet-derived growth factors (PDGFs) to induce the chemotaxis and proliferation of cells necessary for the wound healing process. Then, pro-inflammatory cytokines—such as interleukin 1 (IL-1), IL-6, and tumor necrosis factor alpha (TNF-α) are secreted—and inflammatory cells migrate into the wound site. In proliferative phases, the angiogenesis process is essential for cellular and nutritional support. Vascular endothelial growth factor (VEGF) and PDGFs regulate angiogenesis, which is closely related to osteogenesis [6]. In addition to vessel formation, osteogenesis occurs in the defects, and BMPs, including BMP-2, play critical roles in the differentiation of osteogenic progenitor cells into osteoblasts and in the mineralization process.



growth factors related to bone wound healing. (PDGF, platelet-derived growth factor; VEGF, Vascular endothelial growth factor; BMPs, bone morphogenetic proteins; TGF-β, Transforming growth factor-beta)

PERIODONTAL REGENERATION AND BMPS:

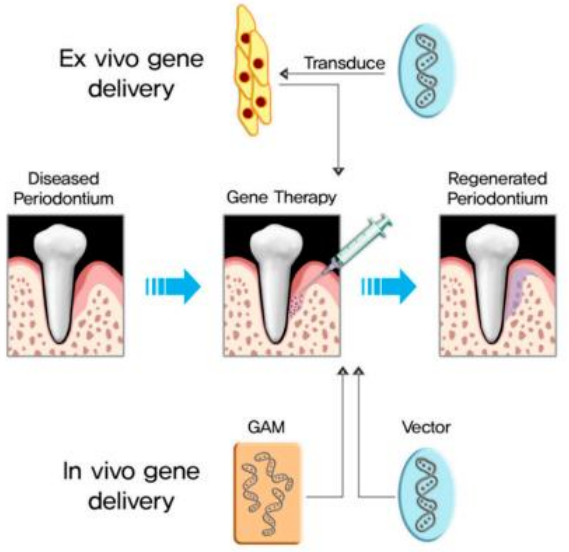
BMPs play an important role in the process of bone modeling and remodeling through chemotatic, mitogenic or differentiating mechanism.Earlier studies have shown that there is a homology of osteogenic proteins among mammals and that bovine BMPs, in conjunction with baboon collagenous matrix, induce bone differentiation in extra skeletal sites of the baboon.In previous experiments, the efficacy of bone-derived BMPs (BMP-2, osteogenin, osteoprotein-1) for regeneration in surgically created large

furcation defects in the mandibular first and second molar was investigated in adult male baboons (Papio ursinus).Histological analysis showed that BMPs, in conjunction with the collagenous matrix, induced cementum periodontal ligament, and alveolar bone regeneration. Another study reported that partially purified osteogenin, isolated from human bone matrix, when reconstituted with allogenic freeze dried deminerlized bone matrix, enhanced new connective tissue attachment, and alveolar bone regeneration in a root submerged environment in a series of human biopsies.A study where rhBMP-2 was used in a prepared periodontal defect in beagle dogs showed significant regeneration of the periodontal tissues.The effect of rhBMP-2 was evaluated in the surgically created critical size, supra alveolar periodontal defects in mandibular premolar teeth in beagle dogs which were implanted with rhBMP-2/ ACS at different concentrations. Extensive alveolar regeneration and limited cementum regeneration were observed. However, ankylosis was observed in all teeth receiving rhBMP-2/ACS without apparent correlation with rhBMP-2 concentration or dose. The ankylotic union was observed in the coronal aspect of supra alveolar defects. Other studies using rhBMP-2 or rhOP-1 in various carriers also provide evidence of ankylosis in large experimental periodontal defects in rodent, canine, and nonhuman primate models. Given the unique action of BMPs on mineralized tissue formation, obliteration of periodontal ligament space and ankylosis are a potential complication for the use of BMPs in the periodontium. The cause of ankylosis is, however, not clearly understood, but may be related to the perturbation of the homeostatic mechanism within the periodontium. In a study BMP-6 (0.13 and 10 μg) in a type 1 collagen sponge carrier was applied into periodontal fenestration defects in rats. Complete osseous healing occurred in BMP-6-treated animals following a 4-week healing interval.Osteogenetic protein-1 (BMP-7) has been evaluated for periodontal wound healing regeneration using surgically induced mandibular molar class II furcation defects in baboons. Defects implanted with rhOP-1 at 0, 100, and 500 μg/g bovine bone insoluble collagen matrix were subject to histometric analysis following an 8-week healing interval. Sites receiving rhOP-1 showed significant cementogenesis, including inserting sharpey fibers. A similar study using a 24-week healing interval showed that rhOP-1 at 0.5 and 2.5 mg/g collagen

matrix induced significantly greater periodontal ligament (PDL) and alveolar bone formation.These observations demonstrate beneficial effects of OP-1 as a candidate therapeutic agent for periodontal wound healing/regeneration. In a pilot study, the potential of growth and differentiation factor-7 (GDF-7)/BMP-12 to stimulate PDL formation was evaluated in a supra alveolar periodontal defect model. This study suggested that GDF-7 has a significant potential to support regeneration of PDL.The effect of BMP-14 (GDF-5) on periodontal wound healing/regeneration has been evaluated using an established canine defect model. In a randomized control trial 20 patients with intra-bony defects treated with rhGDF-5 showed a significant clinical attachment gain, favorable bone, and periodontal regeneration.In a study 30 intra-bony periodontal defects were created in 15 Wister rats to evaluate the regenerative potential of injectable macroporous calcium phosphate cement (CaP) in combination with bone morphogenetic protein-2 (BMP-2). Animals were euthanized after 12weeks and processed for histology and histomorphometry. CaP + BMP-2 showed a significant 2.4-fold increase in bone healing.Periodontal regeneration reaches the next level of predictability by developing gene therapy techniques. Understanding of the molecular pathways underlying the regeneration is increasing; however, translation of this knowledge into regenerative strategies remains in its early stages.

BMP-2 GENE DELIVERY GENE DELIVERY :

It is an alternative method for transferring growth factors into defect sites. The complementary DNA (cDNA) of human BMP-2 can be transferred via a vector into the site, resulting in the production of BMP-2 in vivo, which induces osteogenic differentiation and mineralization of the site. One of the advantages of BMP-2 gene delivery is the modulation of BMP-2 concentration and duration. Previous studies reported that BMP-2 concentrations in BMP-2 gene delivery applications (100–10,000 pg/mL) are much lower than those in rhBMP-2 applications (0.75–2.0 mg/mL) . Depending on the type of vectors carrying the BMP-2 gene, BMP-2 can be released for 2 or 3 weeks at low concentrations. The delivery pattern mimics the action of BMP-2 in the wound healing process, and adverse effects related to high doses of rhBMP-2—such as edema, extensive swelling, implant failure, and immature bone healing—can also be avoided. Gene delivery is divided into in vivo and ex vivo delivery . In vivo gene delivery directly transfers target genes into the host, either locally or systemically. Ex vivo gene delivery is cell-based gene delivery; cells harvested from the host are transduced with a vector carrying the target genes, and the transduced cells are administered into the defect.



Regeneration strategy for the reconstruction of periodontal tissue through gene therapy.

Delivery Type	Advantages	Disadvantages
ex vivo	Gene transfer is limited to the target cell population and not to other cells or tissues Can use gene transfer to genetically modify stem cells, e.g., embryonic stem cells and iPSCs [29,30] High efficacy Low quantity of vectors is necessary for desired therapeutic effects Minimal immune recognition of the gene vectors [33]	Expensive and time-consuming process Complicated manipulation including cell harvesting, cell expansion and transfection The outcome can be influenced by the carrier cells [31,32]
in vivo	Simple process via direct injection into the site or intravenous administration Avoids complicated process related to cells Relatively low cost	Low efficacy High quantity of vectors is necessary for desired therapeutic effects Induction of immune reaction due to direct exposure of vectors Difficult to target the cell population of interest Vector system is potentially toxic [34]

BONE REGENERATION VIA EX VIVO BMP-2 GENE DELIVERY :

Gene-delivered cells are carried by a hydrogel into the defect with or without a bone substitute. BMP-2-producing cells are effective for bone regeneration, but space maintenance for new bone formation is also critical in bone regeneration, especially in large defect healing, innovative approaches are necessary for bone regeneration of peri-implantitis defects. For this purpose, we applied PDLSCs transfected with AdBMP-2 (BMP-2/PDLSCs) for delivery to peri-implantitis defects, which were experimentally induced and where the level of alveolar bone loss was half that of the implant . After 3 months of healing, the BMP-2/PDLSCs produced significantly greater amounts of newly formed bone above the bottom of the defects and resulted in re-osseointegration over the implant . In addition, lace-like immature woven bone, which is frequently observed in

rhBMP-2 application cases , was not observed in the BMP-2/PDLSC groups. Furthermore, the maturity of the newly formed bone was similar to that of the old bone. This study was consistent with Yi et al.'s study, which compared the rhBMP-2 protein with PDLSCs vs BMP-2-producing PDLSCs and revealed the superiority of BMP-2-producing PDLSCs in bone regeneration in calvarial defects.

IN VIVO BMP-2 GENE DELIVERY:

For in vivo gene delivery, direct injection of genetic materials can be applied. However, Zhou et al. reported that directly targeted gene materials do not produce the desired effect due to a rapid clearance rate, rapid enzymatic degradation, nonspecific biodistribution, and low cellular uptake . Accordingly, viral or non-viral vectors are utilized to protect genetic materials and transfer them to cells or defect sites. However, in vivo gene delivery raises concerns related to the direct injection of excessive amounts of adenovirus needed to induce bone regeneration, leading to excessive immune reactions . Recently, plasmids carrying the BMP-2 gene have been increasingly used for gene delivery and have been applied to defects with bone substitutes or polymers for bone regeneration in oral and facial regions.

Enamel Matrix Derivative

INTODUCTION :

Enamel matrix derivative contains proteins belonging to the amelogenin family, which is the hydrophobic constituent of the enamel matrix proteins (Fisher and Termine, 1985). It stimulates cellular proliferation, protein synthesis and mineral nodule formation in several cell types including periodontal ligament cells, osteoblasts, and cementoblasts (Gestrelius *et al*, 1997). Enamel matrix derivative (EMD) is one of the most widely used biologic agents in periodontics. The application of enamel matrix derivatives (mainly composed of amelogenins) may promote periodontal regeneration because it mimics events that take place during the development of the periodontal tissues [69]. During tooth development, cells of the Hertwig's epithelial root sheath deposit enamel matrix proteins on the root surface prior to cementum formation, and these proteins are the initiating factor for cementogenesis. EMD, an extract of porcine immature enamel matrix, is regarded as a candidate protein mixture that is thought to be the induction of proliferation, migration, adhesion, mineralization and differentiation of cells in periodontal tissue. The process of cementum deposition is a prerequisite for the formation of both the periodontal ligament and the alveolar bone. However, recombination between slices of root dentin and follicular cells has demonstrated that an exposed dentin surface is not a sufficient stimulus for cementoblast differentiation and cementogenesis. Instead, it appears that there is an obligatory intermediate short and specific modulating stage in which the HERS cells secrete enamel-related matrix proteins. Enamel matrix proteins are temporarily deposited onto the dentinal root surface and provide an initial and essential step in the formation of a cellular cementum.

COMPOSITION OF ENAMEL MATRIX PROTEINS:

The major fraction of the enamel matrix proteins is composed of amelogenins, a family of hydrophobic proteins that account for more than 90% of the organic constituents of the enamel matrix. The amelogenins have remained remarkably well conserved through evolution, suggesting that they may have great functional importance.

The second largest component of the enamel matrix protein is the enamelins. Enamelins have been found to contain serum proteins, and the more general term "non-amelogenin" is now commonly used to describe this high molecular weight fraction, which includes proline-rich enamelin, tuftelin, and tuft proteins.

Three matrix proteins, corresponding to amelogenin, enamelin, and sheathelin, and two enzymes, corresponding to MMP-20 and EMSP1, have been purified

and the cDNA cloned from developing porcine teeth. Although early immunoassay studies could not identify the presence of growth factors in EMD, nominal levels of transforming growth factor β1 have been detected immunologically. In addition, by using noggin, a bone morphogenic protein (BMP)-binding protein, investigators have identified BMP-2 and BMP-4 in an osteoinductive fraction of enamel extracts. A wide range of in vitro and in vivo studies have demonstrated that EMD and amelogenins stimulate growth of multiple mesenchymal cell types including fibroblasts, cementoblasts, osteoblasts, and stem cells. EMD and amelogenin enhance expression of tissue-specific maturation markers, such as alkaline phosphatase, collagen, and osteocalcin, within osseous tissues. The commercially available EMD (Emdogain®, Biora AB, Malmo, Sweden) is available for the treatment of periodontal defects. Its acts as a tissue-healing modulator mimicking the events that occur during root development and helps to stimulate periodontal regeneration. The amelogenins, which are the hydrophobic constituents of the enamel matrix proteins, aggregate and become almost insoluble at physiologic pH and temperature. They can be dissolved in an acidic or alkaline pH environment and at low temperature. A suitable formulation should thus have a non-neutral pH and allow for gradual reprecipitation of the matrix when physiologic conditions are re-established. Using a buccal dehiscence model in monkeys, investigators evaluated several drug vehicles to determine which model most effectively allowed the EMD to precipitate on the treated root surface. Regeneration of cementum and alveolar bone was measured after 8 weeks. The results showed that propylene glycol alginate (PGA) was more effective than hydroxyethyl cellulose or dextran. PGA appears to enhance EMD precipitation, thus exposing the periodontal ligament cells to the re-established protein aggregate and allowing matrix-cell interactions to take place. The other vehicles that were tested, although stable at neutral pH, appeared to prevent exposure of periodontal ligament cells to the proteins.

In vitro studies

Properties of EMD

Application of EMD results in limited epithelial downgrowth, in contrast with control sites where greater epithelial downgrowth takes place. This histologic observation was reinforced by in vitro studies. Addition of EMD to cell culture media resulted in enhanced proliferation of periodontal ligament cells, as well as increased protein and collagen production and mineralization. In contrast, EMD had no significant effect on epithelial cell proliferation in vitro. It may be concluded that the biochemical environment at the root surface following application of EMD may prevent epithelial downgrowth in a manner similar to the mechanical prevention achieved using a barrier membrane in guided tissue regeneration procedures.

MODE OF ACTION OF EMD :

EMD adsorbs to hydroxyapatite and collagen and also to denuded dental roots. It forms insoluble spherical complexes, and detectable amounts remain at the treated site on the root surface for up to 2 weeks, as was shown by radiolabeled protein in rats and pigs. This appears to be a sufficient period of time to permit recolonization by periodontal ligament cells or undifferentiated cells. In a series of laboratory studies, the effect of EMD on migration of mineralized nodules was examined. Immunoassays were performed to determine the possible presence of existing polypeptide factors. The results showed that, under in vitro conditions, EMD promotes the proliferation of periodontal ligament fibroblasts but not epithelial cells, and increases total protein synthesis of periodontal ligament fibroblasts as well as formation of mineralized nodules by periodontal ligament fibroblasts. In the above mentioned studies, no levels of specific molecules, such as insulin-like growth factor (IGF)-1 and IGF-2, human platelet-derived growth factor BB, tumor necrosis factor, transforming growth factor β, interleukin-6, or platelet-derived growth factor AB were compared with those in the control group.19 EMD has no appreciable effect on osteoclastic differentiation, although it stimulates cell growth and IGF-1 and transforming growth factor β1 production in periodontal ligament cells.

RECENT DEVELOPMENTS IN APPLICATIONS OF EMD:

EMD has shown positive clinical features such as root coverage and promoting the stimulation of soft and hard tissues that surround the tooth in the scope of regeneration. EMD is Considered frequently for application in orthodontics .

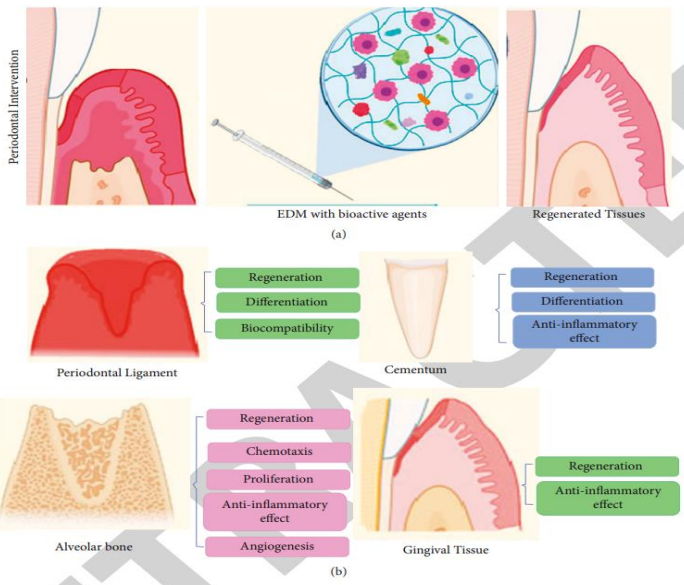


FIGURE 1: The regeneration of the periodontium. (a) EMD is a significant alternative to restore the structure and function of the periodontal complex. (b) EMD in periodontal cells can induce proliferation, differentiation, angiogenesis, and chemotaxis enabling the formation of new tissue.

COMBINATIONS OF EMD WITH GROWTH FACTORS:

Due to its growth factor content, platelet-rich fibrin can facilitate healing of the tissue and is proven to regenerate periodontium. It acts as a regenerative scaffold and promotes the formation of osseous and vascular tissues . Recently, in a randomized clinical trial,

EMD + platelet-rich fibrin and EMD were compared for treating patients with chronic periodontitis having intrabony defects, and both approaches exhibited good clinical outcomes. However, the addition of fibrin rich in platelets did not appear to drastically improve the clinical outcome or the radiographic outcome . Apart from platelet-derived growth factors, other growth factors that have been involved in tooth regeneration include transforming growth factors, vascular endothelial growth factors, connective tissue growth factors, insulin-like growth factors, fibroblast growth factors, and epidermal growth factor .

Trial	Methods and results
Histopathological examination of cementum regeneration on root surfaces using the enamel derivative Emdogain®.	Roots ($n = 40$) from 24 maxillary premolars were evaluated in beagles. Emdogain® has been proven to be effective in the regeneration of cementum on root surfaces in periodontal ligament fractures.
Combining EMDs with autogenous bone graft or singly on intrabony defects in patients with chronic periodontitis.	Deep intrabony defects ($n = 30$) in 12 patients with chronic conditions were treated in a random manner with EMDs and autogenous bone graft, EMDs alone, or open flap debridement alone. The transforming growth factor beta 1 was examined in gingival crevicular fluid before and after surgery. There were no apparent clinical and radiographic differences between the combined group and EMDs, whilst the gingival curricular fluid transforming growth factor beta 1 level increased in the healing phase and was shown to be positively affected by the EMDs.
Assessment of EMD on regeneration of vertical bone around dental implants in an extra-oral model of a rabbit.	There was greater mean bone formation with EMD release from the scaffold, as well as the production of a new bone layer, increased regeneration, and increased bone density in the implant.
A study evaluating the combination of xenogenic collagen matrix and EMD.	It was found that the combinations conferred a better clinical outcome, while coronally advanced flip + EMD and coronally advanced flip + EMD + collagen matrix conferred the best results for complete root coverage.
The combination of matrix protein of the enamel and deprotenized bovine bone mineral with 1% collagen and doxycycline was evaluated in a three-year prospective cohort study in assessing bone defect regeneration related with peri-implantitis.	This combination resulted in a positive effect for bone regeneration.
Periodontal tissue regeneration with a cytokine cocktail of insulin-like growth factor-1, vascular endothelial growth factor A, and transforming growth factor-β1 assessment in a study in dogs.	The cytokine cocktail induced the formation of vascular tissues, cementum, and new bones, but was shown to be less effective at promoting osteogenesis than EMD.
A two-centre prospective clinical study evaluated the two-year outcome of EMD in the regeneration of periodontium for intrabony defects treatment.	Intrabony defect treatment of patients with EMD resulted in positive outcomes and was confirmed with radiographical and periodontal parameters.
A controlled noninferiority phase III and randomized placebo-controlled trials compared trafermin, a rhFGF 2, and EMD in periodontal regeneration in intrabony defects.	Trafermin was recognized to be a safe and effective approach, and it was also found to have superior efficacy when compared to EMD treatments.
A phase I/II trial of a 3D woven fabric scaffold with autologous bone marrow stem cell transplantation for periodontitis.	This approach may be novel for the effective regeneration of periodontitis.
A clinical study reporting on 3-year results following regenerative periodontal surgery of advanced intrabony defects with EMD alone or when combined with a synthetic bone graft.	There was not a significant advantage of comparing EMDs with synthetic bone grafts over EMD alone.
Autologous connective tissue graft or Xenogenic collagen matrix as adjunct to coronally advanced flaps to cover multiple adjacent gingival recessions: a randomized trial assessing noninferiority and superiority in root coverage, and superiority in quality of life in terms of oral health.	The xenogenic collagen matrix shortened the time to recovery and decreased morbidity. It was reported that the devices tested were inferior to the grafts of autologous connective tissue in regard to root coverage.
A clinical study evaluating the treatment results of EMD and/or hydroxyapatite/β-tricalcium phosphate (HA/β-TCP) to treat mandibular class II buccal furcations.	Clinical parameters measured were PPD, gingival index, plaque index, horizontal attachment, relative vertical level (RHCAL and RVCAL), and RGMP (relative gingival margin position). Clinical examinations at 12 months posttreatment revealed remarkable improvements in all parameters other than RGMP.

COMBINATIONS OF EMD WITH DRUGS/BIOACTIVE AGENTS:

Periodontal ligament cells were found to attach in the presence of oral pathogens such as Streptococcus mutants due to the addition of amoxicillin or tetracyclines and calcium phosphate in guided tissue regeneration membranes.

Table 2: Applications of enamel matrix derivatives (EMDs).

Application	Study	Outcome
Periodontal intrabony defect	A multicenter, randomized, placebo-controlled study was conducted on 33 patients with intrabony abnormalities who underwent a split-mouth operation. The effect of EMD in combination with natural bone mineral or bioactive glass was investigated in human histological tests.	The results revealed the production of root cementum and mineralization around the graft particles.
Effect on tissue inflammation	A study investigated the impact of EMD on tissue inflammation, focusing on the cellular process, mediators implicated, and soft tissue repair.	According to the findings, EMD can change inflammatory and healing responses by modifying the expression of proinflammatory markers.
Recession defects	Miller class I and II buccal gingival recessions were investigated utilizing a coronally positioned flap alone and in combination with EMD using the split-mouth method in controlled clinical research.	When compared to a coronally positioned flap alone, subsequent application of EMD resulted in a statistically larger development of keratinized tissue and root coverage that lasted for two years.
Pulp healing and dentin regeneration	An investigation using experimental pulpotomy and pulp capping in healthy premolars slated for extraction for orthodontic reasons was investigated in a blinded, randomized clinical research.	In the teeth that were evaluated, there was much greater pulpal secondary dentine development and dentine bridging, as well as significantly less inflammation.
Furcation defects	Treatment of mandibular class II furcation defects was compared to 90 equivalent defects in the contralateral molars in a multicenter, randomized, controlled, split-mouth clinical research.	Following EMD, there was a considerably higher reduction in horizontal furcation depth and a lower incidence of postoperative pain/swelling.
Wound healing	The extreme structural changes associated with a human gingival wound 10 days following the administration of EMD as an adjuvant to a laterally positioned flap in a patient with gingival recession were investigated in a quantitative study.	Both the cellular and extracellular phases of the EMD and non-EMD sites showed significant differences. At the EMD location, fibroblasts had plump cytoplasm and euchromatic nuclei, as well as a well-developed rough endoplasmic reticulum and many mitochondria. The fibroblasts at the non-EMD location, on the other hand, had a flattened, spindlelike shape.

COMBINATIONS OF EMD WITH AUTOGENOUS BONE GRAFT:

Several preclinical animal and clinical trials have investigated the efficacy of using various bone grafts in combination with EMD for periodontal regeneration Studies have shown that EMD exhibited greater performance in opening flap debridement to treat the tooth intrabony impairment . EMD combined with bone graft material was used in a wide intrabony defect and showed significant regenerative effect for regeneration of damaged tissue . Combined EMD-bone grafts were successful in intrabony defect regeneration; the performance of the regeneration of the EMD-graft combination was comparable with the regeneration performance of human platelet-derived growth factor-BB (recombinant) with bone graft material . Findings of the studies have demonstrated that using EMD in combination with bovine-derived

bovine-derived bone xenograft, freeze-dried bone allograft, and bioactive glass facilitated enhanced bone formation and improved outcomes clinically bone xenograft, freeze-dried bone allograft, and bioactive glass facilitated enhanced bone formation and improved outcomes clinically.

COMBINATIONS OF EMD WITH ALLOPLASTIC BONE GRAFTS:

+e EMD surface coating of a scaffold biomaterial dramatically increases the thickness of enamel matrix proteins . It was also established that a formulation in the liquid could form a better coating of porous alloplastic graft materials compared to the gel form, which allowed the release of enamel matrix proteins in a controlled manner to their neighboring environment . +e combination of EMD with βTCP (β-tricalcium phosphate) was effective in regenerating intrabony defects . +e effect of EMD was comparable to that of guided tissue regeneration and demineralized freeze-dried bone allograft; it was also superior to open-flap debridement for treating intrabony defects.

COMBINATION WITH OTHER APPROACHES:

EMD (5–60 μg/mL) enhanced the osteogenic differentiation and proliferation of human periodontal ligament stem cells on surfaces of titanium implants . It also influenced the angiogenic gene expression and proliferation in endothelial cells on the surface of the titanium implant. Endothelial cells on the surface of the titanium implant . EMD enhanced the gingival fibroblast growth on titanium surfaces along with the increased synthesis of extracellular matrix . A previous report demonstrated that EMD application can be used as an adjunct to mechanical debridement in the nonsurgical treatment of peri-implant mucositis . Randomized controlled trials of peri-implantitis surgical therapies proved that the adjunctive use of EMD enhanced implant survival and augmented marginal bone level.

Aggressive periodontitis (AgP) is a rare but adverse inflammatory condition, which involves periodontal tissue destruction. EMD could be effective in periodontal regeneration in individuals with generalized AgP. Porcine acellular dermal matrix in dogs was examined with or without EMD on recession defects of the gingiva that were treated with a coronally advanced flap; the treatment combined the coronally advanced flap along with EMD and porcine acellular dermal matrix and facilitated regeneration of the periodontium in recession defects of the gingiva.

PRP, PRF and CGF in Periodontal Regeneration

INTRODUCTION ;

PRF (platelet rich fibrin) was first developed in France for use in the field of oral and maxillofacial surgery. Choukroun's platelet-rich fibrin (PRF) is a leukocyte and platelet rich fibrin biomaterial with a specific composition and three-dimensional architecture. PRF is classified as a second generation platelet concentrate as it is prepared as a natural concentrate without the addition of any anticoagulants. PRF is often called Choukroun's PRF as there are other platelet concentrates with similar names such as Vivostat PRF (considered a pure platelet-rich plasma) or Fibrinet PRF (without leukocytes). PRF has a dense fibrin network with leukocytes, cytokines, structural glycoproteins and also growth factors such as transforming growth factor b1, platelet-derived growth factor, vascular endothelial growth factor and glycoproteins such as thrombospondin-1 during P7 day. Leukocytes that are concentrated in PRF scaffold play an important role in growth factor release, immune regulation,9anti-infectious activities, and matrix remodeling during wound healing. The slow polymerization mode of PRF and cicatricial capacity creates a physiologic architecture favorable for wound healing.

HISTORICAL BACKGROUND:

Platelets are used as powerful tools for periodontal regeneration for the past two decades due to the key role of platelets in wound healing process. Although the use of fibrin adhesives is well documented from the past 30 years their use is still controversial due to the complexity in preparation and risk of cross-infection. After that concentrated platelet-rich plasma (cPRP) was developed with a less complex production protocol. It is prepared from the patient's own blood and is activated by the addition of thrombin and calcium. The structure consists of a three dimensional biocompatible fibrin scaffold with a limited volume of plasma enriched in platelets. When PRP is activated the growth factors and proteins are released to the local environment accelerating postoperative wound healing and tissue repair. But the disadvantage of using PRP is that its properties can vary depending on the concentration of platelets, amount of leukocytes, the type of activator used and time of placement of fibrin scaffold after clotting. But there are certain risks associated with the use of PRP.

The presence of bovine thrombin in PRP can result in the development of antibodies to the clotting factors V, XI and thrombin which can adversely affect the coagulation process. In addition, bovine thrombin preparations contain clotting factor V which can result in immune system activation when challenged with a foreign protein. Other drawbacks about the use of PRP include legal restrictions on handling the blood and also controversies in the literature regarding the benefits and clinical outcome of use of PRP. All these have led to the generation of a new family of platelet concentrate called platelet-rich fibrin which overcomes many of the limitations of PRP. PRF is a potent autologous regenerative material with many clinical applications in the field of periodontics as it accelerates both soft tissue and hard tissue healing .

Platelet-rich fibrin after centrifugation

The Technique :

INJECTABLE PRF :

Miron et al. published a modification to PRF: a liquid formulation of PRF injectable PRF (i-PRF) with no use of anticoagulants. As compared to PRP, after 10 days, i-PRF released higher levels of GFs such as IGF-1, EGF, PDGF-AA/AB. Furthermore, i-PRF induced the highest fibroblast migration, while PRP induced higher levels of cell proliferation . Fujioka-Kobayashi et al. noted that modification to centrifugation speed and time influence GF release. As centrifugation speed decreases, GF and leukocyte release from the PRF clot is increased .

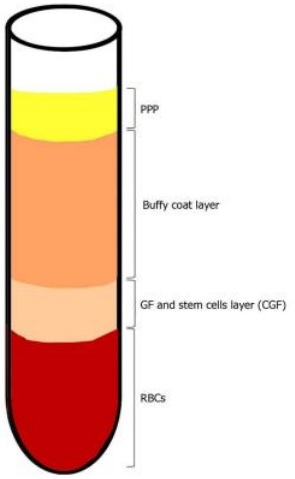
CONCENTRATED GROWTH FACTOR (CGF):

The Newest Platelet Concentrate In 2006, Sacco [26] reported on the newest platelet concentrate—CGF. CGF is produced in a manner that is similar to that used to produce PRF, but it involves a different centrifuge speed (Medifuge, Silfradent, Italy). CGF contains GFs such as VEGF, PDGF, IGF-I and TGFβ1. Compared to PRF, CGF contains a denser and richer GF-fibrin matrix. Furthermore, CGF has a 3D fibrin network in which growth factors are closely

bound to one another. This provides the slow release of growth factors, which helps with wound healing .

THE TECHNIQUE :

As described by Bozkurt et al., IV blood is collected in two 10 mL glass-coated plastic tubes with no anticoagulant addition. The tubes are immediately centrifuged (Medifuge, Silfradent, S. Sofia, Italy) in the following manner: 30" acceleration, 2' 2700 rpm, 4' 2400 rpm, 4' 2700 rpm, 3' 3000 rpm and 36" deceleration until end. At the end of the procedure, four layers are obtained from bottom to top: RBC layer, GF and stem cell layer (CGF), Buffy coat layer, serum layer (PPP) (see Figure 3). Then, the CGF layer is separated using sterile surgical scissors. The CGF clot is then squeezed in a special box at a thickness of 1 mm. The CGF is then placed over the target site.



Blood centrifugation after collection. At the end of the centrifugation period, four layers are obtained: 1. Bottom—RBC layer; 2. GF and stem cell layer (CGF); 3. Buffy coat layer; 4. Top—serum layer (PPP).

POTENTIAL BENEFITS OF USING PRF IN PERIODONTAL REGENERATION :

Platelet-rich fibrin is a second generation platelet concentrate which can enhance both soft and hard tissue healing. Its advantages over platelet-rich plasma include ease of preparation, ease of application, minimal expense, and lack of biochemical modification (no bovine thrombin or anticoagulant is required). This considerably reduces the biochemical handling of blood as well as risks associated with the use of bovine-derived thrombin. PRF also contains physiologically available thrombin that results in slow polymerization of fibrinogen into fibrin which results in a physiologic architecture that is

favorable to wound healing. The cytokines which are present in platelet concentrates play an important role in wound healing.

CLINICAL APPLICATIONS PRF :

It is a powerful healing biomaterial with inherent regenerative capacity and can be used in various procedures such as for the treatment of periodontal intrabony defects,treatment of furcation, sinus lift procedures and as a scaffold for human periosteal cells in vitro, which finds application in the field of tissue engineering.

DRAWBACKS OF PRF :

The main shortcoming of PRF is its preparation and storage. The clinical benefit of PRF depends on time interval between speed of handling between blood collection and centrifugation as PRF is prepared without any addition anticoagulants. Another main disadvantage of PRF is its storage after preparation.40 Also PRF membranes should be used immediately after preparation as it will shrink resulting in dehydration altering the structural integrity of PRF. Dehydration also results in the decreased growth factor content in PRF and leukocyte viability will be adversely affected altering its biologic properties. PRF when stored in refrigerator can result in risk of bacterial contamination of the membranes. These limitations with the use of PRF can be circumvented by sticking onto a standard protocol for preparation and preservation.[67]

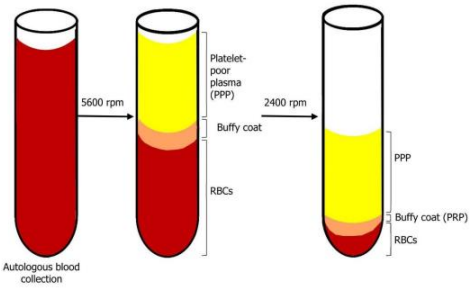
PLATELET-RICH PLASMA (PRP):

In 1997, Whitman et al. published the first article on the use of platelet-rich plasma (PRP), the first generation of APCs, in oral and maxillofacial surgery. During the preparation of PRP, xenogeneic thrombin and anticoagulant were added. This technique uses exogenous materials and might cause an immunologic and infectious response, making its use controversial . PRP plays a vital role in wound healing. The wound-healing process can be divided into three stages: biochemical activation, cellular activation and cellular response. First, there is a conversion of the mechanical injury into biochemical signals. This cascade is triggered by the Hageman factor in the serum. As a result of the disruption of microcirculation, the plasma comes into contact with tissue proteins and the basement membrane, activating the Hageman factor and platelets. The clotting cascade enables fibrin to facilitate homeostasis, and it activates thrombin. Thrombin, calcium chloride and ADP trigger the activation of platelets, leading to the release of alpha granules from platelets, with the subsequent secretion of a large variety of growth and differentiation factors . The complement cascade also includes the release of substances that are

important for wound repair. During this process, bradykinin is produced, which causes vasodilatation and the activation of plasminogen to produce plasmin, which degrades the fibrin. The fibrin degradation causes monocyte migration and vasodilatation. The third stage is the cellular response. In this stage, GFs are released from platelets. These GFs signal the local epithelial and mesenchymal cells to migrate, divide and enhance the synthesis of the collagen matrix. The platelet count in PRP is 338% of the platelet count of the whole blood . PRP enhances bone deposition and the quality of bone regeneration during bone augmentation as GFs from autologous blood are delivered to the treatment site . Moreover, platelet and GF concentrations in PRP are, on average, 3-5 times higher in PRP than in peripheral blood.

THE TECHNIQUE IN THE PRE-OPERATIVE PERIOD :

450 mL blood is collected in a sterile centrifuge tube, containing citrate–phosphate–dextrose solution (as anticoagulant). First, it is centrifugated (Medtronic Electromedic, Elmd-500 Autotransfusion system, Parker, CO, USA) at 5600 rpm. The result of this stage is the separation into two layers: first layer—platelet-poor plasma (PPP); second layer—red blood cells (RBCs) and buffy coat, which contains platelets and white blood cells (WBCs) 1). Only the layer of RBCs and buffy coat then continues to the second stage of separation. The second centrifugation period is processed at 2400 rpm in order to separate the buffy coat into PRP and residual RBCs. When the surgeon needs to use the PRP, thrombin is dissolved in 10 mL 10% calcium chloride in a sterile cup. Then, 7 mL PRP and 2 mL air are aspirated into a 10 mL syringe with a 14 gauge catheter. Then, a 1 mL mixture of thrombin + calcium chloride is aspirated into the syringe. Within 5–30 s, the thrombin enables the polymerization of fibrin into a insoluble gel, platelet degranulation and the release of GFs and cytokines. The gel is injected to the desirable site.[64] It should be noted that there is a difference in platelet quantity: platelet and WBC count is higher in younger people and higher in females compared to males.



Blood centrifugation after collection. After the first centrifugation period, there is a separation of two layers: on top—platelet-poor plasma (PPP), on bottom—red blood cells (RBCs) and buffy coat. The products of the second centrifugation period are: top—PPP; bottom—buffy coat (PRP) and residual RBCs

Hyaluronic Acid in Periodontal Regeneration

INTRODUCTION:

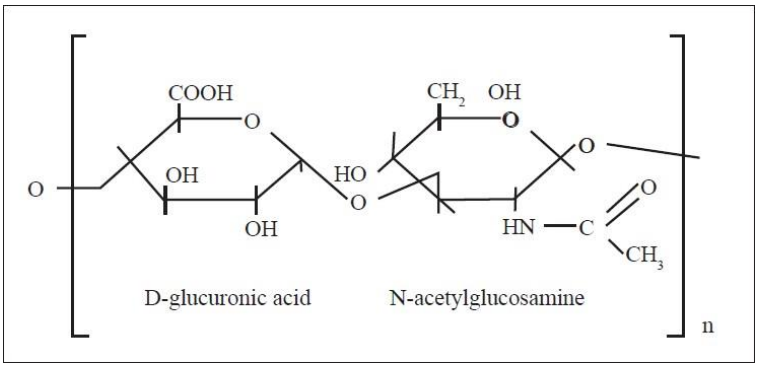
Hyaluronate also identified as hyaluronan or "HA", is a non sulphated, higher molecular mass linear polysaccharide present in connective tissue, synovial fluid, the extracellular matrix and other tissues. It has a variety of biological and physical functions, including extracellular, cellular, growth factor interactions, osmotic pressure regulation, and lubrication of tissue . All of these roles contribute to the tissue's structural and homeostatic integrity. On periodontal tissues invaded by submicrobial flora, HA have anti-oedematous and anti-inflammatory properties.The use of HA in the treatment of inflammatory process is established in medical areas such as orthopedics, dermatology and ophthalmology. It has been used in radio-epithelitis, osteoarthritis of the knee and rheumatoid arthritis and cataract surgery. Rabasseda reviewed its wide use for the treatment of inflammatory conditions of the knee and temporomandibular joint, which has led to the study of its topical application in the treatment of periodontal diseases. In the field of dentistry, preliminary clinical trials have been conducted by Vangelisti and Pagnacco et al. in 1997. Hyaluronate has shown anti-inflammatory, anti-edematous and anti-bacterial effects for the treatment of gingivitis and periodontitis. The anti-inflammatory effect may be due to the action of exogenous hyaluronan as a scavenger by draining prostaglandins, metalloproteinases and other bio-active molecules. The antiedematous effect may also be related to the osmotic activity. Due to its acceleration in tissue healing properties, it could be used as an adjunct to mechanical therapy. However, it is conceivable that Hyaluronan administration to periodontal wound sites could achieve comparable beneficial effects in periodontal tissue regeneration and periodontal disease treatment. Hyaluronic acid has been studied as a metabolite or diagnostic marker of inflammation in the gingival crevicular fluid as well as a significant factor in growth, development and repair of tissues.

HISTORICAL BACKGROUND:

Hyaluronic acid was discovered in 1934 by Karl Meyer and his colleague John Palmer, scientists at Columbia University, New York, who isolated a chemical substance from the vitreous jelly of cow's eyes. They proposed the name hyaluronic acid as it was derived from Greek word hyalos (glass) and contained two sugar molecules one of which was uronic acid.

Structure:

The HA is a non sulphated glycosaminoglycan having naturally occurring 4,000 to 20,000,000 daltons molecular weight. The HA structure is made up of alternating 1-3 and 1-4 bonds connecting "polyanionic disaccharide units of glucouronic acid and N-acetyl glucosamine" .It is a straight chain of polysaccharide found in synovial fluid, connective tissue, embryonic mesenchyma, skin, vitreous humour and a variety of other body organs and tissues. HA can be synthesised by almost all cells in the body, and the process occurs in cell membrane.



Repeating disaccharide unit of hyaluronan

MECHANISM OF ACTION:

Most cells in the body can synthesise HA, which is a major polysaccharide component of connective tissue's extracellular matrix . It helps with tissue hydrodynamics, cell migration, and proliferation, as well as improving the tissue's healing properties . HA helps in chemotaxis, proliferation, and effective differentiation of mesenchymal cells speed up regeneration of bone .

SOURCE,BODY RESERVOIR AND UPTAKE OF HYALURONIC ACID (HA)The quantity of HA in human skin is estimated to be 5 grams. HA can be found in majority of periodontal tissues like gingiva and Periodontal Ligament (PDL). Hyaluronan Synthase (HAS) enzymes (HAS1, HAS2, and HAS3) synthesise high molecular weight Hyaluronan (HY) in gingiva and PDL, cementoblasts in cementum, and osteoblasts in alveolar bone, as well as in smaller amounts in mineralised tissues like alveolar bone and cementum

PROPERTIES OF HYALURONIC ACID (HA):

The HA is hygroscopic in nature, Viscoelastic, has a bacteriostatic effect and is biocompatible, non anti-genic having anti-inflammatory, anti-oedematous and anti-oxidant properties.

FUNCTIONS :

Hyaluronan has many structural and physiological functions within tissues, including extracellular and cellular interactions, growth factor interaction and in the regulation of osmotic pressure and tissue lubrication, which help maintain the structural and homeostatic integrity of tissues.

Modulation Of Inflammation

In the initial stages of inflammation

Enhanced inflammatory cell and extracellular matrix cell infiltration into the wound site

Elevation in proinflammatory cytokine production by inflammatory cells and extracellular matrix cells.

Organization and stabilization of granulation tissue matrix.

Scavenges reactive oxygen species, such as superoxide radical (·O 2) and hydroxyl radical (·OH) thus preventing periodontal destruction.

Inhibition of inflammatory cell-derived serine proteinases. [87]

STIMULATION OF CELL MIGRATION, PROLIFERATION AND DIFFERENTIATION:

The remarkable hydrophilicity of the hyaluronic acid makes the coagulum more receptive and thus more likely to undergo colonization by the cells committed to the reconstruction of the damaged tissue by migration, proliferation and differentiation of mesenchymal and basal keratinocytes.

EFFECT ON ANGIOGENESIS :

Low molecular weight hyaluronic acid has a marked angiogenic effect whereas, surprisingly, high molecular weight has the opposite effect.

OSTEOCONDUCTIVE POTENTIAL:

Hyaluronic acid accelerates the bone regeneration by means of chemotaxis, proliferation and successive differentiation of mesenchymal cells. Hyaluronic acid shares bone induction characteristics with osteogenic substances such as bone morphogenetic protein-2 and osteopontin.

CARRIER FUNCTION :

Hyaluronic acid may act as biomaterial scaffold for other molecules, such as BMP-2 and PDGF-BB, used in guided bone regeneration techniques and tissue engineering research.

BACTERIOSTATIC EFFECT :

Recent studies on regenerative surgical procedures indicate that reduction of bacterial burden at the wound site may improve the clinical outcome of regenerative therapy. The high concentration of medium and lower molecular weight hyaluronic acid has the greatest bacteriostatic effect, particularly on Aggregatibacter actinomycetemcomitans, Prevotella oris Scientific Name Search phylococcus aureus Scientific Name Search strains commonly found in oral gingival lesions and periodontal wounds. Clinical application of hyaluronic acid membranes, gels and sponges during surgical therapy may reduce the bacterial contamination of surgical wound site, thereby, lessening the risk of postsurgical infection and promoting more predictable regeneration.

SYNTHESIS OF HA:

HA is a negatively charged glycosaminoglycan that differs from other glycosaminoglycans. HA synthesis occurs in the cellular plasma membrane in mammals, whereas glycosaminoglycan synthesis often occurs in the Golgi apparatus. Moreover, HA synthesis occurs via three hyaluronan synthase isoenzymes (HAS1, 2 and 3) .[88] HA exhibits a high molecular weight of 10^3-10^4 kDa, a length of 2-25 µm, and it does not contain any sulphate groups . The synovial fluid, epidermis, umbilical cord and other tissues exhibit the highest concentrations of HA, while the blood serum exhibits the lowest concentration . A membrane-bound protein present in plasma membranes produces HA through the transportation of activated monosaccharides to glycosaminoglycan chains, and the release of uridine diphosphate, secreted directly into the extracellular space. Lymphatic drainage into the circulatory system or local metabolism causes HA turnover in tissues. Depending on its removal, HA exhibits a tissue half-life ranging from 12 h to 2-3 days.

HYALURONIC ACID HAS A MULTIFUNCTIONAL ROLE IN PERIODONTICS:

- Topical application of subgingival hyaluronic acid gel can be used as an antimicrobial agent as an adjunct to scaling and root planing.
- Bone regeneration in periodontal bony defects.
- Guided Bone Regeneration.
- Non surgical treatment of peri-implant pockets.
- Peri-implant maintenance of immediate function implants.
- As autologous cell hyaluronic acid graft gingival augmentation in mucogingival surgery.
- As a carrier for newer molecules in various regenerative procedures.
- As a biomaterial scaffold in tissue engineering research.
- Use of HA in Periodontal Regeneration such as in infrabony defects, gingival recession and papilla reconstruction:Topical application of HA in subgingival regions has been found to minimise microbial activity, aid in bone regeneration in deep periodontal bony defects, and is useful in directed bone regeneration, non surgical treatment of peri-implantitis pockets, peri-implant maintenance of immediately inserted implants, and gingival augmentation in mucogingival surgery.

SAFETY:

Hyaluronic acid is biocompatiable and intrinsically safe to use, with no evidence of cytotoxicity has been found. Hyaluronic acid gel,injections or oral (by mouth), should not be used in patients with allergies.

ADVERSE EFFECTS:

Hyaluronic acid side effects although not severe include bruising, swelling, redness, pain, itching and tenderness at the injection site.

AVAILABILITY:

Hyaloss® matrix, trade names of products composed entirely of an ester of hyaluronic acid with benzyl alcohol (HYAFF™), a concentration ranging of from 20 to 60 mg/ml. Hyaloss matrix is a product manufactured as a solid in the form of fibers that forms a gel when hydrated, releasing pure hyaluronic acid for about 10 days. It is highly multipurpose because at room temperature it can form a biodegradable, biocompatible gel that can be adapted by the operator to the desired consistency, by regulating the blood and saline volume.[110]

Gengigel® (Ricerfarma S.r.l., Milano, Italy) contains high molecular weight fractions of Hyaluronic acid in gel formulation with 0.2% concentration for its effect in the treatment of plaque-induced gingivitis as an adjunct to scaling and root planing. The adjunctive use of Hyaluronan with 0.8% after thorough mechanical debridement potentially has major clinical benefits in terms of improved healing after non-surgical therapy.

Gengigel® is available in different presentations to aid treatment efficacy and patient compliance over the longer term. It is available as tubes and applicators for use within the surgery, mouthwash and oral sprays for patients to continue treatment at home. Gengigel as a product for oral use has been evaluated by skin irritation test, sensitizing potentiality and percutaneous absorption test and has been proved to be a safe non irritant product.

ROLE OF HA IN WOUND HEALING:

HA plays a role in numerous physiological and biological processes, serving as a structural component of cartilage and other tissues. To produce proteoglycans, HA interacts with proteins rich in numerous forms of glycosaminoglycans. It increases inflammatory cell and extracellular matrix infiltration, assisting inflammation. Thus, HA exhibits the potential to impact cellular behaviour via influencing the environment surrounding cells . HA is engaged in numerous cell functions which increase tissue healing, such as cell proliferation, locomotion and recognition. This makes HA increasingly susceptible to colonization by tissue repair cells . In its highly purified form, HA has been used in medicine for a number of years, due to its physiochemical characteristics and non-immunogenicity. As HA retains water in large amounts, it affects and improves tissue regeneration; thus, preventing the production of scabs and scars . It has been proposed that HA stimulates angiogenesis, leading to increased levels of wound healing in the bone matrix. At a low molecular weight, HA is angiogenic, whereas at a high molecular weight, HA is anti-angiogenic .

High molecular weight HA enhances osteo-induction or bone production during wound healing . Results of previous studies demonstrated that exogenous HA exerted satisfying wound healing benefits . In cosmetic dermatology, HA is also used as a dermal filler . As it forms an integral part of cell migration, organogenesis and development, HA exhibits potential in tissue engineering . The esterification and crosslinking of HA are two modifications that provide the gel-like structure and rigidity for cell seeding. These biopolymers are biodegradable which help fibroblasts, chondrocytes and mesenchymal stem cells to proliferate . HA has been employed as a chemotherapeutic agent in the treatment of gingivitis. Moreover, osseointegration of dental implants indicates the involvement of HA . As HA exhibits bone induction properties, it may exhibit potential as a biomaterial scaffold in guided bone regeneration and tissue engineering . In 2022, Ibraheem *et al* demonstrated improved wound healing in extraction socket wounds following treatment with HA. Moreover, HA exhibited dose-dependent bacteriostatic effects on a range of microorganisms in the planktonic phase .[102]

GENE THERAPY FOR GROWTH FACTOR DELIVERY:

INTRODUCTION :

Growth Factors Polypeptide growth factors are a class of naturally occurring biological mediators that regulate the proliferation, migration, and extra- cellular matrix synthesis of a variety of cell types including those derived from the periodontium. They were first described in 1960 in blood fluid of fetal calf serum. Recent advances in molecular cloning have proven the application of growth factors in tissue engineering used as an alternative treatment approach for periodontal regeneration. In general growth factors are synthesized as pro-peptide forms which are biologically active and stored in the cytoplasm. Growth factors and their cognates interaction mediate several intracellular signaling pathways and modulate target cell response by altered gene activity, local acting growth factors, regulate the development and function of cells, offer the potential for regenerating tissue types. Growth factors are proteins that may act locally or systemically to affect the growth and function of cells in several ways. The application of growth factors to restore damaged tissues aims at regeneration through biomimetic processes or mimicking the processes that occur during embryonic and post-natal development.[104]

MECHANISM OF ACTION OF GENE DELIVERY SYSTEM :

The gene-delivery therapy for periodontal regeneration identifies a malfunctioning gene and supplies the patient with functioning copies of that particular gene through an in-vivo and ex- Vivo gene delivery system. The transfer of the therapeutic gene into the patient's target cell requires a carrier called a vector, which are either viruses or D.N.A. plasmids. When the scaffold containing the gene constructs is implanted into the tissue defect, the host cells migrate into the implant, take up the gene constructs and start producing the encoded protein.

TYPES OF SIGNALING MOLECULES/Biochemical mediators/growth factors include: Bone morphogenic Proteins, fibroblast growth factor, platelet-derived growth factor, transforming growth factor, epidermal growth factor, cementum derived growth factor, parathyroid derived growth factor and insulin-like growth factor.

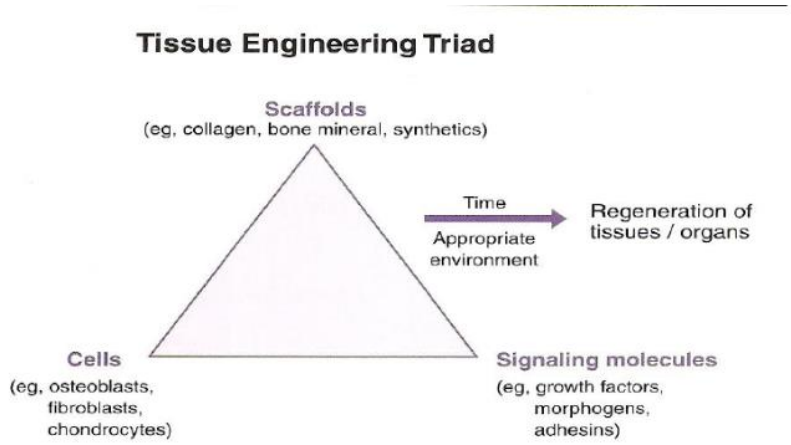
MECHANISM OF ACTION :

The growth factors release may affect target cells either as autocrine, juxtracrine, or intracrine fashion. In certain concentrations growth factors substantially increase the rate of cell turnover through secondary intra- cellular messengers of phosphor di esterase diacylglycerol and C. kinase that can regulate D.N.A synthesis, resulting in mitosis. Few growth factors stimulate cell differentiation and regulate the synthesis of the extracellular matrix. Platelet-derived growth factor acts as a chemo-attractant for a variety of cells. Growth factors operate through highly complex intracellular pathways in order to regulate intra-cellular PH. thereby influencing genetic activity. The activation of receptor by growth factor initiates a cascade of intracellular biochemical changes.[114]

IMPLICATION OF GROWTH FACTORS IN PERIODONTAL TISSUE ENGINEERING :

Platelet-derived growth factor was first discovered by Lynch and his co-workers in late 1805 to promote regeneration of bone, cementum, periodontal ligament. It is the first growth factor to be evaluated in preclinical periodontal and peri-implant regenerative studies. As it contains biological mediators that regulate the proliferation and migration of gingival and periodontal

ligament fibroblasts, cementoblasts, pre- osteoblasts, and osteoblastic cells at a wound site. There are four isoforms of Platelet-derived growth factors namely platelet-derived growth factor -A, -B, -C, -D. The mature parts of A and B chains are 100 amino acids that share 60% of the amino acids. The C & D chains are activated by proteolysis. PDGF stimulates DNA synthesis and cell replication in osteoblasts, as well as increases bone collagen synthesis and the rate of bone matrix apposition. PDGF-BB is most effective on PDL cell mitogenesis and matrix biosynthesis. Recombinant human PDGF BB homodimer (rhPDGF-BB) is a potent recruiter and a strong mitogenic factor for cells crucial to



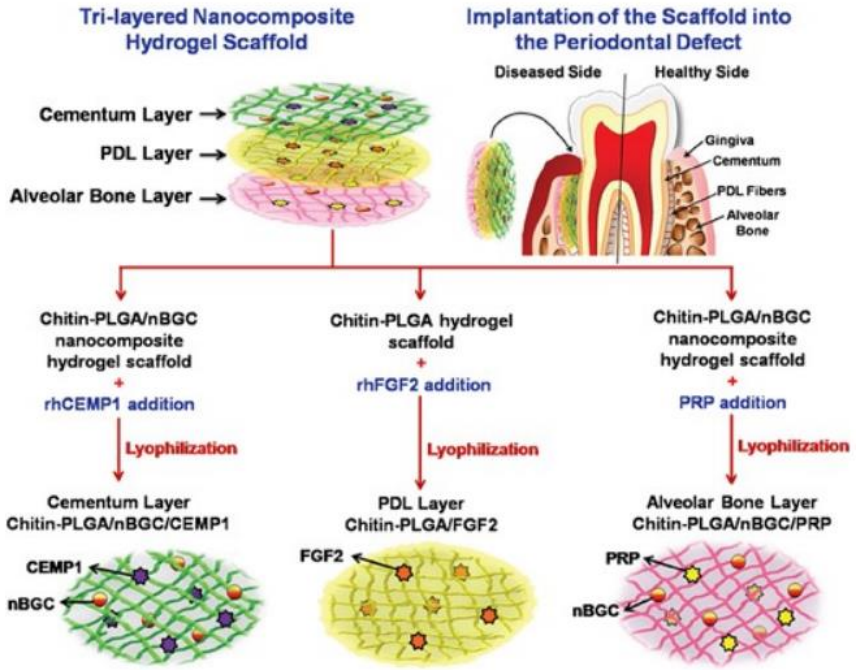
musculoskeletal tissue repair, including mesenchymal stem cells (MSCs), osteogenic cells, and tenocytes. rhPDGF-BB also upregulates angiogenesis. These properties allow rhPDGF-BB to trigger the cascade of bone and adjoining soft tissue repair and regeneration. Howell et .al conducted a study to evaluate the regenerative potential of a combination of recombinant human (rh) platelet-derived growth factor-BB (PDGF-BB) and (rh) insulin-like growth factor-I (IGF-I)in the treatment of periodontal infra-bony defects in humans. They concluded that the local application of rhPDGF-BB and rhIGF-I to periodontal lesions was safe and efficacious in regenerating lost bone. Several studies were conducted by Park et.al11, Cho.et.al, Camelo et.al, Nevin et.al, Jayakumar et .al to evaluate the regenerative potential of PDGF-BB either alone or in combination to allografts including demineralized frozen dried bone graft and beta-tricalcium phosphate and have concluded that PDGF – BB has proven to be a good adjuvant to bone grafts in the regeneration of bone.

PLATELET-DERIVED GROWTH FACTOR GENE DELIVERY : Jin et al. demonstrated that direct in vivo gene transfer of PDGF-B stimulated periodontal regeneration in infra-bony periodontal defects in rats. Descriptive histology and histomorphometry revealed that human PDGF-B gene delivery promotes the regeneration of cementum and alveolar bone, whereas PDGF-1308, a dominant-negative mutant of PDGF-A, has minimal effects on periodontal tissue regeneration Platelet-derived growth factor (PDGF) is an active growth factor, which is a product of two distinct genes PDGF-A and PDGF-B. PDGF stimulates DNA synthesis and cell replication in osteoblasts, as well as increases bone collagen synthesis and the rate of bone matrix apposition.

FIBROBLAST GROWTH FACTO -2 Takayama et al., investigated the role of fibroblast growth factor -2 in periodontal regeneration as a chemo- attractant for periodontal ligament cells during periodontal wound healing.[106] Terranova et al. Kitamura et al. investigated the efficacy of the local application of recombinant human fibroblast growth factor-2 (FGF-2) in periodontal regeneration They concluded that FGF-2 can be efficacious in the regeneration of human periodontal tissue that has been lost by periodontitis. Basic fibroblast growth factor (bFGF or FGF-2) has been demonstrated to have potent angiogenic activity and the potential to induce the growth of immature PDL cells. The mRNA level of laminin in PDL cells, which plays an important role in angiogenesis, is up-regulated by FGF-2 stimulation. Thus it may in turn accelerate periodontal regeneration. Transforming Growth Factor-β is the name given to a group of homodimeric proteins involved in the formation and development of many tissues. Once secreted, the ligand binds to transmembranous heterodimeric receptors, activating a group of intracellular proteins. The phosphorylated intracellular proteins start an intracellular signaling pathway which activates a set of genes.

BONE MORPHOGENETIC PROTEIN GENE DELIVERY:

in an early approach to regenerate alveolar bone in an animal model, the ex vivo delivery of Ad-encoding murine BMP-7 was found to promote periodontal tissue regeneration in large mandibular periodontal bone defects. BMP-7 gene transfers not only enhanced alveolar bone repair but also stimulated cementogenesis and PDL fiber formation. Of interest, the alveolar bone formation was found to occur via a cartilage intermediate When genes that encoded the BMP antagonist were delivered, inhibition of periodontal tissue formation resulted. A recent study by Dunn et al. showed that direct in vivo gene delivery of Ad/BMP-7 in a collagen gel carrier promoted successful regeneration of alveolar bone defects around dental implants. These experiments provide promising evidence that shows the feasibility of in vivo and ex vivo gene therapy for periodontal tissue regeneration and peri-implant osseointegration. Gene therapy for periodontal tissue engineering causes the transfer of genetic information into cells leading to the synthesis of a protein of interest which would aid in the regeneration of lost periodontium. Hence gene therapy could be considered as an advancement towards achieving periodontal regeneration.[110]



Schematic representation of the formation of a trilayered nanocomposite hydrogel scaffold (each layer incorporates different growth factors or preparation-containing growth factors) for the simultaneous regeneration of multiple periodontal tissues growth factor-2; PDL, periodontal ligament; PLGA, poly(lactic-*co*-glycolic acid); PRP, platelet-rich plasma; nBGC, nanobioactive glass ceramic; rhCEMP1, recombinant human cementum protein-1; rhFGF, recombinant human fibroblast growth factor.

ABBREVIATIONS:

1.	AcOH	Acetic acid
2.	API	Active pharmaceutical ingredient
3.	β-TCP	β-tricalcium phosphate
4.	bFGF	Basal fibroblast growth factor
5.	BG	45S5 bioactive glass
6.	BMP-6	Bone morphogenetic factor 6
7.	BSA	Bovine serum albumin
8.	CA	Citric acid

9.	CS	Chitosan
10.	DCM	Dichloromethane
11.	DEX	Dextran
12.	DMF	Dimethylformamide
13.	DMSO	Dimethyl sulfoxide
14.	EA	Ethyl alcohol
15.	GTR	Guided tissue regeneration
16.	HA	Hydroxyapatite
17.	LDH	Layered double hydroxides
18.	NSAID	Non-steroidal anti-Inflammatory
19.	PDGF	Platelet-derived growth factor
20.	PDL	Periodontal ligament
21.	PGA	polyglycolic acid
22.	SRP	Scaling and root planning
23.	UV–vis ACS	Ultraviolet–visible absorbable collagen sponge
24.	BMP(s)	bone morphogenetic protein(s)
25.	EGF	epidermal growth factor
26.	ECM	extracellular matrix
27.	EMD	enamel matrix derivatives
28.	FGF(s)	fibroblast growth factor(s)
29.	HA	hyaluronic acid
30.	KGF	keratinocyte growth factor
31.	FDA	Food and Drug Administration
32.	PDGF(s)	platelet-derived growth factor(s)
33.	PRP	platelet-rich plasma
34.	TCP	tricalcium phosphate
35.	TGF-β	transforming growth factor-β

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