Bone Graft Substitutes: Osteobiologics

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Currently, we are embarking on a chemical revolution in orthopedic medicine. Recent advances in biotechnology have opened the door to a clearer understanding of ways to reproduce and manufacture the various mineral components that mimic human bone. The new group of osteobiologics has given surgeons unlimited access to synthetic bone graft materials. These innovative osteobiologics vary widely with respect to their inductive, conductive, and osteogenic properties and morphologic and mechanical characteristics. In addition, future osteobiologics will have structural and load-bearing characteristics similar to human bone, allowing for their use with or without fixation devices. The ideal osteobiologic that incorporates these properties has not yet been produced, but current technology will evolve to attain these properties in future generations of bone graft materials. Regardless of the structural, histologic, and biochemical makeup of bone, the ideal environment for skeletal healing and graft incorporation is a mechanically stable, uninfected, well-perfused vascular environment.

The primary role of bone graft use in foot and ankle surgery has been to fill traumatic defects and benign tumors or to augment arthrodesis techniques. Historically, autogenous bone from the iliac crest graft was used for this purpose. The technique of autogenous bone harvest and grafting continues to be the historical standard to which all new bone graft substitutes are compared. Autogenous bone has inductive, conductive, and osteogenic properties and structural characteristics that offer surgeons the added advantage of various stabilization techniques. Current and future generations of osteobiologics will diminish the need for autogenous bone graft without losing the predictable osteointegration or choice of fixation.

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Calcium-based ceramics

The use of man made calcium-based material to fill bone voids dates back to 1892, when Dreesman [1] used plaster to fill skeletal defects. Over the past 15 years, calcium-based ceramic materials have been developed that have a porous structure similar to human bone. The porous structure and the ability of the ceramic materials to be integrated and remodeled, forming new bone, have created acceptance for their use as bone graft substitutes. As a group, the calcium-based ceramics have been shown to be safe and biocompatible and generate no local or systemic toxicity when implanted [2,3]. Further, they have been shown to be versatile graft substitutes in the treatment of skeletal tumors, bone defects, cortical and metaphyseal defects, and periarticular fractures [4–9]. Any of the granular bone graft substitutes can be used as graft extenders when combined with autogenous, allograft, or demineralized bone [10]. Further, the combination of bone graft substitutes, each with their unique osteobiologic characteristics, form composite grafts that increases their inductive, conductive, and in the case of bone marrow aspirate, osteogenic potential [11–13].

An important potential drawback to using the ceramic bone materials is that they have physical properties that give them poor mechanical capabilities. Compared with autogenous or allograft bone, most are brittle and unable to resist compressive loads [14,15]. A manufacturing technique to improve the structural performance of the calcium ceramic materials is termed *hot pressing* or *sintering*. In this process, the raw calcium phosphate material is exposed to high pressure and temperature. The result of this manipulation is a more uniform crystalline structure with improved density. The manipulation of the physical structure of the calcium ceramic improves the physical characteristics of the implant, lending improved strength and load-bearing capabilities. A drawback of these graft materials is that they are slowly incorporated after implantation, which is due to the increased density and uniform crystalline nanostructure of the graft material. Because of this characteristic, their use as augmentation for an arthrodesis procedure should be carefully monitored because the slow incorporation can mask the healing process on radiography.

The morphologic structure of graft substitutes plays a significant role in the osteoconductive and mechanical characteristics of the graft when implanted. The size of the pore scaffold influences the quality of new bone and the rate at which new bone is formed. Pore sizes smaller than 100 μm are too small for direct cell invasion in vivo and are therefore considered a minimum size for porosity in graft materials. Such small pore sizes create local hypoxic conditions and favor osteochondral formation instead of direct osteogenesis [16]. Pore sizes of 300 μm show enhanced new bone and capillary formation [16]. Manipulation of pore size has employed several different techniques to synthesize the ideal porous structure that closely resembles native bone. These techniques include methylcellulose scaffolds [17], chemical precipitation techniques [18], hydrothermal conversion of calcium-carbonated coral skeleton to hydroxyapatite (HA) [19], and chemical conversion of bovine bone to HA [20]. Recently, external fixation pins and joint

prostheses have been coated with HA to encourage osseous ingrowth, creating a more mechanically sound bone–implant interface [21–23]. In the case of HA-coated pins, the improved stability at the pin–bone interface has been shown to increase pin life and reduce the rate of infection [23].

The chemical composition of the various highly crystalline HA influences how the graft will behave biologically after implantation. Highly crystalline HA that has undergone sintering is not soluble in the neutral pH of the body and only undergoes osteoclastic resorption [24]. The slow remodeling and incorporation makes them difficult to assess radiographically, and larger grafts may never fully incorporate [25]. These properties should be kept in mind when pure HA grafts are used for arthrodesis procedures because consolidation of the fusion may not be radiographically demonstrated.

Hydroxyapatite

HA grafts are available in the United States as converted exoskeletons of the *Gonioptera* species of coral. The graft is available in pore sizes of 200 μm and 500 μm (ProOsteon 200R and 500R, respectively, Interpore Cross, Irvine, California). The porous structure of the implant closely resembles that of cortical and cancellous bone [26]. The implant is purely osteoconductive and biocompatible. There have been no reports of graft rejection or toxicity associated with implantation. When implanted, the graft lacks the ability to withstand physiologic loads, but as the graft incorporates, the loading tolerances return to those of normal bone [26,27]. Blocks and granules that have a high HA content and dense crystalline structure are soluble only in an acidic medium created by osteoclasts. Therefore, the process of resorption and integration is primarily an active osteoclastic process, with new bone being formed directly on the porous scaffold of the graft [24]. After implantation, there is initial fibrovascular ingrowth, with secondary osteoblastic new bone formation occurring on the porous surface of the graft [27]. For complete osteogenic conversion to occur, the graft must be placed in a favorable vascular environment that is mechanically stable. As the process of osteointegration occurs, the mechanical characteristics of the graft improve [27]. More recently, the hydrothermal conversion of coral to HA has been modified to convert only the outer 2- to 10-μm mantle to HA, leaving a core of unconverted calcium carbonate. The advantage of this modification is that the HA is more lowly remodeled, adding strength, while the inner core is more rapidly resorbed, allowing a reduction in the biologic life of the implant and facilitating osteogenic conversion.

Bucholz and colleagues [28] showed that there was no real radiographic difference in using HA or autogenous bone in metaphyseal defects after tibial plateau fractures. Wolfe and coworkers [29] showed HA to be a safe and effective alternative to autogenous bone in the augmentation of articular reduction of distal radius fractures stabilized with external fixation and wires. Animal studies have supported the use of HA for filing cortical defects [30].

Applications in the foot and ankle include bone cysts in the tibia, fibula, and calcaneus and the smaller bones in the foot. Due to the slow radiographic consolidation of the graft, the use of HA in arthrodesis procedures should be done with caution, although it is not contraindicated. If the graft is used as a structural graft to support articular reduction, such as in calcaneal fractures or tibial plafond fractures, rigid internal fixation is universally recommended. Granules of HA can also be combined with demineralized bone, allograft, or autogenous bone to form composite grafts. The combination of biologic materials enhances the osteoinductive and osteogenic potential of the graft.

Calcium phosphates and calcium composite materials

Calcium phosphate graft substitutes comprise a large group of similar biomaterials with differing chemical compositions and, subsequently, different biologic and mechanical characteristics. Generally, they are composed of HA, β-tricalcium phosphate, biphasic calcium phosphate, mixtures of HA and β-tricalcium phosphate, and unsintered calcium phosphate (calcium-deficient apatite). Calcium phosphate ceramic materials, in general, are structurally weaker than HA and dissolve more rapidly than sintered graft substitutes [31]. Biphasic ceramic grafts that combine HA and tricalcium phosphate (TCP) are now available that slow the rapid rate of resorption and improve the strength of the graft [32,33]. As the calcium phosphate is dissolved, the calcium and phosphate ions released into the biologic medium encourage active new bone formation [32]. Calcium phosphate graft materials are produced when TCP in powder form is precipitated with naphthalene to form a uniform crystalline structure with a pore size in the 100 to 300 μm range. This solid form can then be sintered under high pressure and heat to form a uniformly dense material with a more ordered crystalline structure (β-tricalcium phosphate). The resorption process creates a local graft environment with high concentrations of calcium and phosphate ions that trigger osteblasts to form new bone. In this dynamic process, new bone is formed that replaces the graft material as it is dissolved. Vitoss (Orthovita, Malvern, Pennsylvania) is a macroporous form of β-tricalcium phosphate that is purely osteoconductive and completely resorbed after implantation. It was approved in 2000 as an equivalent to Osteoset (Wright Medical Products, Nashville, Tennessee). It is indicated to fill defects or voids that are intrinsically stable. Calcium phosphate can also be precipitated to form a dallhite, which is a carbonated mineral with very small crystalline structure [34].

Biphasic materials employ the favorable resorption properties of TCP and the structural properties of HA. The biphasic components are synthesized by sintering HA and TCP, creating a chemical composite material. When implanted, the TCP undergoes osteoclastic resorption and passive dissolution, which creates a local environment of calcium and phosphate ions. Secondary osteoblastic new bone formation occurs on the remaining HA matrix. Because of the more rapid

conversion to new bone and enhanced structural properties over calcium phosphate alone, biphasic materials have been shown to be effective in bridging skeletal defects [35].

Collagen composite materials

Calcium phosphate can also be precipitated onto a collagen carrier scaffold. Collagen is bonded into bone in its fibrillar form, comprising the most abundant protein in bone. The surface of the collagen incorporated in bone serves as a scaffold for mineral deposition. The collagen also binds extracellular matrix proteins that are important mediators of mineralization. Healos (Orquest, Mountain View, California) is an HA and bovine collagen composite graft material created by a proprietary accretion process. The spongiform graft material can be shaped, cut, or packed into irregular defects. Collagraft (Zimmer Corporation, Warsaw, Indiana) is another composite material composed of 65% HA and 35% TCP. It is combined with an equal volume of bovine collagen. These materials serve directly as a graft substitutes or can be combined with bone marrow aspirate or platelet gel aspirate to improve their osteoinductive potential [36,37]. Both grafts are novel in that they can be molded or shaped to fit any size or shape defect. These materials have shown to be clinically effective for traumatic defects and spinal arthrodeses [38–41].

Applications in the foot and ankle include grafting of benign bone cysts and traumatic defects of the calcaneus and tibia. These materials must be placed in a mechanically stable environment, mandating rigid internal fixation. These grafts can also be used as "extenders" of autogenous graft material or combined with demineralized bone to form composite grafts, improving their osteoinductive and osteogenic potential.

Calcium sulfate

The first surgical-grade calcium sulfate material to be commercially marketed was Osteoset. This calcium-based material has a uniform crystalline structure and dissolves at a fairly predictable rate. When implanted, this graft readily dissolves in the neutral pH of the body and serves as source of calcium ions, which are incorporated into new bone. Due to the rapid dissolution, the graft cannot act as an osteoconductive scaffold on which new bone can be deposited. Calcium sulfate has been shown to be very effective in filling traumatic defects and benign cysts in bone [42,43]. Kelly and colleagues [42] showed calcium sulfate to be effective in the treatment of long-bone defects up to 4 cm^3. The pellets were used alone or in combination with autograft, allograft, or bone marrow aspirate. At 6 months, radiographs showed that 100% of the calcium sulfate was resorbed and 94% of the defects were filled with trabecular bone [43]. The material can

also be combined with heat-stable antibiotics such as aminoglycosides and vancomicin (Fig. 1) [44,45]. Miclauand coworkers [44] showed calcium sulfate pellets released 17% of the compounded antibiotic at 24 hours, with trace amounts lasting for 3 weeks. In this capacity, the calcium sulfate graft acts as a vehicle to slowly release the antibiotic as the calcium sulfate graft dissolves. This methodology has proved very effective in the treatment of osteomyelitis and septic defects. The antibiotic pellets require adequate soft tissue closure because the dissolution process can create drainage from the surgical site.
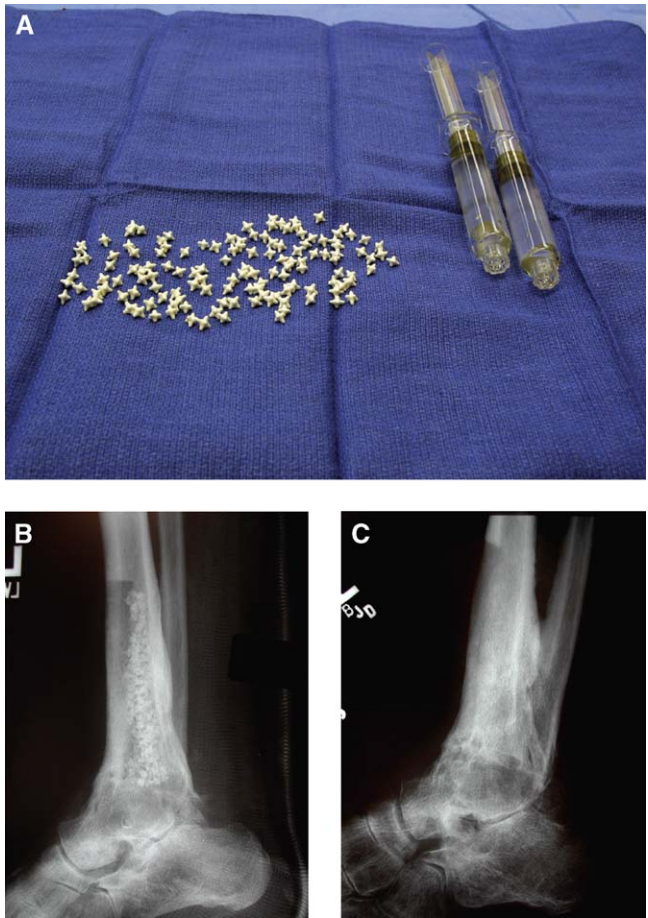

Fig. 1. (A) JAX calcium sulfate (Smith and Nephew, Memphis, TN) with handling gel. The gel can be mixed with antibiotic and packed into septic voids. As the gel is resorbed, the antibiotic is released into the biologic medium and the calcium sulfate is resorbed, forming new bone. (B) Sequestrum in distal tibia from previously infected pilon fracture and attempted septic arthrodesis. Initial sequestrectomy and placement of calcium sulfate bone graft substitute mixed with gel and vancomicin powder. (C) Five-month follow-up, with complete incorporation of the bone graft substitute and new bone formation.

Calcium cements

Calcium bone cements are an evolutionary by-product in bone graft substitute development. In contrast to preformed granules or blocks, the calcium cements are semisolid mixtures that can be pressed or injected into any skeletal defect. The cements are novel in that they are mixed by the surgeon immediately before implantation. Calcium bone cements are composed of various combinations of the mineral components of bone. Similar to the solid calcium phosphate materials, they have different stoichiometry, which influences each graft with respect to dissolution, osteointegration, and mechanical strength. The mineral components are mixed with an aqueous solution to precipitate the cement. After mixing, an isothermal reaction occurs and rapid conversion to a solid occurs within minutes. The intermediary semisolid that is formed allows the cements to be molded or injected into defects or voids. The rate at which the cements harden and their structural properties vary depending on their chemical composition [46,47]. Norian SRS (Norian Corp., Cupertino, California) forms an injectable cement in an isothermal process when the components of monocalcium phos-
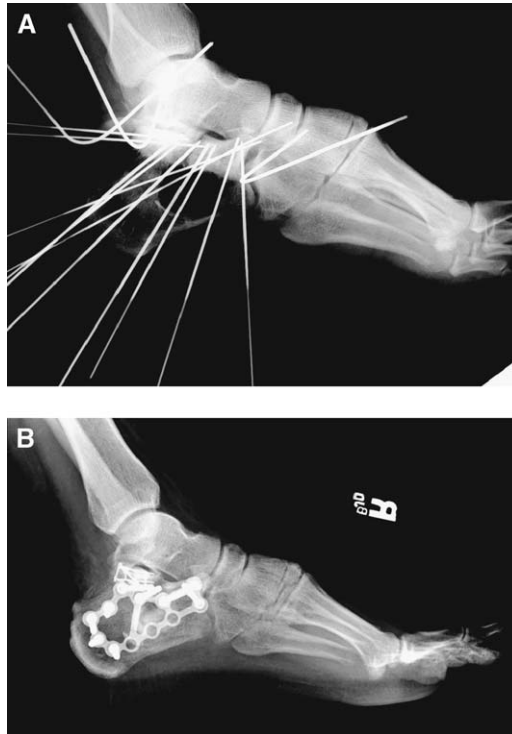

Fig. 2. (*A*) Comminuted intra-articular calcaneus fracture with large subarticular defect after provisional open reduction. (*B*) Six-week postoperative radiograph showing plate and defect filled with bone cement (α-BSM) acting as a subarticular buttress.

phate, calcium carbonate, and TCP are mixed with a sodium phosphate solution. Bone substitute material (α-BSM) (Etex Corp., Cambridge, Massachusetts) is another calcium orthophosphate formed in the same manner. The calcium-based powder is mixed with saline before implantation. Both cement materials, when mixed, harden into a carbonated dallhite in the crystallization process. The dallhite has superior structural performance relative to allograft and has shown to be an effective structural graft in unstable osseous injuries [48]. When implanted, the calcium apatite compounds undergo osteoclastic resorption and remodeling similar to that of cortical and cancellous bone [48].

The primary focus of use for these cements is for structural support in comminuted periarticular fractures, whereby the cement serves as a subarticular buttress (Fig. 2). The cement being used as a void filler or bone grout is slowly resorbed and converted to new bone. In this arena, cements have shown promise in the augmentation of comminuted periarticular fractures with bone loss or instability and in vertebral fractures [49–52]. Thordarson and colleagues [51] demonstrated experimentally that type II-B intra-articular calcaneus fractures fixed with internal fixation and injectable bone cement withstood cyclic loading much better than controls with the same fixation and allograft. Schildhauer and coworkers [52] showed clinically that intra-articular calcaneus fractures augmented with injectable bone cement were able to weight bear as early as 3 weeks post reduction without loss of reduction.

Allograft

There are approximately 150,000 allograft procedures performed each year in the United States [53]. Allograft bone provides an osteoconductive scaffold that is identical to autogenous bone and is osteinductive to a lesser degree [54]. Allograft is historically accepted as the best alternate graft substitute, with no graft site morbidity, predictable incorporation, no local or systemic toxicity, and complete biologic incorporation. The Food and Drug Administration published guidelines in 1977 on donor screening [55]. In 1998, the American Association of Tissue Banks published requirements for donor screening, processing, labeling, and distribution [56]. Even though these guidelines outline every precaution to ensure a safe donor pool, there is no absolute protection against viral disease transmission. Hepatitis B and HIV have initial viremic stages of up to 4 weeks in which the donor may be infectious but not detectable with current testing. Current use of polymerase chain reaction testing has improved screening, allowing for a sensitivity of 1 infected cell in 10^6. Given the current rigorous donor screening and blood testing protocols, the risk of viral transmission with musculoskeletal allograft is estimated to be 1 in 1 million [57]. After grafts are harvested, they undergo processing that includes an initial low-dose irradiation to destroy surface bacteria; physical debridement to remove unwanted soft tissue; pulsatile lavage; an ethanol bath to denature cellular proteins, which kills some viruses and bacteria; and a final antibiotic soak. Preservation is accomplished

with three possible techniques. Freeze-drying to $-70°$C, the most common technique, affects the material properties of the bone to a small degree and requires careful rehydration [58]. If the graft was not harvested or processed aseptically, then a terminal sterilization process can be performed to reduce the risk of disease transmission. Ethylene oxide or irradiation can be used to accomplish this step. Ethylene oxide is generally considered a poor technique for biologic materials because the residual elements are locally toxic [59]. Irradiation is the most commonly practiced procedure in this step, although the virucidal dose of radiation (>30 kGy) affects the mechanical properties of the graft [60]. The incorporation of the allograft with the host bone is dependent on a mechanically stable, uninfected, vascular environment. Recent advances in articular cartilage transplant techniques for the talus have reinvigorated the use of fresh osteoarticular allografts. Mosaicplasty techniques and bulk osteoarticular transplants with fresh allograft have recently been described [61,62]. In addition, allograft can be combined with platelet gel concentrates, demineralized bone, bone marrow aspirate, or autogenous bone to augment the bulk of the graft and increase the osteobiologic properties.

Summary

Future devolvement of osteobiologic materials will no doubt replace materials currently being used. As techniques to improve biointegration and manipulation of the healing environment proceed, future graft substitutes may exceed even autogenous bone in their reliability. Further, as the understanding of the cascade of events that occurs with bone healing and graft incorporation improves, the ability to augment or manipulate the process becomes more of a reality. The lines are increasingly blurred between purely conductive and inductive agents as composite graft materials are developed. We are currently in the "biochemical era" of musculoskeletal medicine and on the leading edge of osteobiologic development. Future technologies will undoubtedly influence and shape the modern evolution of musculoskeletal surgery, ultimately improving surgical outcomes and patient satisfaction.

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