Original Article

Indian Journal of Medical & Health Sciencēs
Volume 8 Number 2, July– December 2021
DOI: https://dx.doi.org/10.21088/ijmhs.2347.9981.8221.2

Potential of Osteoinductive Artificial Bone Graft Materials in Future Bone Graft Markets

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How to cite this article:
Bindiya K Pahuja, Ritu Duggal, Isha Duggal/Potential of Osteoinductive Artificial Bone Graft Materials in Future Bone Graft Markets/Indian Journal of Medical & Health Sciences 2021;8(2):51-57.

Abstract

Bone grafts play a pivotal role in bone regenerative surgeries. Each physician from orthopedics to dentistry knows the three fundamental mechanisms of bone grafting that are osteoconduction, osteo-induction, and osteogenesis. However, there is a huge lacuna in the basic understanding of the variation and range of the function of the graft materials falling under the basic three categories.The popular tissue engineering "biological triad" consisting of cells, scaffold, and growth factors, remains the mainstay of bone grafting techniques. However, the clinical success of bone grafting remains upheld by the basic understanding of other significant factors apart from the 3 basic factors mentioned in tissue engineering triangle. An attempt has been made in the current article to acknowledge the rest of the important factors driving the clinical success of graft surgeries. The article further addresses all the recent modifications made to the tissue engineering triangle by providing special emphasis on Calcium phosphate osteoinductive graft materials due to their unique BMP promoting properties. The application of bone ceramics or calcium phosphates has increased tremendously over the past decade. Their application as targeted delivery vehicles and as synthetic bone graft substitutes has demonstrated success. The diverse natural occurrence of calcium phosphate in various geometric and chemical compositions has complicated its processing and application to some extent. The idea of the current article is to make clinicians and researchers aware of this noble bone substitute to potentiate the use of this material to the maximum utility. The various natural sources of calcium phosphates such as eggshells and seashells should also be explored to obtain perfect cost-effective substitutes for autografts in the future.

Keywords: Bone Grafts; Artificial Bone Substitute; Calcium Phosphate Bone Cement; Tissue Engineering Triangle, Pentagon; The Concept of Bone Regeneration; Guided Bone Regeneration; Oral hard Tissue Formation.

Introduction

The three mechanisms of biology that provide the fundamental basis for bone grafting are either osteoconduction, osteoinduction, or osteogenesis.[1] Among the three bio-mechanism, osteoconduction is the simplest form of bone graft Mechanism.[1,2] In this mechanism the bone graft acts as a scaffold and the homing of cells from osteoblastic lining of the endosteum or periosteum forms the desired bone.[2] These osteoblasts utilize the scaffold material as a framework upon which host elements spread and generate new bone.[2] While on the other hand, Osteoinduction is the process of stimulation of bone formation with more potential of growth than an

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Received on: 18/10/2021
Accepted on: 18/11/2021

osteoconductive material. A bone graft material that is osteoinductive will not only serve as a scaffold for existing osteoblasts, but will also trigger the formation of new osteoblast.[3] In 1970, Urist first described the expression of these triggers as BMPs (bone morphogenetic proteins).[4] These BMPs were labeled as the 'osteoinductive signals', that were isolated from demineralized bone.[4] In osteoinduction, various other materials like enamel matrix derivatives (EMDs) play a role in bone stimulation. EMD has the ability to enhance the osteoinductive effect of demineralized graft materials (DFDBA).[2]

The final bio-mechanism of bone formation via bone graft is osteogenesis, in which the graft materials tend to utilize the host to the fullest. Osteogenesis is the formation or growth of new bone that utilizes viable osteoblasts originating from 'within' the bone graft material.[2,3,4] These osteoblasts contribute to the growth of new bone, through grafting of viable bone in the form of autografts.[5]

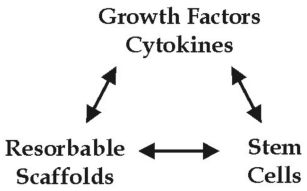

Fig. 1: The tissue engineering triangle.[6]

Background

Tissue engineering has been hyped so much in the last decade and the principles of which are solely based on the "biological triad" i.e. cells, scaffold and growth factors.[1] The scaffold is a mesh work or framework;[2] the cells, are the viable cells required for reconstruction.[3] The growth factors are the signaling molecules that direct the cells to form the desired tissue. Therefore, the participation of three fundamental components was considered pivotal to bone formation unless further essential factors got identified (Figure 2).[12]

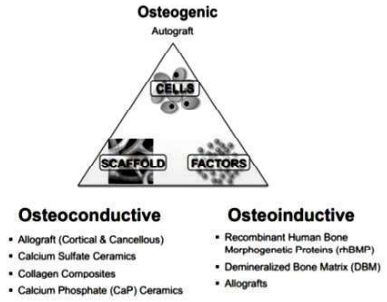

Fig. 2: Three fundamental components for bone formation.

Recent advances in molecular biology has added new heights in the understanding of tissue regeneration leading to the addition of new elements to the biologic triad.[11] A fourth mandatory factor for optimization of the bone formation was found to be the micro-mechanical stability (Figure 3).[11]

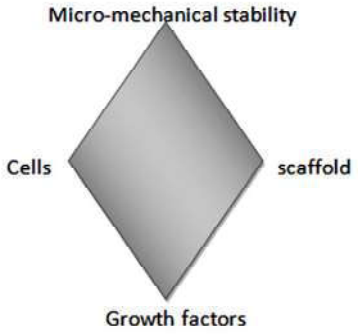

Fig. 3: Four factors needed for optimum bone formation.

The idea behind addition of the 4th factor to the biologic triad is the fact that wound healing requires a significant amount of stability throughout the healing period to perform osteosynthesis.[11] Internal or external stabilization has always remained an integral part of facture healing.[1] The microme chanical stability component and its significance in wound healing cannot be underestimated especially in the case of osteosynthesis, or bone healing.[1] Due to the inclusion of this element, the triangular shape of the triad has transformed into a diamond, and soon after its transformation it has been turned into a pentagon due to the addition of newer factors like vascularity and local environment of the wound area (Figure 5).[5]

There is no doubt in the literature about the fact that vascularity is the major supply of nutrients and raw material for wound healing, and this is established through the Vascular endothelial growth factor (VEGF).[9,11] Neo-vascularization per se plays an important role in the endochondral as well as intramembranous bone formation.[12]

Besides vascularity, 'existing biological variation' of the host and the host related factors like genetics, age, underlying debilitating diseases, can't be ignored[1], which may also influence the bone-forming capacity. Furthermore, the negative impact of preexisting co morbidities such as diabetes, rheumatoid arthritis, osteoporosis, etc cannot be neglected.[11,13] Therefore, the newer model of tissue engineering triangle turned out to be a hexagon finally (Figure 4).

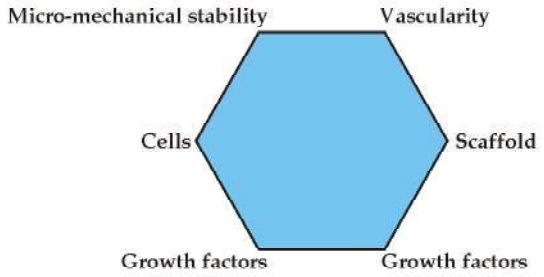

Fig. 4: The hexagonal model of tissue engineering.

Later on, the hexagon was again modified to diamond shaped structure, including all the 6 components based on the significance and the role they play in wound healing. Both the tips of the diamond werelabeled with vascularity, and host factors were made to occupy the body of the diamond.[11]

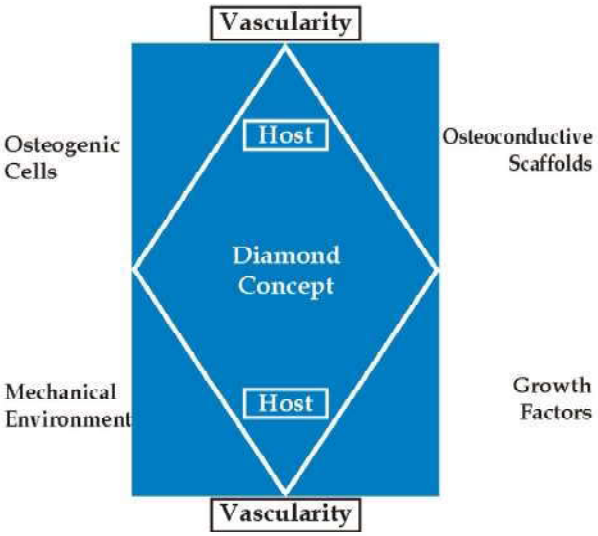

Fig. 5: The diamond model of bone fracture healing interactions.

The elements of Bone grafts in detail

Scaffolds

The 3D scaffold permits sufficient transport of gases, nutrients, and regulatory factors to allow cell survival, proliferation, and differentiation; apart from providing the framework for tissue growth.[7] The resorption rate of the graft should match the physiologic resorption rate of the bone, for optimal outcomes.[8] The material should not collapse and should only get replaced with bone, avoiding soft tissue formation.[8]

Growth Factors/signals

Different bio-active molecules (found in earthen materials like silica), and natural growth factors like BMPs will attach to the cell surfaces producing signals that could ultimately help in cellular proliferation and differentiation for bone formation.[9]

Cells

A lot of cells of different varieties are needed for bone formation.[6] The primary cell form or baseline series of cells of bone formation comes from Mesenchymal stem cells, which then differentiate into osteoblasts in presence of various growth factors like TGF-beta and mature with the help of BMPs.[10] Any source that can provide mature or immature cells to the site of healing is welcomed. Autografts provide differentiated matured osteoblasts and adipose tissue, while bone marrow stem cells could provide immature multipotent stem cells which can be differentiated into bone cells later.[10]

Ironically, all bone graft substitutes are osteocon ductive in nature, despite their least bone growth potential and only an insignificant number of commercially available bone grafts materials are osteoinductive in nature.[14] The functional bone-forming capacity of osteoconductive bone grafts lies in their property to act as a scaffold, but generally, the rapid resorption of the scaffold impairs the regenerative ability of local bone. Destabilization of the graft and untoward inflammatory response may impair the new bone formation.[14]

The phenomenon of osteoinduction, was defined by Friedenstein, as the process of the 'induction of undifferentiated inducible osteoprogenitor cells', that are not yet committed to the osteogenic lineage to form osteoprogenitor cells.[15] Later on Urist found the involvement of bone morphogenic proteins (BMPs) as functional biomolecules responsible for osteoinduction.[4] Demineralized freeze dried bone graft (DMFD) and mineralized freeze dried graft (MFDG) are typical examples of osteoinductive graft materials. However, the only FDA approved artificial graft material that has proven osteoinductive potential is Beta-tricalcium phosphate.[15]

The Calcium Phosphate Bone Graft Substitutes

Calcium phosphates are the largest group of artificial bone graft substitutes. This is mainly due to their close resemblance to the mineral components of bone. Calcium phosphate can occur in various forms in nature .[16,17]

Table1: The various forms of Calcium phosphate.

Name	Abbreviation	Formula	Ca/P ratio
Monocalcium phosphate monohydrate	MCPM	$Ca(H_2PO_4)_2.H_2O$	0.5
Dicalcium-phosphate anhydrate (monetite)	DCPA	$CaHPO_4$	1.0
Dicalcium-phosphate dihydrate (brushite)	DCPD	$CaHPO_4.2H_2O$	1.0
Octacalcium-phosphate	OCP	$Ca_8H_2(PO_4)_6.5H_2O$	1.33
ß-Tricalcium phosphate	ß-TCP	$Ca_3(PO_4)_2$	1.5
Amorphous-calcium phosphate	ACP	$Ca_3(PO_4)_2.nH_2O$	1.5a
α-Tricalcium phosphate	α-TCP	α-$Ca_3(PO_4)_2$	1.5
Hydroxyapatite	HA	$Ca_{10}(PO_4)_6(OH)_2$	1.67
Tetra-calcium phosphate	TetCP	$Ca_4(PO_4)_2O$	2.0

Calcium phosphate ceramics mainly consist of hydroxyapatite (HA) crystals or amorphous hydroxyl apatite, the chemical composition of which resembles the natural bone but physical properties may vary.[18] The calcium phophates are converted to hyroxyapatite by dehydration and sintering at high temperatures.[19] The HA forms the majority of the natural bone (50%), which may account for its excellent osteoconductive and osteo-integrative properties.[19]

How Calcium Phosphate Salts Can be osteoinductive.

Osteoinduction has been demonstrated for various CaP phases, including HA, TCP various blends of the two in the form of BCP, DCPD, DCPA, carbonated apatite (CA) and OCP and in various forms such as sintered ceramics cements.[26,27]

While a growing number of studies confirmed that osteoinductivity can be an intrinsic property of some CaP ceramics, the clinically relevant question remained unanswered i.e. whether we can consider osteo-inductive CaP as a true alternative to natural bone grafts?

Probably the best proof for clinical relevance of CaP ceramics with intrinsic osteo inductivity was provided by Yuan et al, in which an osteo-inductive TCP ceramic was compared to an autograft and to a rhBMP-2 construct in a critical size defect.[28] The osteo inductive ceramic was shown to be successful in bridging an ovine critical sized iliac wing defect by newly formed bone.[28]

Nobody could explain how chemical composition, macrostructure, surface micro and nano structural properties altogether could produce osteogenic effects. Some say it is the biodegradation of the graft materials or the ions that leach out in the environment, others say the stem cells get accumulated near the foreign graft materials.[21] Osteo-induction requires a perfect blend of permutation and combination of all the factors necessary for bone formation.[30] The determinants of osteointegration also include the surface properties, texture, and consistency of the graft materials in toto.[31] In the present world, only the calcium phosphates cement have shown some osteo-induction in vivo. Depending upon the composition of calcium phosphate they take the place between bioactive and bio-tolerant bone graft substitutes.[33]

Another contrasting fact about CAPs grafts is their potential to always induce intra membranous bone formation, while on the other hand, BMP grafts materials can induce both kinds of bones.[8,29] It is quite obvious in the literature of embryonic development that the intra cartilaginous bone could never respond to pressure, in contrast to BMP 2 induced formation that mainly occurs via the endochondral pathway.[29] Because BMPs are differential factors rather than true bone graft factors, it is found that the material induced ectopic bone formation is relatively slow with CAP's as compared to the BMP 2 driven cases.[29]

Many CAPs have been studied and clinically implemented in the past, the first among which is HA. Mechanical properties of HA are compared to the cancellous bone which is brittle and has a tendency to wear under tension but is resistant to compressive loads.[20] The ideal porosity of > 100 mm of HA allows the optimal gluing, multiplication, and differentiation of its bone-forming cells and hence allows the bone formation.[22,29] Although, the pore size is important, but its significance should does not be overrated as there is no graft material which does not function at all.[21] With the introduction of newer subtypes of CaP cements like ß tricalcium phosphate (ß-TCP) [(3-$Ca_3(PO_4)_2$], octocalcium phosphate and other similar graft materials, the bone graft industry has found absorbable materials possessing more bio-mimicking properties.[29] It is further stated that the more is the Ca/P ratio, the less is the resorption rate of that particular graft material.[16] Researches have

tried many combinations and permutations of grafts materials to resolve the mystery of bone resorption and to potentiate efficient bone forming substitutes, for example, a combination of HA and TCP has been tried which resulted in biphasic calcium phosphates BCP. This graft has shown superior bone-forming capacity with slow resorption rate.[23] The resorption rate of BCP exactly matches the physiologic resorption of bone.[23]

Another combination of HA and calcium sulphate has been introduced in the form of Cerament™. It forms an advanced type of syringe graft material that is putty in consistency and can easily be injected.[22] The superior handling properties intra operatively, have been responsible for the gain in popularity of this graft material.[22]

To further overcome the drawbacks of HA, nanocrystalline HA was introduced, in which a larger surface to volume ratio is conferred. This property not only significantly reduced the sintering temperature of HAp(ID1) ceramic but also led to the increased resorption rate. However, this has not gained popularity in clinical observation.[23]

Amorphous calcium phosphate (ACPs), which may later get organized to crystalline forms, have proved efficacy as a crystalline HA itself. ACPs are never considered promising minerals for bone formation, partly because their processing may require cumbersome methods, which may be a cause behind their less popularity and partly because their handling is not easy.[18] The physiologic pore size optimal for the ingrowth of new tissue lies somewhere between 150 and 500µm. Larger pore size may not provide sufficient stimulus to bone growth, and smaller pore size, on the other hand, may not provide space for cell homing.[16]

Limitations of artificial bone grafts

The bone graft substitutes are falling short of natural bones in three major qualities (1) Their physical handling properties, (2) deficient osteogenic potential, and (3) lack of physical and biological properties innate to natural bone.[24]

Calcium phosphates can never equate to natural bones in any form or structure due to the lack of elasticity provided by the presence of a collagenous organic component in bone. The elasticity is essential to provide strength and resilience to the bone as well as to reduce brittleness.[23] If we talk about the compressive strength of the bone, then the calcium phosphate cement may provide some bare minimum strength which may equate to the strength of the cancellous bone, but this could nowhere come close to the strength of cortical bone.[23,24]

HA grafts possess high compression strength, and beta TCP grafts have lower compression strengths.[25] Hence, it can be inferred that the selection of calcium phosphate graft substitutes is based on their chemical composition (as shown in Table 1) as well as on their physical properties but not on the mechanical properties.

Bioactive Glass

Bioactive glass, also known as bioglass, refers to a group of synthetic silicate-based ceramics and was originally constituted by silicon dioxide (SiO_2), sodium oxide (Na_2O), calcium oxide (CaO), and phosphorus pentoxide (P_2O_5) in the 1970s. This was later modified to a more stable composition by the addition of potassium oxide (K_2O), magnesium oxide (MgO), and boric oxide (B_2O); the key component, silicate, constitutes 45-52% of its weight.[32] The optimized constitutions lead to a strong physical bonding (kind of glue) between bioglass and host bone. This property is attributed to the leaching and accumulation of silicon ions while coming in contact with host tissue.[32] These earthy ions are highly loved by the minerals of the body, which in turn may produce hydroxyapatite coating on the surface of bioglass.[33] This coating of hydroxyapatite may further absorb proteins from the surrounding environment, leading to bone formation.[33]

Bioglass (NovaBone Product) containing SiO_2, Na_2O, CaO and P_2O^5, is a commercially available bioglass. The degradation of bioglass increases the pH of the surrounding tissue and makes it more alkaline, which may, in turn, lead to the accumulation of ions in the host micro environment.[34] According to biological principles, the accumulation of ions is not liked by cells and is also repulsive to growth factors, and that is plausibly the cause of the non-popularity of these grafts. Bioglass could glue to the bone, but it allows no osteoinduction (Fig. 6).[34]

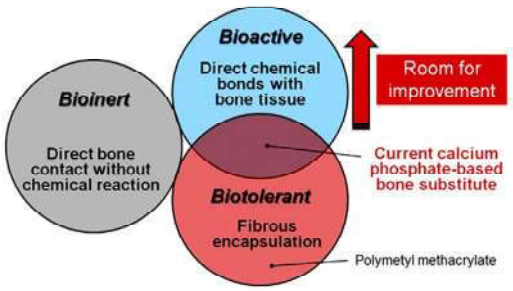

Fig. 6: Properties of different bone graft materials.[34]

The question is how do these graft materials get eliminated from the body?

The graft materials can be eliminated either by cell-mediated elimination, hydrolysis, or by chemical degradation. Biodegradable is defined as something that can be cleared off the body through enzymatic degradation.[17] Bio-resorption on the other hand is degradation by the process of hydrolysis. Bio-absorbable substances get degraded by chemical degradation methods, and their byproducts are generally excreted through one of the body's organ systems. It is unclear so far about which route (among the 3) of elimination these bone graft materials prefer.[17]

Bio inorganics

Bio inorganics are the trace elements present in the body,yet known to play a major role in bone formation.[4] For example strontium ranelate which acts as an anti-osteoporotic agent, and fluoride, being an anti-cariogenic agent, are too small to be easily ignored but carry great importance when it comes to bone graft industry.[15]

While strontium ranelate generally can't be consumed via the oral route, because of its low oral bioavailability and high first-pass metabolism, many local routes have been tried, for example, insertion at the location of bone defects placement, but how significantly such compounds are showing an uptrend in the bone graft industry is yet to be found.[15]

Conclusion

An attempt has been made to summarize the mechanism of action of bone graft materials and the potential of CaPs as an artificial osteoinductive bone graft material. The application of bone ceramics or calcium phosphates has increased tremendously over the past decade. Their application as targeted delivery vehicles and as synthetic bone graft substitutes has demonstrated success. The diverse natural occurrence of calcium phosphate in various geometric and chemical compositions has complicated its processing and application to some extent. The clinicians and researchers must bring up this noble bone substitute to the point of maximum utility in the medical graft market. The various natural sources of calcium phosphates such as eggshells and seashells should also be explored to obtain perfect cost effective substitutes for autografts in the future.[35]

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