REVIEW ARTICLE
JIVA 21 (3) December 2023
https://doi.org/10.55296/JIVA/21.3.2023.7-19

Received on 24/11/23
Accepted on 03/12/23

BIOSYNTHETIC SUBSTITUTES IN BONE ENGINEERING FOR AUGMENTING HEALING IN ORTHOPAEDIC PATIENTS- A REVIEW

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ABSTRACT

Bone is a hard connective tissue which helps to maintain the body structure and functions. Several factors like aging, drugs, hormonal changes, and physical activities may lead to bone injuries and fractures. Fracture without bone loss will heal if proper immobilisation techniques are used. But if there is an extensive bone loss fracture fails to heal and get filled with fibrous tissue. To address these problems bone graft materials are used. Autologous bone graft is considered as the ideal graft material. However, complications related to its harvesting process including donor site morbidity and pain, limit its use in bone regeneration. Allograft and xenografts can also be used, but there are some problems like immune reaction and chance of rejection. Biosynthetic bone grafts are developed as an alternative to conventional bone grafting materials. These grafts generally contain one or more of three critical components: (1) osteoinductive factors that induce the various stages of bone regeneration, (2) osteoconductive matrix to provide physical support and direction to the repair process, and (3) osteogenic stem cells that are capable of differentiating and facilitating the bone formation process. This article will cover common biosynthetic substitutes for augmenting bone healing.

Keywords: Fracture, Autograft, Allograft, Xenograft, Biosynthetic bone graft

INTRODUCTION

A fracture is a partial or complete break in continuity of the hard tissue like bone and cartilage. Fractures of varying magnitude are common in a clinical scenario. In a healthy individual, fractures without bone loss tend to heal, when the fracture fragments are immobilized in proper apposition either internally of externally. If there is extensive bone loss, even with proper immobilization and alignment, the fracture fails to heal. These sites tend to be filled with soft fibrous

tissue, which does not provide the desired mechanical support. The possible method to overcome this situation is to use bone tissue substitutes. Bone tissue substitutes are used to reduce the gap between the fracture fragments, thereby help in bone healing.

Over the years a lot of materials have been used as bone tissue substitutes. They can be classified as autograft, allograft and synthetic graft. Ideal properties of a bone graft include osteointegration, osteoconduction, osteoinduction and osteogenesis. Osteointegration is the ability of the material to chemically bond to the surface of the bone without an intervening layer of fibrous tissue. Osteoconduction is the ability of the material to support the growth of bone over its surface (Costantino and Friedman, 1994). Osteoinduction is the ability of the material to induce differentiation of pluripotent stem cells from the surrounding tissue to an osteoblastic phenotype. Osteogenesis is the formation of new bone by osteoblastic cells present within the graft material (Cypher and Grossman, 1996).

AUTOGRAFT

Autograft, as the name suggests, is the transferring of bone from one region of the patient to the fracture site of the same patient (Lane *et al.,* 1999). The main advantages of this graft are good biocompatibility, no cytotoxicity, no immunogenicity or rejection (Kurz *et al.,* 1989). Autograft provides good osteointegration, osteoconduction, osteoinduction and osteogenesis, because of which it is considered as a gold standard for bone tissue substitutes (Lane *et al.,* 1999). Autograft has some limitations such as lack of availability of viable bone for transplant in case of large bone defects, donor site morbidity, complications associated with surgery like blood loss, wound complications, local sensory loss, muscle injury, chronic pain and chances of infection (Kurz *et al.,* 1989). Venugopal (1994), used autogenous rib graft for the treatment of metacarpal fracture in calves and found out that, autogenous bone grafts were better than the splint and pop alone and the graft was gradually replaced during the process of healing.

ALLOGRAFT

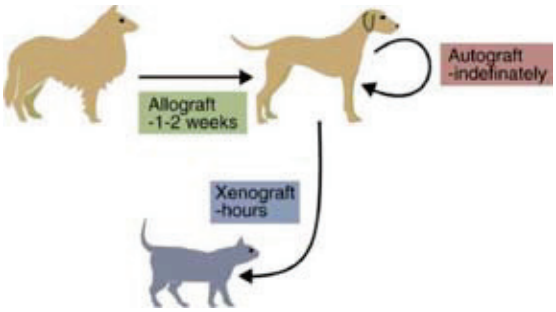
Allograft is the transferring of bone from one individual to the fracture site of another individual of the same species. These were primarily collected and processed from cadavers. Allograft promotes good osteointegration, osteoconduction and osteoinduction. The main advantages of this technique are the availability of bone tissue substitutes even for larger bone defects. Additionally, there is no donor site morbidity,surgery

associated complications and chances of infection. The main limitations of this technique are chances of post-operative infection, fracture, non-union, possibility of disease transmission, immunogenicity and graft rejection (Mankin *et al*., 1996).

XENOGRAFT

As xenograft comes from another species, antigenicity is significantly greater than that of allografts. Naturally, it requires more sterile processing, which can result in reduced osteoinductive properties. Owing to the abundance of donors, xenograft may be less expensive and more readily available than others. Also because of the extensive sterilization processes, the shelf life will be generally long.

BIOSYNTHETIC BONE GRAFTS



(Shibuya and Jupiter, 2015).

They are the synthetic composite grafts that are intended to mimic the natural components required for bone healing. Synthetic bone grafts are biocompatible materials that undergo remodelling and support new bone formation. Ideally, they should possess strength similar to cortical or cancellous bone (Lane *et al.,* 1999).

The synthetic graft ideally should contain primitive osteoprogenitor stem cells with receptors that respond to inductive signals and have the capability of proliferating and differentiating into osseous forming cells, sufficient osteo-inductive growth factors to stimulate these osteoprogenitor cells and an osteoconductive material to provide a favourable environment and scaffold for the cell growth (Lane *et al.,* 1999).

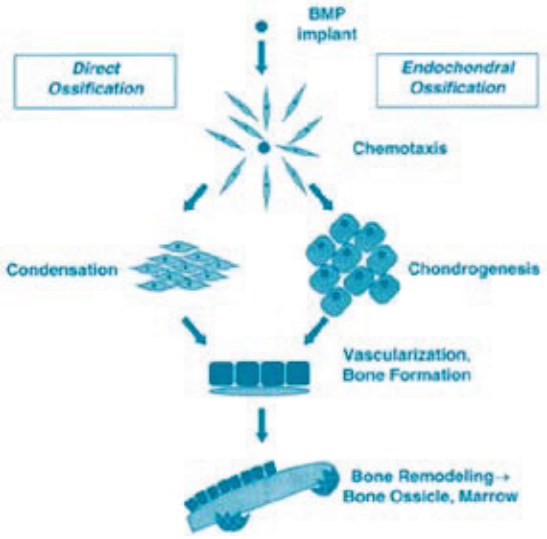
OSTEO-INDUCTIVE GROWTH FACTORS

Osteoinduction is mediated by numerous growth factors provided by the bone matrix and these growth factors seem to play a critical role in bone healing. Therefore these peptides have become an important area of investigation in an effort to enhance fracture healing (Lane *et al.,* 1999). Different types of osteo-inductive growth factors are bone morphogenetic protiens, transforming growth factor beta, platelet derived growth factor, insulin like growth factor and fibroblast growth factor.

BONE MORPHOGENETIC PROTIENS (BMP)

Bone morphogenetic proteins are low molecular weight glycoproteins that function as morphogen. The ability

of devitalized bone, when implanted in an animal, to induce a cellular response resulting in new bone tissue formation has been known for decades. This unique activity was observed and researched extensively by an orthopedic surgeon, Dr. Marshall Urist. He subsequently demonstrated that this activity could be extracted from the organic component of bone using chaotropic agents, and that a protein was responsible for this activity. He thus named this activity as "bone morphogenetic protein".

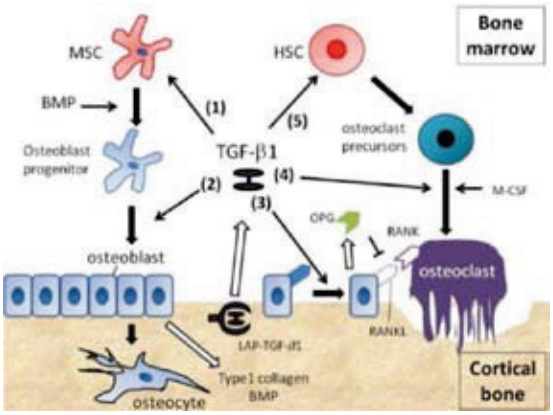


(Wozney, 2002)

Cellular events after implantation of bone morphogenetic protein (BMP): These proteins induce both endochondral (through cartilage intermediate) and direct (intramembranous) bone formation. The end result in each case is woven bone that then remodels and becomes populated with hematopoietic bone marrow (Wozney, 2002).

TRANSFORMING GROWTH FACTOR-BETA (TGF-β)

It is a part of the TGF superfamily. TGF-beta is a multifunctional growth factor that has been shown to mediate normal cellular physiology and tissue embryogenesis. The largest source of TGF-beta in the body is the extracellular matrix of bone, and the second largest reservoir is platelets (Sporn and Roberts, 1989).

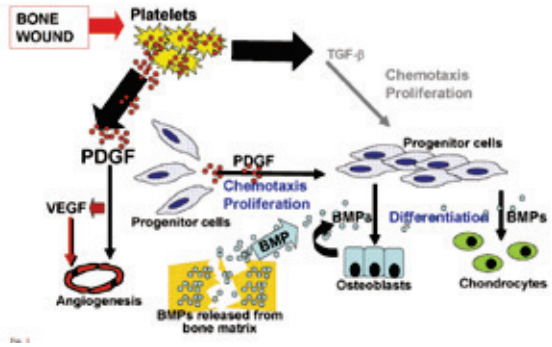


(Kasagi and Chen, 2013)

TGF-β1 stimulates the proliferation of MSCs, which differentiate into chondrocytes. High concentration of TGF-β1 enhances osteoblast proliferation, however, it down regulates the expression of RANKL of osteoblast. Low concentration of TGF-β1 promoted osteoclast maturation and TGF-β keeps hematopoietic stem cells (HSC) in hibernation state.

PLATELET DERIVED GROWTH FACTOR (PDGF)

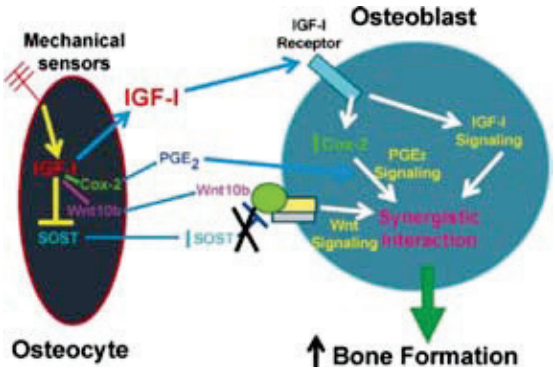
Alpha granules containing PDGF are produced by platelets for the purpose of angiogenesis, chemotaxis, and mitogenesis. In addition, PDGF upregulates vascular endothelial growth factor (VEGF), further enhancing angiogenesis. Transforming growth factor-beta (TGF-b) helps in chemotaxis and cell proliferation during wound-healing. The attraction of osteoprogenitor cells (chemotaxis) and their increase in number (mitogenesis) provide a pool of osteo-regenerative cells that will respond to the BMPs. BMP is a differentiating factor. Consequently, BMPs and PDGF are primary and powerful co-regulatory controls for healing and regeneration of bone (Hollinger *et al.,* 2008).



(Hollinger *et al.,* 2008)

INSULIN LIKE GROWTH FACTORS (IGF)

Osteocytes upregulate the expression of osteocyte-derived IGF-I which down regulate Sost expression in osteocytes and up regulate Cox2 expression in osteocytes. Osteocyte-derived Sost interferes negatively with the binding of Wnt to its receptor/coreceptors, resulting in suppression of the canonical Wnt signaling in osteoblasts, the loading-induced Sost suppression would lead to increased osteoblastic activity and bone formation (William *et al.,* 2013).



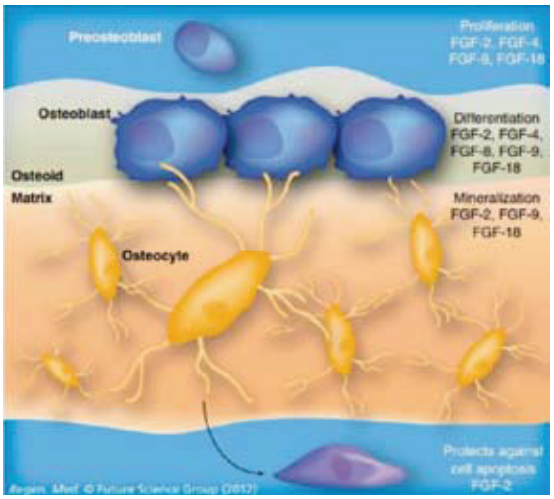
(William *et al.,* 2013)

FIBROBLAST GROWTH FACTOR (FGF)

Originally detected as substances in the brain and pituitary gland, FGFs isolated from these areas were found to promote the growth of fibroblasts. FGFs mediate their effects via activation of signaling pathways, including RAS/MAPK. (Yun *et al.,*2012).

OSTEOPROGENITOR CELLS

The direct infusion of progenitor cells leads to more rapid and consistent bone recovery (Kahle *et al*., 2010). Bone tissue engineering has a number of potential



(Yun *et al.,*2012)

cells, such as osteoblasts, preosteoblasts, and stem cells (Shi *et.al*., 2019).

STEM CELLS

The stem cells in bone regeneration are divided into adult stem cells, embryonic stem cells, extraembryonic stem cells, and induced pluripotent stem cells (Chan *et.al.,* 2015).

ADULT STEM CELLS

Advantages of adult stem cell are multipotency, osteogenic capacity, and less ethical constraints. Disadvantages of adult stem cells are limited self-renewal capacity, lower proliferation and differentiation rates. These include mainly bone marrow derived mesenchymal stem cells (BMSCs), adipose derived stem cells (ADSCs), muscle derived stem cells (MDSCs), synovium derived stem cells (SDSCs) etc.

BONE MARROW DERIVED MESENCHYMAL STEM CELLS (BMSCs)

BMSCs are generally obtained from the marrow cavity by bone marrow aspiration. BMSCs can differentiate into chondrocytes, osteoblasts, osteocytes and adipocytes. Limitations of BMSCs include extremely low cell proportion in bone marrow cells and reduced differentiation capacities with age (Li *et al*., 2014).

ADIPOSE TISSUE DERIVED STEM CELLS (ADSCs)

Adipose tissue can be easily obtained using minimally invasive surgical procedures like liposuction. Adipose cell yields are significantly high. ADSCs can differentiate into osteogenic, chondrogenic, adipogenic, and myogenic cells. Limitations of ADSCs include improper isolation of ADSCs at source because of contamination of the cells which reduce proliferation and differentiated of the cells (Mizuno, 2009).

MUSCLE DERIVED STEM CELLS (MDSCs)

MDSCs is found in skeletal muscles. They have good self-renewal and differentiate as mesodermal progenitors (Gharaibeh *et al*., 2008).

SYNOVIUM DERIVED STEM CELLS (SDSCs)

The synovium is usually obtained from the articular cavity. SDSCs has higher proliferative rate and colony-forming property than BMSCs, but osteogenic differentiation capacity of SDSCs was lower than BMSCs (Nimura *et al.,* 2008).

OSTEOCONDUCTIVE MATRICES

`It will function as the platform for cells that will form new tissue. Microstructural properties of osteoconductive matrix reflect the anatomical three-dimensional (3D) microstructure of native bone. Ideal scaffold should display biocompatibility, osteoconductivity and architectural details like mechanical strength, surface topography, optimal porosity, and pore interconnectivity. Scaffolds can be divided into metallic, ceramic, polymeric, and composite (Przekora, 2019).

METALLIC BIMATERIALS

Metallic biomaterials are produced mainly using stainless steel, titanium-based alloys, magnesium alloys, nickel–titanium alloys, and cobalt-based alloys. The main drawback of metallic scaffolds for orthopaedic applications is their poor biodegradability and high stiffness, resulting in a stress-shielding effect followed by bone atrophy and implant loosening (Fousova *et al.,* 2017).

CERAMICS

Ceramic materials, such as calcium phosphate cements, bioactive glass (BG), hydroxyapatite (HA), α-tricalcium phosphate (α-TCP), β-tricalcium phosphate (β-TCP), and calcium silicate, possess the ability to create direct bonds with the host bone after implantation, which is called osseointegration. Ceramic materials are characterized by good bioactivity and biodegradability. Ceramic material will show, low mechanical strength, high brittleness, slow resorption rate, biocompatibility, bioactivity, osteoconductivity, and osteoinductivity (Przekora, 2019).

BIOACTIVE GLASS

The idea that certain types of glass might be bioactive and could chemically bond with bone was first introduced by Hench in 1967 (Hench *et al*., 1971). Hench glass is still used in clinical practice. Gadhafi *et al.*(2016) conduct a study on clinical evaluation of bioglass for augmenting bone healing in dogs and was found that there was early formation of bridging callus as well as early healing due to osteoconductive and osteointegrative property of bioglass.

ALUMINIUM OXIDE

Aluminium oxide is used in medical field for the first time in 1969. Since then,

there were over 200 million aluminium oxide joints and 300 thousand aluminium oxide acetabulum that had been used in total hip replacement. Aluminium oxide ceramic offers excellent chemical stability, resisting attack by most corrosive agents, except hydrofluoric, phosphoric, hydrochloric and sulfuric acids. And the hydrophilic character makes it form water films easily on the crystal surface. Some people think that the good frictional performance is related to this film (Huang *et al.,* 2014).

CALCIUM SULFATE

Calcium sulfate, also known as plaster of Paris, is a kind of osteoconductive and biodegradable ceramics composed of $CaSO_4$ and has been applied in filling void defects since 1892. It was first used by Dreesman. Calcium sulfate has a rapid resorption rate and weak internal strength, which limits its use only to fill small bone defects with rigid internal fixation, the ingrowth of vascular and new bone formation happens in conjunction with the resorption of the graft.

CALCIUM PHOSPHATE CERAMICS (CPC)

CPCs are the most useful synthetic bone graft substitute known to date possessing osteoconductive and osteointegrative properties. CPCs are constituted by calcium hydroxyapatites, which is a chemical composition similar to the mineral phase of calcified tissues. Absorption rate and mechanical properties, are strictly related to the Ca/P ratios (Zwingenberger *et al.,* 2012).

BETA TRICALCIUM PHOSPHATE (β-TCP)

β-TCP was first reported in 1920 by Albee and Morrison. With the chemical formula of $Ca_3(PO_4)_2$, β -TCP has Ca/P ratio of 1.5 and is thus lower than that of hydroxyapatite that may partially accelerate its degradation and absorption. Like HAp, TCP has even more interconnected porous structures that can directly benefit fibrovascular invasion and bony replacement, but at the same time weaken mechanical properties (Ogose *et al.,* 2006).

CORALLINE HYDROXYAPATITE (CHA)

CHA has been the only implant material found to date of the bone graft substitutes that had a structure analogous to osteon-evacuated bone. It is characterized by its uniform network of interconnected channels and pores, which make it similar to the mineralized inorganic scaffolding of living bone. Marine invertebrates make coral by taking calcium and phosphorus from seawater to build an exostructure. Calcium carbonate exoskeleton is then

converted into pure hydroxyapatite through a hydrothermal exchange process. The genus, Goniopora, found in the South Pacific Ocean is quite similar in chemical composition and structure to cancellous bone (Elsinger and Leal, 1996).

HYDROXYAPATITE (HA)

Hydroxyapatite is the naturally occurring mineral form of calcium apatite with the formula of $Ca_{10}[PO_4]_6[OH]_2$ and comprises about 50% of the weight of the bone, which accounts for its excellent osteoconductive and osteointegrative properties (Bhatt and Rozental, 2012).

CATIONIC SUBSTITUTIONS OF HYDROXYAPATITE

Calcium in the HA shall be substituted by cations (Cazalbou *et al.,* 2004). The common cations used to substitute calcium in synthetic hydroxyapatite are magnesium, strontium, zinc, silver, titanium, manganese, copper, cobalt and lanthanide substitution (cerium, europium, yttrium, samarium) (De Maeyer and Verbeeck, 1993).

ANIONIC SUBSTITUTIONS OF HYDROXYAPATITE

Anions shall be used to substitute phosphate or hydroxyl group in HA (Cazalbou *et al.,* 2004).

FIBERS INCORPORATED IN HYDROXYAPATITE SCAFFOLD

These include mainly both natural fibers and synthetic fibers. Natural fibers have good cellular adhesion and remodelling property. They carry a high risk of immune response. Synthetic fibers are less immunogenic and more customizable than natural fibers. They carry high risk of local toxicity. These include mainly collagen (Glowacki and Mizuno, 2008), gelatin (Barbani, *et al*., 2012), chitosan (Danilchenko, *et al.*, 2011), poly caprolactone (Park *et al.,* 2011) etc.

POLYMERS

Polymeric materials used in BTE are natural or be synthetic. Polysaccharides (chitosan, cellulose, agarose, starch, alginate, hyaluronic acid, lignin) and proteins (collagen, fibrinogen, silk, fibrin, and gelatin) are natural polymers that show good biocompatibility, osteoconductivity, and low immunogenicity. Synthetic polymers are polylactic acid (PLA), polycaprolactone (PCL), polyglycolic acid(PGA) etc (Kazimierczak and Przekora, 2020).

COMPOSITE

Composite materials consist of two or more components possessing various features to obtain biomaterials

with properties that differ from particular components. The most popular composite materials are: metal–ceramic, polymer–ceramic, metal–polymer, and polymer–polymer. Composites of HA and various polymers are considered as the most biomimetic materials since they are proven to significantly enhance bone formation in vitro and/or in vivo (Kazimierczak and Przekora, 2020). The properties of composite materials are biomimetic properties, good mechanical strength, biocompatibility, osteoconductivity, osteoinductivity, bioactivity, and biodegradability.

CONCLUSION

A comprehensive overview of the biosynthetic bone grafts used for augmenting bone healing has been provided. Fracture with extensive bone loss may go for non-healing or may get healed by soft fibrous tissue which will not provide sufficient mechanical strength. Bone tissue substitute were used in such cases, which fill the gap between fracture fragments and promote healing. There are different types of bone tissue substitutes like autograft, allograft, xenograft etc which are traditionally used. These traditional grafts have many disadvantages like lack of availability to fill large defects, immunogenicity, chance of disease transmission etc. To overcome the disadvantages of this graft biosynthetic bone graft was introduced, which have synergistic effect of osteoconductive scaffold, osteoprogenitor cells and osteoinductive growth factors. Therefore, use of composite grafts was practical solution to the difficult problems of treating bone loss.

FUTURE PROSPECTS

Currently bone tissue engineering is used to create bone tissue substitute which resembles normal bone. Further we can use bone tissue engineering to create characteristic new bone tissue substitutes which are better than native bone, aimed at specific purposes. New domains should be explored in scaffold preparation and biomaterial based regenerative medicine strategies in orthopedics.

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