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Review Article

A boomy century of periodontal regeneration with biological mediators

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ABSTRACT

With the advanced and improved knowledge of bone regeneration on the molecular level, some of key molecules that alter the complicated physiological method were identified, and are already in scientific use or beneath research to enhance bone restore. Of those molecules, BMPs were the maximum considerably studied, as they are robust osteoinductive elements. They result in the mitogenesis of mesenchymal stem cells (MSCs) and different osteoprogenitors, and their differentiation in the direction of osteoblasts. Other growth factors except BMPs which have been implicated through out the bone regeneration, with one-of-a-kind features with respect of cell proliferation, chemotaxis and angiogenesis, are also being investigated or are presently getting used to reinforce bone restore, which include platelet-derived growth factor, transforming growth factor- β, insulin-like growth factor-1, vascular endothelial growth factor and fibroblast growth factor, amongst others. One present day technique to enhance bone regeneration and soft-tissue recovery with the aid of using nearby application of growth factors is the use of platelet-rich plasma, an extent of the plasma fraction of autologous blood with platelet concentrations above baseline, that is wealthy in most of the aforementioned molecules. This overview focuses and target on the biological mediators that regulates key cellular events which have a capacity to induce the method of tissue repair and regeneration.

1. Introduction

Periodontitis is an inflammatory disease of supporting tissues of teeth caused by group of specific micro-organisms resulting in progressive destruction of periodontal ligament and alveolar bone with pocket formation, recession or both.[1] The maximum serious effect of chronic inflammation is bone destruction because of the extension of inflammation spreads from the marginal gingiva into the supporting structures of periodontium.

Periodontal disease may also adjust the morphologic capabilities of the bone and decreases the bone height. Bone loss patterns related to periodontal disease are varied, and type of bone loss is unique in different areas of oral cavity.[2] Resective and Regenerative are the two procedures which may be used to get rid of the bone defects triggered due to periodontitis. Resective surgery seeks to obliterate the periodontal defects with the aid of using elimination of gingival and bony walls partition by gingivectomy, osseous resection and apically positioned flaps however Regenerative surgery seeks to eradicate the anatomical defects with the aid of using developing a new bone, periodontal ligament and coronally displacing gingival attachment and margin. Regeneration is described as the reproduction or reconstitution of a lost or injured part, with form and function of lost structures restored. Periodontal regeneration consists of regeneration of alveolar bone, cementum, PDL, and gingiva. Vertical or angular defects are amenable to regenerative periodontal surgery with the aid of using a number of bone graft materials and

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bone substitutes or bioactive molecules. Often, a couple of substances may be used with larger huge defects to reap an excellent result. The use of bone grafts for reconstructing osseous defects caused by periodontal diseases dates back to Hegedeus (1923) and turned into revived by Naber's or O Leary in 1965.[3]

Bone grafting is the most common aspect of regenerative remedies these days and is typically crucial for restoring all types of periodontal supporting tissues. A huge variety of bone grafting materials, inclusively bone grafts and bone graft substitutes, had been implimented and evaluated clinically, inclusive of autografts, allografts, xenografts, and Alloplasts (synthetic/semi synthetic materials). Bone replacement grafts (bone grafts and bone graft substitutes) offer a structural framework for clot development, maturation, and remodelling that helps in bone formation in osseous defects. Bone grafting materials should own attributes of biocompatibility (missing an immunogenic response) and osteoconductivity (imparting a shape and floor topography that allow cellular attachment, proliferation, as well as migration). Osteoinductive grafting substances, which include a demineralized bone matrix (DBM), offers a biologic stimulus (proteins and growth factors) that induces the development of mesenchymal stem cells and different osteoprogenitor cells in the direction of osteoblast lineage. They are used as a scaffold to permit bone formation and increase the potential of wound healing and act as a mineral reservoir which allows in new bone formation.

2. Growth Factors

The application of native growth factors has been studied to boost the healing and regeneration capability of periodontal surgery. Growth factors-enhanced grafts are created with the aid of using recombinant DNA technology. They include either human growth factors or morphogens (BMPs in conjunction with a carrier medium, reminiscent of collagen). Growth factors are present at low concentrations in bone matrix and plasma, however perform necessary biological functions. Growth factors attaches to transmembrane receptor molecules on human cells surface and induce protoplasm cascade reactions by means of intracellular environment, which can translate this activity to protein kinase which is a macromolecule enzymes which creates a series of events leading to transcription of mRNA and end result is the formation of intracellular and extracellular protein.[4] These factors, stays in extracellular matrix of bone which includes transforming growth factor-beta (TGF-β), insulin-like growth factors I and II, platelet-derived growth factor (PDGF), fibroblast growth factor, and BMPs.

Growth/ differentiation factors constitute a large family of polypeptidic molecules that modulate cell responses like cell attachment/adhesion, cell survival, proliferation, chemotaxis and differentiation. Insulin like growth factor (IGF-1) and TGFβ mostly modulate the synthesis of the cartilage matrix while basic fibroblast growth factor (bFGF) that has a powerful and strong mitogenic factor to stimulates the differentiation of chondrocytes.

Levander observed, in 1938, ectopic bone formation around periosteal-placed and surface placed free bone grafts in non-skeletal sites. Urist later showed that protein extract which are macromolecules in nature from demineralised bone matrix was ready to induce bone formation and named, in 1971, the accountable factors bone morphogenetic proteins. The tissue forming capability of bone morphogenetic proteins is closely regarding the delivery matrix of the agent which is not a species specific.

Bone morphogenetic proteins are a category of transforming growth factor-β superfamily, that could be a massive cluster of proteins have an affect on cell growth, migration and differentiation, and play a regulative role in tissue physiological state and repair in adult organisms. The transforming growth factor β in taxonomic category includes osteogenic proteins, cartilage-derived morphogenetic proteins and growth differentiation factors and bone morphogenetic protein-like molecules. A minimum of 30 bone morphogenetic proteins are identified. Bone morphogenetic protein-2 to bone morphogenetic protein-8 have high osteogenic potential.

Bone morphogenetic proteins will induce an area immediate action, attaches to extracellular antagonists at the site or location of secretion, or move along with the extracellular matrix proteins and afterward the target cells. In vitro, mesenchymal stem cells differentiate into osteoblasts which exhibit an excellent number of bone morphogenetic macromolecules receptors. Mesenchymal stem cells additionally synthesize the bone morphogenetic protein antagonist's noggin, gremlin, follistatin and sclerostin that have a capability of interference in osteogenesis as mesenchymal stem cells which further differentiate into osteoblasts. Bone morphogenetic protein-blocking factors are necessary in physiologic state of bone turnover and regulation. Bone morphogenetic protein-9 may be extremely osteogenic as a result of it is unable to attach to those regulative molecules (i.e., noggin). Osteoblasts produces bone morphogenetic proteins similarly to their antagonists by a fragile regulatory mechanism throughout bone formation and bone remodelling process.

3. Potential Role of Growth Factors In Periodontal Regeneration

1. To stimulate and have an additive effect on cell proliferation. E.g., Platelet-derived GF (PDGF)
2. To boost the function of cells and cell differentiation. E.g., BMP
3. To stimulate matrix synthesis. E.g., TGF-β
4. To act as co-factor for gene expression.

4. Biological Principles of Growth Factors

Biological explanation for the utilization of many growth factors is that these biologically active molecules are ready to regulate and perform proliferation, accelerate activity and / or stimulate differentiation of key cells concerned within the periodontal regenerative approach, such as cementoblasts, periodontal ligament fibroblast and osteoblast, encouraging the victorious regeneration of lost tissue.

Among varied growth factors widely used. Out of which Bone Morphogenic Proteins needs special mention because they give rise to osteogenic precursor cells into osteogenic cells and have shown tremendous bone growth in several clinical research.[5] Different growth factors with the exception of BMP includes

1. Transforming growth factor-β
2. Platelet derived growth factor
3. Fibroblast growth factor
4. Insulin-like growth factor
5. Vascular endothelial growth factor

5. Transforming Growth Factor β

The term transforming growth factor beta could be applicable to the taxonomic group of growth and differentiating factors. Bone morphogenic protein (BMP) macromolecules is a member of this family and contains a minimum of 13 BMPs. TGF-β1 and TGF-β2 are proteins that have relatively molecular weight of roughly 25kd. Similar to PDGF, they are synthesized and found in macrophages additionally as in different cell types. When they are released by platelet degranulation or actively secreted by macrophages, they start acting like a paracrine growth factor and have an effect on cells similar to fibroblasts, marrow stem cells and preosteoblasts. Each of those target cells has the potential and power to synthesize and secrete its own TGF-β proteins. Therefore, it represents a mechanism for sustaining a long-term healing process and even starts developing into a bone remodelling factor.

Transforming growth factor β has 5 isoforms, that have numerous biologic effects and functions. It is found at the highest concentration in platelets however quantitatively most swarming in bone, being present at a concentration of roughly 200 μg / kg of tissue. It is produced by osteoblasts, stimulates the expression of bone matrix proteins and suppresses the degrading activity of matrix metalloproteinases and numerous other enzymes. Transforming growth factor-β additionally induces the differentiation or proliferation of osteoblastic cells whereas inhibiting the formation of osteoclast precursors associated in higher concentrations, could exert a restrictive result on mature osteoclasts. The foremost necessary and vital functions are chemotaxis and mitogenesis of osteoblast precursors. They even have the power and potential to stimulate osteoblast deposition of the collagen matrix of wound healing and bone. In addition, it also inhibits osteoclast formation therefore, favouring bone formation over resorption.

Smads are the signalling pathways from the membrane of the effector cell to the nucleus for the transforming growth factor-β taxonomic category. Smad-proteins are found in many animal species, that has enabled researchers to use additional straight forward models to know the transcriptional events of cells affected once cytokine stimulation. In distinction to bone morphogenetic proteins, transforming growth factor-β does not induce ectopic bone formation. Transforming growth factor-β release (transforming growth factor-β1, -β2 and -β3), additionally because of the release of bone morphogenetic proteins 1–8 and growth differentiation factors 1, 5, 8 and 10, are swarming throughout the healing of fractures.

Signalling molecules have an importance throughout fracture healing could be categorized into three groups:

1. The pro-inflammatory cytokines (interleukin-1, interleukin-6 and tumour necrosis factor-α),
2. The transforming growth factor-β superfamily (bone morphogenetic proteins and transforming growth factor-β) and various other growth factors (platelet-derived growth factor, fibroblast growth factor and insulin like growth factors I and II) and
3. The angiogenic factors [vascular endothelial growth factor, angiopoietins 1 and 2 and matrix metalloproteinases (that degrade bone and cartilage and alter the vessel invasion)].

The cytokines interleukin-1, interleukin-6 and tumour necrosis factor-α occur initially within the repair cascade. These cytokines are produced by macrophages and mesenchymal cells which are present in the periosteum layer and counter to injury with a peak in expression throughout the initial 24 hours, however, also active within the cartilaginous and remodelling section of a fracture. These cytokines exert chemotactic activity on inflammatory cells, increase cellular matrix formation and stimulate angiogenesis.

6. Platelet Derived Growth Factor (PDGF)

PDGF, a glycoprotein that has a molecular mass of roughly 30kd. It was first described within the alpha granules of platelets, however also can be synthesized and secreted by cells like macrophages and endothelium. The PDGFs are a family of dimeric disulfide – bound GFs that exert their biological effects by activating a pair of structurally connected with tyrosine kinase receptors, the PDGF-α and PDGF-β receptors.

There are approximately 0.06ng of PDGF per one million platelets, a incontrovertible fact that emphasizes this molecule's great potency. Its mechanism is to activate

plasma membrane receptors on specific target cells and results in the development of high-energy phosphate bonds on internal cytoplasmic signal proteins that then activate the signal proteins which initiate a selected activity within the specific target cell. The most specific activities of PDGF are mitogenesis, angiogenesis and macrophage activation.

Platelet-derived growth factor could be a potent mitogen for mesenchymal cells from the periosteal layer. Platelet-derived growth factor is synthesized by platelets, monocytes, macrophages, endothelial cells and osteoblasts. The platelet derived growth factor is consisting of two polypeptide chains (A and B) and these kinds of chains form either a heterodimer or a homodimer. Of the three platelet-derived growth factors (platelet-derived growth factor AB, AA or BB), platelet-derived growth factor BB is biologically most potent. The foremost recently discovered PDGF-C and -D.[6]During the initial stages of fracture healing, platelet-derived growth factor could be a powerful and strong chemotactic agent for inflammatory cells and stimulation for osteoblasts and macrophages. Platelet-derived growth factor (PDGF) was hypothesized on the bone healing of unilateral tibial osteotomies in rabbits and discovered that it had a stimulatory results on fracture healing.

PDGF was the primary GF to be evaluated in preclinical periodontal and peri-implant regenerative studies. Proliferation, migration and matrix synthesis were determined on cultures of periodontal cells excited by PDGF, together with gingival and PDL fibroblasts, cementoblasts, preosteo-blasts and osteoblastic cells. It has been shown that PDGF includes a chemotactic effect that enhances collagen synthesis, and increase hyaluronate synthesis by gingival fibroblasts and fibroblast proliferation. Furthermore, if additionally, to a culture with osteoblast-like cells, PDGF can regulate the ALP and osteocalcin expression. Lynch et al 1991 applied PDGF along with insulin-like growth factor-1 (IGF-1) in dogs, and therefore the results incontestable have an excellent effectiveness in periodontal regeneration.[7] Furthermore, the results of clinical trials discovered that the synergistic effect of these 2 growth]factors results in the stimulation of bone regeneration in periodontal defects in humans, too.[Even if used alone, PDGF will considerably stimulate the formation of new cementum and formation of collagen. Molecular cloning along with biological research and large-scale purification have allowed the production of recombinant human PDGF, that has been mixed with β-TCP and created commercially available to clinicians (GEM 21s®, Osteohealth, Shirley, NY, USA).

7. GEM 21 (Osteohealth Company): Human Platelet Derived Growth Factor in Tri-Calcium Phosphate

GEM is growth factor enhanced matrix approved by FDA in 2005 advertising a brand-new dental bone filling device. It is a modern aggregate of rhPDGF- and β-TCP. GEM 21S(Osteohealth/ Luitpold Pharmaceuticals, Inc, Shirley, NY) is an artificial grafting device for bone and periodontal regeneration consists of a purified recombinant growth factor and a synthetic calcium phosphate matrix. Its mechanism is that PDGF stimulates migration and enhance proliferation of osteoblasts, fibroblasts and cementoblasts, results in the formation of new bone, PDL and cementum. Randomised controlled trial recommended that GEM 21S produced a significantly greater extent of radiographic bone fill, but did not significantly gain in attachment level as compared to control (tri-calcium phosphate alone). The radiopacity of GEM 21S is comparable to that of bone and diminishes as GEM 21S is resorbed. The radiopacity of GEM 21S have to be taken into consideration when comparing radiographs as it is able to mask underlying pathological conditions.

7.1. GEM 21S has two components which is sterilized

1. Synthetic beta-tricalcium phosphate (ß-TCP) [Ca_3 (PO4)] is a distinctly porous, resorbable osteoconductive scaffold or matrix that gives a framework for bone ingrowth, aids in inhibiting the collapse of the soft tissues and enhance the stabilization of the blood clot. Pore diameters of the scaffold are particularly designed for bone ingrowth and varies from 1-500 μm. The particle size ranges from 0.25-1.0 mm.
2. Highly purified, recombinant human platelet-derived growth factor-BB (rhPDGF-BB).

7.2. GEM 21S is supplied and available in a single use kit. Each GEM 21S kit composed of

1. One cup containing 0.5 cc of ß-TCP particles (0.25 to 1.0 mm); and
2. One syringe that contains a solution of 0.5 ml rhPDGF-BB (0.3 mg/ml).

The contents of the cup of ß-TCP are supplied sterile with the aid of using gamma irradiation.

7.2.1. Indications

GEM 21S is indicated to treat the subsequent of periodontally associated defects:

1. Intrabony periodontal defects;
2. Furcation periodontal defects; and
3. Gingival recession associated with periodontal defects.

McGuire et al., 2006 discovered similar scientific results to SCTG, when rhPDGF-BB +β -TCP was used to cover gingival recession. In an RCT, approach with rhPDGF-BB stimulated a significant increase in the rate of CAL

gain, reduced gingival recession at 3 months post-surgery, and improved bone fill as compared to a beta-TCP bone substitute at 6 months.[8]

8. Fibroblast Growth Factor (FGF)

Fibroblast growth factor is produced by monocytes, macrophages, mesenchymal cells, chondrocytes and osteoblasts. Fibroblast growth factor is critically necessary in chondrogenesis and bone resorption. The target specific cells are mesenchymal and epithelial cells along with chondrocytes and osteoblasts. Two isoforms exist: α-fibroblast growth factor and β-fibroblast growth factor. The α-fibroblast growth factor plays an important role in chondrocyte proliferation, and β fibroblast growth factor (the more strong and powerful isoform) is produced natively in bone at an early stage of fracture healing and is essential for the maturation of chondrocyte.[9]

Fig. 1:

The angiogenic and fibroblast stimulatory properties of FGF-2 at the stage of wound healing and its chemotactic and proliferative effects directly results on PDL cells recommended its use for periodontal regenerative therapeutic approaches.[10] In preclinical studies, this GF become evaluated for the treatment of different types of periodontal bone defects, in dogs and non-human primates.

9. Insulin Like Growth Factor (IGF)

These are a family of single chain serum proteins that may share 49% homology in sequence with pro-insulin. IGF-1 and IGF-2 are two polypeptides from this group. IGF-1 acts as a progression factor and potentially stronger which further stimulates bone formation and have a direct effect on PDL cells. IGF-1 is likewise critically essential for bone remodelling and maintenance of skeletal mass and plays an important role in age-related osteoporosis. Insulin-like growth factor-II acts in the later stages of endochondral bone formation. IGF-1 is capable of inhibiting apoptosis in fibroblasts by activating the multiple signal transduction pathways. It has additionally been proven to regulate DNA and protein synthesis in PDL fibroblasts in vitro and to enhance soft tissue wound healing in vivo.

The role and role of insulin-like growth factors in bone formation has been disputed. Sources of insulin-like growth factor are bone matrix, endothelial cells, osteoblasts and chondrocytes. The insulin-like growth factor-binding proteins modulate the action of insulin-like growth factor in a cell-specific manner. In the later stages of fracture healing (i.e., endochondral ossification), and in bone remodelling, cartilage and bone are degraded by matrix metalloproteinases. This permits an angiogenic elements to regulate vessel ingrowth by either the vascular endothelial growth factor-dependent pathway or the angiopoietin-dependent pathway.

10. Vascular Endothelial Growth Factor

Vascular endothelial growth factor is present in four isoforms (A, B, C and D) and the protein is produced with the aid of using variety of cells, inclusively macrophages, smooth muscle cells and osteoblasts. Vascular endothelial growth factor induces the migration and proliferation of endothelial cells with the aid of using the usage of transmembrane adhesion proteins (integrins) and transmitting the signals from the extracellular surroundings to the cellular genes. Vascular endothelial growth factor additionally induces relaxation in the cell-to-cell contact of endothelial cells, which may further result in hyperpermeability of blood vessels. In addition, the stimulated endothelial cells produce matrix degrading enzymes and facilitate cell migration. Vascular endothelial growth factor become these days proven to be an important critical aspect for boosting and directing cellular motility.

Vascular endothelial growth factor, which mixed with a coralline scaffold both coated with a control-plasmid DNA (a small cellular inclusion consisting of a ring of DNA that is not in a chromosome but is capable of autonomous replication), VEGF-plasmid DNA, loaded with mesenchymal stem cells (BMSC) transfected with control plasmid or with both stem cells and the VEGF plasmid confirmed to enhance recovery in large bone defects, where bone substitutes will not be vascularized and replaced by a fresh bone.[11] The application of gene transfer, which is a modern technology, represents a unique opportunity for the local administration of growth factors.[12]

11. Healing Promotive Factors

Healing promotive factors such as growth factors were considerably used to treat bony defects and for Osteoinduction. Some growth factors such as vascular endothelial growth factor (VEGF), TGF-β, PDGF, and BMPs such as BMP-2, BMP-7, and IGF are present in the healthy bone matrix and are expressed during bone healing.[13,14] These factors can alter vascularization and induce proliferation and differentiation of the osteoblasts and their precursors, so that they may be beneficial in

enhancing the healing processes.

'Orthobiologics' and the general idea to stimulate the native 'biology' with the aid of applying growth factors (mainly BMPs, due to the fact that those are the maximum amazing osteoinductive molecules) can be tremendous for bone regeneration or may be for acceleration of physiological bone healing to decrease the length of fracture treatment. Their scientific use, either alone or combined with bone grafts, is continuously increasing. However, there are numerous problems regarding their use, safety (because of the supraphysiological concentrations of growth factors required to obtain the desired osteoinductive effects), excessive cost of treatment, and more importantly, the capability for ectopic bone formation.

12. Growth Factros Delivery System

Several matrices and delivery systems have been used and evaluated for their efficacy and biocompatibility as carrier for GFs. Two common types of polymeric materials used in GF delivery strategies are natural collagen-derived materials and synthetic polymers of lactic and glycolic acid (i.e., Poly [lactide-co-glycolide]). Extracellular matrix-derived macromolecules inclusively of collagen were used for decades as a biomaterial application, and it is now possible to produce synthetic analogues of extracellular matrix proteins using recombinant DNA technology.[15]

Carriers may be of various kinds like solids, gels or combinations. A range of recent new injectable materials such as hydrogels is likewise being developed for GF delivery applications and have been of unique interest. These injectables are mainly appealing due the fact that in clinical application, they could permit for minimally invasive delivery of inductive molecules.[15]

13. Bone Morphogenetic Proteins

The demineralization of the cortical bone allograft improves the osteoinductive capability, and exposing proteins to accelerate the bone formation. These proteins are together known as bone morphogenetic proteins (BMP). They are composed of a collection of acidic polypeptides belonging to the transforming factor β gene super family which have been cloned and sequenced. BMP stimulates recent bone formation through Osteoinduction that further inducing a pluripotential stem cells to distinguish into osteoblasts. BMPs produce more than one consequence on bone through:

1. Behave like as mitogens on undifferentiated mesenchymal cells and osteoblast precursors;
2. To generate the expression of osteoblast phenotype (e.g., enhancing alkaline phosphatase activity in bone cells;
3. Also behave as a chemo attractant for mesenchymal cells and monocytes along with binding to extracellular matrix type IV collagen.

In the 1960s, Urist et al discovered the bone morphogenetic proteins (BMPs): soluble bone matrix glycoproteins that results in differentiation of osteoprogenitor cells into osteogenic cells. Their capability to behave as a bone inductive agent, which will be utilized in number of applications wherein bone technology was needed and ultimately be recognised. Urist advanced the idea the of autoinduction in 1965 and, 6 years later, brought the term Osteoinduction, an essential principle of bone regeneration energized through the action of BMPs. Wozney et al. cloned BMP genes in 1998. Following the isolation and expression of BMP complementary DNA, two recombinant human proteins – rhBMP-2 and rhBMP-7 (OP-1) – are recent commercially available.

13.1. Reddi's classification[16] of BMPs distinguishes 4 subgroups

1. BMP-2 and 4
2. BMP-3 (osteogenin
3. BMP-5, 6, 7 (osteogenic protein-1), 8 (osteogenic protein-2), 8B (osteogenic protein-3), 9, 10, and 11.
4. BMP-12 (growth differentiation factor-7 or cartilage-derived morphogenetic protein-3), BMP-13 (growth differentiation factor-6 or cartilage-derived morphogenetic protein-2), BMP-14 (growth differentiation factor-5 or cartilage-derived morphogenetic protein-1), and BMP-15.

14. Structure of BMPs

The structure BMPs usually composed of 3 parts: signal peptide, pro-peptide, and a mature region. The pro-peptide and mature region include seven conserved cysteine residues features of TGF-β superfamily. BMPs are synthesized inside the cell in a precursor shape with a hydrophobic secretory leader and a pro-peptide sequence joined to the mature region. Proteolytic cleavage frees the mature region and dimerize with different BMPs. Dimeric molecules may be either homodimers, where both subunits are the same/or heterodimers composed of two distinct subunits. Structural and chemical variations among the homo-dimeric and heterodimeric forms may be responsible for variations of their biologic capability and binding features.

BMPs are dimers and are held collectively through a crucial intermolecular disulfide linkage. The dimeric conformation is crucial for bone induction and morphogenesis. Each of the two monomers is biosynthesized as a precursor molecule of over 400 amino acids. However, mature BMP monomer derived through proteolytic processing is an approximately 120 amino acid polypeptides. BMPs are pleiotropic signals. Pleiotropy is the property of a gene or protein to act in a multiplicity of

Table 1: Subtypes of bone morphogenetic proteins and their respective primary and common functions

BMP Subtype	Alternative Name	Functions
BMP-1	——-	• Not a member of the TGF- superfamily and now no longer technically a BMP • Cleaves procollagens I, II, and III to supply fragments that self-accomplice into mature collagen fibrils
BMP-2	BMP-2a	• Role in cartilage and bone formation for the duration of embryogenesis and also apoptotic signal molecules • Possible position in morphogenesis • Active position within side the induction of osteogenesis from lineage-specific differentiation of mesenchymal progenitor cells and in mature osteoblasts • Expressed particularly in lung, spleen, and colon
BMP-2	Osteogenin	• Role in cartilage and bone formation and act as chemoattractant • Induces synthesis and secretion of TGF- 1 with the aid of using monocytes (certain factors associated with collagen synthesis and related matrix constituents)
BMP-3b	GDF-10	• Expressed particularly in lung, ovary, and small intestine • Participate in endochondral bone formation in mature animals • Expressed only in cerebellum, lung, pancreas, testis, and femur • Regulatory developmental molecule
BMP-4	BMP-2b	• Involvement is particular towards bone induction, limb formation, fracture repair, and tooth development • Induces formation of embryonic hematopoietic tissue
BMP-5	———-	• Expressed only in lung and kidneys • Role in initial developmental skeletal patterning • Expressed mainly in lungs and liver • Act importantly in bone formation
BMP-6	Vgr-1	• Actively participate in the induction of osteoblast lineage-specific differentiation of mesenchymal progenitor cells • Higher concentration found in cartilage of the foetus
BMP-7	OP-1	• Role in cartilage and skeletal patterning, lens formation, and acts as an initial inducer of glomeruli formation • Possible osteoinductive potential for epithelial osteogenesis • Function as bone homeostasis and calcium regulation • Expressed mainly in brain, kidney, and bladder
BMP-8	BMP-8a, OP-2	• Role in the maintenance of spermatogenesis
BMP-8b	OP-3	• Expressed at high levels during pregnancy in the decidual cells of the uterus • Found only in mice and prevent male adult germ cell apoptosis • Expressed with increased levels in the placental trophoblasts
BMP-9	GDF-2	• Actively participate in the induction of osteogenesis from lineage-specific differentiation of mesenchymal progenitor cells and in mature osteoblasts • Role in hepatic reticuloendothelial and nervous system
BMP-10		• Role in cardiac development (trabeculation of embryonic heart)
BMP-11	GDF-11	• Role during embryogenesis for development of mesodermal and neuronal (dorsal root ganglia and dorsal lateral region of the spinal cord) tissues.
BMP-12	GDF-7, CDMP-3	• Involved in chondrogenesis • Role in tendon/ligament formation and repair
BMP-13	GDF-6, CDMP-2	• Involved in chondrogenesis • Role in tendon/ligament formation and repair • Expressed mainly in long bones during embryonic development
BMP-14 (Cartilage derived morphogenetic)	GDF-5, CDMP-1	• Involved in chondrogenesis • Role as neurotrophic, survival-promoting molecule for dopaminergic neurons • Enhances tendon healing and bone formation mainly in long bones during embryonic development
BMP-15	GDF-9b	• Role in ovarian development and function

Legend: BMP=Bone Morphogenetic Protein; CDMP=Cartilage-Derived Morphogenetic Protein; GDF=Growth/Differentiation Factor; OP=Osteogenic Protein; Vgr= Vegetal Related.

steps. BMPs act on the three key steps in the sequential cascade of bone morphogenesis including chemotaxis, mitosis and differentiation of transient stage of cartilage and the permanent induction of bone.

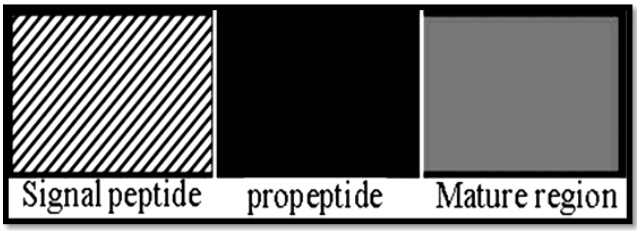

Fig. 2: Structure of bone morphogenetic protein (BMPs)

Bone Morphogenetic Proteins (BMPs) are effective osteoinductive proteins and features efficaciously been carried out for the stimulation of fracture healing, treatment of non- and delayed unions in various research papers. Two BMPs are commercially available in their recombinant human (rh) form: rhBMP-2 and rhBMP-7 (additionally named osteogenic protein-1, OP-1). BMPs elicit their biological movements through their interaction with each other especially types I and II BMP receptors. There are two type of BMP receptors, types IA and IB.[16] BMPs receptors are protein kinases that phosphorylate cytoplasmic substrates referred to as Smads 1, 5 and 8. The phosphorylated Smads 1, 5 and 8 partners with a co-Smad are considered as Smad 4 and moved towards the nucleus to turn on BMP-response genes. The phosphorylation of Smads 1, 5 and 8 by BMP receptors is inhibited through inhibitory Smad 6. Therefore, the BMP signalling system is an intricately regulated homeostatic machine likewise a thermostat in an air conditioner.[16] BMP-BMP receptor signalling system in the mesenchymal stem cells results in bone induction and morphogenesis.

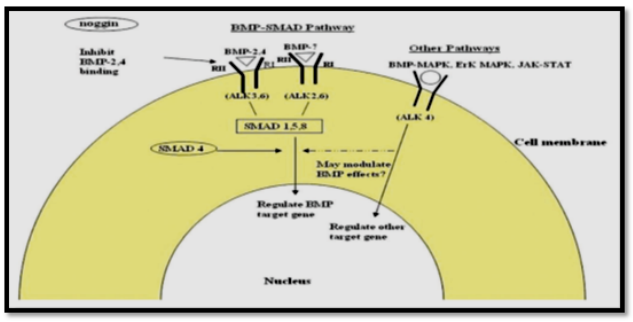

Fig. 3: Signalling mechanism of bone morphogenetic protein activin receptor-like kinase (ALK)

Bone morphogenetic protein (BMP), type I and type II receptors (R I and R II), mitogen-activated protein kinase (MAPK), extracellular signal-related kinases (ErK), Janus kinase (JAK), signal transducers, and activators of transcription (STAT)

15. BMPs- Recombinant Technologies

Recombinant human BMP-2 is extraordinarily osteoinductive. Mesenchymal stem cells (MSCs) have the capacity to alternate or "differentiate" into numerous varieties of cells which further makes a new variety of tissues. Bone Morphogenetic Proteins (BMPs) direct MSCs to distinguish into an osteogenic bone line. It binds to mesenchymal stem cell receptors resulting in proliferation and differentiation into osteoblasts In vitro research shows that mesenchymal stem cells incubated with rhBMP-2 has higher alkaline phosphatase activity and undergo matrix mineralisation. When implanted in vivo, rhBMP-2 induces Osteoinduction through recruiting mesenchymal stem cells, then inducing the proliferation and differentiation of those cells into an osteoprogenitor lineage. The bone formed has precisely the equal composition as bone elsewhere in the body.

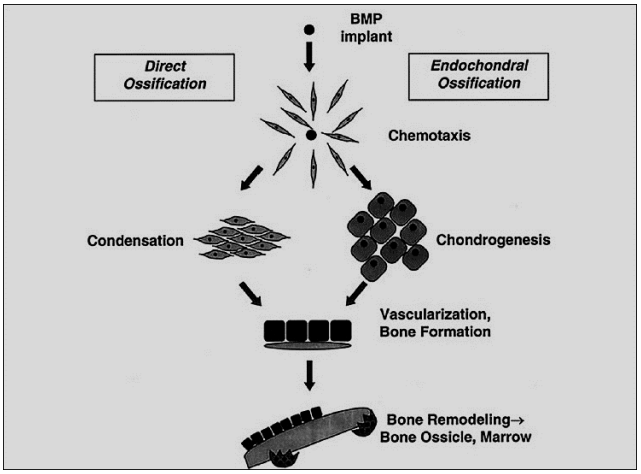

Fig. 4: BMP- differentiation of mesenchymal stem cells

In the human body, tissue clearance of BMPs is very fast. Therefore, a carrier is required to retain an effective amount of concentration of rhBMP-2 at the site of implantation. It also provides a scaffold for newly recent bone formation. Initial studies and research in animal segmental defect models explored numerous types of materials. Type I collagen has proved to be maximum bioactive, biocompatible and bioresorbable carrier, and all rhBMP products presently in the market place contains a variety of collagen Type-I.

Certain complications have been discovered during clinical trials with rhBMP-2 at therapeutic dosages, despite the fact that BMPs – by virtue of their nature – have the ability to result in heterotopic bone formation. As with all exogenous protein molecules, antibody formation is mainly concerned. Antibodies to rhBMP-2 and the bovine collagen carrier have been reported to occur in approximately 6%

and 5-20% of patients, respectively, however titres have been low and transient and no clinical sequelae have been observed. These proteins gives rise to both endochondral and intramembranous bone formation. The end result in each case is woven bone that then remodels and will become populated with hematopoietic bone marrow.

When recombinant human BMP-2 (rhBMP-2) was used in supra-alveolar periodontal defects, the gains in bone and cementum were 3.5 mm and 1.6 mm, respectively as compared to 0.8 mm and 0.4 mm for controls. Histologic evaluation revealed areas of periodontal regeneration associated with certain areas of ankylosis. In contrast to these findings, BMP-7 augmentation resulted in a significant increase in periodontal regeneration without any ankylosis. Healing through ankylosis has been a concern so most of the recent research utilizing rhBMPs has involved implant site preparation.[17]

The rhBMP commercially available and accredited by FDA in the United States presently are: rhBMP-2 Infuse (Medtronic Sofamor Danek, Memphis, Tennessee) and OP1 (Stryker Biotech, Hopkinton, MA). Some other BMPs products are presently being evaluated for commercial use include BMP-X (Sulzer Biologics, Wheat Ridge, Colorado), BMP -9, and combinations. A rhBMP-2 Infuse® is available in market place in packaging of all the components needed to prepare the bone forming component Infuse®: rhBMP-2 lyophilized powder to be reconstituted, sterile water, absorbable collagen sponge, syringe with needles, and preparation instructions. The rhBMP-2 is supplied as a lyophilized powder in vials containing 4.2 mg or 12 mg of protein. After proper reconstitution, the two sets result in the same formulation and concentration (1.5 mg/cc) of rhBMP-2.

According to the manufacturer, the Infuse® bone graft have to be prepared at time of surgery always 30 minutes before the application of bone graft at the surgical site. The sterile water may be removed from the bottle and inject in the vial containing rhBMP-2, then mixed without stirring. The original packaging is opened and place the absorbable collagen sponge in sterile field. With the help of second syringe, the reconstituted bone graft is removed from ampule and is applied uniformly in the sponges. The moist sponges should rest for at least 15 minutes (time for incorporation of the protein to the sponge) and it should be placed at the surgical site within two hours (for avoiding the drying of the sponge).

16. Clinical Application of BMPs

The concept of osteoinductive composite of BMPs and scaffolding have been used to fabricate a tissue engineered bone was evidenced.[18] In this experiment a vascularized muscle flap was placed in a mold mimicking the head of the femur of rat and was injected with BMPs and collagenous matrix. It is noteworthy that a true transformation of muscle into bone mirroring the shape of the femur was accomplished demonstrating the proof of principle for tissue engineering of bone.[18] The outstanding regenerative potential of bone is common knowledge. However, in the repair of massive segmental bone loss due to tumours, trauma or fractures due to metabolic diseases such as diabetes and osteoporosis, it is common orthopaedic practice to aid and abet the healing site with autogenous bone graft. The limited supply of autograft bone, the associated donor site morbidity including infections and pain is a major challenge. The availability of recombinant BMPs and biomimetic biomaterials and stem cells has set the optimal stage for tissue engineering to enter the operating suites in orthopaedic surgery.

An auspicious beginning was made by the use of BMP 7 in treatment of tibial non-unions.[19] In addition to orthopaedics BMPs have been used in clinical dentistry in the realms of maxillofacial surgery, bone augmentation, and integration of dental implants.[20] BMPs -2, -4, -7 and -12 have all been evaluated for periodontal and peri-implant bone regeneration. BMP-2 is one of the commonly studied BMP for bone and periodontal regeneration treatment. Several preclinical studies demonstrate significant improvement of alveolar bone regeneration in different types of periodontal defects after treatment with rh BMP-2 via different carriers.

Another important therapeutic application of BMPs is for maxillary bone regeneration to allow replacement of lost teeth by Osseo integrated dental implants. This approach involves the re-generation of peri-implant bone after implant fixation or bone height improvement in areas below the maxillary sinus. Preclinical and clinical research papers have been shown to improve bone formation after getting treatment done with BMP-2. However, the use of different carriers and the association of barrier membrane (GTR technique) or different biomaterials appears to be crucial element in affecting the treatment outcome.

The ability of BMP-12 to restore tendon and PDL tissues has been proven and documented in vitro and in vivo studies. A preliminary study in dogs comparing the rhBMP-12 with rhBMP-2 for the treatment of periodontal defects. The consequences confirmed that less bone and greater functionally oriented PDL among the new bone and cementum after BMP-12 treatment, indifferent with a more parallel fibre arrangement of BMP-2-treated defects.[21] Moore YR, Dickinson DP 2010 conducted a comprehensive literature research to re-view the therapeutic effects of Growth/differentiation factor-5 (GDF-5), which is a a member of the bone morphogenetic protein family. They stated that GDF-5 appears to be a promising therapeutic agent for periodontal wound healing/regeneration because it supports/accelerates bone and tendon/ligament formation in numerous musculoskeletal settings consisting of periodontal tissues.[22]

Despite those affective advances, many scientific situations are still demanding. In addition to optimization of the dose of BMPs, pharmacokinetics of release, the optimal delivery from biomimetic biomaterials and sterilization consisting of irradiation rays needs to be investigated. The latest approval through the Food and Drug Administration of recombinant BMP2 for spine fusion appears to be the first use of a recombinant human morphogen in orthopaedic surgery for tissue engineering of bone.

17. Bone Morphogenetic Protien Gene Delivery

Several matrices and delivery systems were used and evaluated for their efficacious potential and biocompatibility as carrier system for BMPs. Three important techniques for growth factor delivery: gene therapy, cell therapy, and protein therapy.

Gene therapy and stem cell-based therapy constitute the primary advancement, however, currently are still in their infancy especially for safety and efficacy in human. Protein therapy has been demonstrated and confirmed one of the most practical approach, particularly incorporating osteoinductive morphogens (BMPs) in spite of having some limitations. It was demonstrated that the clinical efficacious potential along with capability of rhBMPs will depend upon the carrier system, for efficacious delivery of adequate protein concentrations at the desired site. Another carrier functions are to maintain the factor at the site of implantation and thus increase its local concentration.

Ideal characteristics features of the delivery system or carrier include biocompatibility, biodegradability, structural integrity, absence of immunogenicity, absorption, rate of release, cost, and handling. The qualities of best carrier may vary and always depends on the specific implantation site and are intended as a therapeutic outcome. Different types of carrier systems are available such as solids, gels or combinations. Moreover, BMPs preparations has been used along with occlusive or porous resorbable or non-resorbable space providing devices for guided bone regeneration. Ideally BMPs are soluble and if delivered in a buffer solution, the clearance value is very fast. Less than 5% dose remains at the site of application whereas combinations of these proteins along with gelatin foam or collagen showed an increase in retention. An absorbable collagen sponge (ACS) was the first BMP carrier technology to be approved by the US Food and Drug Administration (FDA).

The absorbable collagen sponge is a bovine type I collagen matrix is soak loaded with a BMP solution before surgical implantation. The rhBMP/ACS assembly has proven the clinical efficiency for some of indications; however, it is far at risk of tissue compression. The collagen matrix keeps 65% of the BMPs all through preliminary impregnation and releases it in two levels a preliminary segment within hours of implantation and a second segment depends on nature and geometrical features. BMPs blended with porous elements of hydroxyapatite or fibrous collagen membrane causes an intramembranous ossification, while fibrous glass membrane or insoluble bone matrix support indirect bone formation through cartilaginous intermediate.

There are few disadvantages related to the carriers including loss of bone induction with BMPs blended with hydroxyapatite alone – possibly because of the shortage of resorption of hydroxyapatite and the tight binding affinity among BMPs and hydroxyapatite; moreover, immunogenicity and danger of disease transmission with the usage of demineralized bone matrix and acidic breakdown products of synthetic polymers which would possibly show unfavourable to wound healing. A major problem with delivery of growth factor proteins is the restrained bioactivity (half-life) of proteins because of degradation and risk associated with achieving a controlled release. Therefore, localized growth factor delivery remains a risk in clinical practice. Use of the gene therapy strategy as one of the aspects to address the problems related to conventional protein delivery.

18. Platelet Rich Plasma (PRP)

Researchers constantly try to enhance bone-grafting strategies and to offer the method to attain a quicker and denser bony regeneration. Marx et al. 1998 have proposed the using platelet rich plasma (PRP) as a possible approach to attain a higher concentration of growth factors (GFs).[23] PRP is a blood derivative, generated through differential centrifugation, wherein platelets are concentrated in a small plasma volume. The use of PRP is primarily based totally on the basis that platelets represent a reservoir of vital GFs which includes platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), insulin-like growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF), which as soon as released, can also additionally definitely modify the wound-healing process. PRP is an autologous modification of fibrin glue, derived by methods that concentrate autologous platelets, and has been defined and utilized in number of applications with obvious scientific success. It is an easily available source of growth factors to help in bone and soft tissue healing.

Separating PRP from patient blood and also including the bone graft materials is a brand newer approach.[24] PRP has been used alone or in aggregate with autografts and allografts for the therapeutic approach of periodontal defects, extraction socket preservation, alveolar ridge augmentation, mandibular reconstruction, sinus floor elevation and maxillary cleft repair. Results have proven with a more extent in volume and denser bone in comparison to autografts used alone for bone regeneration.[25] The development within the bone healing ability is assumed to be because of the growth factors found in PRP, and various clinical research have pronounced wonderful consequences from PRP use on bone regeneration. A current meta-

Table 2: Types of carrier systems for the delivery of bone morphogenetic proteins

Organic (natural or synthetic)	Organic polymers-allogenic/ xenogenic collagen (Absorbable collagen sponge), fibrin, poly- α hydroxyl acids, hyaluronan, methyl methacrylate
Inorganic (natural or synthetic)	Autogenous bone, Hydroxyapatite, calcium phosphates, calcium sulfate, β tricalcium phosphate, and bioglass technologies
Combinations of the above	

analysis on potential scientific research concluded that there has been high heterogeneity amongst research reporting on PRP in periodontal regeneration which made it tough to draw clear conclusions. PRP may provide a few useful consequences on clinical and radiographic results for regeneration of periodontal intrabony defects.[26]

19. Platelet Rich Plasma

PRP is a simple method to concentrate platelets or enrich natural blood clot. A natural blood clot consists of 94% red blood corpuscles (RBCs), 5% platelets and 1% white blood corpuscles (WBCs) even as PRP consists of 95% of platelets. PRP acquired from autologous blood is used to deliver growth factors in higher concentration to the site of bone defect or a region requiring augmentation. The drawbacks of PRP encompasses biochemical blood handling along with an additional anticoagulant. Due to legal regulations on blood handling, a new family of platelet concentrate, which is neither a fibrin glue nor a classical platelet concentrate pays more attention in France.

20. Plaletlet Rich Fibrin

The PRF is described by Choukroun et al 2001 in France and is a second-generation platelet concentrate with an improvement over conventionally prepared PRP. This brand-new biomaterial referred to as platelet-rich fibrin (PRF), looks as an autologous cicatricial matrix that is neither like fibrin glue nor like a classical platelet concentrate. Platelet-rich fibrin (PRF) belongs to a new generation of platelet concentrates geared with simplified preparation without biochemical blood handling. PRF includes fibrin matrix polymerized in a tetra molecular structure, with incorporation of platelets, leucocytes, cytokines, and circulating stem cells. Clinical research revealed that this biomaterial could be a beneficial matrix for the improvement of a coherent healing, without any inflammatory excess. PRF in the form of a platelet gel may be used along with bone grafts, which has certain advantages like promoting wound healing, bone growth and maturation, wound sealing and haemostasis, and imparting better handling properties to graft materials. It can also be used as a membrane. Many scientific trials endorse the aggregates of bone grafts and PRF to enhance bone density.

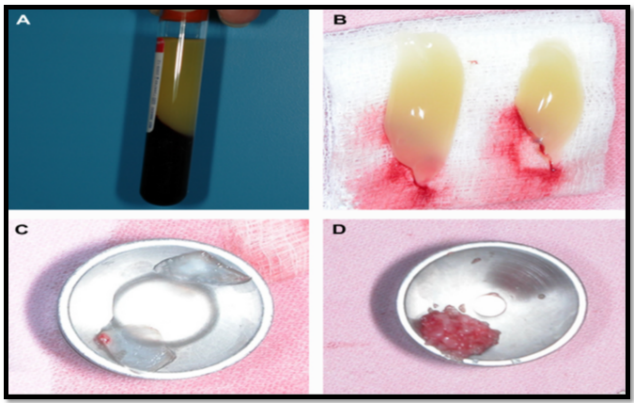

Fig. 5: Preparation of platelet-rich fibrin (PRF); **A:** PRF formed in the middle part of the tube. The upper part contained acellular plasma and the bottom part contained red corpuscles; **B:** The fibrin clot was easily separated from the lower part of the centrifuged blood; **C:** The PRF clot was gently pressed between 2 layers of sterile dry gauze to form a membrane; D**:** PRF can be minced as a grafting

21. Advantages of PRF over PRP

1. No biochemical handling of blood.
2. Simplified and cost-effective process and use of bovine. thrombin and anticoagulants not required.
3. Favourable healing due to slow polymerization.
4. More efficient cell migration and proliferation.
5. PRF has supportive effect on immune system.
6. PRF helps in homeostasis.

22. Disadvantages of PRF

1. Amount available is low because of autologous blood.
2. After the immediate collection of blood, the handling process of blood requires fast processing.

23. Preparation of PRP

The PRP application described by most authors requires initiating the coagulation process with a mixture of 1,000 US units of TBT powder suspended in 10 mL of sterile saline with 10% calcium chloride. The protocol for PRP activation requires the use of an individual 10-mL syringe for each mix. Each mix draws 6 mL of PRP, 1 mL of the 10% calcium chloride, 1 mL of thrombin, and 1 mL of air to act as a mixing bubble. The syringe is agitated for a few seconds to initiate clotting and is then ready to be applied

to the bone grafts. Once added to the grafts, the fibrin, fibronectin, and other cell adhesion molecules establish a network that may serve as osteoconduction in bone growth. After centrifugation three layers are obtained.

1. Upper straw colored fluid PPP (platelet poor plasma).
2. Middle buffy coat rich in platelets.
3. Lower layer rich in RBCs.

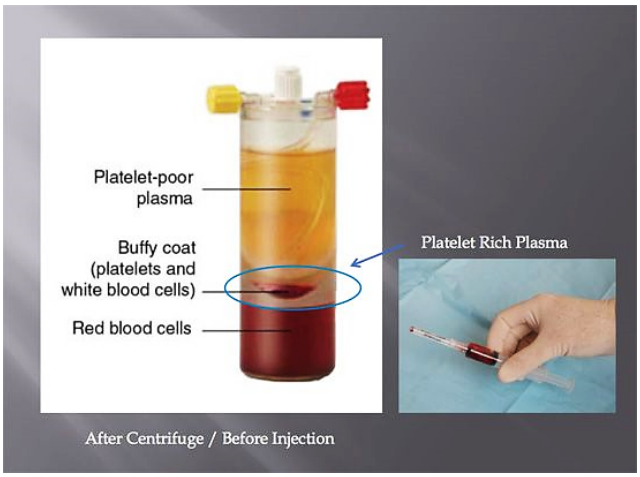

Fig. 6: Three layers of PRP

Fig. 7: Centrifuge machine

24. Platelet Rich Plasma Procurement Techniques

It can be done by using various techniques:

1. Gradient density cell separators
2. Concentrating cell separators.

24.1. Using gradient density cell separators

The well-known cell separators are ELMD-500 (Medtronic Electromedic, Autotransfusion System, Parker, CO) require a massive amount of blood (450 ml) and always to be operated under hospital institution. Blood is drawn right into a bag containing citrate-phosphate-dextrose anticoagulant. Firstly, the blood is centrifuged in a well-known cell separator, at 5,600 rpm to separate the platelet poor plasma (PPP) from the red blood cells (RBC) and the PRP (additionally termed "buffy coat," which incorporates platelets and leukocytes). Then centrifuged at 2,400 rpm to achieve PRP from the slurry of RBC and PRP. The procurement of PRP with this method may be done in 30 minutes. PRP is procured within 6 hours after being drawn from the patient. Platelet counts of 500,000-1,000,000 in the PRP are usually procured with the plasmapheresis method and ultimately the remaining erythrocytes and PPP may be moved in the circulation or discarded.

24.1.1. Advantage

1. RBCs may be returned back to patient venous blood.

24.1.2. Disadvantages

1. Need more amount of blood
2. Need large hospital/ medical setup.

24.2. Using concentrating cell separators

Platelet concentrating cell separators are most commonly used seeking that device can be used may be accommodated in dental clinic setup. This method allows the obtaining of PRP through smaller amount of blood, appropriate amount of platelet concentration and no requirement of RBC and PPP reinfusion. Two such separators commercially available are Harvest Smart Prep Platelet Concentrate System (HSPCS) (Harvest Technologies, Plymouth, MA) and the 3i Platelet Concentrate Collection System (3i PCCS) (3i Implant Innovations, Palm Beach Gardens, FL). Both these platelet-concentrating cell separators are equal and comparable overall performance and simplicity, and that they constitute a tremendous benefit because they require much less time to obtain PRP (15 minutes versus 20 minutes) and less operator intervention and training along with less technique sensitive.

24.2.1. Advantages

1. Do not require large hospital/ medical institution or setup.
2. Need less quantity of blood.

24.2.2. Disadvantage

1. RBCs cannot be returned back to patient's venous blood.

25. Properties of PRP

1. Increase tissue vascularity through accelerated angiogenesis.
2. Increase collagen synthesis and process of osteogenesis
3. Increasing the rate of epithelial, as well as granulation tissue production.

4. Antimicrobial effect
5. Reaction with different material: PRP does now no longer to react or interfere with everywhere other types of restorative material such as glass ionomer cements or composite resin used as filling material are not affected by it.
6. Biocompatibility: Any material which is identified and can be used in humans or animals should be biocompatible without having any toxic/poisonous or injurious effects on biologic tissues and its functions. PRP brings a biologically active substance along with the release of growth factor.
7. Tissue regeneration: PRP provide regeneration of tissue with the release of growth factors.

The properties of PRP are primarily based at the manufacturing and release of more than one growth and differentiation factors after platelet activation. These factors are crucial in the regulation and stimulation of the wound healing process, and play a vital function in regulating cellular processes such as mitogenesis, chemotaxis, differentiation and metabolism. Growth factors have interaction with each other and ultimately forming a cascade of different signal proteins with more than one pathway that results in the activation of gene expression and protein production. Recent scientific reports reviewed that PRP results in more rapid epithelialization, denser and more mature bone along organized trabeculae, and greater bone regeneration.

25.1. Mechanism of action of PRP

Platelet rich plasma usually work through three mechanisms:

1. Increase in local cell division (producing a greater number of cells): According to Nathan E Carlson 2002 after the injury, platelets start sticking to exposed collagen proteins and release various granules containing adenosine diphosphate, serotonin and thromboxane and all may contribute to the haemostatic mechanism and the clotting cascade.
2. Inhibition of excess inflammation by reducing the initial stage macrophage proliferation.
3. Degranulation of the agranules in platelets that contain the synthesized and pre-packaged growth factors.

The active secretion of these growth factors is initiated by the clotting process of blood and initiate within 10 minutes after clotting. More than 95% of the pre-synthesized growth factors are secreted within 1 hour. PRP has been proven to remain sterile and the concentrated platelets viable for up to 8 hours once developed in the anticoagulated state.

26. Clinical Applications of PRP

Because PRP enhances osteoprogenitor cells in the host bone and in bone grafts, it has found clinical applications in

1. Continuity defects
2. Sinus lift augmentation grafting
3. Horizontal and vertical ridge augmentations
4. Ridge preservation Grafting
5. Periodontal/peri- implant Defects
6. Cyst enucleations/Periapical Surgeries
7. Healing of Extraction wounds
8. Endodontic surgeries and Retrograde procedures
9. Ablative surgeries of the Maxillo-Facial region
10. Blepharoplasty

The use of PRP helps to fulfil some of these requirements, particularly as an aid to bone regeneration. In fact, in vitro studies have proven that platelet-derived growth factors stimulate the proliferation of both human trabecular bone cells and human osteoblast-like cells. Initial in vivo experiments involving PRP were reported in the field of oral maxillofacial surgery and in dentistry, particularly in periodontal therapy. Marx and co-workers in 1998 studied the potential effect of autologous PRP on a bone graft reconstruction of mandibular continuity defects stated that the combination of PRP and bone grafts resulted in a markedly faster maturation and histomorphometrically denser bone regeneration.[23]

Another therapeutic approach involves the combination of PRP with different bone matrices. One potential benefit of the combination of PRP with both mineral and organic bone matrices is the improved handling and adaptation of the matrix to the injured tissue because the fibrin acts as a biological glue to hold together the matrix particles. However, initial vascular invasion is a key element in bone allograft or xenograft incorporation, which reduces complications and incomplete integration. Aghaloo et al. 2004 evaluated a natural deproteinized bovine bone, known as Bio-Oss, with and without PRP in rabbit cranial defects and evaluated a marked histomorphometric improvement at one, two and four months with the addition of PRP as compared to Bio-Oss alone.[27]

27. Safety concern of PRP

Because it is an autogenous preparation, PRP is inherently safe and therefore free from concerns over transmissible diseases such as HIV, Hepatitis, West Nile fever, and Cruetzfeld-jacob disease (CJD) (“mad vow disease”). However, Sanchez et al. 2003 have elaborated on the potential risks associated with the use of PRP.[28] The preparation of PRP involves the isolation of PRP after which gel formation is accelerated using calcium chloride and bovine thrombin. It has been discovered that the use of

Table 3: Growth factors realesed from platelets

Growth factor	Source cells	Target	Action
PDGF	Platelets, macrophages, monocytes, endothelial cells, smooth muscle cells	Fibroblasts, smooth muscle cells, glial cells, Macrophages/Neutrophils	Stimulates chemotaxis/ mitogenesis in fibroblast/glial/smooth muscle cells; regulates collagenase secretion/collagen synthesis; stimulates macrophage/ neutrophils chemotaxis
TGF-b	Platelets, T-lymphocytes, macrophages/monocytes, neutrophils	Fibroblasts, marrow stem cells, endothelial cells, epithelial cells, preosteoblasts	Stimulates/inhibits endothelial, fibroblastic, and osteoblastic mitogenesis; regulates collagen synthesis/ collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis.
PDEGF	Platelets, macrophages, monocytes.	Fibroblasts, endothelial cells, epithelial cells	Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis.
PDAF	Platelets, endothelial cells.	Endothelial cells	Increases angiogenesis and vessel permeability; stimulates mitogenesis for endothelial cells by direct or indirect actions; several cytokines and growth factors up-regulate PDAF, including IGF-1, TGF-alpha and beta, PDGF, bFGF, PDEGF, and IL-1 beta
IGF-1	Osteoblasts, macrophages, monocytes, chondrocytes	Fibroblasts, osteoblasts, chondrocytes.	Stimulates cartilage growth, bone matrix formation, and replication of preosteoblasts and osteoblasts; acts as an autocrine and paracrine factor; in combination with PDGF can enhance the rate and quality of wound healing.
PF-4	Platelets	Fibroblasts, neutrophils	Chemoattractant for neutrophils and fibroblasts; potent anti-heparin agent.

PDGF – platelet-derived growth factor; TFG-b – transforming growth factor beta; PDEGF – platelet-derived epidermal growth factor; PDAF – platelet-derived angiogenesis factor; IGF-1 – insulin-like growth factor 1; PF-4 – platelet factor 4; bFGF – basic fibroblast growth factor.

bovine thrombin may be associated with the development of antibodies to the factors V, XI and thrombin, resulting in the risk of life-threatening coagulopathies. Bovine thrombin preparations have been shown to contain factor V, which could result in the stimulation of the immune system when challenged with a foreign protein.

28. Conclusion

Recent advances in biotechnology have given an access to brilliant type of bone grafting materials. However, the best grafting material has yet to be identified. Latest research and studies make a speciality of proteins and carriers for delivering growth factors at the surgical site. Growth factors are present at low concentrations in bone matrix and plasma to execute a vital biologic function. Growth factors associated with transmembrane receptor molecules on mammalian cells and induce cytoplasmic cascade reactions results in transcription of mRNA and intracellular and extracellular protein release.

29. Source of Funding

None.

30. Conflict of Interest

The authors declare no conflict of interest.

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