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Bone Graft Substitutes for Bone Defect Regeneration. A Collective Review

Review Article

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Abstract

Objective: The purpose of this review article is to illustrate the current state of development of bone graft substitutes that could be used for bone defect regeneration, as well as to analyze their efficacy for clinical use.
Methods: An electronic search of the PubMed, was performed for articles written in English. The focused question was "ideal graft substitute material to choose in clinical practice"?. The searches were limited to articles including: bone graft, bone defect scaffold, bone substitutes, and bone regeneration. An attempt was made to identify clinical studies. During the data collection, the data were extracted from the studies including scaffold material, properties and advantages of bone substitutes, as well as clinical results if the study has been provided clinically.
Results: In spite of several acceptable scaffold options available for bone regeneration, these options still need to bridge the gap between research and clinical practice. There is little information available about the cellular basis for bone regeneration in humans. Several problems limit the broad usage of such options, including lack of randomized controlled human studies, and dubious long term results.
Conclusion: The studies should be nurtured and monitored by a combination of clinical experience. Future trends may focus on the effective combinations of osteoinductive materials, osteoinductive growth factors and cell-based tissue regeneration tactics using composite carriers. There is no single ideal graft material to choose in clinical practice, therefore researches are ongoing in all relevant fields, to establish modern bone regeneration protocols that may lead to the innovation of ideal graft substitutes.

Keywords: Bone Graft; Scaffold Bone Defect; Bone Substitutes; Bone Regeneration.

Introduction

Bone is the second most transplanted tissue in the body [1, 2], with approximately 3.5 million bone graft procedures performed each year [3]. There are many cases in which bone grafts are needed in large quantity such as for reconstruction of large bone defects caused by trauma, tumors, infections, and congenital defects, and also in cases where the regeneration is compromised (osteoporosis, necrosis and atrophic non-unions) [4, 5]. All these facts emphasis that, large bone defects are still a challenge for maxillofacial surgeons.

Generally four elements are needed of bone grafts for bone regeneration: Osteoconduction, osteoinduction, osteointegration, and osteogenesis [6, 7]. Osteoconduction is the ability to support bone growth on a surgical site, during which pores, channels, and blood-vessels are formed within bone. Osteoblasts from the margin of the defect that is being grafted utilize the bone graft material as a framework upon which to spread and generate new bone. Osteoinduction involves the stimulation of osteoprogenitor cells to differentiate into osteoblasts that then begin new bone formation. The most widely studied type of osteoinductive cell mediators are bone morphogenetic proteins (BMPs) [8]. A bone graft material that is osteoconductive and osteoinductive will not only serve as a scaffold for currently existing osteoblasts but will also trigger the formation of new osteoblasts. Osteointegration is the direct contact of living bone to graft material [9]. Finally osteogenesis is the formation of new bone by osteoblasts within the graft material [5-7].

There are three main types of bone grafts, autografts, allografts and bone graft substitutes [5]. The autograft is considered as the standard in bone grafts. The ilium is the most frequent donor site accounting for one-third of all cases [10]. However, its use is limited by complications such as pain, additional operating time, infection, scarring, blood loss, and donor site morbidity [10, 11].

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Allograft from cadavers or living donors is the most commonly used alternative to autografts [12]. The main advantage of allografts is increased availability in various shapes and sizes and the avoidance of donor-site morbidity [6]. On the other hand they have no viable cells to offer osteogenic properties, which may result in incomplete integration with the host tissue [13]. They lack the osteogenic capacity of autografts and carry the risk of infectious agents or immune rejection [14]. Both types of the bone graft options come with inherent limitations; which has led to the need for the development of new bone graft substitutes. Substitute materials are advantageous because of their unlimited availability but unfortunately they lack both osteoinductive and osteogenic properties, so suffer from poor integration and eventual mechanical failure [5, 13]. Therefore, the next generation of bone graft substitutes must be developed. Using tissue engineering techniques, it is possible to design new scaffolds aiming to decrease the disadvantages of traditional grafts and improve graft integration, osteogenicity, osteoconductivity, and osteoinductivity. The aim of this review article is to illustrate the current state of development of these substitutes that could be used as alternative for their application in bone defect regeneration, as well as to analyze their efficacy for clinical use.

Biological properties of bone

Proceeding from the need of bone graft substitutes, the field of tissue engineering seeks to address this need using different aspects of medicine, biology, and material science. However, before a suitable bone tissue replacement can be engineered, the biological properties of the bone must be better understood.

Bone tissue consists of bone extracellular matrix and bone cells, extracellular matrix is comprised of both organic and inorganic components [15]. The organic components are formed of type-I collagen fibrils, osteopontin and osteocalcin. Within the bone extracellular matrix, osteopontin is known to promote cell attachment through covalent binding with fibronectin and type I collagen. Both osteopontin and osteocalcin have an alliance with calcium and may support the nucleation of calcium phosphate during mineralization [16]. The inorganic components of the matrix are calcium, carbonate, and phosphate ions, arranged in a crystalline-like structure. Matrix mineralization starts with nucleation of calcium phosphate crystals, and followed by crystal growth [17]. Non-collagenous proteins can be nucleation points for crystallization [18]. There are three types of bone cells in bone tissue: osteoblasts, osteoclasts, and osteocytes. Osteoblasts are responsible for bone formation through the synthesis and secretion of an organic extracellular matrix, and also synthesize a variety of growth factors including transforming growth factor-β (TGF-β) and bone morphogenic proteins (BMPs) that can aid in both the recruitment and differentiation of stem cells [15]. When matrix is no longer actively being formed, the osteoblasts become embedded within the extracellular matrix and become osteocytes. Osteoclasts are responsible for bone resorption [19]. Communication between the three types of bone cells regulate the formation and resorption of bone.

Bone grafts into the recipient site

It is known that an ideal bone graft should have osteogenesis, osteoinductivity, osteoconductivity, and osseointegration characteristics [1, 20, 21]. Therefore it is important to understand the mechanism of action for each graft. Bone grafts are used to bridge a critical size bone defect, they become incorporated into the recipient site. Incorporation of the bone graft involves two essential steps: (1) the union between the edges of the graft with the edges of native bone segments, (2) gradual resorption of the graft, associated with its replacement by new bone [22-24]. The space occupied by that the graft should ultimately turn into viable bone and permanently accessible to the physiological remodeling mechanisms. This process is typically very slow, and cannot always be accomplished. There are many factors which regulate the incorporation process, these factors may be relevant to the type of graft, it porosity, recipient site, and the graft - bone interface. Incorporation is better in autogenous grafts, because of their porous nature, which allow easy vascular and cellular invasion. The graft structure has a large surface area that is covered by osteoblasts, making it osteoconductive and osteogenic, also through the extensive vascular invasion, the bone matrix can be demineralized and its proteins exposed through the actions of osteoclasts. Lack of integration between the graft and adjacent bone segments, makes the process slow and rarely complete [22]. Finally, graft incorporation can be summed up into five main steps [2]: (1) hematoma formation, release of bone inducing factors and cellular recruitment, (2) inflammation and development of fibro-vascular tissue, connecting the graft to the adjacent bone, (3) vascular invasion of the graft, (4) focal resorption of the graft by recruited osteoclasts, (5) new bone formation, union between the graft and the surrounding bone, and graft remodeling.

Bone graft substitutes

Selection of bone graft substitutes should be based on its characteristics and properties of the biomaterials, which include mechanical, chemical, toxicological, and morphological properties. The overall success is based on tissue compatibility of a biomaterial, as well as the nature, degree, frequency, and its constituents to the intended tissues into which it will be utilized. During the few last years, studies have been focused on the optimal scaffold design which changed the requirements and properties of the biomaterials used. Since 2002, Hench has defined three different generations [25], starting with the earliest and following the order in which they occurred. First generation of bone graft substitutes desired to match the biomaterial with the physical properties of the tissue to be replaced, while maintaining inertia with the immediate small-scale environment of the tissue. This generation contains metals (stainless steel, titanium), alloys (alumina, zirconia), and polymers (silicone, polypropylene, polymethylmethacrylate). A common manifestation for this generation is the formulation of fibrous tissue at the tissue graft interface that would finally enclose the graft then leading to loosening. The material cannot be phagocytosed since it is encapsulated by fibrous connective tissue, isolating it from the surrounding tissues [26]. In order to avoid the formation of this fibrous layer and improve osseointegration, tissue engineering has developed the second generation of bone graft substitutes, by covering the biomaterial with hydroxyapatite, Beta-tricalcium phosphate, or bioactive glass. This generation used synthetic or natural polymers to stimulate a controlled chemical slump, leading to passive products which can be resorbed [27]. Third generation of bone graft substitutes have been developed, to get material nearer to the autograft, using patient material which induces, at molecular level, cellular response

by combination of the bioactivity and biodegradability of second generation devices. This generation has been founded on the notion of bone tissue engineering which lead to creation of a device that support bone regeneration through the cooperation between bone osteoprogenitor cells and growth factors (natural components) for stimulation of cells into a scaffold made of various natural or synthetic biomaterials [28].

Tissue engineering has established specific scaffold properties, which guarantee biocompatibility, porosity, Micro & Nano scale structure, rate of degradation , and growth factor delivery [29]. Biocompatibility is linked to a scaffold material which does not formulate undesirable local or systemic responses [30]. Porosity is related to interconnectivity, this means that it improve osteogenesis. A highly porous scaffold expedites cell husbandry and migration, while smaller pores allow tissue ingrowth [14]. Although the mechanical strength of the scaffold decreases with porosity, this estimation should be tied with the mechanical needs of the bone tissue which have to be replaced. Also pores promote the diffusion from and to the scaffold and simplify vascularization [14]. Micro & Nano scale structure promote cell functions leading to improved osteoinduction and osseointegration [31]. The proportion of degradation of the scaffold must stay tuned to stock the structure until the new bone has adequate mechanical strength [32]. Lack of this state, may lead to the scaffold fracture after a mechanical loading, before the completion of bone healing process. Delivery of growth factors such as transforming growth factorβ (TGFβ), insulinlike growth factors (IGF), platelet derived growth factors (PDGF), and bone morphogenetic proteins (BMP), increase the potential of osteoprogenitor and osteoblast functions to enhance bone growth by encouraging MSCs to migrate into the scaffold, proliferate, differentiate, and begin extracellular matrix production [33]. There are many techniques to deliver these growth factors such as controlled release from biodegradable scaffolds, osmotic pumps, bolus injection, and surface adsorbed protein release. The confrontation of these techniques is a short half-life which leads to loss of their bioactivity, in addition to restrictive control of administered dose.

Scaffolds and bone substitutes

A wide variety of biomaterials are currently used as scaffolds, in modern clinical practice, including collagen, hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), calcium-phosphate, and glass ceramics [34, 35]. Bone substitutes can be defined as "a synthetic, inorganic or biologically organic combination biomaterial which can be inserted for the treatment of a bone defect instead of autogenous or allogenous bone" [36]. In the past, inoperative scaffolds have been used as space possessors during the restoration processes. Currently no one single scaffold can display osteoinductive, osteogenic, promote vascularisation and has suitable mechanical properties. Subsequently, the challenge is to form a scaffold with biologically active molecules, living cells, and promote the regeneration of bones. Currently used scaffold biomaterials can be classified into different groups:

Polymers scaffolds

Polymer materials are divided into natural polymers and synthetic polymers. Natural polymers, have likable properties, and facilitate the addition of chemicals, proteins, peptides, and cells to scaffolds texture. In addition, the porosity and mechanical strength can be controlled by inconstant polymer concentrations.

The most widely studied of natural polymers are collagen/gelatin, chitosan, silk, alginate, hyaluronic acid, and peptides (Table 1). Collagen can be isolated from animal tissues [37], it is characterized by biodegradability, biocompatibility and bioreabsorbability, also it has low antigenicity and ability of being crosslinked [38, 39], but it is difficult to control the rate of degradability [40]. Scaffold properties can be modified by using various concentrations of collagen [41]. Collagen is the main protein of the extracellular matrix, provides support to connective tissues such as skin, bones , blood vessels, and ligaments [42, 43], so collagen sponges have been used for the treatment of long bone fractures (Collagraft®) [44]. Yannas et al. has inspected, collagen scaffold in dermal regeneration process [45]. Kohara and Tabata induced bone formation by using gelatin sponges with bone morphogenetic proteins, also concluded that the use of gelatin scaffold mixed with multiple osteoinductive agents could be active to induce bone formation [46].

The synthetic polymers contain polyesters, polyanhydride, polyorthoester and polycaprolactone. The most commonly used synthetic polymers are the polyesters such as poly glycolic acid, poly lactic acid, and their copolymer of poly lactic-co-glycolic acid (Table 2). These polymers lack the desired bioactivity as documented by Holzwarth and Ma [47]. However they have the ability to be converted into specific material with chemical and mechanical properties needed for the required application [48-50]. Although synthetic polymer has shown clinical outcomes comparable to autograft, it was found to be connected with bad incorporation and biodegradation forms [51, 52]. Synthetic polymer implements poorly on radiographic estimation with elevated rates of graft breakdown, nonunion, displacement, and even as a spacer is debatable [53-55]. However since both the polymers (natural and synthetic) have certain advantages, a combination of them can be used to produce composite scaffolds with safely better biological and mechanical properties. Yang combined polycaprolactone with chitosan to create bioactive nanofibers [56]. This new hybrid scaffold takes advantage of the physical properties of the synthetic polymer and the bioactivity of the natural polymer while minimizing the disadvantages of both. A collagen and poly lactic acid scaffold with parallel collagen fibres embedded within a PLA matrix has been fabricated by Dunn et al. They suggested that resorbable polymeric composite scaffolds are potentially useful for reconstruction of the anterior cruciate ligament of the knee [57].

Ceramic scaffolds

Ceramics are crystalline texture of inorganic, nonmetallic mineral salts. This group is classified to two subgroups: bioinert or bioactive, also bioactive ceramics are categorized as resorbable or nonresorbable (Table 3) [58, 59]. Hydroxyapatite (HA), Calcium Phosphate (CP), Tricalcium Phosphates (TCP), and glass ionomer cesments, are all designated in this group [60]. The most commonly inspected are calcium phosphate ceramics, tricalcium phosphate and hydroxyapatite, because of their chemicophysical uniformities to the bone mineralization phase [61], they form a perfect osteoconductive scaffold for bone regeneration. Suetsuna et al. analyzed the records of 36 patients, where HA scaffold (40–45% porosity) sunk slightly into the vertebral body, for one level herniated cervical discs, no graft related complications were observed [62]. On the other hand Kim et al., using a HA scaf-

Table 1. Properties and advantages for natural polymers scaffolds.

No	Scaffold Materials	Properties and advantages
Natural polymers		Biocompatible, control of porosity, and mechanical strength by changing concentrations [95-98].
1	**Collagen/gelatin**	Biocompatible, stimulates proliferation and differentiation of cells, and poor mechanical properties [99-101].
1.1	Collagen added with poly-ε-caprolactone (PCL) and alkaline phosphatase	Increase cell proliferation, and cause mechanotransduction [102-104].
1.2	Collagen added with CaO–P_2O_5–SiO_2 and phosphatidylserine (PS)	Higher PS ratios, increase permeability and decrease compressive strength. PS could enhance osteogenic potential but osteoinduction and osteoconduction has not been fully defined [105-107].
1.3	Gelatin added with Chitoolisaccharide mixing	Support ectopic calcium deposition [108].
1.4	Gelatin added with magnesium calcium phosphate (MCP).	Increase proliferation and osteocalcin but it is not clear if the magnesium released could have an important impact [109, 110].
1.5	Collagen-poly(lactic-co-glycolic acid (PLGA) hybrid added with bone morphogenetic protein-4 (BMP4)	Induce osteogenic differentiation of osteoblasts[111, 112].
1.6	Collagen added with silica	Increase osteogenic and angiogenic potential [113, 114].
2	**Chitosan**	Biodegradable, biocompatible, antibacterial, and bio-adhesive properties [115].
2.1	Chitosan added with poly(butylene succinate) (CHPBS) and marrow stromal cells	Elevate rate of bone formation [116].
2.2	Chitosan added with collagen and Beta-glycerol phosphate	Scaffolds were 3 times stiffer than pure chitosan. Also it is highly beneficial for the osteogenic capabilities of the scaffold [117, 118].
2.3	Chitosan added with collagen and glyoxal	Mechanical changes conferred to the hydrogel upon crosslinking are not beneficial to the induction of bone formation [119].
2.4	Chitosan added with silk fibroin and HA	Unclear whether the presence of silk fibroin and HA help promote osteogenesis [120, 121].
2.5	Chitosan added with tricalcium phosphate and platelet rich plasma	Promising system where MSCs do not need to be conditioned in osteogenic medium in order to produce robust bone growth [122, 123].
2.6	Chitosan added with alginate	Addition of proteins tends to improve osteoactivity [124].
2.7	Chitosan added with IGF1 and BMP2	Improve bone formation, but the effect was higher with IGF1. It is unclear if the results are due to changes in the porosity or to the presence of growth factors [125].
2.8	Chitosan added with HA and peptide	Beneficial to the differentiation process, and should be explored in composites of other materials [126].
3	**Silk fibroin**	Impressive mechanical properties, environmental stability, biocompatibility, controlled proteolytic biodegradability, and morphologic flexibility [127].
3.1	Silk added with CaP	Enhance osteogenesis, as it did not have any effect on the scaffold's mechanical properties [128].
3.2	Silk added with HA	Enhance the osteoconductive activity and mechanical properties [129-131].
3.3	Silk added with hexafluoroisopropanol	Critical component of bone regeneration [132].
4	**Alginate**	Improve the osteogenic outcomes [133].
4.1	Alginate added with fibrin	Does not address whether the presence of fibrin, has a role in enhancing osteogenesis [134].
4.2	Alginate added with calcium phosphate cement	Subtle changes in porosity, can be adjusted to obtain more satisfactory results [135].
4.3	Alginate added with proteins and peptides	It is not clear if the coordinated release of proteins from the scaffold had any effect on the osteogenesis [136, 137].
4.4	Alginate added with nanoscale calcium sulfate	Enhance the angiogenesis, as it is a necessary for bone formation [138].
5	**Hyaluronic Acid Hydrogels**	Hydrophilic, nonimmunogenic, and has been found in the cytoplasm of osteoprogenitor cells [139].
5.1	Hyaluronic Acid Hydrogels added with simvastatin	Improve viscoelastic properties and the addition of SIM improved osteogenesis *in vitro*, although the results were not as notable *in vivo* [140, 141].

5.2	Hyaluronic Acid Hydrogels added with growth factors	Osteoinductive and angiogenic factors can have a synergistic effect on bone mineralization [142-144].
5.3	Hyaluronic Acid Hydrogels added with growth factors and heparin	Non-Heprasil loaded hydrogels resulted in a more efficacious outcome *in vivo*, emphasizing the therapeutic importance of an increased release of BMP2 [145].
5.4	Hyaluronic Acid hydrogel added with an integrin ligand	Enhances the osteogenic potential of rhBMP2 [146]].
6	**Peptide Hydrogels**	Biocompatible and biodegradable [147-152].

Table 2. Properties and advantages for synthetic polymers scaffolds.

No	Scaffold Materials	Properties and advantages
Synthetic Polymers		
1	Aliphatic polyesters such as (polyglycolic acid, polylactic acid, and polycaprolactone)	Their degradation products are present in the human body and can be removed by natural metabolic pathways [153, 154].
1.1	PCL scaffolds incorporating HA particles	Increase in the amount of new bone formation in the PCL/HA scaffolding implants as compared to neat PCL counterparts [155].
1.2	PCL added with poly(diisopropyl fumarate)	The changes in mechanical properties imparted by the addition of PDIPF to PCL could have an effect on bone formation, but that effect is cell type dependent [156].
1.3	PCL added with poly(diisopropyl fumarate) and hydroxyapatite	The change in mechanical properties and presence of a mineral component can work together to enhance osteogenesis [157].
1.4	PCL added with calcium phosphate cement	BMSCs were able to attach to the composite, proliferate, and undergo osteogenesis [158].
1.5	Poly (L-lactide-co-ε-caprolactone) added with Poly (L-lactide-co-1,5-dioxepan-2-one)	Support osteogenic differentiation [159].
1.6	Dipeptide polyphosphazene-polyester blend	Good osteocompatibility [160, 161].
1.7	PCL coated with collagen and either chondroitin sulfate or a high sulfated hyaluronan.	Synergistic effect in osteogenic media and exposed to electrical stimulus [162].
2	**Copolymers**	Controllable physicochemical properties [163].
2.1	Copolymer added with BMP2 related peptide (P24) or simvastatin	Scaffold with simvastatin was more effective at inducing bone formation than the bone scaffold alone [164, 165].
2.2	Copolymer added with PEG–PCL–PEG , collagen and nano hydroxyapatite	Although the composite is better at regeneration than the self-healing process, they do not address how the individual components of their copolymer contribute to this effect [166, 167].
2.3	Copolymer added with poly (ethylene oxide terephthalate)/poly (butylene terephthalate)	Controlling surface roughness of a scaffold gives a level of control over MSC differentiation [168].
2.4	Copolymer added with poly (lactide-co-ε-caprolactone) (PLCL) scaffolds, collagen and HA	Promising results, and it should be tested with other types of cells that have not been committed towards the osteogenic route, such as MSCs [169].
2.5	Copolymer added with electroactive poly (ester amide) containing conjugated segments of amino-capped tetraaniline	Combining appropriate materials with physical stimulation such as electrical impulses can produce superior responses [170].

Table 3. Properties and advantages for ceramic scaffolds.

No	Scaffold Materials	Properties and advantages
Ceramic		Excellent biocompatibility and bioactivity but presents particular problems with its mechanical properties in terms of fracture and fatigue [59, 171, 172].
1	Bioglass	Control rate of degradation, excellent osteoconductivity, bioactivity, and capacity to deliver cells, but they present limitations in certain mechanical properties such as low strength, toughness, and reliability [173].
2	Calcium Phosphate	Osteoconductivity, bioactivity and resorbability *in vivo* due to their complex chemical composition (Ca/P ratio) and physical properties such as crystallographic structure and porosity [174].
2.1	Calcium Phosphate added with hydrogel microbeads and chitosan combined with BMP-2.	Increase in cell proliferation and a good level of histocompatibility [175-177].
3	Ceramic with combination of calcium aluminate added with melatonin and platelet rich plasma	Adhesion, viability, and proliferation of osteoblasts [178].
4	Bioglass-calcium phosphate composite	Biocompatibility, biodegradability and osteogenic [179].
5	Corals	Highly controlled pore sizes and promote the differentiation of human MSCs into osteoblasts [180].

fold (30% porous), found that all implants carried out had fusion at 6–12 months, with good clinical results and no graft collapse [63]. In another study, HA but with plating was used, and complete fusion occurred in 98-100% [64]. Dai and Jiang investigated clinical controlled trial of βTCP, contained in interbody cages, for patients with cervical radiculopathy [65]. A total of 62 patients were randomized into an anterior plating or nonplating treatment group, and followed for 2 years. At 6 months, successful fusion was noted in all patients. The authors concluded interbody cage containing βTCP to be an appropriate treatment for cervical fusion. However, the debate is still ongoing about their mechanical strength and resorbability.

Calcium sulphate can be used safely in benign metaphyseal bone defects, but it has a quicker resorption rate with more inconsistent results [66]. Biphasic calcium phosphates, which combine 40% TCP with 60% HA, may produce further physiological equation between mechanical support and bone regeneration. Yamada et al. accomplished a histological research of βTCP, HA with various ratios of the two so as to recognize which was the most conducive to osteoclastic activity [67]. This study suggests 60/40 ratio to give a more natural surface than either βTCP or HA alone. Biphasic macroporous ceramic scaffold appears effective in the treatment of metaphyseal defects for bone cysts and benign bone tumors, also the addition of osteoprogenitor cells from a bone marrow aspirate may hasten bone regeneration. Siegel et al. [68] observed 51 patients with benign bone tumors treated with the combined scaffold of βTCP and osteoprogenitor cells aspirated from bone marrow. At six months after operation, all implanted grafts showed radiological features comparable to the framing cancellous bone, as well the trabeculation and resorption rates were similar. In addition El-Adl et al. [69] reported on 34 patients with benign bone tumors treated with TCP/HA scaffold and bone marrow aspirate. This study demonstrated that the rate of bone regeneration was directly related to the size of defect. Several studies have estimated the wall cyst confusion by injection of a ceramic. Mik et al. marked out 55 patients who were treated for bone cysts using calcium sulphate pellets [70]. This study showed that 80% of patients had a complete or partial response after treatment. Furthermore Joeris et al. used percunateous tricalcium sulphate for bone regeneration and concluded that 96% of patients were with good results [71].

Coral in the presence of a phosphate donates calcium hydroxyapatite (known as coralline HA) and removes all immunogenic protein [72]. Agrillo et al. using granulated coralline HA within a carbon fiber cage demonstrated complete fusion in all 45 patients at 12 months with no complications [73].

Metallic and Composite scaffolds

Several metallic scaffolds are used to provide support for bone

Table 4. Properties and advantages for metallic and composite scaffolds.

No	Scaffold Materials	Properties and advantages
1	**Metallic**	Poor stimulation of new bone growth due to the elastic moduli, which does not correspond with natural bone tissue [110, 181].
1.1	Ti-based metals	Elasticity, mechanical properties, shape memory effect, porous structure and biocompatibility [182, 183].
1.2	3D microporous NiTi with Ti	Stimulate cell attachment and proliferation [183].
1.3	Ti with TiO_2	osteoconductivity and osseointegration [184, 185].
1.4	Metallic added with silica glass layers	Enables release of proteins and drugs into body fluid [187].
1.5	W4-Mg and Fe-Mn alloys	Viability and proliferation under certain conditions, bone formation, and biocompatibility *in vivo* [187, 188].
2	Composite	Strong as bone and has the same modulus of elasticity and capable of drug or growth factor delivery.
2.1	Polymer/ceramic	Biocompatibility, sufficient mechanical strength, osteogenic differentiation, and bone growth [189-193].
2.2	Metal/ceramic	Bioactivity, osteoconductivity, osteoinductivity, biocompatibility, and biodegradability [194-196].
2.3	Polymer/metals	Mechanical stability, biocompatibility, and partial biodegradability [197, 198].

defect regeneration, such as titanium [74], stainless steel [75], and aluminum [76], these metals are mechanically strong but are not biodegradable and release toxic metallic ions that lead to inflammatory cascades, allergic reactions, and tissue loss [77]. The essential abuse of metallic scaffolds is the deficiency of biological realization on the material surface. To overcome this problem, tissue engineering presents different ways to protect the mechanical properties and improve the biocompatibility of the surface. For example, Hydroxyapatite has been used to provide the necessary bioactivity to the titanium mesh with a porous network to assist osteoconduction [78, 79], also in cases where there is existing gap, titanium scaffolds often has been complemented with delivery of TGF-B and BMP-2 [80, 81], as well as stem cells have been cultured *in vitro* onto titanium scaffolds to induce the formation of calcified nodules and increase the production of mineralized extracellular matrix onto the scaffold [82]. Different clinical studies have utilized porous Ti scaffolds for tissues reconstruction. Kuttenberger et al. have applied laser-perforated titanium micro-mesh into 20 patients with defects in the craniofacial region [83]. The results showed loss of the mesh and excellent long-term stability during 8 years follow-up. Also Bystedt et al. concluded that implantation of porous titanium granules seem to function well as augmentation material in the sinus floor [84]. As well Jaquiéry et al. have used titanium meshes and autogenous bone graft into 26 patients with small and midsize orbital defects. This study indicated that titanium meshes provided stability and can support the orbital content [85]. Although there is a paucity of literature regarding the clinical outcomes and result of porous titanium scaffolds, longer follow-up periods and a larger sample of patients is still required in order to obtain reliable clinical success rates.

Combining two or more materials such as ceramics and polymers [86-88], the structure and biochemical properties can be modified to achieve more favorable characteristics, like biodegradability [89, 90]. For example, hydroxyapatite/PLGA composites possess the osteoconductive properties of hydroxyapatite and biodegradability of PLGA [91]. Polycaprolactone-tricalcium phosphate (PCL–TCP) scaffolds combined with recombinant human BMP7 has been demonstrated to completely bridge a critical size of tibial defect in a sheep model [92]. Also adult stem cells have been used to generate new tissue in combination with scaffold matrices [93]. Currently tissue engineering has developed smart delivery system which act in a sequential manner (one agent appears while another disappears) to achieve sequential delivery of BMP-2 and BMP-7, where nanocapsules of PLGA release one of the growth factors and then co-entrapped in chitosan fiber or PCL 3-D plotted scaffolds [94]. Smart systems can be used for controlled drug delivery using a group of polymers that physically or chemically respond to environmental stimuli such as light, temperature or pH. The mechanism of delivery suggests that upon decrease of temperature of the target site, swelling of nanospheres leads to release of their content, maximizing delivery at the target site.

Results

In spite of several acceptable scaffold options being available for bone regeneration, these options still need to bridge the gap between research and clinical practice. There is little information available about the cellular basis for bone regeneration in humans. Several problems limit the broad usage of such options, including lack of randomized controlled human studies, regulatory necessity, dubious long term results, as well as technique specific limitations.

Conclusion

The studies should be nurtured and monitored by a combination of clinical experience, and knowledge of basic biological principles. Future trends may focus on the effective combinations of osteoinductive materials, osteoinductive growth factors and cell-based tissue regeneration tactic using composite carriers. There is no single ideal graft material to choose in clinical practice, therefore research is ongoing within all relevant fields, to establish modern bone regeneration protocols that may lead to ideal graft substitutes.

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