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Biologic and clinical aspects of integration of different bone substitutes in oral surgery: a literature review

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Many bone substitutes have been proposed for bone regeneration, and researchers have focused on the interactions occurring between grafts and host tissue, as the biologic response of host tissue is related to the origin of the biomaterial. Bone substitutes used in oral and maxillofacial surgery could be categorized according to their biologic origin and source as autologous bone graft when obtained from the same individual receiving the graft; homologous bone graft, or allograft, when harvested from an individual other than the one receiving the graft; animal-derived heterologous bone graft, or xenograft, when derived from a species other than human; and alloplastic graft, made of bone substitute of synthetic origin. The aim of this review is to describe the most commonly used bone substitutes, according to their origin, and to focus on the biologic events that ultimately lead to the integration of a biomaterial with the host tissue. (Oral Surg Oral Med Oral Pathol Oral Radiol 2016;122:392-402)

Statement of Clinical Relevance Bone regeneration is required to treat maxillary and mandibular defects caused by accidents, violence, tumors, or atrophy resulting from long-term edentulism. The choice of the most suitable biomaterials could guide clinicians with regard to bone graft reconstructions

The reconstruction of large maxillary and mandibular bone defects, caused by accidents, violence, tumors, or atrophy resulting from long-term edentulism, represents an important clinical challenge for maxillofacial and oral surgeons. To better repair jaw defects, it is important to have knowledge of the biologic properties of native bone tissue and to perform tissue engineering procedures by utilizing bone substitutes that are able to mimic it. Tissue engineering was initially defined as “an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biologic substitutes that restore, maintain or improve tissue or organ function” 1 and then as a discipline with “the grand aim of understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use.” 2 These procedures do not simply position a material having a similar function to fill the spaces but also to allow for regeneration of tissues structurally and functionally similar to the ones lost. In the oral cavity, regenerative techniques applied to bone tissue are required to counteract vertical and horizontal bone loss following a trauma or teeth extraction and to provide adequate bone quantity and quality to allow dental implant insertion for prosthetic rehabilitation. Bone tissue is a specialized mineralized connective tissue consisting of anorganic calcium phosphates and organic components, in which bone-forming and boneresorbing cells are embedded. In general, the presenceof three fundamental elements is necessary to obtain bone regeneration: (1) a source of cells that are able to differentiate and to secrete a mineralized matrix; (2) growth factors or biophysical stimuli, to guide the regenerative process in a positive way; and (3) a scaffold that is able to mechanically support the invasion of the bone defect by cells from the periphery and to be a substrate for their growth and proliferation.3 Mesenchymal cells (i.e.,

osteoblast precursors in the recipient site) are the cellular elements responsible for the regenerative event. Mesenchymal cells migrate toward the bone substitute conveyed by the bloodstream and by the newly formed vessels and differentiate into osteoblasts responsible for the integration of the graft. In bone regeneration, growth factors produced by the organism play a key role. Growth factors are represented by a large number of molecules locally released by the cellular elements already existing in the recipient site or conveyed through the bloodstream which stimulate mesenchymal cells to migrate from the periphery of the defect and to differentiate toward osteoblastic cell lineage. This process is called osteoinduction. The scaffold is represented by the grafted bone substitute, whatever its biologic origin may be. Its function is to recreate a three-dimensional spatial structure that harbors and mechanically supports cells and tissues, including blood vessels, which colonize thegrafted biomaterial during the healing phases. Such a characteristic, referred to as osteoconduction, is common to all bone substitutes and does not provide a biochemical interaction between the graft and the recipient site. An ideal bone substitute should be biocompatible; well tolerated; without any antigenic, teratogenic, or carcinogenic characteristic; bioactive; handy; sterile or sterilizable; hydrophilic; and should have good mechanical and chemical properties.4 Biocompatibility is defined as "the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy." 5 Bioactivity is the ability to form a bond at the interface with the adjacent tissue. However, the time for such binding and the strength, the mechanism, and the thickness of the area where this interaction occurs differ among materials of different origins. A biomaterial should also have suitable mechanical properties, ensuring a proper mechanical support lasting for an appropriate time, to maintain the three-dimensional structure required for tissue regeneration. Moreover, it must be sterile or readily sterilizable and have suitable chemical and physical properties, including porosity, hydrophilicity, and suitable surface topography. The geometry, ultrastructure, and mechanical properties of biomaterials are determine the successful healing of bone defects, and their ability to be resorbed in vivo is important for allowing simultaneous replacement of the material itself with newly formed bone. It has been suggested that the chemistry and the geometry of the biomaterial in contact with these cells are critical factors that induce cell differentiation into bone-forming cells.6,7 To design a scaffold architecture, the right balance between the high degree of porosity and the mechanical stability of the material needs to be achieved. Following the natural shape of bone marrow, the scaffold must have open and interconnected porosity.8,9 Pores are necessary for bone tissue formation, as they allow the migration and proliferation of osteoblasts and mesenchymal cells, as well as vascularization. Moreover, the presence of pores accelerates the biodegradation of the material by increasing the area in contact with body fluids. To ensure cell viability and function, an ideal scaffold needs to exhibit porosity in different length scales: nanopores to allow molecule transportdessential for any nutritiondwaste removal, and signaling; micro-pores to ensure cell migration and capillary formation; and millimeter-wide pores to allow the development of nerves and blood vessels.10 Moreover, the degree of interconnectivity is as critical as the pore size, since a final porous surface could improve the physical contact between the grafted biomaterial and the surrounding host tissue, thus ensuring greater mechanical stability.11 Osteoblasts have been shown to be sensitive to the morphology of the material to which they adhere. For example, osteoblasts perform greater migration, attachment, and proliferation in the presence of pores with a mean diameter of 200 to 400 mm,12,13 probably because the curvature of these pores provides optimal compression and tension on cell mechanoreceptors, whereas porosity greater than 300 mm is recommended for vascularization. In general, researchers indicate an open porosity above 50 vol% and pore sizes in the range of 200 to 800 mm as optimal for bone tissue growth.14,15 Any regenerative process implies a sequence of biochemical, cellular, and tissue events and interactions, which are more significantly dependent on the biologic characteristics of the grafted tissue rather than on the surgical technique performed. The integration of a bone graft results in a remodeling process that is comparable with the physiologic bone healing events that follow a bone fracture.16,17 Bone remodeling occurs through

different steps, beginning with the destruction of the vascular structure at the site of the lesion, reducing the supply of nutrients and determining an initial bone resorption process. The hematoma generated activates signaling molecules and growth factors, which stimulate the proliferation and differentiation of osteoprogenitor cells. Bone substitutes used in oral and maxillofacial surgery could be categorized on the basis of their biologic origin and source as autologous bone graft when obtained from the same individual receiving the graft; homologous bone graft, or allograft, when harvested from an individual other than the one receiving the graft; animal-derived heterologous bone graft, or xenograft, when deriving from a species other than human; and alloplastic graft, made of bone substitute of synthetic origin. On the basis of this knowledge, many bone substitutes have been proposed for bone regeneration, and researchers have focused their attention on studying the interactions between grafts and host tissue, as the biologic response of host tissue can be related to the origins of different biomaterials. Thus, the aim of this review is to describe the most commonly used bone substitutes according to their origin and to focus on the biologic and molecular events that finally lead to the integration process with the host tissue.

AUTOLOGOUS BONE GRAFT

Autologous bone graft can be obtained from intraoral and extraoral donor sites, which can be cortical, medullar, or corticomedullar, and both fresh and frozen forms can be used. Autologous bone graft is often used in cases of severe bone atrophy to obtain adequate ridge augmentation for the insertion of dental implants. Autologous bone graft is the only bone substitute that has osteogenic potential, as it maintains viable cells from extraction from the donor site to grafting if properly treated and shares the same biologic origin as the recipient organism. This means that rejection will not occur, and neoangiogenesis and bone regeneration are strongly promoted. It also ensures good physical support for the membranes and the flap. The disadvantages of autologous bone graft are its limited availability and the need for a second surgery, with consequent increase in morbidity. Moreover, residual disability in the withdrawal area may persist, and there is a risk of seizures and osteitis or osteomyelitis. To investigate the efficacy of different bone substitutes and to assess the success of the grafting procedure itself, several authors studied the success rate of dental implants inserted in regenerated sites. In a systematic review by Aghaloo and Moy,18 it was found that implant survival rates for up to 102 months was 92% following maxillary sinus augmentation with autologous bone alone or combined with other biomaterials, 81% for implants inserted in sites regenerated with allograft and allograft/xenograft materials, and 95.6% for implants in xenograft bone substitutes alone.18 However, this method of indirectly evaluating the success rate of the bone substitute was not entirely accurate, and other studies aimed to analyze the biologic integration between the host tissue and the bone substitute. In a recent in vivo study conducted by Tetè et al., the performance of autologous bone with respect to heterologous bone substitutes was evaluated.19 Intraand extraoral autologous bone grafts and a porcinederived heterologous biomaterial were used as bone substitutes before implant-prosthetic therapy in the posterior maxilla. Bone tissue samples from sites regenerated through different methods were taken at the time of implant insertion (i.e., about 6 months after the regenerative therapy), and histologic and immunohistochemical analysis was performed to identify the expression of specific molecules involved in bone graft healing and integration. Results from this study indicated that sites treated with autologous bone, after the same period of healing, show a better integration with host tissue because of a greater neoangiogenic and osteoproliferative triggered response, as outlined by the higher levels of bone sialoprotein and vascular endothelial growth factor (VEGF) expression in comparison with levels recorded for porcine-derived heterologous bone graft. In addition, the sites treated with the heterologous biomaterial show clearer signs of inflammatory response and apoptosis, evidenced by higherlevels of inducible nitric oxide synthase and Bax, and a more limited integration, visible both directly at histologic analysis and indirectly through the evaluation of the above-mentioned molecule expression levels. With limitation to the repair of localized alveolar defects, the choice of intraoral sites as donor sites for bone grafts has shown clear advantages over extraoral sites: easy surgical access, contiguity between donor and recipient sites, avoidance of permanent skin scars, and minimum discomfort to the

patient. The maxillary bone and the interforaminal segment of the jaw, with the exception of the alveolar bone, share an intramembranous embryologic origin, whereas most of the skeletal bones have an endochondral origin. According to previous findings, intramembranous bone has a resorption rate that is lower than that of endochondral bone when used as graft. Other authors have emphasized that, by using a graft of ectomesenchymal origin, such as that of the jaw, the incorporation in the maxillofacial region is undoubtedly greater, as it contains a high concentration of growth factors, and vascularization seems to be more rapid in the presence of intramembranous bone tissue.3 However, more recent findings have attributed the better integration and slower resorption process to the bone graft microarchitecture, more specifically to the ratio between the cortical and the cancellous bone components, rather than to its embryologic origin.20 Among the extraoral donor regions, the iliac crest and the calvaria are the most described and allow harvesting of large amount of bone tissue for more extensive repair of bone defects. In its outer portion, an iliac crest graft is made almost entirely of cortical bone, with abundant trabecular bone below. Bone extracted from the medullary portion of the iliac crest maintains high osteoproliferative characteristics. The use of this material in interventions for maxillary sinus augmentation allows for positioning long and large-diameter implants and those with a more distal extension, thus decreasing the loading tensions of the resulting prosthesis. In a long-term study, conducted through immunohistochemical analysis on samples obtained from sites treated with autologous bone grafts from the iliac crest, a good degree of integration was observed between the graft and the native bone tissue after a mean healing period of 4 months. As such, integration, even after the insertion and occlusal functionalization of an implantsupported prosthesis and long-term observation, is maintained.21 Even though matrix metalloproteinase (MMP) 2 and VEGF expression is significantly increased approximately 4 months after the intervention, revealing a healing phase with high vascular proliferation, the expression of all molecules decreases after 10 years to return to basal levels, suggesting the presence of only weak rearrangementphenomena of the graft and indicating that the graft is incorporated in the physiologic process of bone remodeling. However, several studies reported a 12% to 60% resorption rate of the initial iliac crest bone graft height in the 5 years following implants loading.22 The best results in terms of low resorption rates were reported for vertical ridge augmentation through calvarial bone graft.23 The bone tissue obtained from the calvaria is widely used in maxillofacial reconstructions, as it has complication and postoperative morbidity rates lower than those of other extraoral donor sites.24 According to previous studies, the most common complications of this type of surgery are represented by hematoma, seroma, wound dehiscence, infection of the scalp, dural tear, and arachnoid bleeding.25,26 The incidence of dural perforation is very low and does not involve permanent neurologic complications, thanks to the availability of new surgical aids, such as the piezoelectric terminal.27 Beside their low rate of resorption over time, calvarial bone grafts provide excellent primary stability upon insertion of dental implants. Microcomputed and histologic evaluations of humans have demonstrated areas of extracellular matrix (ECM) that are extremely stained and organized in concentric lamellae edging few vascular canals and increased expression of MMP9, bone sialoprotein, and VEGF. These data confirmed that calvarial grafts adequately integrated with the bone tissue of the recipient site. The host tissue revealed low inflammatory and apoptotic responses, as also indicated by low expression of inducible nitric oxide synthase and proapoptotic Bax.19,28 Previous studies have suggested that although both the iliac crest and the calvaria can be clinically considered suitable sites for bone tissue extraction in the case of large oral rehabilitation, some differences can be detected microscopically.29 Both grafts appear to promote an adequate neoangiogenic response in the treated sites, as demonstrated by immunohistochemical analysis for MMP9 and VEGF expression, but in terms of new bone formation and the absence of inflammatory events, the calvaria can be considered the most suitable donor site for bone grafts. In fact, the use of calvarial grafts in the posterior maxilla would seem to not only determine not only a volume recovery, thus obtaining a bone quantity ideal for subsequent implant rehabilitation, but also favor the regeneration of bone tissue with quality more similar to that of the donor site than that of the recipient site.30 In addition to the choice of the correct bone substitute, correct functionalization of the recipient bed is needed as well. Perforations or

decorticalizations of the receptor bed seem to be of fundamental importance not only in guaranteeing graft integration but also in preventing graft volume loss during the healing period, as these factors could positively influence the processes of revascularization and osteogenesis.31 The disadvantages of calvarial bone grafts include the proximity of the donor and recipient sites in the same operative field, since two simultaneous surgical interventions are impossible; the friability of the graft material, which may fracture during surgery; the volume of trabecular bone, which is limited in adults; and the possibility of scar tissue, posing the risk of baldness.

HOMOLOGOUS BONE GRAFTS

Even if autologous bone is still considered the "gold standard" in bone regeneration, the limited quantity available and the postoperative morbidity following extraction from the donor site represent the limitations of this approach, and this has researchers to look for nonautologous sources for bone substitutes.32 Homologous bone is obtained from living or nonliving donors that belong to the same species of the receiving subject. Once extracted, homologous bone is treated and subsequently stored, in various shapes and sizes, in bone banks.33 Various types are known: 1. Fresh frozen bone (FFB), extracted and then treated with low temperatures to reduce the antigenicity and the risk of infection 2. Freeze-dried bone allograft (FDBA), treated with dehydration and freezing and not demineralized; for this type only the osteoconductive effect has been recognized 3. Demineralized freeze-dried bone allograft (DFDBA), with osteoconductive and inductive potential Preparation of allogenic FFB is easy: It is simply removed; frozen at 80————————————C; not irradiated, lyophilized, or demineralized; and stored in authorized bone banks.34 It maintains its healing capabilities, that is, its osteoinductive and osteoconductive properties similar to autologous bone, because of the presence of a mineral constituent expressing bone morphogenetic proteins (BMPs), although it is acellular.35,36 However, it cannot be considered an osteogenic biomaterial, even if some scientific evidence suggests that the cryoprotective substance dimethyl sulfoxide, used for the bone graft during freezing, allows osteoblasts, osteoclasts, osteocytes, and periosteal cells to survive and accelerates the biologic integration phases.37,38 For years, the use of FFB was discussed because of the risks of viral infections. However, the careful selection of the donors makes the risk of transmission of viral diseases extremely rare (less than 1 in 1,000,000), and the pretreatment used appears to alterthe protein's major histocompatibility complex type 2, making the material nonantigenic.39,40 In fact, donors must provide the following information: hematocrit count; inflammation markers; hepatic and renal functionality; liver electrophoresis; human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and Treponema serologic markers; and thoracic radiography and electrocardiography results. On the day of the intervention, other examinations are performed, including serum concentration of alanine transaminase and serologic markers and viruses.41 Bone samples are usually extracted from the femoral epiphysis in total sterility. Any residual fibrous tissue is removed, and the bone samples are immersed in an antibiotic solution of vancomycin, polymyxin, ceftazidime, and lincomycin for at least 72 hours at 4————————————C, followed by freezing at 80————————————C, using the cryoprotective dimethyl sulfoxide, which allows storage for up to 5 years.37 Clinically, as well as histologically, FFB seems to be a successful biomaterial.41,42 Several studies have shown perfect integration of the biomaterial in preexisting bonedwithout any distinction from newly formed bone, lined by osteoblasts, depositing osteoid and parts previously remodeled with a mineralized matrix presenting osteocytes, newly formed blood vessels, and an almost insignificant rate of inflammationdwhen FFB is used for both postextraction alveolar socket preservation and maxillary sinus augmentation.43,44 FDBA is generally extracted from live healthy donors undergoing orthopedic surgery of the hip. It is prepared by collecting trabecular bone and/or cortical bone under sterile conditions; the bone graft is subsequently washed in distilled water and fragmented into particles of variable size, from 500 mm to 5 mm, immersed in 100% ethanol to elute the lipids, frozen in nitrogen, dehydrated, and reduced to smaller particles (250e750 mm).45 The dehydration process promotes long-term maintenance and reduced antigenicity. Thus, it is possible to keep both the organic and the inorganic components in such a way as not

to eliminate calcium and phosphate. FDBA seems to be able to grant clinical results comparable with those of DFDBA, but it does not appear to have the osteoinductive potential of DFDBA, which is related to BMPs that may remain after commercial preparation. A histologic study on monkeys showed a trend toward greater and faster bone formation with FDBA in comparison with DFDBA.46 In contrast, another study on humans indicated a significantly greater percentage of vital bone tissue and lower percentage of residual graft particles at the moment of dental implant insertion in sites grafted with DFDBA versus FDBA.47DFDBA is a kind of homologous bone that undergoes demineralization and lyophilization and is obtained from deceased donors within 24 hours after death. It is then stored in a dedicated bone bank. After bone material is extracted, it is washed with hydrogen peroxide at 3% for 5 to 15 minutes in an ultrasonic bath, delipidated with ethanol at 70% for 1 hour, heat treated at temperatures higher than 300———————————C for 15 to 18 hours, lyophilized and dehydrated into liquid nitrogen up to a temperature of 90———————————C, sterilized with ethylene oxide or gamma rays, and finally demineralized in hydrochloride for 36 to 48 hours.34 The use of gamma rays is controversial, but low doses of radiation are considered safe and not to inhibit the process of bone formation.48 However, before use, the graft material is subjected to a series of tests to verify that the demineralization process has destroyed all pathogens to prevent the risk of antigenicity and disease transmission.33,49 During DFDBA preparation, the inorganic component of the bone is eliminated, whereas the organic component remains and could be responsible for the osteoconductive characteristics of the biomaterial, even if this property was found to be strictly dependent on the donors' age, as decreased amounts of BMPs are more evident after the age of 50 years.50

ANIMAL-DERIVED HETEROLOGOUS BONE SUBSTITUTES

Heterologous bone substitutes of animal origin were found to be as clinically efficient as autologous bone for their osteoconductive potential. However, not much is known about their capability to be fully resorbed or about the time needed for entire substitution by newly formed bone.51 A biomaterial exhibiting no or scarce resorption could compromise correct bone regeneration because of its lower osteogenesis capability compared with native autologous bone during the remodeling phase.52 Heterologous bone substitutes can be used, either alone or in combination with other bone substitutes, in all cases of bone graft, ridge augmentation, maxillary sinus elevation, and, in general, correction of even large bone defects. They should ideally combine the osteoregenerative features of autologous bone, eliminating at the same time the limits imposed by the need for a second surgical intervention for extraction. In addition, they should be biologically safe; possess osteogenic, osteoinductive, and angiogenic potentials; have a long shelf-life and no size restrictions; and be costeffective.53 Different heterologous biomaterials have been proposed for clinical use in oral surgery. Bovine-derived bone substitute is one of the most well-documented substitutes in the literature. In vitro histologic andclinical studies have described the osteoconductive properties of bovine-derived biomaterials, the characteristics to support mineralized ECM deposition by adequately differentiated stem cells, and their capability for integration with host bone tissue.19,54,55 However, it has been reported that the granules of biomaterial undergo slow or poor resorption and therefore tend to be surrounded by newly formed bone tissue rather than being reabsorbed and entering the physiologic processes of bone remodeling.19,56 According to some authors, such in vivo behavior could, in part, result from the specific treatment of deproteinization at high temperatures and sterilization that these biomaterials undergo before being available for clinical use. In fact, the absence of proteins is critical to avoiding unwanted immunologic reactions, allergic reactions, and any possible risks of crossinfections that may follow the placement of a bovine-derived biomaterial in the human body. The deprivation of the organic component is carried out by heating the material to high temperatures for more than 15 hours to eliminate all organic components and all possible antigens, followed by sterilization at 160———————————C. Even though it allows for and ensures the removal of the entire organic component, this treatment also modifies the mineral structure of bone hydroxyapatite (HA), and thus the resulting biomaterial usually possesses a reduced resorption potential. In addition, in recent years,

the risk of prion disease transmission associated with bovine-derived heterologous bone substitutes has led to the search for other sources for bone substitutes.57,58 Recent studies show that biologic deantigenation, by a proteolytic process through digestive enzymes at about 37————————————————C for 7 days, selective for the organic component, could leave unaltered the ability of the biomaterial to be reabsorbed in vivo.59 For this purpose, other animal-derived bone substitutes have been proposed as valid alternatives to bovine-derived ones. Equine-derived bone substitute is described as having the capability to induce osteoblast differentiation, to be resorbed in vitro by osteoclasts, and to be successfully used in mandibular ridge augmentation.60,61 Immunohistochemical analysis to evaluate VEGF expression and osteoprotegerin (OPG)/receptor activator of nuclear factor kappa-B ligand (RANKL) ratio showed, respectively, new blood vessels and new bone formation in sites treated with equine-derived heterologous grafts.62 Light microscopic analysis strongly evidences that sites treated with equine-derived bone substitutes show good integration between the biomaterial and the surrounding host tissue. Moreover, after a mean healing period of 6 months, the existence of large areas of newly formed bone tissue associated with intenseremodeling phenomena around the grafted biomaterial, occurring as a result of high levels of VEGF, can be observed.63 The strong relationship between new bone formation and ingrowth of new vessels was also highlighted in a study by Di Stefano et al., in which a high intensity of VEGF expression was found in areas treated with the equine-derived bone substitutes.64 Porcine-derived bone substitutes may be successfully used to obtain osteogenesis in guided bone regeneration techniques, even if scant features of particle resorption can be observed at light microscope analysis.19,65 However, differences in vascularization resulting from a low level of VEGF expression significantly affect the clinical performance of this bone substitute, and its ability to be resorbed is important in influencing longterm integration and long-term predictability of bone regeneration.

ALLOPLASTIC BONE SUBSTITUTES

Another valid alternative to autologous bone graft is synthetic biomaterial capable of simulating the typical characteristics of native bone tissue and which could be used as a three-dimensional scaffold to support cell growth and subsequent bone tissue formation. Alloplastic bone substitutes represent a large and heterogeneous group of chemically diverse synthetic calcium-based biomaterials, including calcium phosphate, calcium sulfate, bioactive glasses, and polymers. Acting as a matrix for osteogenic cell migration, these biomaterials offer excellent osteoconductive properties, support growth and proliferation in vitro, and promote the deposition of mineralized ECM by osteoblastic cells.54,66 The construction of these bone substitutes is based on their ability to mimic the properties of natural bone and serves as a three-dimensional template for initial cell ingrowth and subsequent tissue formation. Beside the chemical composition, the geometry, ultrastructure, and mechanical properties of these biomaterials are the determinants for successful healing of bone defects, and their capability to be resorbed in vivo is important for allowing simultaneous replacement of the material itself by newly formed bone. Several strategies were proposed to stimulate bone tissue growth within the scaffold. These include the production of biomaterials with a specific surface nanotopography, the development of biomimetic materials, the formation of mineralized layers, and the use of bioreactors for cells. The principle behind all these approaches is to mimic the natural environment in which bone cells normally grow.32 Biomimetism is defined as the creative initiation of several specific biologic systems inspired by natural phenomena.67 Biomimetic materials are able to increase cell adhesion and proliferation on the same scaffold.68The geometric design of a biomimetic matrix enhances the activation of signaling molecules, which then leads to the induction of bone formation.69 According to studies by Ripamonti et al., the geometry of the biomaterial, in particular the concavity created in the bioceramics HAbased and/or biphasic calcium phosphate, triggers cell differentiation when introduced into the muscle tissue of primates.70,71 The same authors report that “the driving force of the intrinsic induction of bone formation by bioactive biomimetic matrices is the shape of the implanted substratum. The language of shape is the language of geometry, and the language of geometry is a sequence of repetitive curves that describes the remodeling cycle of osteonic bone in

primates.” 72 Among the materials based on calcium phosphate, b-tricalcium phosphate (b-TCP; Ca3(PO4)2; Ca/P ¼ 1.5) and HA (Ca10(PO4)6(OH)2; Ca/P ¼ 1.67) are considered the most suitable ceramic materials for bone reconstruction. Two- and three-dimensional computed tomography, histologic and histomorphometric analyses found new bone formation similar to that of autologous bone when b-TCP was used as the filling biomaterial in maxillary sinus augmentation.73 New bone formation was described when b-TCP was used in postextractive alveolar sockets prior to dental implant placement. A good integration was observed between the grafted biomaterial and the newly formed bone tissue, as well as expression of osteonectin, a noncollagenous protein related to osteoblasts differentiation and metabolism.74 Other studies have shown that the process of chemical dissolution of b-TCP particles results in a local decrease of pH caused by the metabolites of degrading b-TCP, the effect that favors bone tissue regeneration.75,76 HA is a biomaterial with good biocompatibility and bioactivity. It can be of natural origin, or it may be synthetically produced. It can be further classified as dense, microporous, or macroporous, according to the porosity percentage, and as crystalline or amorphous.77 Crystalline HA is more resistant to fracture compared with the amorphous form. The biomaterials with a crystalline structure differ from each other in their characteristics, depending on the origin of the product. The presence of small crystals, such as those found in natural bone tissues, are desirable because the interactions at the interface with the bone improve with increased surface area, thus enhancing the material’s potential to create a bond with the host bone tissue. The chemical or thermal treatment of the biomaterial outlines the shape of the crystal. In fact, high-temperature treatment (over 1000————————————C) leads to growth of the crystal, which may alter the surface characteristics although it does not change the basic structure. In an in vitro study, stem cells obtained from human dental follicles were cultured on a sample of syntheticHA. Light and electron microscope analysis performed at different experimental times revealed the colonization of the biomaterial by cells of polygonal shape within the first week of culture, suggesting the high bioactivity of the biomaterial itself. Moreover, via scanning electron microscope, it was possible to observe the initial ECM and three-dimensional colonization of the biomaterial.66 In an experimental study on animals, the clinical efficiency of nonresorbable porous HA was tested as a filling biomaterial for maxillary sinus augmentation and simultaneous dental implant placement. By comparing results with those obtained from sites in which autologous bone was grafted, no significant differences were recorded in terms of bone-to-implant contact where HA was used as biomaterial. However, because of its characteristic limited resorption, the recommendation is to limit its use as unique biomaterial for maxillary sinus augmentation and instead to use it in association with autologous bone.78 In a study on humans, a commercially available highly porous nanocrystalline HA was described as possessing osteoconductive and biomimetic properties and having the capability to be completely resorbed and to enter the physiologic bone remodeling cascade during the healing process.79 The nanoporosity of the biomaterial seems to allow absorption of bone-specific molecules and growth factors, such as alkaline phosphatase, BMP-2, collagen type I, osteocalcin, and osteopontin, thus permitting osteoblast precursor recruitment and differentiation in the grafted area, together with osteoclast adhesion, which ensures graft substitution with mature bone tissue during healing. However, HA is generally a brittle material and is characterized by low fracture toughness. These drawbacks could be overcome by using different toughening materials, such as alumina, titanium, and carbon nanotubes (CNTs) to improve its mechanical characteristics.80,81 It has been reported that CNT/HA composites also induce improved in vitro osteoblast proliferation and differentiation.82 Starting from this evidence, researchers at the Department of Pharmacy at University “G. d’Annunzio” (Chieti-Pescara, Italy) proposed improvement of preformed HA features by using graphene oxide (GO). As a matter of fact, although GO is an insulating material,83 it keeps its extraordinary mechanical stiffness and strength.84 These mechanical features assimilate graphene to CNTs, even if graphene likely lacks the potentially toxic metal catalysts that are entrapped within CNTs and requires extensive and time-consuming purification processes.85 In fact, only a few studies have been published on the possible applications of GO in biomedical and regenerative tissue engineering, such as induction of differentiation of mesenchymal stem cells toward osteoblastic lineage86 and formation of in situ HA via precipitation of calcium phosphate.87

Thus, future studies should aim to set up a simple and inexpensive protocol for the preparation of GO-coated HA and to investigate the biofeatures that the coating can induce on preformed HA. Preliminary results have shown that the coating is homogeneously distributed on the surface of the HA granule, as evidenced by transmission electron microscopy, scanning electron microscopy, Raman microscopy, and optical microscopy measurements, and demonstrated an increase of HA resistance to mechanical stress. Moreover, in vitro tests on human fibroblasts and preliminary observations of in vivo behavior have shown that no toxic effects of GO-coated HA samples are caused by such hybrid material (unpublished data). Biphasic calcium phosphate ceramics are highly biocompatible and osteoconductive, they can be degradable, and their mechanical strength is sufficient for bone reconstruction in noneload-bearing applications.88 Their specific characteristics are based on the balance between the less-soluble HA and the moresoluble TCP. By varying the HA/TCP ratio, the mechanical and biologic performance of the ceramic to induce bone and cartilage tissue production can be tailored.73 In a study by Mangano et al., a custom-made threedimensional scaffold, with programmed porosity and made by a mixture with a HA/TCP weight ratio of 30/ 70, was used to regenerate bone tissue in the maxillary sinuses in sheep. The scaffold was demonstrated to support a progressive process of tissue regeneration and to undergo gradual degradation during the healing phase. The scaffold was, in fact, completely colonized by a highly vascularized fibrous tissue enriched by several foci of newly deposited bone, mostly located at the periphery of the grafted area, as shown by histomorphometric and immunohistochemical analyses.89 In 2002, Yuan et al.6 compared the effect of HA and biphasic calcium phosphate grafted in intramuscular sites in goats. Both biomaterials showed macroporosity of 55% and pore distribution between 100 and 800 mm, but biphasic calcium phosphate also showed microporosity. After 12 weeks, bone tissue formation was observed around the biphasic calcium phosphate samples but not around the HA samples, leading to the conclusion that the biomaterial itself was found to be osteoinductive in function of its architecture.90 Daculsi and Layrolle,7 in 2004, used a biphasic calcium phosphate bioceramic with the same characteristics just mentioned and observed that its macrostructure and microstructure are important not only for osteoconduction but also for osteoinduction.91 Calcium sulfate is another material of synthetic origin and is totally resorbable. In the past, calcium sulfate was used, but its resorption occurred at such a high speed that the material was immediately replaced by connective tissue and not by bone; today, heat-treated calcium hyposulfate is preferred, which, in contrast, has a slower phase of resorption, thus promoting its replacement with bone.92 Histologic analysis on healing of bone defects in rabbits treated with calcium sulfate showed complete resorption of the material after 4 weeks in favor of new bone apposition. Calcium sulfate could be used in the form of cement or granules, and both types have shown high biocompatibility, bioactivity, tolerability, biodegradability, and osteoconductivity, even if the granules may have some benefit from the clinical point of view.7 In a histologic study, calcium sulfate was demonstrated to lead predictable bone formation, which is useful for monitoring implant placement. Complete resorption of the biomaterial after a healing period of 9 months has also been shown.93 Bioglass, or bioactive glass, is a class of innovative ceramic biomaterial exhibiting some characteristics of biologic tissues. The surface forms a biologically active HA layer, which provides an interface for bone and soft tissue binding. Many bioactive silicate glasses are based on a 45% in weight of silicon dioxide and calcium oxide/phosphorus pentoxide (CaO/P2O5) with a molar ratio of 5:1. Bioglass with a low CaO/P2O5 molar ratio cannot bind to bone.94 In a histologic and histomorphometric study, it was reported that by using a mixture of 80% to 90% of bioactive glass ceramics and 10% to 20% of autologous bone as biomaterial for maxillary sinus augmentation, new bone formation could be observed within 6 months, whereas by using bioglass alone, it could be histologically documented only after 12 months. These results demonstrated that the best results are obtained by combining bioglass particles with autologous bone, and this could also represent a method to overcome one of the major obstacles in applying bioglass in bone regeneration (i.e., its poor mechanical properties).95 In a recent study, bioglass coupled with Poly(methyl methacrylate) via a nanoscale interaction were tested in vivo and in vitro: The addition of the synthetic polymer seems to enhance the mechanical properties of the bioglass itself and also results in prolonging the time of degradation, which could be useful for ensuring mechanical

support of soft tissues during bone regeneration.96 Polymeric biomaterials have also been proposed. Polylacticepolyglycolic acid is a polymer with good properties of biocompatibility and biodegradability. Its resorption takes place through hydrolytic reactions, leading to the formation of lactic acid ($C_3H_6O_3$) and glycolic acid ($C_2H_4O_3$), which, in turn, through the Krebs cycle, are eliminated in the form of carbondioxide and water, nontoxic substances to the body. It is possible to control this phenomenon by acting on the density, the molecular weight, and the percentage of polymer present, thus obtaining materials with theoretical times of degradation that may vary from 5 to 7 weeks to a maximum of 2 to 3 years.96

CONCLUSIONS

Knowledge of biological response, as revealed by recipient sites and in terms of integration, bone regeneration, and neovascularization upon graft insertion can be useful in choosing the right bone substitute to be used to repair maxillary and mandibular bone defects caused by accidents, violence, tumors, or atrophy resulting from long-term edentulism prior to implantsupported prosthetic rehabilitation. Moreover, researchers should focus on producing newly designed biomaterials or enhancing existing ones, ensuring their chemical and mechanical properties make them as similar as possible to native bone tissue, grant mechanical resistance and stability over time, and enable them to be completely incorporated after the healing phase in the bone remodeling phenomena.

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