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REVIEW ARTICLE

Commercially available bone graft substitutes: the impact of origin and processing on graft functionality

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ABSTRACT

Development of effective and cost-efficient bone tissue engineering grafts has been the key area of research for regenerative medicine, yet an ideal grafting material has remained elusive due in large part to the highly dynamic nature of bone. A wide array of materials, both natural and synthetic, have been implemented as potential candidates for commercially available products, yet the gold standard for grafting material still remains autogenous bone. We review currently commercially available bone graft materials and relevant graft characteristics that impact the effectiveness of tissue repair, emphasizing the advantages and disadvantages of materials based on composition and origin. Examined materials were selected through a web-based search for readily accessible and clinically applicable graft materials. Grafts were then categorized according to material source to examine advantages and disadvantages associated with allogenic, xenogeneic, synthetic materials. Lastly, the application of bioactive molecules onto these basal grafts is explored to illustrate the enhancement and regulative capacity of these additives on traditional osteobiologic materials.

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Bone; graft; biomaterial; scaffold; osteobiologic; allogenic; xenogeneic; synthetic; regenerative medicine

1. Introduction

Far from the static structure that it is sometimes portrayed, bone comprises a highly dynamic system regularly undergoing remodeling based on skeletal force distribution. This process relies on specialized cells, namely osteoclasts and osteoblasts, capable of resorbing existing substrate and depositing new bone respectively. Osteoclasts, which are multinucleated cell bodies formed from hematopoietic precursors of monocytic and macrophagic lineage, operate to degrade existing structures enzymatically exposing mature osteocytes embedded within matrix (Lemma et al. 2016). Osteoblasts, which form epithelioid structures along the surface of existing bone, modulate secretion of bone organic matrix and mineralization at this interface (Blair et al. 2017). During this deposition process, osteoblasts become encased within the newly formed mineral construct and mature to osteocytes, which comprise over 90% of the cellular content of bone and has demonstrated the ability to regulate the balance between remodeling agents (Bellido 2014; Hasan et al. 2018). The operation and coordination of function for these critical structural remodeling agents is heavily reliant on the flux of chemical signals produced by the extracellular matrix (ECM) in the form of proteins and growth factors, which stimulate highly specific reactionary cascades. These signaling cascades are largely responsible for the recruitment and differentiation of precursor cells through-out the repair process (Majidinia et al. 2018).

The complex interaction of the described mechanisms can be credited for the impressive regenerative capacity of bone, with functional repair and restoration possible for even large tissue trauma. However, for injuries that exceed the healing capabilities of the tissue, what is known as a critically-sized defect, spontaneous regeneration and repair will not be possible. It is therefore necessary for such cases to implement a graft material to facilitate cellular migration and signaling through the defect region permitting effective repair (Noori et al. 2017). For this reason, the development of effective bone graft materials has been a major research focus, resulting in a wide range of scaffold designs with varying advantages and disadvantages.

In designing an optimal graft material for bone tissue engineering applications, the product should display key osteobiologic characteristics, such as osteoconductive, osteo-inductive, and osseo-integrative

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attributes, to be capable of facilitating and promoting growth of new bone tissue, as well as integration with native tissue. Biomaterials that mimic or utilize the natural architecture of bone therefore offer superior function for not only encouraging the migration of local progenitor cells, but also to serve as a substrate for tissue development. Additionally, the combination of micro- and nano-scale topographical elements have been observed to significantly impact the interaction with, and activity of, exposed cells (Zhu et al. 2017; Jackson et al. 2018). The current gold standard for grafting material is the use of autologous bone, tissue harvested from a donor site of the individual receiving the graft, as this does not pose concerns of immune response or disease transmission while presenting an optimal construct for tissue in-growth (Azi et al. 2016). However, autologous grafts are limited with respect to available source material and raise concerns of donor site morbidity (Lee et al. 2018). For these reasons, the use of allogenic, xenogeneic, and synthetic graft materials offer attractive alternatives with regard to availability and cost parameters. Furthermore, the application of bioactive agents such as proteins and growth factors closely associated with osteogenesis or genetic manipulation through both viral and non-viral methods have demonstrated the potential to enhance existing scaffold technologies, as well as act as effective stand-alone treatments (Hasan et al. 2018).

This article will explore commonly employed, commercially available bone graft and bioactive materials of both organic and synthetic origin found through a rudimentary web-based search of PubMed and Medline databases. The examined materials, assessed based on origin and matrix composition, will be separated into allogenic, xenogeneic, synthetic, and bioactive graft classifications. Evaluation of the advantages and disadvantages associated with each graft type will be driven by comparison of material processing methods and tissue interaction post-implantation. The application of explored commercially available materials in combination with experimental elements, such as cell-based delivery platforms or polymer binder additions, will not be addressed further in this article (Rao and Stegemann 2013; Lei et al. 2018).

1.1. Allograft products

Allografts comprise of scaffolds and particles derived from human cadavers, thereby maintaining architecture and extracellular proteins identical to that observed in the native bone tissue. For this reason, this category of grafting material demonstrates strong osteoconductive and integrative capabilities, as well as varying degrees of osteo-inductive potential based on the processing method utilized (Drosos et al. 2015; Kadam et al. 2016). The primary concern with allografts is the risk of disease transmission or immune response due to same species transplantation. To address this, the harvested samples are most commonly subjected to a freeze-drying procedure to eliminate the cellular component of the tissue. Removal of this element permits a drastic reduction in the risk factors associated with allografts. The remaining extracellular matrix can then be applied as a scaffold material or reduced to particles of specific size ranges for void filling applications. By varying the duration and number of freeze-dry cycles, the resulting scaffold can have significantly altered mechanical stability and surface protein characteristics (Kadam et al. 2016), making it suitable for new bone repair and regeneration.

Further processing of harvested human allograft bone can be conducted using an acid extraction to produce demineralized bone matrix (DBM), the general process of which can be observed in Figure 1. DBM is comprised of the organic elements of the bone, including proteins and other growth factors, which maintain the osteoconductive and osteo-inductive characteristics while removing the mineral structural components of the matrix. This permits the product to be implemented in a variety of means including granular particles, powders, or putties for filling void spaces (Kadam et al. 2016). Additionally, the process of demineralization reduces antigenic structures that may cause an immunological response, though this will still vary depending on the extent of the demineralization (Drosos et al. 2015).

Commonly used and characterized commercially available allograft materials include both freeze-dried and DBM products, as well as different material forms for some products. [The products: Grafton®, MinerOss®,

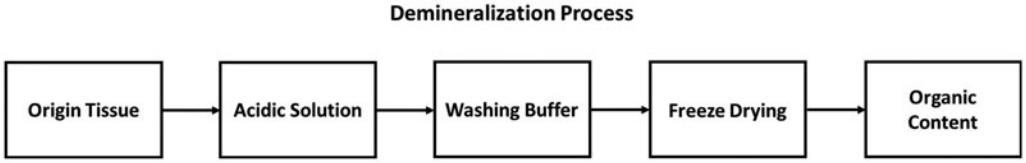

Figure 1. Generalized flow chart diagram for demineralization of bone tissue. Process moves from origin bone tissue to low mineral/high organic content product.

RaptOs®, Cancellous Chips, Puros®, and RegenerOss®, were selected though a basic web-based search of commonly implemented allogenic grafts.]

1.1.1. Grafton® DBM (BioHorizons)

Grafton® DBM is an allogenic graft material produced and distributed by BioHorizons that provides a scaffold matrix encompassing both osteoconductive and osteoinductive properties. As noted in Kadam et al. (2016), Grafton® DBM has been implemented in a wide variety of applications including sclerosis, cervical spine, and lumbar fusion applications. It is intended to be applied as in cases requiring bone graft extensions, substitute, or filler that are not directly related to structural stability or weightbearing sites. This is due to the DBM grafts maintaining low mechanical strength as compared to the compression forces observed in weightbearing skeletal structures. For this reason, a particularly attractive application of Grafton® DBM is in oromaxillofacial surgical applications, such as in alveolar ridge augmentation. The graft material is designed to be absorbed and replaced by native tissue during normal remodeling of the defect region. A prospective randomized clinical trial comparing Grafton® DBM with an autologous graft material harvested from the iliac crest bone (ICBG) was conducted to determine efficiency in fusion with local bone. The study conducted by Kang et al. (2012), assessed the 2-year follow-up of 41 patients that had received either the Grafton® DBM ($n = 28$) or ICBG ($n = 13$) for final fusion rates. There was no significant difference between the two groups (Kang et al. 2012), Indicating that the Grafton® DBM material may be capable of facilitating comparable repair to autogenic graft materials for bone injuries where fusion is required.

1.1.2. MinerOss® chips (BioHorizons)

MinerOss® particles are an allogenic graft product produced and distributed by BioHorizons that are derived from either cortical bone, cancellous bone, or a blend. The freeze-dry process used for this product results in a mineralized particulate material with both strong osteoconductive properties and enhanced surface area for tissue interaction. These particle materials (particle size ranging from 600 μm to 1250 μm) are intended, as per the product page, for implementation as a defect filler in ridge/sinus augmentation and socket grafting to act as a mineral matrix for native tissue in-growth. A study was conducted to assess MinerOss® particles as a primary grafting material for a sinus augmentation procedure and was followed for a post-operative period of 6 months. Bone core biopsies harvested during implant placement permitted histological evaluation of graft-tissue integration. Implants placed in graft-filled defects ($n = 39$) demonstrated satisfactory stability, with only one implant failing, and histologic analysis revealed strong osseo-integration characteristics (Avila et al. 2010).

1.1.3. RaptOs® (citagenix)

RaptOs® is an allogenic graft block product produced and distributed by Citagenix derived from cortico-cancellous bone. As per product page, the graft material is intended for filling bony void space in non-weightbearing osseous defects, since compressive mechanical forces of skeletal bone exceed those observed in allogenic products produced through freeze-dry processes. In a study conducted by Kaya et al. (2015), RaptOs® was evaluated histologically for bone reparative characteristics in a tibial defect model alongside two other graft materials of different origins, BioOss® (xenogeneic) and β-tricalcium phosphate (synthetic). Generated in both tibias of 28 Wistar rats, the defects measured 10 mm in length, 3 mm in depth, and 2 mm in width. Each rat was one of the three grafts in both legs, or left void for control samples, with one site receiving a pretreatment with a commonly employed antibiotic, rifampin. 21 days post-operatively rats were sacrificed, and samples were harvested for histological sectioning. Defects treated with RaptOs®, without inclusion of the antibiotic, demonstrated partial unions and early stage development of woven bone, as indicated by the presence of collagen fibers within the site. Despite the low cellular activity observed, these samples did maintain a consistently higher degree of cell activity as compared to the unfilled control samples and displayed the presence of bone marrow along the periphery of the material (Kaya et al. 2015). This study indicated that the human allograft product was capable of acting as a supplemental matrix within the defects to permit early-stage repair.

1.1.4. Cancellous chips (musculoskeletal transplant foundation)

Cancellous chips are a common allograft material and for human use can be procured readily from organizations such as the Musculoskeletal Transplant Foundation. The process of production of this graft material, described earlier, involves the use of freeze-dry cycles and irradiation to counteract the disease transmission and immune reactivity risks associated with allografts. The degradation of surface proteins and growth factors within the bone matrix during this process results in the final porous scaffold product

exhibiting severely reduced or no osteo-inductive capabilities. Therefore, cancellous bone chips are primarily utilized as osteoconductive filler matrices within non-weightbearing osseous defects. A study conducted by Hall et al. (2018), assessed cancellous chips derived from canine bone as a predicate material against a synthetic graft material in a critically-sized axial defect in the proximal humorous of 13 hound-type dogs. The administered cancellous chips ranged from 1–4 mm and were acquired from Veterinary Transplant Services, Inc. The humeri harvested at sacrifice (3 samples at 6 weeks, 5 samples at 13 weeks, and 5 samples at 26 weeks) were examined histologically to evaluate new mineralized bone and fibrous tissue formation. Analysis of 13-week and 26-week samples revealed that cancellous chip treated defects did exhibit enhanced healing and integration with native tissue at the periphery of the implanted material, yet fibrous tissue formations were observed at the center region of these defects. These formations were attributed to the poor inductive ability of the allograft material resulting reduced capacity to facilitate repair of critically-sized defects (Hall et al. 2018). Additional analysis of compressive mechanical strengths of samples and percentage of residual material compliment this finding with cancellous chip treated samples showing lower mechanical strength and greater volumes of remaining material as compared to the synthetic graft. As allograft products such as cancellous chips have been observed to require as much as 1 to 3 years for complete healing of the treated injury, it is possible that the 26-week time point utilized in this study may account for the low level of repair observed in this study (Hall et al. 2018).

1.1.5. Puros® (zimmer biomet)

Puros® is a mineralized cancellous bone allograft produced and distributed by Zimmer Biomet and utilizes a Tutoplast® processing method. This process provides a scaffold structure with preserved internal porous structure, surface proteins, and matrix growth factors of the natural bone. The preservation of matrix proteins and growth factors enable the grafting material to have osteo-inductive capabilities in addition to the osteoconductive properties of the basal structure, making such a material an attractive alternative to autologous grafts. A study conducted by Reddy et al. (2016) assessed Puros® with relation to the effectiveness of autologous bone grafts for treating periodontal intra osseous defects over 6 months. Patients included in the study ($n = 10$) were divided at random into either Group A, receiving Puros® treatment ($n = 5$), or Group B, receiving autologous bone graft ($n = 5$). The primary assessment was conducted through radiography of the defect region by evaluating changes in the defect depth (DD) at 1, 3, and 6 months post-operative intervals. Each timepoint was compared to baseline measurements. DD was determined based on parameters associated with the cementoenamel junction (CEJ), the region of the interface between enamel and cementum, including the relation to the most apical point of the defect and to the most coronal point of the alveolar crest. Defects treated with Puros® demonstrated significant decreases in depth size over each analyzed time point and was found to be comparable to autologous bone in the percentage of defect filled at the 6-month interval. It was concluded that both the Puros® and autologous bone promote predictable periodontal regeneration (Reddy et al. 2016).

1.1.6. RegenerOss® (zimmer biomet)

RegenerOss® is a partially demineralized, freeze-dried allogeneic product produced and distributed by Zimmer Biomet that undergoes processing methods designed to remove unwanted cellular elements while preserving lipids in the tissue. The resulting product is recommended for primarily oral and maxillofacial surgical procedures such as alveolar ridge augmentation, sinus floor elevation, and tooth socket preservation, as per product page. As with previously discussed allograft materials, RegenerOss® is not capable to provide sufficient mechanical stability alone for use in weightbearing bony defects. Available product particle sizes can range from 200–800μm and therefore offer variation in both surface area and topographical elements that may aid in promoting osteoconductive capabilities. In a case-controlled study by Eskan et al. (2017), 14 patients were treated with the allogeneic bone graft in conjunction with a bioresorbable matrix membrane for covering the defect region, and then placed into one of two groups, those receiving primary wound flap closure and those with the primary wound left exposed. The primary objective of the study was to assess the impact of initial wound exposure with relation to regenerative and reparative capacities, as well as to evaluate the effectiveness of the allograft material with membrane cover. Treatments were analyzed based on alveolar ridge widths, with baseline measurements conducted at initial surgical entry and final values taken after 4 months healing time during dental implant placement. Despite a lack of significance between study groups in alveolar ridge width increase over the 4-month period, all defects treated with the RegenerOss® product in conjunction with the bioresorbable matrix

membrane demonstrated a significant increase in mean alveolar ridge width, indicating effective application of the allograft for osteo-reparative functions in this model (Eskan et al. 2017).

1.2. Xenograft products

Xenografts comprise of scaffolds and particles derived from non-human species and therefore encompass a wide array of structural and protein compositional characteristics. Xenografts have been an attractive alternative to human-derived graft materials primarily due to manufacturing costs and enhanced availability of source material (Qiao et al. 2018). Additionally, the risk of disease transmission is greatly reduced since the transplant material is no longer human in origin, yet this also results in the material having a greater risk of evoking an immune response due to foreign proteins and elements. To address this risk, raw xenogeneic materials are subjected to the processes discussed earlier with allogenic grafts, namely freeze-drying and demineralization procedures. Materials that have undergone extensive freezing and lyophilization cycles to remove the organic components of the tissue matrix are considered "anorganic" and offer an inexpensive substitute for apatite structures that possess strong osteoconductive characteristics (Lee DSH et al. 2014). The general process utilized for this decellularization of tissue can be observed in Figure 2. As these constructs do not maintain effective/intact proteins within the matrix, products prepared through this method do not generally demonstrate osteo-inductive capabilities. Currently, the most prolifically utilized xenograft materials are of bovine origin; however, grafts derived from porcine tissue have shown promise, due to architectural and compositional similarities to human bone (Qiao et al. 2018).

The explored commercially available xenogeneic grafts consist of products from both bovine and porcine origins, with varying processing methods. The products: MinerOss XP®, BioOss®, InterOss®, and Gen-Os®, were selected through a basic web-based search for commonly employed xenograft materials.

1.2.1. MinerOss XP® (BioHorizons)

MinerOss XP® is a porcine-derived bone particulate graft material produced and distributed by BioHorizons. Similar to the previously discussed MinerOss® allogenic chips, MinerOss XP® is designed to act as a filler agent for bony defects that are non-weightbearing, as in cases as ridge and sinus augmentation. Source tissue undergoes extensive washing and fat stripping processes to remove the organic elements, including surface and matrix proteins, to eliminate factors that may elicit a reaction in native tissue surrounding the implant site. The resulting anorganic matrix is highly porous and maintains strong osteoconductive functions, providing an environment favorable for new bone formation. The efficiency of MinerOss XP® to form new bone (osteoid) was examined against a bone grafting material of bovine origin, which is currently more commonly utilized for xenograft applications, in a case study conducted by Guarnieri et al. (2017). The study consisted of a comparative histological assessment of new bone formation in two sockets that had received either the bovine or porcine-derived graft material. Core samples from the sockets were taken 6 months initial extraction and material application, during implant placement. Histological evaluation of the samples indicated that the porcine-derived product resulted in an increased formation of new bone as compared to the bovine-derived material, with percentage osteoid being 32.19% and 26.85% respectively. Additionally, the porcine-derived material demonstrated a reduced level of residual grafting material, an important consideration for xenogeneic graft materials as it is indicative of ability of the host to breakdown and resorb the graft (Guarnieri et al. 2017). Both materials utilized in the study demonstrated osteoconductive attributes and did not impede bone formation at the defect site.

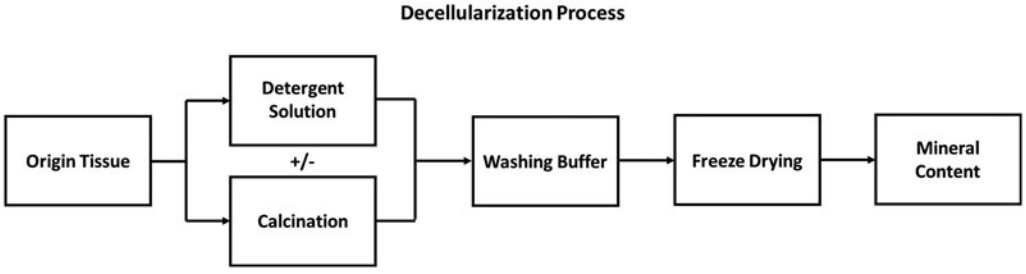

Figure 2. Generalized flow chart diagram for decellularization of bone tissue. Process moves from origin bone tissue to high mineral/low organic content product.

1.2.2. BioOss® (geistlich)

Produced and distributed by Geistlich, BioOss® is a deproteinized bone mineral particulate product of bovine origin. Deproteinization through common processes such as calcination, the removal of non-mineral elements by thermal degradation, or chemical treatments offer resulting graft products that consist primarily of the mineral phase structures (Su et al. 2018). These structures have inherently high porosities with varying pore sizes and intricate connecting channels, which are essential attributes for material intended to provide osteo-conductive effects. BioOss® has demonstrated significant enhancement of new bone development when implemented in non-weightbearing bony defects, particularly when incorporated as a supportive element to autologous bone particulate. In a systematic review by Aludden et al. (2017) the comparative impact of BioOss® as a standalone grafting material was assessed in relation to the bovine-derived products' coupling with autologous bone particulate in a selection of human lateral ridge augmentation procedures conducted between January 1990 and May 2016. The study evaluated effectiveness of treatment options based on two primary outcome criteria, the "survival of the suprastructure" and the "survival of the implant" (Aludden et al. 2017). If the suprastructure integrity, the newly formed bone matrix within the defect site, was determined to be compromised, this was defined as a "total loss" as the implant site could not then be assessed. Permitting that suprastructure was intact, the implant site was evaluated based on integration with native tissue and impact on surrounding tissue. To further support primary outcome classifications, measurements of histologically assessed new bone formation, ridge dimensional elements, and patient-reported outcomes were also incorporated into the study. It was determined that the variation in study design of the non-comparative evaluations of BioOss® treatments that were evaluated by Aludden et al. (2017) complicated the ability to accurately compare the individual study results in a systematic review. Therefore, the review heavily relied on secondary assessment characteristics to compare the two treatment modalities. Histological results and ridge dimensional assessments, both two dimensional and volumetric, indicated that there was not a significant difference between the treatments. Furthermore, comparison of characteristics of BioOss® mixed with autologous bone particulate against the application of purely autograft material did not yield a significant variation in implant survivability, thereby indicating the potential of BioOss® and similar xenogeneic-based graft materials as effective alternatives.

1.2.3. InterOss® (sigma graft)

InterOss®, similar to BioOss®, is an anorganic bovine-derived bone particulate graft material developed and distributed through SigmaGraft. The process utilized for deproteinization consists of initial chemical treatment of the origin tissue with NaOH and H_2O_2 solutions, followed by calcination at 350 °C. The resulting highly porous mineral structure is then capable of providing an osteoconductive substrate for application in non-weightbearing bony defects. The design and function of this material closely mimics the previously described BioOss® graft material, which is the basis for a comparative study conducted by Lee et al. (2014) of the SigmaGraft research and development department. The study primarily focused on comparing the physical and chemical characteristics of both materials including the surface area, porosity, and protein residue measurements. Results of the evaluation of the products indicated that the mineral composition and surface area of structures were not significantly different. Likewise, the crude protein residue content was not significantly different between the two materials, though it was speculated that the relatively lower content observed in InterOss® may have been a result of the extended annealing process (Lee et al. 2014). Another comparative study of these two graft materials was conducted by Kim et al. (2017) to evaluate the impact of these materials when applied to a complex *in vivo* system. The preclinical study utilized a critically-sized mandibular alveolar ridge defect in canines and was assessed at 4, 8, and 12 weeks post treatment. 54 defects in 27 animals received either treatment with InterOss®, BioOss®, or left empty. Histological and microcomputed tomography were used to evaluate the new bone development at defect sites and indicated that both materials were effective at facilitating new bone growth in relation to the negative control group, though there was no significant difference between products (Kim et al. 2017).

1.2.4. Gen-Os® (tecnoss dental)

Gen-Os® is a porcine-derived cortico-cancellous xenogeneic graft product developed and distributed by Tecnoss Dental primarily for oromaxillofacial applications. Conservation of origin tissue matrix structure enables graft particles to serve as highly porous substrate material for facilitating osteoconductive functions, much like previously described xenogeneic grafts.

Additionally, Gen-Os® processing preserves the collagen content of the origin tissue, which promotes osseo-integrative and osteo-inductive capacities. The inherent hydrophilic nature of the product due to the collagen content is also emphasized as a potential carrier mechanism for select drugs (Figueiredo et al. 2013; Fischer et al. 2015). A study evaluating the effectiveness of Gen-Os® as treatment in a select population of healthy chronic periodontitis patients, with individuals receiving graft product after debridement or only receiving open flap debridement. Treatments were assessed through clinical rankings, including plague index, gingival index, and pocket depth, and radiographical measurements of bone density. Comparison of baseline values with results at 6 and then 12 months post operation demonstrated that Gen-Os® significantly enhanced both clinical and radiological outcomes (Attia 2017).

1.3. Synthetic graft products

Synthetic bone graft substitutes are those that are derived from non-organic sources and consist of a broad spectrum of materials with varying characteristics. Though many of these materials are polymeric-based, these constructs are susceptible to high variability during synthesis and will not be further discussed in this article. Focusing primarily on commonly employed ceramic-based grafting materials, these materials consist of dense mineral structures that can be modified to adjust porosity and surface topography. Such materials have demonstrated promise in bone tissue regenerative application due to their ability to mimic the structure of native bone and provide an osteoconductive substrate for tissue in-growth. However, these materials do not inherently contain proteins or growth factors that would allow for osteo-inductive functions and therefore must be coupled with other materials or biological agents to elicit such activity.

The selected synthetic graft materials consist of ceramic-based mineral products intended to act as osteo-conductive constructs. The explored products include hydroxyapatite nanocrystals, Chronos®, and Vitoss® Synthetic, and were produced via a rudimentary web-based search for commercially available synthetic bone grafts.

1.3.1. Hydroxyapatite nanocrystals (berkley advanced biomaterials)

Synthetic hydroxyapatite nanocrystals (nHA) are a commonly employed calcium-phosphate (CaP) salt that is identical in composition to naturally forming hydroxyapatite (HA), which is a primary mineral element in bone (Sadat-Shojai et al. 2013). nHA can be readily synthesized through simple chemical processing to generate bulk quantities for a variety of applications. Additionally, pre-synthesized nHA products are commercially available, such as products generated and distributed by Berkley Advanced Biomaterials, for application as a standalone material or in conjunction with other materials for enhancement of properties. The nano-scale of this CaP significantly increases the surface area as compared to micro-scale HA, thereby heavily impacting the osteo-conductivity of the material. However, the high surface energy associated with the nanoparticles results in a propensity for particles to agglomerate and form macrostructures with significantly different characteristics (Fu et al. 2017). These varying microscale topographical landscapes can result in substantially different cell-material interactions and influence both cytocompatibility and cell differentiation characteristics (Jackson et al. 2018). nHA has demonstrated strong biocompatibility and is readily internalized by native cells, resulting in modulative effects on gene expression (Ha et al. 2015; Santos et al. 2017). These particles can also be readily incorporated with various material structures as a surface coating or integrative component, permitting design of composite graft materials that facilitate new bone formation (Bow et al. 2019).

1.3.2. ChronOs® (DePuy synthes)

ChronOs® is a synthetic β-tricalcium phosphate (β-TCP) bone grafting material produced and distributed by DePuy Synthes. β-TCPs are a commonly implemented material for bone grafting applications due to inherent biocompatibility, as well as resorbable and osteo-conductive functions (Arbez and Libouban 2017). As with the previously described synthetic nHA material, β-TCPs like ChronOs® are often used in conjunction with other mineral or bioactive components. In materials such as Syntoss®, a bone graft substitute produced by Dental Solutions Israel, β-TCP is combined with HA for an osteo-conductive porous structure (no available peer reviewed publications for Syntoss®). However, as a standalone grafting material ChronOs® has demonstrated effectiveness as a readily available bone void filling agent in non-weightbearing structures. In a study conducted by Bonardi et al. (2018), ChronOs® was examined alongside autogenous bone grafts and BioOss® in a human maxillary sinus bone augmentation procedure. The results indicated that ChronOs® was not significantly different from autogenous bone grafts in new bone formation, residual material, or area of

connective tissue; however, ChronOs® did display significantly less residual material than those treated with BioOss®. ChronOs® therefore offers an attractive alternative bone grafting material, particularly when coupled with autogenous bone particulate which can provide an osteo-inductive characteristic (Bonardi et al. 2018).

1.3.3. Vitoss® synthetic (stryker)

Vitoss® is a synthetic cancellous bone developed and distributed by Stryker as a bone graft substitute for bony voids in intrinsically non-weightbearing structures. This CaP grafting material is designed to be highly porous with complex inter-pore channels to mimic the structure of natural bone. The matrix composition and structure enable Vitoss® to exhibit both biocompatible and osteo-conductive characteristics, and when coupled with bioactive agents can serve as an osteogenic substrate. In a prospective multi-cohort study conducted by Epstein (2015), Vitoss® is compared to NanOss, a bioactive material composed of nano-scale CaP and porcine-derived collagen matrix, to assess the effectiveness of the materials in patients receiving laminectomies followed by posterior cervical fusions. The first cohort ($n = 72$), were treated with a combination of autografts, bone marrow aspirate, and Vitoss®, while the second cohort ($n = 20$) received the NanOss® in place of Vitoss®. Findings indicated that both examined material treatments yielded comparable fusion times and did not demonstrate significantly different fusion characteristics (Epstein 2015).

1.4. Bioactive graft products

Bioactive materials comprise of a wide array of compounds ranging from osteogenic-related proteins, such as bone morphogenic protein-2 (BMP-2), to genetic manipulation of native cells using nucleic acid, i.e. plasmid DNA (pDNA) and chemically-modified RNA (cmRNA), or viral-based approaches. For this reason, the application of bioactive materials varies dramatically based on the type of compound with many requiring a delivery mechanism to enhance effectiveness or duration of effect. This coupling with existing technologies permits development of finely-tuned grafts capable of facilitating bone repair via a tailored set of mechanisms, and therefore offers a highly attractive alternative to traditional bone graft substitutes. For this reason, the application of bioactive molecules in combination with traditional osteobiologic substrates has garnered the focus of many researchers in the field of bone tissue engineering. However, many of these agents are restricted in commercialization potential due to inherent costs and time associated with developing materials classified as drugs by the U.S. Food and Drug Administration (FDA) (Hasan et al. 2018). Furthermore, treatments utilizing bioactive materials may in some cases increase the risk of side effects in the local tissues or lead to tumorigenic growth. Therefore, limited products in this category are readily available commercially, which consequently results in products themselves being expensive, and are restricted to specific applications.

The bioactive graft materials selected for this review consist of growth factor-based molecules added to traditional osteobiologic grafts, such as those discussed above, in order to enhance or better regulate osteogenic activity. The products described here include Infuse™ and GEM 21S®, which were produced via a rudimentary web-based search for commercially available FDA-approved bioactive materials.

1.4.1. Infuse™ (medtronic)

BMP-2 has been demonstrated to be closely associated with osteogenesis (Schuberth et al. 2009), particularly with relation to mineralization, and is the only FDA-approved osteo-inductive growth factor currently available for bone grafts (James et al. 2016). The Infuse™ product produced and distributed by Medtronic, consists of an absorbable collagen sponge scaffold seeded with recombinant human BMP-2 generated using a hamster oocyte production cell line for protein recombination. Chinese hamster ovary (CHO) cell-derived rhBMP-2 maintains potent osteo-inductive potential, but, as a consequence of production costs, remains an expensive product. Comparative studies examining the osteo-inductive potential of CHO cell-derived rhBMP-2 with a relatively cheaper manufacture process utilizing Escherichia Coli (E. Coli)-derived rhBMP-2, which permits increased protein yield, have thus far indicated that the CHO cell-derived protein boast superior osteo-inductive potential (Jin et al. 2019). As per the Infuse™ product page, the bioactive product is indicated for only specific applications in spinal fusion procedures and acute tibial shaft fractures. The primary reason for the observed limited application of the product are likely related to the potential side-effects associated with administering growth factor doses *in vivo*. As discussed in James et al. (2016), adverse effects of BMP-2 can range from surgical site inflammation to ectopic bone formation. This is particularly concerning when considering applications related to spinal fusion and further stresses the importance of adherence to product guidelines.

1.4.2. GEM 21S® (lynch biologics)

GEM 21S® is a growth-factor-enhanced matrix (GEM) material produced and distributed by Lynch Biologics for use in dental therapy applications. The bioactive product is comprised of purified recombinant human platelet-derived growth factor-BB (rhPDGF-BB), derived from yeast cultures, seeded to a β-tricalcium phosphate (β-TCP) (Singh and Suresh 2012), similar in design to the previously discussed ChronOs®. PDGF-BB is strongly associated with angiogenesis and has been demonstrated to be produced by osteoclasts during osteogenesis for recruitment of precursor cells (Xie et al. 2014). The combination of the growth factor with an osteoconductive matrix, β-TCP, is intended to promote healthy bone repair by recruitment of progenitor cells and formation of vasculature within the scaffold. In a study conducted by Young et al. (2009), the GEM 21S® product was examined for protein release dynamics in vivo using a calvarial defect in rats. It was observed that the protein underwent a rapid burst release, with complete depletion of the protein by 72 hours post-implantation (Young et al. 2009). Despite the release rate of the protein it was observed that the rhPDGF-BB was still bioactive in the surrounding tissue. A separate comparative study examined the use of GEM 21S® with a collagen membrane for dental recession defects to determine if the product was capable of enhancing root coverage as compared with a collagen membrane alone (Singh and Suresh 2012). Though root coverage in the GEM 21S® treated samples appeared improved, the researchers noted that no significant difference was observed between the study test groups.

2. Conclusion

The bone graft materials discussed in this article (Table 1) represent a small portion of currently available biomaterials for bone tissue engineering applications; however, these products demonstrate the fundamental osteobiologic characteristics for materials designed to act as effective bone tissue engineering grafts. Though, as the different processing methods used to generate these products result in an array of grafts that display highly variable reparative functions, an ideal bone substitute graft is still yet to be developed. This is further echoed in the low mechanical structural integrity of these materials restricting application primarily to non-weightbearing injury sites. For these reasons, the previously described materials serve as the more commonly applied bone grafts substitutes and are utilized as basal elements of more complex scaffold composite designs. As the majority of these products exhibited osteo-conductive and biocompatibility qualities, coupling bioactive components, such as growth factors, with the materials can offer enhanced functions including osteo-inductive and osseo-integrative characteristics (Zhao et al. 2017). Furthermore the implementation of cell-based or gene therapy-based approaches can serve to generate osteogenic environments capable of facilitating finely-tuned bone repair (Hasan et al. 2018).

Table 1. Graft Material Overview. List of bone graft materials detailing general information and characteristics. In addition to graft type and source, material content, and application references are listed.

Graft Material	Company	Graft Type	Graft Source	Inorganic Content	Organic Content	In Vitro Application Reference(s)	In Vivo Application Reference(s)
Grafton DBM	BioHorizons	Allogenic	Homo Sapien	✗	✓	Kumaran et al. 2010	Bomback et al. 2004; Brecevich et al. 2017; Kadam et al. 2016; Kang et al. 2012
MinerOss	BioHorizons	Allogenic	Homo Sapien	✓	✗	Greenspan 2012	Avila et al. 2010; Potres et al. 2016
RaptOs	Citagenix	Allogenic	Homo Sapien	✓	✗	–	Kaya et al. 2015; Kolerman et al. 2019
Cencellous Chips	Musculoskeletal Transplant Foundation	Allogenic	Homo Sapien	✓	✗	–	Hall et al. 2018
Puros	Zimmer Biomet	Allogenic	Homo Sapien	✓	✓	Greenspan 2012	Reddy et al. 2016
RegenerOss	Zimmer Biomet	Allogenic	Homo Sapien	✓	✓	–	Eskan et al. 2017
MinerOss XP	BioHorizons	Xenogeneic	Procine	✓	✗	–	Guarnieri et al. 2017
BioOss	Geistlich	Xenogeneic	Bovine	✓	✗	Jackson et al 2018; Xu et al. 2019	Aludden et al. 2017; Bow et al. 2019; Kumar et al. 2018; Sohn and Moon 2018; Xu et al. 2019
InterOss	Sigma Graft	Xenogeneic	Bovine	✓	✗	Lee et al. 2014	Kim et al. 2017
Gen-Os	Tecnoss Dental	Xenogeneic	Porcine	✓	✓	–	Attia 2017; Figueiredo et al. 2013; Fischer et al. 2015
Nano-Hydroxyapatite	Berkley Adv. Biomaterials	Synthetic	N/A	✓	✗	Fu et al. 2017; Ha et al. 2015; Jackson et al. 2018; Santos et al. 2017	Bow et al. 2019; Pujari-Palmer et al. 2016
ChronOs	DePuy Synthes	Synthetic	N/A	✓	✗	Arbez and Libouban 2017	Bonardi et al. 2018; Kanter et al. 2016
Vitoss	Stryker	Synthetic	N/A	✓	✗	–	Epstein 2015; Walsh et al. 2013
nanOss	rti Surgical	Synthetic	N/A	✓	✗	–	Epstein 2015; Walsh et al. 2013

Utilizing polymer-based additives to matrix compositions can result in grafts with both hard mineral and pliable elastic regions, thereby mimicking mechanical diversity in natural bone. Additionally, hydratable polymers may provide optimal means for carrying and eluting drugs at the site of interest. Drugs capable of preventing infection of the treated site or stimulating the native tissue to facilitate enhanced reparative characteristics, can be readily incorporated into multi-composite structures comprised of any number of the discussed materials and a polymeric binding agent to develop a scaffold material that could far exceed the capabilities of even autologous grafts. Such novel combinations of available technologies provide an ever-expanding arsenal of graft options for treating bone injuries and represent the impressive potential of bone tissue engineering. However, determining the optimal graft technology for replacement of autografts will require continued concentrated research efforts in both benchtop and clinical trial settings to ensure an effective and superior osteobiologic product.

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