Chapter 1 | Bone Graft Substitutes: Past, Present, and Future

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INTRODUCTION

The field of medicine as a formal discipline has been traced by many to Imhotep and his descriptions of ailments and treatments found written on papyrus and translated in the mid-1800s by Edwin Smith [**1**]. Among the medical descriptions included in Imhotep's writings are cervical dislocations, skull fractures, and compound fractures [**1**]. Indeed, mummies found in Egyptian tombs have been found with crude braces constructed from wood planks and linen straps on their limbs representing some of the earliest accounts of orthopedics [**2**]. The use of autografts, allografts, and bone graft substitutes also has interesting origins. The use of each graft type dates back several hundred years to apparently crude yet inspired methods and theories, which nonetheless set the stage for what we today consider state of the art. The following is a brief history of each graft subgroup.

Autografts were first used as far back as the early 1800s when, after a trephination (i.e., the practice of drilling holes in the skull to release pressure), Walther repaired the defect by refilling the hole with the original bone plug [**3**]. This repair resulted in good healing and informally began the practice of autografting. In the late 1800s, more reports of autografting emerged: Seydel used tibial periosteal flaps to close a cranial defect and Bergmann used a fibular graft to close a tibial defect [**4**]. By the early 1920s, more than 1600 autograft procedures had been documented [**4**]. However, early structural limitations of cancellous autograft tissue delayed its full emergence, which did not occur until more modern tools of external and internal fixation were available [**4**]. One of the primary reasons for the success of autografts is their ability to be

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osteoinductive, which is due to the presence of blood, cells, growth factors, and proteins within the graft that stimulate and facilitate healing. Although it is within the past 30 years that intense attention has been paid to these growth factors as healing tools, the notion that the body's own fluids could provide stimulus for healing and bone growth dates back further than that. Early attempts at nonunion treatments involved sawing both ends of the fracture to expose fresh bone, rubbing both ends of the bone together, and splinting the wound to allow some limited motion in hopes of stimulating inflammation and thus healing [**5**]. Although early surgeons may not have realized it, this procedure may have stimulated the recruitment of growth factors and inflammatory elements. A similar approach to nonunions was described by Physick in 1802, when he repaired a fracture nonunion by running a seton, or a small bundle of fibers, through and between both ends of the fracture with the hopes of stimulating an exaggerated immune response and healing [**6**].

Allograft use has been reported as far back as the late 1800s when Macewen reported on the implantation of a tibial graft from one child to another [**7**,**8**]. In the early 1900s, cadaveric and fresh allografts were used as in the case of a transplant of cadaver cartilage to a patient and another of a fresh bone allograft from parent to child for the treatment of spina bifida [**3**,**9**]. The earliest collections of allograft tissue, or bone banks, were established in the beginning of the 20th century when Bauer refrigerated bone samples for 3 weeks and then implanted them in dogs. Allografts were prepared for storage at this point by chilling or heating, but it was soon determined that boiling the bone samples rendered them inferior in healing to autografts because the endogenous proteins and factors were undoubtedly destroyed during heating [**9**]. The big leap forward in bone banking came during World War II when new methods of bone storage preparation were studied, including freezing, freeze-drying, deproteinating, irradiating, autoclaving, demineralizing, and chemically treating the harvested bone. Initially prompted by the U.S. Navy to help combat war injuries, the expansion of bone banking continued with a new focus on civilian needs. Many of today's currently held beliefs and understandings about bone bank tissues came from the naval projects [**8**]. It was about this time that the use of fresh allograft tissue declined sharply in orthopedic procedures, giving rise to the need for better allograft treatments and bone graft substitutes in general.

Some of the first evidence for the use of bone graft substitutes, crude as it may be, has been found in prehistoric skulls with gold and silver plates and even remnants of coconut shells found in place of cranial defects [**3**]. In more recent times, several synthetic materials have been used as either bone graft substitutes or internal fixation devices. Several metals, including platinum, vitallium, tantalum, stainless steel, and titanium, have been used for joint replacements or fracture fixations. Polymers including polyethylene, silicon rubber, acrylic resins, polymethylmethacrylates, and others have been used, as have ceramics, in place of bone grafts. In their infancy, these materials were more suited for replacement rather than regeneration of bone tissue. However, the current generation of bone graft substitutes has been designed with replacement

and regeneration in mind. Materials are either designed with living tissue structures in mind or are combined with factors, proteins, and other tissues to encourage rapid and complete healing. Some of the more successful materials have been around for decades. For example, calcium sulfate, also known as gypsum or plaster of Paris, was used in the late 1800s by Dreesman to fill bone voids [**10**], and it is still used today as a bone graft substitute with very good clinical results. The newest generation of bone grafts and bone grafts substitutes, of which this book is the focus, continues a long tradition.

Between 1998 and 1999, the number of bone graft procedures in the United States climbed from 300,000 to 500,000 with the estimated cost of these procedures approaching $2.5 billion per year [**11,12**]. Also in 1998, nine of ten procedures used autograft or allograft tissue [**11**]. The autograft, tissue harvested from the patient (commonly the iliac crest but other regions as well) and implanted within the patient at another site, is the current gold standard of bone grafts because of its inherent osteoconductivity, osteogenicity, and osteoinductivity [**13**]. Osteoconductivity describes a graft that supports the attachment of new osteoblasts and osteoprogenitor cells onto its surface and has an interconnected pore system that allows these cells and others to migrate. Osteogenicity describes a graft that supports the apposition of the graft with the preexisting bone. Osteoinductivity describes a graft that can induce nondifferentiated stem cells or osteoprogenitor cells to differentiate into osteoblasts. Although autografts provide the best replacement tissue to a defect site, the harvesting procedure requires an additional surgery at the donor site, which can result in its own complications, most commonly pain and risk of infection. This donor-site morbidity occurs in approximately 20 % of all cases [**13–15**]. Supply limitations are also a problem for the autograft, further limiting its desirability. There are several categories of bone graft substitutes encompassing varied materials, material sources, and origin (natural vs. synthetic). Accordingly, a bone graft classification system, described in **Table 1.1**, has been developed that describes these groups based on their material makeup.

TABLE 1.1 Description of Classification System for Bone Graft Substitutes.

Class	Description
Allograft-based	Allograft bone used alone or in combination with other materials
Factor-based	Natural and recombinant growth factors used alone or in combination with other materials
Cell-based	Use cells to generate new tissue either alone or seeded onto a support matrix
Ceramic-based	Includes calcium phosphate, calcium sulfate, and bioactive glasses used alone or in combination
Polymer-based	Degradable and nondegradable polymers used alone and in combination with other materials

Note: Many of the currently available bone graft substitutes fall within one or more of the above-described groups.

Allograft-Based Substitutes

Before the 1980s, allograft tissue was primarily used as a substitute for autografts in large defect sites, but since then, allograft tissue use has expanded from approximately 5000–10,000 cases in 1985 to almost 150,000 in 1996 [**16**]. The coordination of donor screening and tissue processing methodologies has reduced the risk of disease transmission from allograft tissue; thus, it has become a more attractive alternative to autograft. With the increase in acceptance of allograft tissue, several products have emerged that are allograft-based but also used in combination with other materials. See Chapters 2, 4, and 5 for an in-depth discussion of allografts as bone graft substitutes.

Factor-Based Substitutes

The factors and proteins in bone regulate cellular activity by binding to receptors on cell surfaces and thereby stimulating the intracellular environment. This activity generally translates to a protein kinase that induces a series of events that result in the transcription of mRNA and ultimately into the formation of a protein to be used intra- or extracellularly. The simultaneous activity of many factors acting on a cell results in the controlled production and resorption of bone. These factors, residing in the extracellular matrix of bone, include transforming growth factor-β (TGF-β), insulin-like growth factor (IGF) (I and II), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and the bone morphogenetic proteins (BMPs). These factors have been isolated and some have been synthesized, allowing for the examination of function of the factors alone and in combination. The ability to isolate appropriate factors from bone, synthesize them in large quantities, and reapply them in concentrated amounts to accelerate bone healing has produced many possibilities for bone graft substitutes. Much work has been done and continues in the research setting, and some products have appeared on the market for clinical use.

Although growth factors have provided advantages in bone healing, they also present some distinct disadvantages, such as high manufacturing cost, risk of contamination, potential immunological response, protein instability [**17–19**], and the risk of uncontrolled bone growth or cancer [**20**]. An alternative approach to protein- or factor-based therapies is small-molecule therapy, a relatively new area of research that is growing rapidly. "Small molecules" for tissue repair are lower-molecular-weight organic compounds than their full protein counterparts (typically <1000 Da) and are capable of diffusing across cell membranes to reach intracellular targets [**21,22**]. Small molecules exhibit beneficial qualities beyond some of the limitations of protein growth factors, including being more stable, soluble, nonimmunogenic, affordable, and requiring a lower effective dose [**23**] while still affording the same beneficial effects as the full protein. See Chapters 7 and 8 for an in-depth discussion of growth factors and bone graft substitutes.

Cell-Based Substitutes

As regenerative medicine capabilities emerge, various sources of stem cells will be required to meet patient-specific demand. A few commonly studied stem cells for use in conjunction with bone graft substitutes include mesenchymal stem cells (MSCs), adipose-derived stem cells (ADSCs), and induced pluripotent stem cells (iPSCs). To differentiate MSCs in vitro to the osteogenic lineage, stem cells are cultured in the presence of certain additives. After culture in these additives, phenotypic assays and staining can confirm the osteoblast-like cell phenotype of the stem cell [**24**]. The addition of TGF-β and BMP-2, -4, and -7 to culture media has also been used to guide the stem cells toward the osteogenic lineage. ADSCs are an attractive source of stem cells because supply limitations and ease of harvesting is less of a problem given the ready access of adipose tissue deposits found under the dermal layers. A significant breakthrough in 2006, Yamanaka et al. discovered how adults cells treated with the right factors could be engineered back to a pluripotent state capable of producing any cell in the body. These cells induced back to an earlier lineage became known as iPSCs [**25**]. With the advances in stem cell technology, the interaction between stem cells and their potential use in bone graft substitutes for clinically relevant applications are, and continue to be, evaluated and developed. Chapter 6 discusses cell-based approaches in greater depth.

Ceramic-Based Substitutes

Many of the currently available bone graft substitutes contain ceramics, including calcium sulfate, bioactive glass, and calcium phosphates. The use of ceramics, especially calcium phosphates, is motivated by the fact that the primary inorganic component of bone is calcium hydroxyapatite (HA), a subset of the calcium phosphate group. Hence, depending on the structure and porosity of the scaffold, calcium phosphates can come close to mimicking the natural matrix of bones. It is of no surprise that the most widely used bone graft substitutes contain HA-based biomaterials because of their unique properties [**26**]. Calcium phosphates are also osteoconductive, osteointegrative, and in some cases osteoinductive [**27**]. For example, MSCs cultured and seeded onto HA constructs have been shown to successfully differentiate into osteoblasts, resulting in bony tissue growth on the HA surface [**28**]. In addition to calcium phosphate composition, structure and crystallinity also play a role in how osteoblasts proliferate and differentiate when in contact with calcium phosphate and can be modified as needed during the fabrication process. Higher crystallinity HA used for in vitro culturing of rat osteoblasts caused an early increase in proliferation with a subsequent dropoff as culture time increased [**29**]. However, when rat osteoblasts were cultured on lower crystallinity HA scaffolds, which more closely mimic natural bone in overall crystallinity, proliferation was gradual yet increased as culture time increased. In addition, lower crystallinity calcium phosphates are more soluble in body fluid or in vitro analogues than higher crystallinity calcium phosphates, leading to a higher ion concentration near the scaffold [**29**] and a plate-like precipitation on the scaffold

surface, resulting in increased bone repair activity [30]. Although ceramics generally have many positive attributes, their use in scaffold formation often requires exposure to high temperatures, which can complicate adding biological molecules, and ceramics generally tend to have brittle failure properties, making them challenging in certain bone graft applications. To combat the brittle nature and to facilitate the addition of biological molecules, they are frequently combined with other materials to form a composite (see *Polymer-Based Substitutes*). See Chapter 10 for a detailed discussion of calcium-based ceramics as bone graft substitutes.

Polymer-Based Substitutes

The final group of bone graft substitutes are the polymer-based groups. Polymers present some options that the other groups do not. For example, there are many polymers that are potential candidates for bone graft substitutes representing different physical, mechanical, and chemical properties. These polymers used today can be loosely divided into natural polymers and synthetic polymers, which can be divided further into degradable and nondegradable.

Natural polymers, such collagen or chitosan, are derived from living sources such as animals or plants, whereas synthetic polymers do not exist in nature as such. A degradable polymer is ideally used in a tissue engineering application where the natural extracellular matrix will eventually replace the scaffold. However, the tissues surrounding the scaffold must be able to metabolize or excrete the products from the polymer's degradation, otherwise an immunological response may occur. Poly(lactide-*co*-glycolide) (PLGA) is an attractive, synthetic, degradable polymer for bone graft substitute applications because it breaks down with the addition of water to lactic acid and glycolic acid, two safe and naturally occurring metabolites in animals. Although synthetic polymers may have optimal mechanical properties and affordability, they can lead to toxicity or chronic inflammation. Natural polymers are advantageous because they can mimic the endogenous extracellular matrix and surrounding tissues can recognize and metabolize their products through common pathways. However, some natural polymers can cause immunological responses, may have variability among different supply sources, and may offer inferior mechanical properties to synthetic polymers. Hydrogels, another representation of polymeric structures, are networks of natural or synthetic hydrophilic polymer chains capable of containing over 99.9 % water by mass. Collagen hydrogels are attractive candidates for use as scaffolds in tissue engineering because cells can adhere and grow on the collagen fibers within the hydrogel, similar to the cell's natural environment. As with ceramics, the functionality of polymers can be enhanced if used in combination with other materials, such as ceramics, to form composites.

To mimic natural and physiological conditions, in many cases composites, or substances containing two or more constituent materials, are optimal for the application. From an engineering perspective, composite materials can often harness benefits beyond which each of its constituent materials would possess on its own, in essence

providing the best of both worlds. In summary, one constituent material could not perform without failure for a particular application without the other. In terms of an orthopedic example, bone tissue is a naturally occurring composite in which collagen proteins provide an elastic or flexible phase to a more rigid and stiffer calcium phosphate matrix. In the end, bone tissue has evolved to become a strong enough support system to carry the weight of the human body, yet flexible enough to endure the daily stresses and loads that act upon it with rare failure. Polymer-ceramic composites, like bone, provide the opportunity to impart the benefits of each material while counteracting their limitations. Toward this end, polymer-ceramic composite scaffolds have successfully been used in vitro and in vivo to differentiate stem cells into osteoblasts [**31**]. Chapter 9 discusses polymers as bone graft substitutes in detail.

REGENERATIVE ENGINEERING AND FUTURE WORK

Although significant advances in bone graft substitutes have been made in recent years, research progress continues to bring various technologies and theories together to produce clinical solutions for orthopedic repair. The human body is undoubtedly a highly organized and efficient machine. As more is learned about genetic and cellular pathways and questions are answered, new questions arise to replace the old ones. Advances in biomaterials such as osteoinductive ceramic-polymer composites may not only provide a superior healing potential than conventional methods but also a more affordable and available alternative, resulting in a better quality of life for more patients.

Tissue engineering has been developing over the last 25 years. However, recent advances in tissue engineering technologies have paved the way for a new perspective—regenerative engineering [**32–35**]. Regenerative engineering has been defined as "the integration of tissue engineering with advanced material science, stem cell science, and areas of developmental biology for the regeneration of complex tissues, organs, and organ systems" [**32**]. As the field of material science has progressed, new materials can be chosen to satisfy the required mechanical properties, degradation rates, and chemical functionality of the application. Advances in stem cell technology may allow patient-specific cells to be directed down the appropriate lineage on a scaffold construct to heal the proper tissues [**33**]. Lastly, a better understanding of the genetic expression of regenerative-capable animals such as newts and salamanders may give insight to the morphogenesis required to form complex human tissues. Many of the concepts introduced here are expanded in Chapter 15.

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