

## TISSUE REGENERATION: CURRENT CONCEPTS IN PERIODONTICS

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### ABSTRACT

Tissue Engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to improve or replace biological functions. Tissue engineering can hence be defined as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use." Tissue engineering was once considered a sub field of biomaterials but is now considered a field in its own right. It also constitutes an integral part of all surgical disciplines. This article reviews the past and present concepts of tissue engineering along with a brief discussion of the trends that can be expected in the future. It also discusses the various components of tissue engineering and the different commercially available products and their sources.

**KEY WORDS : Tissue Engineering, Periodontal Regeneration, Bone Regeneration**

### INTRODUCTION

**Tissue engineering** is a term closely related to repair or replacement of portions of or whole tissues. It has also been applied to efforts to perform specific biochemical functions using cells within an artificially created support system. The term '**regenerative medicine**' is at times used synonymously with it.

It is defined as **an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ**<sup>1</sup>.

Use of barrier membranes to direct bone regeneration was first described in the context of orthopaedic research in 1959. The theoretical principles basic to guided tissue regeneration were developed by Melcher in 1976, who outlined the necessity of excluding unwanted cell lines from healing sites to allow growth of desired tissues. Based on positive clinical results of regeneration in periodontology research in the 1980s, research began to focus on the potential for re-building alveolar bone defects using guided bone regeneration. Barrier membranes were first applied in the mouth in 1982 in the context of regeneration of

periodontal tissues via GTR, as an alternative to resective surgical procedures to reduce pocket depths. This article discusses the various components of tissue engineering and also the different commercially available products.

### Current Tissue Engineering approaches include:

1. Design and growth of human tissues *in vitro* for later implantation to repair / replace defects<sup>2</sup>.
2. Implantation of cell-containing / cell-free devices inducing regeneration of functional human tissues<sup>3</sup>.
3. Establishing primary cell-lines, placing cells on or within structural matrices and implanting the new system inside the body (**Shinoka et al., 1995**)<sup>4</sup>.

### Periodontal wound healing: why is it different?<sup>5</sup>

In a periodontal apparatus, the wound margins are the avascular tooth and the gingival flap. Following surgery, clot forms at the tooth gingival interface. In periodontal

wounds, the wound margins are not opposing vascular margins but rather the rigid, nonvascular cementum on one hand and the connective tissue and epithelium of the gingival flap on the other.

The sequence of events in periodontal wound healing occur as,

- Absorption and adhesion of plasma proteins onto the root surface
- Development of a fibrin clot (within minutes)
- Accumulation of neutrophils and monocytes on the root surface- early phase of inflammation (within hours)
- Migration of macrophages into the wound followed by formation of granulation tissue- late phase of inflammation (within 3 days).

Development of connective tissue attachment at the root surface occurs within 7 days. However areas of fibrin clot in various stages of maturation may be observed.

### Mechanism of Bone healing<sup>6</sup>

The alveolar bone, forming an integral part of the periodontium undergoes healing in the following sequential cascade (**Fig. 1**):

- Chemotactic activation and migration of undifferentiated mesenchymal cells.
- Anchorage dependent cell- matrix attachment via fibronectin.
- Mitosis and proliferation of mesenchymal cells.
- Chondroblast differentiation and cartilage deposition.
- Cartilage mineralization, angiogenesis, vascular invasion and chondrolysis.
- Osteoblast differentiation and bone matrix deposition.
- Bone mineralization and hemopoietic marrow differentiation in newly formed ossicles.

### Periodontal Healing: Repair vs. Regeneration

Periodontal healing can occur through repair or regeneration. Periodontal repair implies healing after periodontal surgery without restoration of the attachment apparatus. However, the

preferred mode is via regeneration, which means healing after periodontal Surgery that results in the restoration of the attachment apparatus, namely, cementum, alveolar bone and periodontal ligament.

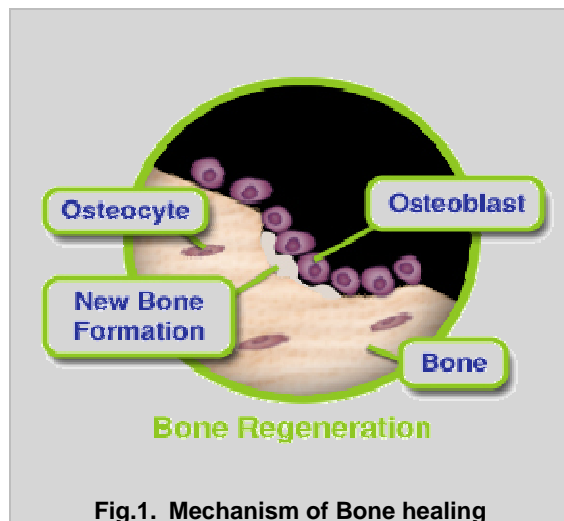


Fig.1. Mechanism of Bone healing

Stem cells responsible for regeneration of periodontal tissues reside within the the periodontal ligament. The innate regenerative potential has been investigated and appears to be dependent on the wound management.

**Melcher<sup>7</sup>** posulated the biological concepts at the base of periodontal regeneration. It was established that periodontal ligament cells have regenerative capacity while the alveolar bone and gingival epithelial cells do not<sup>8</sup>. This suggests that if the periodontal ligament cells are given preference, regeneration may consistently occur. Variables affecting periodontal regeneration include<sup>9</sup>,

- Bacterial contamination
- Innate wound healing potential
- Surgical procedure and technique
- Local site characteristics

**Cortellini and Tonetti<sup>10</sup>** suggested decision trees to guide the clinician with regard to therapy. Biologic observations in *Critical size Supraalveolar Periodontal Defect Model* are used to elucidate factors affecting regeneration of periodontal defects. These include-

- Wound maturation
- Tissue occlusion
- Primary intention healing

- Wound failure and membrane exposure
- Defect characteristics
- Space provision and innate regenerative potential

### Basic principles of tissue engineering

Tissue engineering consists of three key components (Fig. 2):

1. **Cells as building blocks**<sup>11</sup> : Cells are obtained from endogenous and exogenous sources. In many tissues, the endogenous sources are sufficient. Exogenous cells and mitogenic factors are necessary only when proliferation of precursor cells is impeded or their pool is diminished by previous surgery or disease. Examples of exogenous cells include, autologous parenchymal cells, allogeneic parenchymal cells, xenogenic cells, syngenic / isogenic cells, primary cells, secondary cells and marrow stromal stem cells. Certain defects requiring exogenous cells include articular and certain bony defects.
2. **Matrices / Scaffolds**<sup>11</sup> : Cells are implanted into an artificial structure capable of supporting three - dimensional tissue formation. These structures (scaffolds) are critical, both *ex* and *in vivo*. Scaffolds serve the following purposes:
  - i.Allow cell attachment, migration and proliferation
  - ii.Deliver and retain cells and biochemical factors
  - iii.Enable diffusion of vital cell nutrients and expressed products
  - iv.Insoluble regulator of cell function

*In vivo*, scaffolds also performs the following functions:

- i.Structurally reinforce the defect site to maintain the shape of the defect and prevent distortion of surrounding tissue.
- ii.Serves as barrier to prevent in growth of surrounding tissue, which may impede the process of regeneration.

The scaffold should be designed such that it should have adequate pore size : high porosity and high strength for immediate load bearing. The modulus of elasticity of matrix should match that of the surrounding tissues. Matrices should serve as analogues of extracellular matrix and should be biodegradable.

#### Examples of matrices:-

##### ABSORBABLE

###### 1. Synthetic polymers

*Polylactic acid*

*Polyglycolic acid*

###### 2. Natural polymers

*Collagen (types 1,2,3,4)*

*Collagen-glycosaminoglycan copolymer*

*Fibrin*

*Chitosan*

###### 3. Natural mineral

*Anorganic bone*

##### NONRESORBABLE

###### 1. Synthetic polymer

*Polytetrafluoroethylene*

###### 2. Synthetic ceramic

*Calcium phosphate*

3. **Soluble Regulators** : These are involved in cellular events in tissue repair: mitogenesis, migration, matrix synthesis and remodeling. They stimulate angiogenesis and serves as chemoattractant for specific cell types. Certain growth factors can act as soluble regulators and promote tissue regeneration.<sup>12</sup>

- i. **Platelet derived growth factor (PDGF)** is a group of polypeptides secreted by platelets during fracture healing and has both systemic and local actions. It is a powerful mitogen for connective tissue cells<sup>13</sup> but lacks potent bone induction properties. PDGF isoforms have strong chemotactic effect on connective tissue cells<sup>14,15</sup>. It has direct and indirect effect on bone resorption by upregulation of collagenase transcription<sup>16</sup> and increase in IL-6 expression in osteoblasts<sup>17</sup>. It also affects neovasculogenesis (important in wound healing). PDGF along with other

growth factors is involved in tissue regeneration<sup>18</sup>. *In vivo* application of PDGF increased bone regeneration in calvarial defects when a resorbable membrane was used as a carrier<sup>19</sup>.

Another factor, **Transforming Growth Factor (TGF)** regulates proliferation and differentiation of multiple cell types. Their isoforms are involved in influencing osteoprogenitor cells to form osteoblasts<sup>20,21</sup> and with matrix formation influencing osteoblasts to lay down bone matrix through osteogenesis. TGF $\beta$ 1 is the more abundant isoform<sup>22</sup>. They also activate fibroblasts, endothelial, osteoprogenitor, chondroprogenitor and mesenchymal stem cells. *In vitro*, TGF- $\beta$  promotes extracellular matrix production in periodontal ligament fibroblasts. TGF- $\beta$ 1 stimulates proliferative activity of periodontal ligament fibroblasts.

ii. **Fibroblast growth factor (FGF)** mediates effects by signaling via membrane spanning tyrosine kinases. Mutations in the receptors involved in FGF expression are associated with ossification abnormalities<sup>23</sup>. FGF is involved in angiogenesis, mesenchymal cell mitogenesis and bone development. Most abundant are FGF-1 and 2. Both stimulate osteoblast proliferation *in vitro* but do not increase collagen production or alkaline phosphatase in differentiated osteoblasts<sup>24-26</sup>.

iii. **Insulin like growth factor (IGF)** plays an important role in skeletal development. It is involved in late stage differentiation and activity of osteoblasts.

iv. **Bone morphogenic proteins (BMP)** are osteoinductive factors with bone enhancing potential belonging to TGF- $\beta$  superfamily. They regulate cartilage and bone formation during embryonic development and regeneration in postnatal life. Recombinant human bone morphogenetic protein-2 (rhBMP-2) induced bone formation and connective tissue attachment in animal models<sup>27</sup>. These facts indicate that periodontal regeneration may involve a complex, coordinated expression of signaling isoforms.

v. **Platelet rich proteins (prp)**<sup>28</sup> are soluble regulators defined as platelet concentration above baseline (published by Marx). The platelet count in PRP can exceed 2 million platelets per microliter. They consist of growth factors, WBC and phagocytic cells, native fibrogen concentration, vasoactive and chemotactic agents and high concentration of platelets. Functions of PRP include:-

- a. "Jump-starts" osteogenesis by releasing growth factors at the local site.
- b. Early consolidation of the graft.
- c. Speeds up mineralization by 1.62 to 2.18 times.
- d. Improves trabecular bone density by 15% to 30%.
- e. Allows early placement of implants into the grafted site by enhancing osteoconduction.
- f. Accelerates endothelial, epithelial, and epidermal regeneration.
- g. Stimulates angiogenesis, collagen synthesis, soft tissue wound healing, hemostatic response, decreases dermal scarring, and reverses the inhibition of wound healing caused by glucocorticoids.
- h. Antimicrobial effect.
- i. Augments extracellular matrix deposition (early wound closure).

All three components of tissue engineering, when present together, can promote regeneration of diseased tissue. Bone grafts and certain barrier membranes are used for this purpose.

### Bone Grafting: Rebuilding the ruins

Grafting in simple terms, is a surgical procedure that replaces missing tissue with material from the patient's own body, an artificial, synthetic, or natural substitute. Grafting in the periodontium is possible because of its high regenerative capacity. In bone grafting, eventual replacement of the graft material by native bone growth is seen. The biologic mechanisms that provide a rationale for bone grafting are osteoconduction, osteoinduction and osteogenesis<sup>29</sup>. Certain

drugs are also seen to enhance bone growth such as allendronate, etc.

The gold standard of bone grafting is taking the patient's own bone : **Autogenous bone graft**. Autogenous bone grafting has excellent fusion rates and no risk of rejection. Sources of autogenous bone graft include bone from healing extraction wounds, bone from edentulous ridges, bone trephined from within the jaw without damaging the roots, bone removed during osteoplasty and ostectomy, bony exostoses, mandibular ramus, parasymphyses and symphysis, and the maxillary tuberosity.

Obtaining autograft may not always be possible. It also inflicts surgical trauma on the patients body. Hence other techniques have been developed that use biological products as bone graft extenders or as bone graft replacements, one common example of which is the use of **allograft bone**.

**Deminerlized Bone Matrix (DBM)** is an allograft with proven osteoinductive properties<sup>30</sup> and biocompatibility<sup>31</sup>. It is harvested from cadavers or deceased individuals who have donated their bone for use in the treatment of living patients.

The use of **cadaver bone** is legal and common. Bone banks provide cadaver bones which are sterilized and tested to suit the patient's body. Osteotech, Inc. is the largest US supplier of cadaver bone. After extensive donor screening, bone tissue is processed by American Red Cross (ARC) and Musculoskeletal Transplant Foundation (MTF), which have been accredited by the American Association of Tissue Banks (AATB).

Bone undergoes a proprietary viral inactivation process that greatly exceeds the levels of known clinically relevant viruses that conceivably contaminate bone. The process reduces the likelihood of survival of an HIV virus to less than 1 in 2.8 billion.

**Tissue bank** is a faculty providing a complete spectrum of human tissues that has clinical usefulness as fresh or preserved allografts. Elimination of trauma and morbidity associated with secondary surgical procedures

used to harvest autografts, reduced costs and availability in unlimited quantity of allografts are advantages of tissue banks. Some commercial tissue banks are Rocky Mountain Tissue Bank and Pacific Coast Tissue Bank<sup>32</sup>.

Considerable effort has been made to develop osseous implant materials that are readily available, osteoinductive, biocompatible and similar in structure to human bone. Type I collagen fulfills some of these criteria for bone formation. This type of collagen stimulates osteoblast proliferation and differentiation of bone marrow cells. Calf bone / boplant (**xenograft**), treated by detergent extraction, sterilized and freeze dried, have been used for treatment of osseous defects.

There are several substances such as ceramics, calcium phosphates, and other synthetic materials which have similar biomechanical properties and structure to that of cadaver bone (**Alloplasts**). However, they are not biologically active. These products are currently only recommended for use as bone graft extenders. Synthetic hydroxyapatite (HAP), beta-tricalcium phosphate ( $\beta$ -TCP) and their composite are promising biomaterials specifically in the orthopaedic and dental fields, as their chemical composition is similar to that of bone. Due to the need for safer bone graft applications, these bone graft substitutes are gradually gaining increased acceptability. Hydroxyapatite and Hydroxyapatite and Beta – tri calcium phosphate based Bone Graft materials are both bio compatible and bio active. Hydroxyapatite phase is the major constituent. The porous and crystalline structure provides osteoconductivity and resorbability. Bioactive glass is also seen to favour formation of mineralized bone<sup>33</sup>.

### Barrier Membranes / Scaffolds

Exogenous cells lack the ability to grow in favoured 3D orientations and thus define the anatomical shape of the tissue. The scaffold therefore is a very important component for tissue engineering.

The therapeutic use of autologous platelet-rich plasma constitutes a relatively new biotechnology that has been a breakthrough in

the stimulation and acceleration of soft-tissue and bone healing. The efficiency of this process lies in the local and continuous delivery of a wide range of growth factors and proteins, mimicking the needs of the physiological wound healing and reparative tissue processes. Its use as a scaffold requires further research.

**Acellular regenerative tissue matrix** is donated human soft tissue that is processed to remove dermal cells (leaving behind a regenerative collagen matrix). It provides the components needed to allow the body to restore the missing tissue with excellent cosmetic results. As the intact acellular matrix is recognized as normal by the host, it decreases the likelihood of specific immunologic and non specific inflammatory reactions. This tissue matrix allows clinician to perform soft tissue regenerative procedures without discomfort and trauma to a second site.

Acellular regenerative tissue matrix is a processed tissue that comes from donors who are extensively screened and tested for presence of diseases including HIV and hepatitis. The processing procedure has been demonstrated to reduce HIV and hepatitis C surrogate virus to non-detectable levels. Additional testing for presence of pathogens is performed prior to and following processing to ensure that it is disease-free before release for patient care.

The processor accepts tissue from organization that meet the standards of the AATB and and the US FDA (Food and Drug Administration).

The processing involves separation of the epidermis from the basement membrane by passing through a buffered salt solution. Multiple cell types within the dermis are then solubilized and washed away using a patented series of non-denaturing detergent washes that rapidly diffuse into the dermis. Tissue matrix is then preserved using a freeze-drying process.

Dermal matrix graft shows promising results in procedures of gingival augmentation<sup>34</sup>. When compared with polytetrafluoroethylene membrane, both membranes were found suitable for

alveolar ridge augmentation<sup>36</sup>. Its crosslinked collagen membrane prototype did not provide any further advantage in animal model<sup>36</sup>. Also no statistical difference was seen in its use with PRPs<sup>37</sup>.

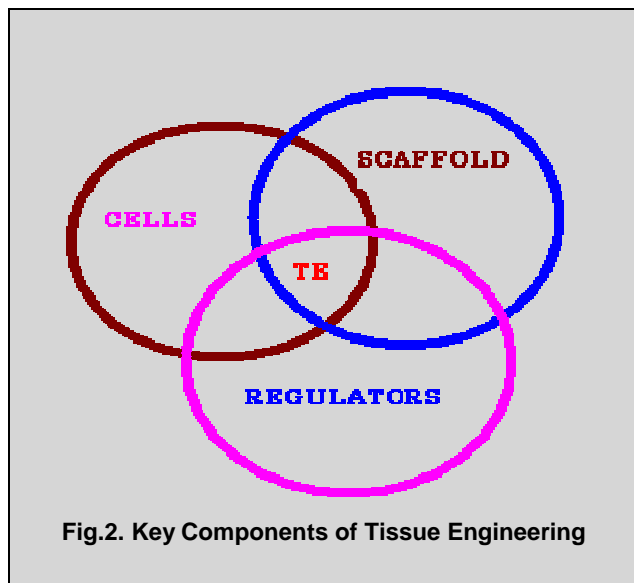


Fig.2. Key Components of Tissue Engineering

#### Sources of various commonly used products for tissue regeneration (Table 1)

##### Other products

1. **HealiGuide-** Sterile Bioresorbable Collagen Membrane for Guided Tissue Regeneration (GTR) of Periodontal Bone.
2. **OsseoGraft** – Sterile Bioresorbable Demineralized Bone Matrix Xenograft (Type 1 Collagen) in granule form for Bone Void Filling.
3. **OsseoMold\_-** Sterile Bioresorbable & Moldable Matrix Xenograft for Bone Void Filling.
4. **Osteograft/N.-** Targeted formation of bone structures: Osteograft/N has been supplying the essential calcium-phosphate minerals for effective formation of new bone for more than 10 years. Natural hydroxyapatite is the basic material of Osteograft/N – perfectly suited for augmentation of bone defects.
5. **Osteohealth-** Bio-oss collagen.

Although tissue engineered substitutes and grafts have been used with some success for

several years, most clinical applications of TE constructs need to be considered as experimental at this time due to the following limitations:

1. Cell source
2. Stable 3-D constructs
3. Vascularization
4. Interfacial stability
5. Sterilization
6. Cost
7. Survivability
8. Regulatory considerations

### Future Directions

Achieving and maintaining an integrated assembly of fully differentiated, mature cell phenotypes throughout the *in vitro* and postimplantation *in vivo* stages are major challenges to TE. Recent developments in two fields may help meet these objectives:

1. New techniques have been developed for real-time monitoring of partial pressures of O<sub>2</sub>. These may be used to provide feedback data in bioreactors in the *in vitro* phase of cell growth and differentiation and be used to monitor with minimal invasion of the metabolic state of TE constructs during their incorporation phase *in vivo*.
2. A noninvasive biophotonics system for real-time *in situ* monitoring of cell viability and cell death, cell phenotype, cell differentiation and cellular responses to positive and negative stimuli, including severe toxins. The Bio-Raman spectroscopic cellular fingerprints obtained by this system do not require labels or markers and provide data on single or clustered cells growing in 2-D or 3-D organoids. Application of this method using fibre optic arrays to monitor TE constructs before and after implantation may overcome some of the clinical limitations of TE technology listed above.
3. Novel spectroscopic techniques have been developed to monitor structural changes in collagen fibril alignment in loaded tendons and are being applied to TE constructs.

4. Gene therapy uses purified preparations of genes or fraction of gene to treat diseases. Gene delivery involves two basic modalities:

- a. *In vivo*- Gene constructs like plasmid DNA or viral particles are entrapped within the scaffold which on implantation, cause host cells to migrate into the implant; take up the gene constructs producing encoded proteins.
- b. *Ex vivo*- Cultured cells are transfected (non viral delivery systems) or transduced (viral delivery systems) with gene constructs *in vitro* before transplantation into tissue defects.

**Jin et al**<sup>38</sup> demonstrated that direct *in vivo* gene transfer of PDGF-B stimulated tissue regeneration in large periodontal defects.

**Dunn et al**<sup>39</sup> demonstrated successful regeneration of alveolar bone defects around implants by *in vivo* gene delivery of BMP7 in a collagen gel carrier.

5. Creation of functional tissues and biological structures *in vitro* has been attempted using tissue culture. Tissue culturing promotes survival, growth and inducement of functionality. The basic requirements of cells must be maintained in culture, which include oxygen, pH, humidity, temperature, nutrients and osmotic pressure. In many cases bioreactors are employed to maintain specific culture conditions.

### CONCLUSION

In conclusion, the rapidly expanding fields of stem cell research and Tissue engineering are now converging to form the new discipline of regenerative medicine, the aim of which is to restore normal structure and function of the periodontal tissues. Although, regenerative medicine strategies using stem cells are already in clinic, most are still at the bench or animal testing stages. As new therapeutic approaches emerge, extensive, rigorous clinical trials must be undertaken to ensure that this very promising area of research delivers justifiable hope and not hype.

TABLE 1.products of Tissue regeneration

NATURAL SOURCES	COMMERCIALY AVAILABLE PRODUCTS
<b>I. ANIMAL SOURCES</b>	
<b>1. BOVINE SOURCE</b>	<ol style="list-style-type: none"> <li data-bbox="651 661 1406 782">1. <b>G-graft</b>- has hydroxyapatite with collagen granules, as naturally present in the body. It is derived from <b>bovine bone</b> and can be used at most sites requiring bone graft.</li> <li data-bbox="651 822 1406 889">2. <b>Bio-Oss</b>- is a natural bone substitute material obtained from mineral portion of <b>bovine bone</b>.</li> </ol>
<b>2. PORCINE SOURCE</b>	<ol style="list-style-type: none"> <li data-bbox="651 943 1430 1064">1. <b>DynaMatrix</b>- is a biological extracellular membrane (ECM) that remodels. Derived from <b>porcine</b> small intestinal submucosa, DynaMatrix is a strong pliable tissue providing scaffold for host cells.</li> <li data-bbox="651 1104 1430 1360">2. <b>Emdogain</b>- It was introduced in 1996. When applied to root surfaces of affected teeth after cleaning by scraping, Enamel matrix derivative, and the amelogenin component, induce formation of acellular cementum and contribute to the regeneration of periodontal tissues by stimulating proliferation of mesenchymal cells, inhibiting proliferation of epithelial cells and promoting secretion of certain growth factors, such by periodontal ligament cells.</li> </ol>
<b>3. MARINE SOURCES</b>	<ol style="list-style-type: none"> <li data-bbox="651 1413 1373 1534">1. <b>Bone medik</b>- coralline hydroxyapatite bone graft substitute, nonosteogenic and similar in structure and composition with human cancellous bone. It is derived from the exoskeleton of <b>marine coral</b>.</li> <li data-bbox="651 1548 1398 1628">2. <b>Periocol</b>- is a Type 1 collagen membrane derived from <b>fish sources</b>. It is non-toxic, non-allergenic, non-immunogenic and biocompatible.</li> </ol>
<b>II. HUMAN SOURCES</b>	<ol style="list-style-type: none"> <li data-bbox="651 1682 1414 1776">1. <b>Dembone</b>- is Demineralized Freeze-dried Cortical Bone Powder. It is prepared from cortical bone harvested from carefully screened <b>human donors</b>. It contains BMPs.</li> <li data-bbox="651 1817 1414 1911">2. <b>MinerOss</b>: blend of mineralized allograft cancellous and cortical chips that provide an osteoconductive scaffold for bone regeneration.</li> <li data-bbox="651 1951 1414 2107">3. <b>Alloderm GBR</b>- is a collagen based barrier membrane prepared from dermis that is processed to remove its cellularity and subsequently cryoprecipitated to remove its antigenicity. Structurally, it is similar to alloderm used for recession coverage.</li> </ol>



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