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Review

Current bone substitutes for implant dentistry

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ABSTRACT

Purpose: Alveolar ridge augmentation is essential for success in implant therapy and depends on the biological performance of bone graft materials. This literature review aims to comprehensively explain the clinically relevant capabilities and limitations of currently available bone substitutes for bone augmentation in light of biomaterial science.
Study selection: The biological performance of calcium phosphate-based bone substitutes was categorized according to space-making capability, biocompatibility, bioabsorption, and volume maintenance over time. Each category was reviewed based on clinical studies, preclinical animal studies, and *in vitro* studies.
Results: Currently available bone substitutes provide only osteoconduction as a scaffold but not osteoinduction. Particle size, sensitivity to enzymatic or chemical dissolution, and mechanical properties affect the space-making capability of bone substitutes. The nature of collagen fibers, particulate size, and release of calcium ions influence the biocompatibility of bone substitutes. Bioabsorption of bone substitutes is determined by water solubility (chemical composition) and acid resistance (integrity of apatite structure). Bioabsorption of remnant bone substitute material and volume maintenance of the augmented bone are inversely related.
Conclusion: It is necessary to improve the biocompatibility of currently available bone substitutes and to strike an appropriate balance between bioabsorption and volume maintenance to achieve ideal bone remodeling.

Contents

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1. Introduction

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Implantation of prostheses to replace lost teeth is essential in prosthodontic therapy. Primary reasons for tooth loss include tooth fracture or severe periodontal disease [1], which leads to severe alveolar bone resorption compromising bone quantity, morphology, and quality [2]. In addition, the labial bone in the upper anterior region is normally thin (approximately 100 μm) and subject to considerable resorption after tooth extraction [3]. As a result, the volume of bone housing surrounding the implant often cannot withstand peri-implant bone resorption over time [4,5], which is known as "socialization" [6]. Therefore, pre-prosthodontic alveolar ridge augmentation is a requisite for successful and predictable prosthesis implantation.

Guided bone regeneration (GBR) is the most versatile and effective technique for alveolar ridge augmentation [7,8]. Several studies have demonstrated that GBR can yield >5.0 mm of bone augmentation both vertically [9–11] and horizontally [12,13], with a good long-term prognosis [14]. Recently, GBR has been reported to provide alveolar bone augmentation with a height of >8.5 mm on average in severe defects or alveolar bone atrophy with an average defect height of 10.0 mm [15]. The following four treatment principles of GBR have been proposed for predictable horizontal or vertical bone augmentation beyond the envelope of skeletal bone [16]: (1) maintenance and (2) stability of space for infiltration and proliferation of osteogenic cells, (3) promotion of angiogenesis, and (4) primary wound closure. Bone graft materials primarily function as scaffolds contributing to maintenance and stability of space for osteogenic cells, and the host response to the scaffolds is therefore one of the determining factors for successful outcomes in GBR [17,18].

The traditional classes of bone graft materials include autogenous bone, allografts, xenografts, and alloplasts [19]. Allografts consist of freeze-dried human bone with or without demineralization (DFDBA, demineralized freeze-dried bone allograft; FDBA, freeze-dried bone allograft). Xenografts are formed from bovine bone-derived materials that are divided into two types according to whether they are subjected to a chemical or thermal deproteinization process (CD-BB, chemically de-proteinized bovine bone; TD-BB, thermally de-proteinized bovine bone). Various types of alloplasts have been developed with a view toward control of biological performance based on physicochemical properties. All alloplasts utilize hydroxyapatite (HAp) or other calcium phosphate compounds such as beta-tricalcium phosphate (β-TCP). The particular calcium phosphate material used affects the performance of the bone substitute. However, only HAp and β-TCP have been sufficiently clinically evaluated as bone substitutes to date. Collectively, allografts, xenografts, and alloplasts are known as "bone substitutes." This classification is based on differences in biological origins and thus places emphasis on differences among the materials with regard to undesirable immunoreactions or transmission of unknown pathogens from the graft material. However, this origin-based classification does not reflect the actual function and effectiveness of current bone substitutes. For further advancement of alveolar ridge augmentation, it is essential to identify the inherent capabilities and limitations of currently available bone substitutes. The present review aims to organize existing knowledge regarding physicochemical and biological properties of current bone substitutes, providing important information to guide clinical decision-making and to generate new perspectives on bone substitutes for future advancement of alveolar ridge augmentation.

2. Requirement for bone substitutes in implant dentistry

2.1. Versatility of autogenous bone as a bone graft material

Typically, autogenous bone is regarded as the gold standard among bone graft materials in the origin-based classification system. The rationale for the gold standard status is that only autogenous bone inherently contains osteogenic cells (cell) on or within the bone matrix. Mesenchymal stem cells within the bone marrow are believed to survive ischemia during grafting, which causes changes in oxygen tension, pH, and cytokine environment. However, several studies have demonstrated that most endogenous cells (probably osteocytes, osteoblasts, and mesenchymal stem cells) on or within autogenous bone undergo apoptosis or necrosis during bone grafting. Flow cytometry analysis demonstrated that the proportion of viable and apoptotic cells in bone chips collected from maxillary bone was <5% and >95%, respectively, regardless of the type of instrument, such as piezoelectric devices, scrapers, and rotary mills, used to collect the graft [20]. Moreover, 80% of osteocyte lacunae within a bone block showed debris or were empty at the end of grafting surgery [21]. Histological examination after maxillary sinus augmentation in humans using calvarial or iliac autogeous bone particles demonstrated that the proportion of nonvital bone was 20%–25% after 5 months of healing [22].

Meta-analyses comparing bone graft materials via histomorphometrical evaluation of human bone biopsies from sinus augmentation demonstrated that compared with bone substitutes, autogenous bone enabled faster initial bone formation, but the final amount of bone formation did not differ from that observed with bone substitutes [23,24]. A combination of autogenous bone with a bone substitute led to the greatest final amount of bone formation within the sinus cavity [23,24]. A preclinical study in a large animal model demonstrated that bone substitute particles yielded a larger bone volume than autogenous bone chips in severe conditions, such as peri-implant bone defects [18]. Moreover, a meta-analysis did not detect superiority of autogenous bone over bone substitutes in the clinical outcomes of maxillary sinus augmentation and alveolar ridge augmentation [25]. These observations support the following conclusions: (i) although autogenous bone may have higher bone formation capability than bone substitutes, the actual benefit is limited to favorable recipient conditions; and (ii) bone substitutes not only reduce or eliminate the risk of donor site morbidity endemic to autogenous bone [26] but also have a theoretical advantage in augmentation under severe recipient conditions.

2.2. Basic properties of bone substitutes required for bone formation

In tissue engineering, scaffolds are defined as three-dimensional porous solid biomaterials designed to perform some or all of the following functions during tissue formation: (i) promote cell-biomaterial interactions, cell adhesion, and extracellular matrix

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(ECM) deposition; (ii) permit sufficient transport of gases, nutrients, and regulatory factors to allow cell survival, proliferation, and differentiation; (iii) biodegrade at a controllable rate, which approximates the rate of tissue regeneration; and (iv) provoke a minimal degree of inflammation or toxicity *in vivo* [27]. The attachment of mesenchymal stem cells and osteoprogenitors initiates bone formation on bone substitutes. Thus, the material used should support the proliferation and differentiation of mesenchymal stem cells and osteoprogenitors derived from bone tissue at the transplanted site, allowing ECM deposition and matrix mineralization by osteoblastic cells (osteoconductivity) (Fig. 1A). In addition, the material must minimize the risk of biological adverse events (biocompatibility) (Fig. 1B). Moreover, the material must prevent the collapse of the introduced space for bone formation (space-making capability) (Fig. 1C) and preferably be replaced with newly formed bone tissue (regeneration) through passive chemical dissolution and bone remodeling by osteoclasts (bioabsorbability) (Fig. 1D).

3. Factors for the space-making capability of bone substitutes

The space-making capability of bone substitutes should be considered separately from the bone formation and remodeling occurring in the relatively early phases after new bone formation. One of the factors greatly reducing the volume of a mass of packed bone substitute granules or particles during bone formation is pressure from adjacent tissues such as the mucosal or gingival flap or Schneider's membrane as a result of tissue shrinkage [28] or gravity [29], which can be simulated by intracranial pressure from the side of the dura mater in a rat calvarial defect model (approximately 10 mmHg) [30]. Substantial morphological stability of bone substitutes is necessary, as both a mass and individual particles, to resist such pressure.

3.1. Enzymatic or chemical dissolution

Similar to macrophages and neutrophils [31,32], tissue-forming cells such as osteoblasts [33], gingival fibroblasts [34], and periosteal cells [32] produce various isoforms of collagenase and can degrade the collagen matrix. Tissue fluids contribute to degradation of bioabsorbable polyester [35] or calcium phosphate compounds [36–38] used as bone substitutes through hydrolysis. However, the bone substitute should be retained during new bone formation to support osteoblast function. Sensitivity to enzymatic or chemical dissolution also greatly influences the space-making capability; if passive chemical dissolution is too fast, bone substitutes disappear before new bone formation, leading to defective space formation. For example, resistance to dissolution affects the space-making capability. Heat-denatured atelo-collagen has much lower resistance to collagenase and less space-making capability than fibrous atelo-collagen [39]. In a collagen composite consisting of both types of collagen, as the proportion of heat-denatured collagen increases, the space-making capability is markedly reduced regardless of mechanical strength. Octacalcium phosphate (OCP) has much higher water solubility than HAp and β-TCP [40], and it thus promoted new bone formation to a greater extent than HAp and β-TCP, but was inferior in volume maintenance of the implanted region in a rat calvarial defect model [41]. Macroarchitectural design aspects of the material such as porosity or pore size can also control the space-making capability by influencing the solubility of the material in the local tissue. Alpha-tricalcium phosphate (α-TCP) has much higher water solubility than HAp and β-TCP [42]. Inlay or onlay grafting of relatively dense particles or blocks of alpha-tricalcium phosphate (α-TCP) into rabbit calvarial bone resulted in degradation with new bone formation and without reduction of the space established for bone regeneration [43,44].

3.2. Mechanical properties

Mechanical properties of bone graft materials can also affect their space-making capability [45]. The mechanical properties of autogenous bone particles vary widely and are determined by the harvest site and patient age [46,47] (Table 1). Freeze-drying reduces the mechanical strength of bone tissue by 20% [48]. DFDBA has an extremely low elastic modulus compared with other bone substitutes [47]. Bovine bone mineral xenografts have an elastic modulus similar to that of mandibular cortical bone, whereas

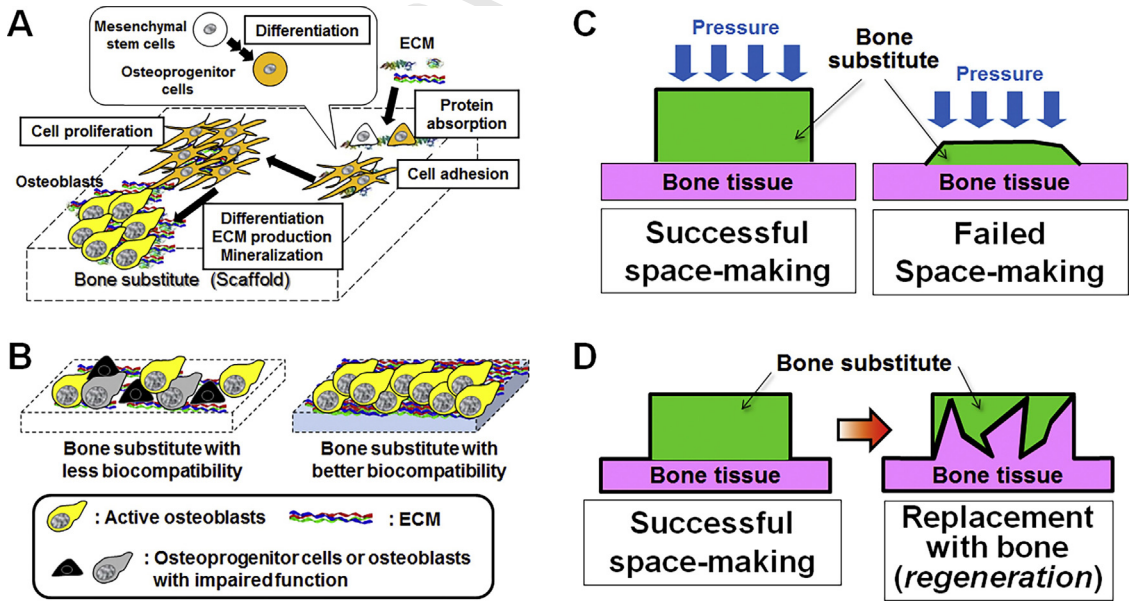

Fig. 1. Basic properties of bone substitutes required for bone formation. (A) Osteoconductivity. ECM: extracellular matrix. (B) Biocompatibility. (C) Space-making capability. (D) Volume maintenance by replacement with bone over time (regeneration).

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Table 1
Elastic modulus of autogenous bone and currently available bone substitutes.

Bone graft materials	Elastic modulus (GPa)
Autogenous bone [46,47]	Edentulus jaw: 15–18 Mandibular cortical bone: 30–39
Freeze-dried bone allograft (FDBA) [48]	Theoretical 20% reduction as compared to fresh bone
Demineralized freeze-dried bone allograft (DFDBA) [47]	1–2.5
Chemically deproteinized bovine bone xenograft (CD-BB) [47]	28
Thermally deproteinized bovine bone xenograft (TD-BB) [47]	30–33
Synthetic hydroxyapatite in porosity-free bulk (HA) [49,50]	80–120
β-Tricalcium phosphate in porosity-free bulk (β-TCP) [50]	110

alloplastic materials such as HAp and β-TCP in nonporous bulk have considerably higher elastic moduli than other bone substitutes [49,50]. However, bone substitutes are generally used in particle, and not block, form in alveolar ridge augmentation because shapeability is required to mold three-dimensional ridges on the irregularly shaped alveolar bone. Mechanical resistance to compression depends more on granule size than on the actual mechanical properties of the material.

3.3. Particle size

Osteoclast-like multinucleated giant cells appear to prefer small particles (<1 mm) in both autogenous bone [51] and bone substitutes, such as bovine bone mineral [52]. However, larger particles of the bone substitute can produce a larger amount of bone augmentation. In a vertical bone augmentation model in rabbit calvaria using polytetrafluoroethylene chambers, larger autogenous bone particles (diameter, 1–2 mm) produced a larger augmented bone volume than smaller particles (diameter, 150–400 μm) [51,53]. Relatively moderate-sized or large particles of silicate-substituted calcium phosphate (diameter of 250–500 or 1000–2000 μm, respectively) tended to maintain the volume of initial bone formation better than smaller particles (90–120 μm) in femoral condyles of sheep [54]. A multicenter randomized controlled clinical histomorphometric human study indicated that bovine bone mineral granules with a large size (1–2 mm) generated 1.4 times higher volume in sinus augmentation than smaller granules (0.25–1 mm) [55]. Larger particles tend to be retained in newly formed bone tissue, owing to the longer time required for dissolution or remodeling [56,57]. A block graft of autogenous bone had a lower bone resorption rate after successful engraftment for GBR than particulates [58]. These observations indicate that compared with smaller particles (<1 mm), larger particles (≥1 mm) possess greater mechanical resistance as a lump for space-making (Fig. 2) and that the space-making capability is more important for initial bone formation than the balance between bone resorption and formation.

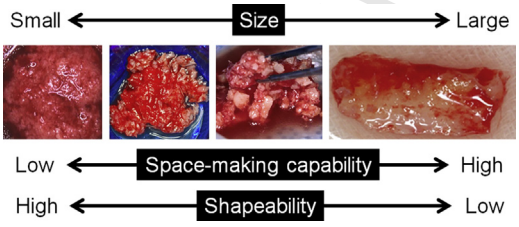

Fig. 2. Relationship among material size, space-making capability, and shapeability. Images show autogenous bone particles or blocks. Larger size of autogenous bone particles is associated with better space-making capability but lower shapeability. This relationship is adapted for all bone substitutes.

4. Need for improved biocompatibility of currently available bone substitutes

Biocompatibility is defined as "the ability of a material to perform with an appropriate host response in a specific application" [59]. Traditionally, in terms of biocompatibility, bone graft materials are classified as biotolerant, bioinert, or bioactive. Biotolerant implant materials remain in the body with fibrous encapsulation by evoking a tissue reaction. Bioinert implant materials have direct contact with the adjacent bone tissue without any chemical reaction. Bioactive implants establish chemical bonds with adjacent bone tissue, which leads to direct deposition of bone matrix on the implant material. This conceptual classification is based upon histological observations of local effects after implantation into bone tissue.

Cytocompatibility is a primary factor in biocompatibility and should be determined by cell culture experiments. For instance, polymethyl methacrylate (PMMA) is categorized as a biotolerant material. PMMA has excellent mechanical properties with high space-making capability and guaranteed volume maintenance, but it is well known to exert severe cytotoxicity. PMMA-based bone cement and dental resins decrease the viability and inhibit the proliferation and differentiation of osteoblastic cells [60–63], odontoblastic cells [64,65], and gingival fibroblasts [66,67]. In contrast, calcium phosphate-based bone substitutes allow direct bone deposition by osteoblastic cells [44,68,69] and are thus categorized as bioactive. However, laboratory studies have demonstrated that these bone substitutes decrease cell viability and function, although not to the extent observed for PMMA-based materials. Previous studies on various bone substitutes using rat calvarial osteoblastic culture demonstrated that the percentage of viable cells decreased to 20%–50% on CD-BB [70], DFDBA [71], and β-TCP [72]. The amounts of various interleukins released from human osteoblastic cells increased markedly on CD-BB [70]. Under controlled culture conditions, the alkaline phosphatase (ALP) activity of osteoblastic cells was markedly suppressed on bone substitutes [70–72], indicating that bone substitutes actually are both bioactive and biotolerant, and thus that their cytocompatibility should be further improved (Fig. 3). Furthermore, apoptosis was induced in osteoblasts cultured on polystyrene in the presence of bone substitutes, such as CD-BB, DFDBA, and β-TCP, without physical contact between the cells and the materials [70–72]. These observations suggest that substances eluted from bone substitutes negatively affect osteoblastic viability and function, even in the absence of any physical contact.

4.1. Nature of collagen fibers

Degradation of the extracellular matrix affects the survival of adherent cells [73]. Collagen fibers not only function as starting points for bone mineralization but also play a critical role in cellular adhesion and proliferation. Fragmentation of collagen

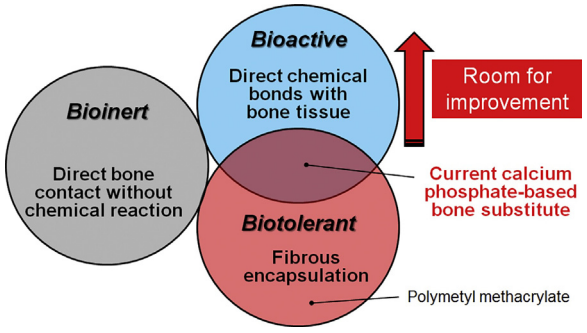

Fig. 3. Relationship among traditional classifications of biocompatibility: biotolerant, bioinert, and bioactive. Bioinert material is only independent, whereas biotolerant and bioactive materials partially overlap. Currently available calcium phosphate-based bone substitutes belong to the overlapped region. Polymethyl methacrylate is a typical biotolerant material.

fibers in dermal connective tissue, disturbed fibroblastic adhesion, and collapse of connective tissue by increased production of matrix metalloproteinases from fibroblasts is characteristic of aged human skin [74]. Chondrocyte survival is dependent upon the interaction of cell surface receptors with type II collagen [75], and cartilage matrix degradation induces chondrocyte apoptosis [76]. Rat calvarial osteoblastic cells had lower viability and ALP activity on a soluble collagen sponge than on an insoluble fibrous collagen sponge [77,78]. Interestingly, the cell viability differed depending on the microarchitecture of the scaffold even when the scaffolds were formed from analogous soluble collagen I fibers [79].

De-proteinization is a critical process performed to remove the antigenicity of xenograft bone substitutes. Immersion in a strong acid or base or thermal cycling at approximately 1000 °C is generally used for de-proteinization of bone tissue [80–82]. Such processes destroy the arrangement of collagen fibrils as well as the crosslinking [82,83]. De-proteinization with milder reagents preserves the inherent collagen fibroarchitecture of cancellous bone. A xenograft bone substitute de-proteinized in this manner permitted greater attachment, ALP activity, and matrix mineralization by an osteoblastic cell line (MC3T3-E1) for bone regeneration in cortical bone defects in a rabbit radius model [83,84] in comparison with CD-BB prepared via conventional de-proteinization. The influence of the nature of collagen fibers within the bone substitute on cell viability would be of interest as a future research topic.

4.2. Micro- or nanoparticulates

Micro- or nanoparticulates have the potential to induce adverse biological effects even though these materials are formed from biocompatible ceramics, calcium phosphate, or bioabsorbable polymers. Microparticulates with a diameter of $<10\,\mu m$ evoke inflammatory responses in phagocytes such as macrophages as a result of cellular uptake via endocytosis [85,86]. Moreover, nanoparticulates can evoke cytotoxicity in several types of cells [87]. Tissue-forming cells such as fibroblasts or osteoblasts also take up nanoparticulates, which in turn triggers intracellular oxidative stress via lysosomal accumulation [88,89]. Calcium phosphate-based bone substitutes such as synthetic HAp and β-TCP are advantageous for their capability to be shaped into arbitrary three-dimensional forms with interconnective porous structures. Such bone substitutes are made by sintering of mixtures of micro- or nano-particulates and a binder [90] and hence easily generate particulate debris if wear resistance is not adequate. In addition, other factors such as composition, shape, surface topography, electric potential, and types of functional groups play roles in the cellular response to micro- or nano-particulates [87,91–94].

4.3. Excessive release of calcium ions

Pure water-soluble calcium phosphates such as β-TCP release calcium ions into local tissues. Calcium ions control osteoblastic viability, proliferation, and differentiation [95–97] via intracellular calcium signaling after influx into the cells through calcium channels [98]. In addition, calcium ions may induce osteoblastic apoptosis by increasing cytosolic calcium ion concentrations and triggering downstream events leading to apoptosis [99]. In contrast, bioabsorption has been shown to be positively associated with early bone formation *in vivo* and promotion of osteogenic differentiation of osteoprogenitor cells *in vitro* [100]. In a previous study, a composite of β-TCP particles with collagen sponges enhanced ALP activity and mineralizing nodule formation of rat calvarial osteoblasts with increased intracellular calcium levels [101], and promoted bone regeneration in critical-sized rat calvarial defects to a greater extent than a composite of CD-BB particles with collagen [102]. Therefore, pure water-soluble calcium phosphate possibly induces both apoptosis and activation of osteoblasts depending on the local concentration of the released calcium ions. Moreover, certain calcium phosphates exhibiting partial hydrolysis were shown to promote bone formation via activation of the osteoimmunological network by releasing the proper concentration of calcium ions into local tissue [103]. A human clinical study with histomorphometric analysis of bone biopsy demonstrated that sinus cavities augmented with CD-BB particles showed faster bone formation than those augmented with β-TCP particles [104]. In contrast, biphasic calcium phosphate (BCP) particles consisting of nonresorbable crystalline HAp and β-TCP induced greater formation of osteoids within a sinus cavity than CD-BB particles [105]. These observations indicate that release of calcium ions from bone substitutes positively and negatively contributes to bone formation depending on the calcium ion concentration in the local tissue. Controlling the amount of calcium ions released may facilitate or hamper the application of pure water-soluble calcium phosphates as bone substitutes [106].

4.4. Improving the biocompatibility of current bone substitutes

DFDBA and FDBA were expected to possess osteoinductivity owing to the presence of endogenous growth factors in the bone matrix, but this property has not been confirmed [69,107]. A clinical study demonstrated that addition of DFDBA to a mixture of CD-BB and autogenous bone for simultaneous alveolar bone augmentation together with dental implant placement did not exert additional benefits in terms of preventing marginal bone loss around dental implants over time [108]. Additionally, preclinical studies in larger mammals indicated that bone healing in small three-wall bone defects, where growth factors and stem cells are easily supplied, was better when using autogenous bone chips than when using bone substitutes such as CD-BB or β-TCP [109]. Previous studies have reported delayed initial bone formation in human sinus augmentation with bone substitutes, such as CD-BB or β-TCP, compared to autogenous bone, although the final bone formation was similar [23,24]. These findings suggest that commercially available bone substitutes are not expected to exert additional positive effects on initial bone formation beyond those provided by autogenous bone. Increasing the cytocompatibility for osteoblastic cells might thus provide a necessary increase in the osteoconductivity of bone substitutes. For instance, incorporation of the antioxidant amino acid derivative, *N*-acetyl cysteine, into PMMA-based materials reduced intra- and extracellular levels of

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cytotoxic agents released from the materials, resulting in improved cytocompatibility for osteoblasts [63], gingival fibroblasts [67], and odontoblastic cells [64,65,110]. Implantation of *N*-acetyl cysteine-ameliorated PMMA-based bone cement in the rat femur bone marrow space markedly increased bone volume, bone-cement contact ratio, and interfacial strength, in contrast to the limited and poor bone formation observed for non-ameliorated material [63]. Thus, remediation of toxic products by *N*-acetyl cysteine might upgrade the biocompatibility of PMMA-based materials from biotolerant to bioactive. Similarly, mixture of CD-BB, DFDBA, and β-TCP with *N*-acetyl cysteine improved the viability and ALP activity of calvarial osteoblasts cultured on these materials [70–72].

5. Factors affecting bioabsorbability and volume maintenance of bone substitutes

The term "bioabsorption" has been extensively used in the field of polymers and biomaterials but has not been well defined. Several synonyms such as "bioresorption," "biodegradation," and "bioerosion" have also been used [111]. Favorable bioabsorption of bone substitutes should involve the replacement of the implanted material by newly formed bone tissue via bone remodeling, i.e., "regeneration" and not "reconstruction" [112]. Bioabsorption during the bone formation phase is associated with space-making capability and biocompatibility, and is predominantly mediated by passive chemical dissolution of the bone substitute. Maintenance of the augmented bone volume over time is important in pre-prosthodontic alveolar bone augmentation to control the three-dimensional alveolar bone morphology [15].

5.1. Pore size and porosity

The presence of interconnected pores or channels is the basic premise for use of inorganic bone substitutes. The pore size (diameter) must be at least 100 μm, which allows diffusion of nutrients for cell survival and intrusion of the minimum osteon [113,114]. A pore size of 200–350 μm offers optimal circumstances for in-growth of newly formed bone [115]. FDBA, CD-BB, and TD-BB preserve the microstructure of the endogenous cortical or cancellous bone frame. This native three-dimensional structure provides such materials with a distinct advantage. Alloplastic calcium phosphate-based bone substitutes are prepared on a spectrum of bulk to porous forms during the manufacturing process. Greater porosity results in faster and greater bone in-growth and replacement [116]. In addition, the pore space affects the flow of blood and interstitial fluid in local bone tissue [117]. Blood and interstitial fluid transport oxygen and nutrients into the local tissue and are therefore essential for bone regeneration [118]. Greater pore size, porosity, and interconnectivity of scaffolds facilitates blood and interstitial fluid flow as well as subsequent cell infiltration and vessel ingrowth into the material [119]. However, increased pore size and porosity also reduce the mechanical resistance of such materials and thus must be balanced against the space-making capability and maintenance of volume.

5.2. Water solubility

A critical factor determining the bioabsorption rate during the remodeling phase is the chemical composition and water solubility of the bone substitute. Osteoclasts can degrade bone substitutes in a manner similar to hydrolysis by secreting hydrogen ions. *In vivo* and clinical histology studies have confirmed that osteoclasts and osteoclast-like cells can form resorption pits on pure water-soluble calcium phosphate [44,120,121]. A recent culture study demonstrated that osteoclasts could directly attach on BCPs and that the number of cells increased with increased β-TCP ratio [122]; this indicates a role of extracellular calcium ions in the differentiation and activation of osteoclasts [123,124]. However, histology of human bone biopsies from sinus augmentation demonstrated that chemical dissolution was predominant for resorption of β-TCP particles embedded in newly formed bone rather than cellular absorption by osteoclasts [125]. This chemical dissolution might lead to apoptosis of osteoclasts and inhibition of osteoclastic resorption through generation of excess calcium ions [126–129].

5.3. Integrity and crystallinity of the apatite structure

In association with water solubility, the integrity and crystallinity of the apatite structure in bone substitutes also affect osteoclastic activity. The integrity and crystallinity of the apatite structure determine acid resistance and directly affect the bioabsorption of bone substitutes other than pure water-soluble calcium phosphate. The native bone matrix consists of not only pure HAp but also calcium-deficient apatite and carbonates such as calcium carbonate and carbonate apatite (CAp) (Table 2). Bone tissue contains approximately 34%–44% HAp by mass with some CAp content. Highly crystalline and stoichiometric HAps (calcium/phosphate ratio = 1.67) have high acid resistance; however, non-stoichiometric HAps and carbonates have only moderate acid resistance and high solubility [36,130]. Alloplastic HAps are generally pure or closely stoichiometric; such HAps do not allow

Table 2
Contents of hydroxyapatite (HAp) and carbonate apatite (CAp) and extent of chemical dissolution or enzymatic and acid resistance of autogenous bone and currently available bone substitutes.

Bone graft materials	HAp content (wt%)	CAp content (wt%)	Chemical or enzymatic dissolution	Acid resistance
Autogenous bone [148]	Young cortical bone: 44 Young cancellous bone: 26 Old cancellous bone: 34	Young cortical bone: 1.4 Young cancellous bone: 0.6 Old cancellous bone: 2.4	Slightly	Low
Freeze-dried bone allograft (FDBA) [80]	49	7.5	Slightly	Low
Demineralized freeze-dried bone allograft (DFDBA) [80]	Unknown (theoretically 0)	Unknown (theoretically 0)	Completely	–
Chemically deproteinized bovine bone xenograft (CD-BB) [80]	93.6	3.4	Slightly	Moderate/high
Thermally deproteinized bovine bone xenograft (TD-BB) [80]	≒100	0	Hardly	High
Synthetic hydroxylapatite in porosity-free bulk (HA) [80]	≒100	0	Hardly	High
β-tricalcium phosphate in porosity-free bulk (β-TCP)	–	–	Completely	Low

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formation of resorption lacunae by osteoclasts [126,127]. In contrast, the CAp content activates osteoclasts to secrete acid via stimulation of carbonic anhydrase activity [131]. Moreover, the CAp content of osseous apatite is directly related to reduced acid resistance as well as acquisition of water solubility [132]. Allograft and xenograft bone substitutes can be dissolved in tissue fluids and release calcium ions into local tissue to a certain extent [36], although the bioabsorption rate is far inferior to that of β-TCP.

5.4. Influence of manufacturing process on integrity and crystallinity of apatite structure

Mineral composition and apatite crystallinity in bone tissue can be affected by thermal treatment during the manufacturing process [133] (Fig. 4). CD-BB is prepared by de-proteinization with stepwise sintering treatment (up to 300 °C) and subsequent chemical treatment with sodium hydrate solution [80]. In contrast, TD-BB is prepared by calcination of bone at high temperatures (approximately 1000 °C) [80,81]. Calcination at temperatures above 400 °C eliminates carbonate in the bone matrix and enhances HAp crystallinity [80,134]. However, extremely high temperatures (above approximately 1200 °C) decompose HAp and generate water-soluble calcium orthophosphates as impurities [135]. According to the transformational theory of HAp, TD-BB loses carbonate, whereas CD-BB maintains approximately half of the original endogenous carbonate content [80] (Table 2). Culture studies have demonstrated that osteoclasts attach to and form resorption pits on CD-BB [136,137], whereas osteoclasts attach but only form limited resorption pits on TD-BB [138]. Histological observations in a mini-pig experiment demonstrated that newly formed bone tissue could surround and incorporate CD-BB particles [139]. A human histological study in sinus augmentation demonstrated that CD-BB allowed formation of CD44-positive vital osteocytes with lacunae inside [140], but the number of osteoclasts decreased as healing progressed after sinus augmentation [141]. This indicates that CD-BB can be remodeled, but very slowly. This extremely slow bioresorption of CD-BB is supported by a previous report, wherein an increase of bone tissue area with remnants of CD-BB particles in a bone core biopsy was noted at 10 years of clinical follow-up after sinus augmentation [142]. In accordance with the transformational theory of HAp, TD-BB degradation was never observed even at 52 weeks after surgery in a critical-sized mini-pig metaphyseal defect model [143].

FDBA allografts are not subjected to sintering during the manufacturing process; thus, FDBA does not undergo any changes in the composition of inherent osseous apatite. However, such allografts must be sterilized during manufacturing to prevent transmission of pathological viruses via implantation despite rigorous donor screening. Typically, lyophilization is used for extensive virus inactivation, as it can preserve the biological and mechanical properties of the treated tissue better than sterilization techniques such as autoclaving, gamma irradiation, and treatment with ethylene oxide [144–146]. However, lyophilization alters the mechanical properties of the treated tissue [147]. In general, FDBA has a higher content of carbonate than autogenous bone [80,148] (Table 2). The order of the amount of calcium ions released during immersion in 0.1 N hydrochloric acid for >40 days was FDBA > autogenous bone ≫ CD-BB > TD-BB [149]. Based on these observations, it can be assumed that lower acid resistance corresponds to greater bioabsorption.

5.5. Inverse relationship between bioabsorption and volume maintenance

Overly extensive bioabsorption can impair the volume maintenance of augmented tissue. FDBA did not prevent volume reduction of the alveolar ridge after implantation in a tooth extraction socket beyond the effects of DFDBA implantation, despite leaving a much higher proportion of remnant particles [150]. Clinical X-ray evaluation of volume changes after sinus augmentation over time demonstrated that autogenous bone chips reduced the total volume of the augmented tissue more than CD-BB at 6 months after surgery [151]. The total volume of augmented tissue in the sinus cavity at 6 months after surgery was lower when a combination of CD-BB and calcium sulfate paste with low acid resistance was used than with the use of CD-BB alone [152]. Similarly, morphometric measurements of panoramic radiographs demonstrated that sinus augmentation with β-TCP particles resulted in a greater decrease in height compared to autogenous bone, and the height continued to decline even 4.5 years after surgery [153]. Another clinical study revealed that bioabsorbable calcium phosphates such as β-TCP did not maintain the volume of augmented tissue over time [154]. The dominance of chemical dissolution over bone remodeling for bioabsorption of bone substitutes even after they are embedded in newly formed bone [125] might contribute to the inverse relationship between bioabsorption and volume maintenance (Fig. 5). On the one hand, the presence of residual solid bone substitutes leads to maintenance of the volume and shape of the augmented tissue; however, on the other hand, such augmented tissue shows impairment of physiological properties [155]. An ideal bone substitute is a

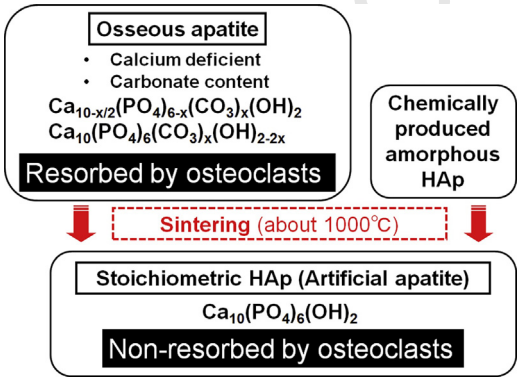

Fig. 4. Thermal-based transformation of hydroxyapatite (HAp) crystals from osseous apatite or chemically produced amorphous HAps.

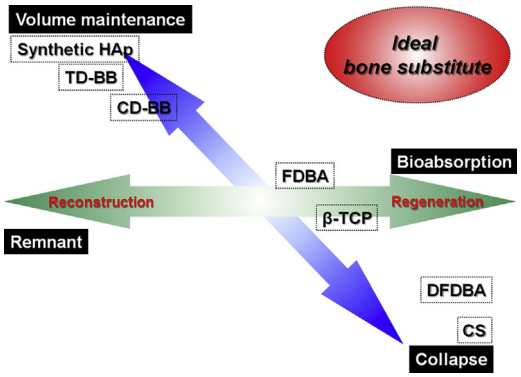

Fig. 5. Distribution map showing association between bioabsorption and volume maintenance. Currently available bone substitutes exhibit an inverse relationship between these two properties. The upper right corner indicates an ideal bone substitute that has both properties. The bone substitute is selecting depending on whether volume maintenance (reconstruction) or displacement (regeneration) is more important. Synthetic HAp: synthetic hydroxyapatite; TD-BB: thermally de-proteinized bovine bone; CD-BB: chemically de-proteinized bovine bone; FDBA: freeze-dried bone allograft; β-TCP: beta-tricalcium phosphate; DFDBA: demineralized freeze-dried bone allograft; CS: collagen sponge.

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material that can provide complete bioabsorption in combination with volume maintenance of the augmented tissue over time (indicated as "ideal bone substitute" in Fig. 5). However, such a bone substitute does not yet exist. Controlling the bone remodeling cycle, i.e., osteoclastic resorption coupled with bone formation, will be critical for engineering suitable bioabsorption in bone substitutes. Further advances in alveolar ridge augmentation will also likely require inclusion of growth factors and stem cells, given the lack of osteoinductivity of the scaffold alone [112,156,157].

6. Conclusion

This review describes the capabilities and limitations of commercially available bone substitutes based on the insights of biomaterial science from outcomes of clinical and preclinical studies. In summary:

- Autogenous bone is still the gold standard and accelerates initial bone formation to a greater extent than bone substitutes. However, autogenous bone is only effective under favorable recipient conditions and thus requires supplementation with bone substitutes in bone augmentation under severe recipient conditions.
- Currently available bone substitutes induce only osteoconductivity as a scaffold and not osteoinductivity.
- Space-making capability is required for bone augmentation. Particle size, sensitivity to enzymatic or chemical dissolution, and mechanical properties affect the space-making capability of a material.
- The biocompatibility of current bone substitute materials should be improved. Reduced biocompatibility is thought to result from degradation of the extracellular matrix (irregular arrangement of endogenous collagen fibers), generation of micro- or nano-particulates, and excessive release of calcium ions.
- Bioabsorption of remnant bone substitutes and volume maintenance of the augmented tissue over time are inversely related. Bioabsorption during the bone remodeling phase after bone formation is induced by passive chemical dissolution of the material and cellular absorption by osteoclasts, although passive chemical dissolution seems to be predominant.
- Important factors that determine bioabsorption are water solubility and acid resistance, which are determined by chemical composition and the integrity of the apatite structure, respectively.
- Bone substitutes have the following order of bioabsorbability: DFDBA $\gg$ FDBA $>$ (autogenous bone) $\gg$ CD-BB $>$ TD-BB.
- At present, no complete bone substitute exists that simultaneously exhibits suitable biocompatibility, bioabsorption, and volume maintenance.

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