

# Breakthroughs in Bioscience

Developed by the Federation of American Societies for Experimental Biology (FASEB) to educate the general public about the benefits of fundamental biomedical research.

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## Bone Builders: The Science of Grafts, Biomaterials, and Bone Engineering

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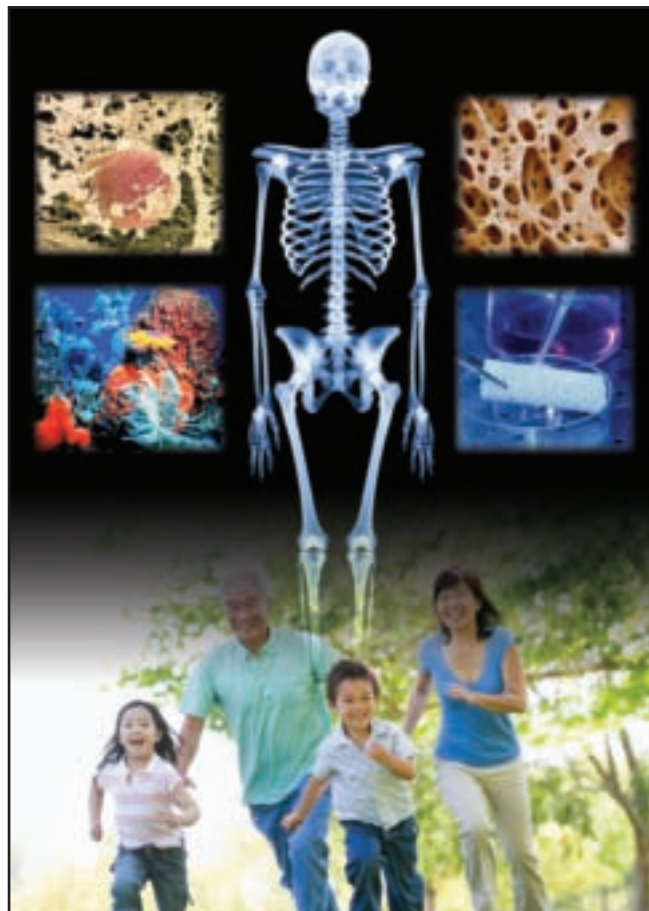
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**COVER:** The science of bone grafting and bone biomaterials has provided extraordinary therapies for patients who have suffered bone loss through traumatic injury, bone cancer, or birth defects. In fact, bone and bone materials are the most commonly transplanted materials after blood transfusions. From late seventeenth century skull grafts to modern bone bioengineering, scientists have taken their cues from fundamental physiology and even sea coral to develop replacements for bone. *Images from Science Photo Library, iStockphoto, and the National Oceanic and Atmospheric Administration.*

# Bone Builders: The Science of Grafts, Biomaterials, and Bone Engineering

In the late seventeenth century, the Catholic Church threatened to excommunicate a Russian nobleman named Butterliijn who had recently been treated for a skull wound obtained after a sword-fight with a Tartar soldier. According to a Dutch physician, Job Janszoon van Meekeren, who published a case study of the incident, the hole inflicted on Butterliijn's head was patched with a piece of the skull from a dog by an anonymous and inventive surgeon. The merging of human and animal tissue was considered unacceptable to the Church, but this first reported bone grafting operation was a clear success: the wound had healed so well, physicians were unable to remove the graft so that the hapless nobleman could return to church. More than one hundred years later, in the 1820s, a surgeon named Phillips von Walther achieved similar excellent results when he repaired holes he had cut in patients' skulls to relieve pressure by replacing the plugs he had removed (Figure 1). These were the beginning steps towards solving a medical problem confronted by physicians even today: how do you replace missing pieces of bone?

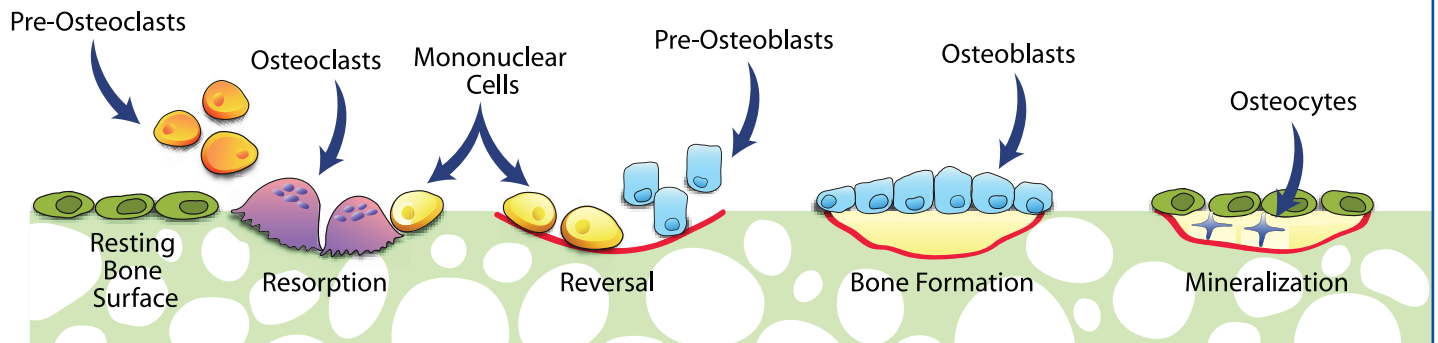


**Figure 1 – First recorded bone graft:** The Phillips von Walther performed a successful allograft in 1820 when, following surgery to relieve intercranial pressure by removing a piece of skull, he repaired the hole with the piece of bone removed from the patient's own head. This sort of bone grafting surgery had been successfully performed over a thousand times by the early twentieth century. *Source: Manchester Daily Express/SSPL*

According to the American Academy of Orthopaedic Surgeons, musculoskeletal diseases and conditions are among the most commonly reported debilitating illnesses, with nearly 36 million Americans affected. In the intervening centuries since van Meekeren documented the case of the wounded Russian, much progress has been made in perfecting bone grafts and finding biologically compatible materials, or biomaterials, to repair diseased bone or to fill

bone voids caused by traumatic injury, birth defects, or bone cancer surgery. Bone biomaterials are also used to promote spinal fusion, in which bones in the spinal column are joined together through new bone growth in the spaces between existing bones. This is done to provide more stability to the spine or when a disc in between spinal bones is no longer viable. Breakthrough discoveries and decades of research to understand how bone is formed, and what materials

## Basic Bone Biology



Bone is a type of rigid, connective tissue that functions in support, movement, protection of vital organs, production of blood cells, and storage of minerals. Bone consists of inorganic materials, primarily a form of calcium called hydroxylapatite, and organic materials, including a protein called collagen and a variety of specialized bone cells. Osteoblasts are the main type of bone-forming cell and they originate from stem cells that are found in the bone marrow. Osteoblasts produce a complex, organic matrix called osteoid, which is mineralized or converted to a hard, inorganic substance, through deposits of calcium. As the bone mineralizes around them,

osteoblasts become trapped in the bone matrix and are then known as osteocytes. Osteocytes no longer function to make bone, instead serving a communication function, sending signals between different types of bone cells.

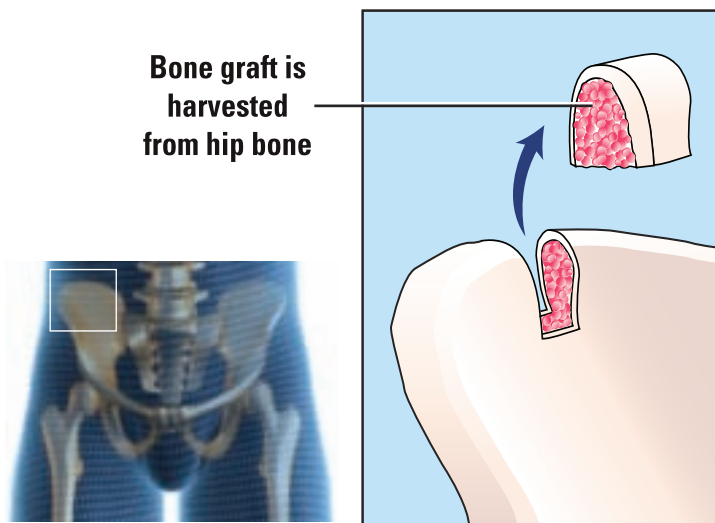
Bone is constantly undergoing reformation or remodeling, through new growth and a destructive process called resorption. In other words, as new bone is laid down, old bone is destroyed, creating a continual rebuilding of bone. As described above, osteoblasts are the main player involved in growth, but a different kind of cell, the osteoclast, is involved in resorption, which

prevents the new growth from significantly changing the size and shape of the bone over time. The middle phase of remodeling is known as reversal, in which the resorption of bone by osteoclasts stops and the bone is prepared by mononuclear cells for the growth process involving osteoblasts. Remodeling of bone helps regulate the levels of calcium in the body (which is alternately stored and released during growth and resorption), repairs microdamage caused by everyday stress on the bones, and helps shape and support the skeleton during periods of growth.

offer characteristics similar to bone tissue, have led to remarkable advancement in bone grafting and bone biomaterials, as well as forming the foundation of the next generation of cutting-edge bone engineering.

### Bone Grafting: From Holes in the Head to Common Transplants

Every year, more than 800,000 people in the United States receive bone biomaterials, including bone grafts and bone graft substitutes. Bone is the most commonly transplanted tissue after blood transfusions. Unlike most tissues, bone is able to regenerate and reform without scarring, given the proper conditions and materials. (See sidebar, “Basic Bone Biology”) Researchers and scientists have learned to exploit this property over many centuries to create



**Figure 2 – Harvesting of bone for autografts:** Bone used in autografts is taken from the patient’s own body, usually from the thigh bone or crest of the hip. Because the bone is being harvested from the patient, the size of the bone available for autografts is very limited. *Figure designed by Corporate Press.*

modern day bone grafts and bone biomaterials used as substitutes for bone grafts.

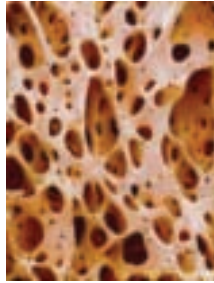
A bone graft is bone that is transplanted from one area of the skeleton to another or from one person to another. Bone grafts obtained from other bones in the patient’s own body, such as the crest of the hip bone or the lower part of the thigh bone, are known as autografts (Figure 2). Bone

grafts that come from a donor, such as a cadaver or someone undergoing a hip replacement, are known as allografts. When the bone material comes from another species, such as a pig or a cow, it is known as a xenograft.

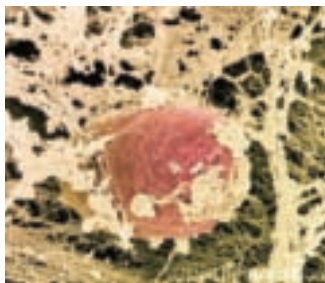
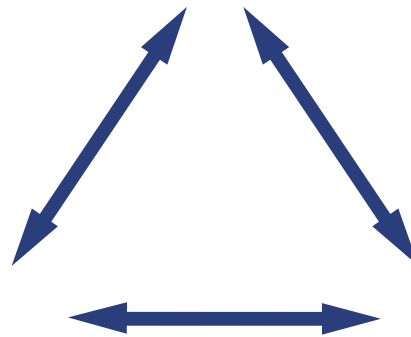
The current gold standard in bone grafts is autografts. The first autograft was that described above by von Walther, and by the 1920s over a thousand bone

# Making an Ideal Bone Graft: Osteoconductivity, Osteogenicity, and Osteoinductivity

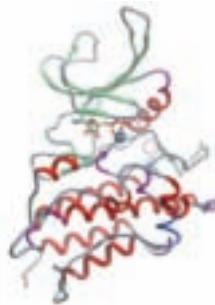
What makes the perfect bone graft? There are three characteristics inherent to natural bone and autografts: osteoconductivity; osteogenicity; and osteoinductivity. The search for bone graft substitutes has led scientists to try to find materials or combinations of materials that have all three properties.



**STRUCTURE – Osteoconductivity:** the ability of a material to serve as scaffolding to support new bone formation and growth. Ideal osteoconductive materials are strong enough to provide support while also having a porous structure through which new blood vessels can form and new cells can migrate.

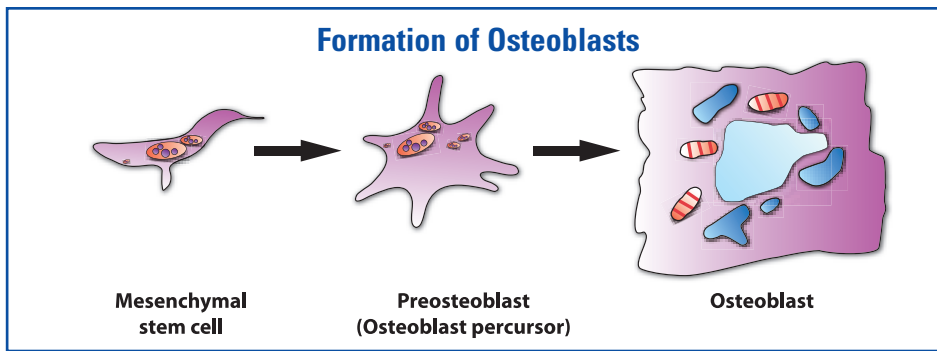


**VITALITY – Osteogenicity:** the presence of osteoblasts, a cell responsible for bone formation, or mesenchymal stem cells, which can transform into osteoblasts. Osteogenicity is the living, regenerative property of bone and bone grafts.



**GROWTH – Osteoinductivity:** the presence of growth factors, such as BMPs (protein structure shown), which recruit bone forming stem cells and encourage their transformation and growth into osteoblasts. Osteoinductive materials promote new bone formation and growth.

The properties necessary for ideal bone grafts are somewhat analogous to those needed to grow plants. The osteoconductivity would be represented by the pot and the soil, the osteogenicity by the seeds, and osteoinductivity by the fertilizer and water needed to encourage growth.



**Figure 3 – Formation of osteoblasts:** Osteoblasts, the specialized cells critical to bone growth and reformation, are formed from mesenchymal stem cells found in bone marrow. These stem cells differentiate (become more specialized) into osteoblast precursors and then osteoblasts under the influence of growth factors, such as bone morphogenetic proteins or BMPs. *Image by Corporate Press.*

autografts had been reported, most replicating von Walther's repair of holes in the patient's head. Because they come from a patient's own body, there is less chance of disease transmission or the immune reactions experienced when transplanting foreign tissue. Autografts also promote rapid healing and successful fusion with the existing bone. This is because autografts have three important elements for new bone growth: osteoconductivity, osteogenicity, and osteoinductivity. Osteoconductivity refers to the ability of the graft to act as a scaffold with pores through which blood vessels can grow and fluids containing cells, proteins, and other substances for making new bone can enter. Osteogenicity (also called osteogenic potential) refers to the presence in the graft of viable mesenchymal stem cells. Mesenchymal stem cells (MSCs) are a type of adult stem cell which have the capability to form many types of cell types, including bone cells, fat cells (adipocytes), cartilage (chondro-

cytes), muscle (myocytes), and fibroblasts. Under proper conditions, MSCs can mature into a type of bone forming cell called osteoblasts (Figure 4). Specific bone growth factors can promote transformation of the mesenchymal stem cells to osteoblasts.

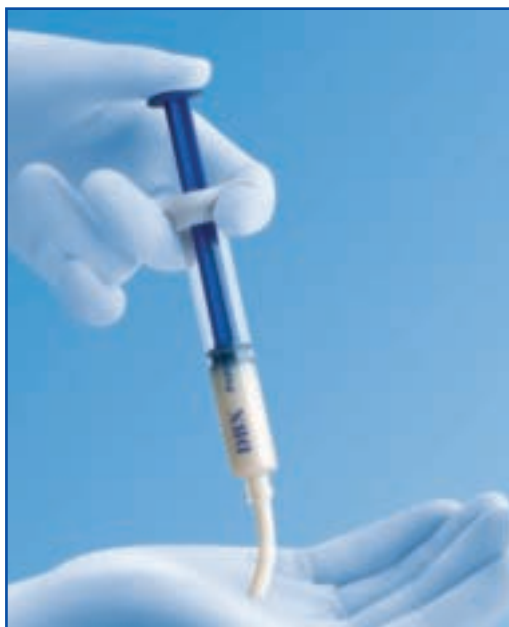
Although bone autografts work well, they are not without challenges. Because doctors need to remove bone from the patient before they can transplant it, autografts involve an extra surgical procedure with its associated blood loss, postoperative pain, and possible complications, such as injury to nearby nerves or major blood vessels. Many patients experience pain at the site from which the bone graft is taken for many months after surgery. Also, the amount of bone tissue that can be harvested is limited, particularly in older patients and infants.

Allografts, which use bone material donated by another person, solve many of the problems related to autografts. Supply is not as much of a problem with

allografts, because materials from cadavers or living donors is readily available, although harvesting the bone and testing it for possible viruses, such as HIV and hepatitis B and C viruses, is costly. However, bone allografts are not without their own problems. In addition to the possibility of disease transmission, allografts don't always fuse as well to existing bone or heal as readily as do autografts, largely because of the way they are treated prior to transplantation. To preserve bone allograft material for future use, the bone is frozen or freeze-dried. These treatments usually kill the cells that can form new bone. As a result, bone allografts provide primarily an osteoconductive matrix in which the body of the person receiving the graft—the host—has to do all the work of forming new bone. In other words, the allograft serves as a scaffold, but the stem cells and growth factors needed to grow new bone may have to come from the graft recipient or be provided. For this reason, new bone takes longer to form in an allograft, and fractures may occur in the first year or two after implantation.

## From Coral to Glass: The Search for Bone Graft Substitutes

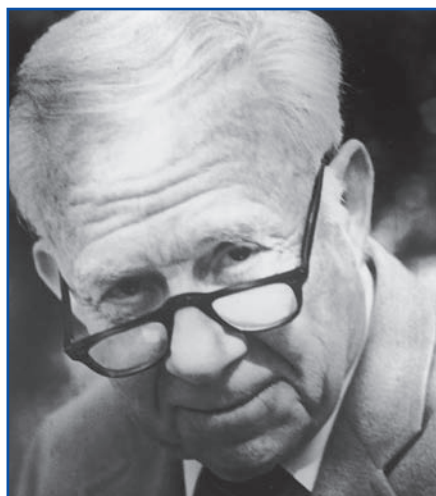
The limitations of autografts and allografts have motivated scientists to develop alternatives, both by enhancing the ability of allografts to fuse with existing bone and through development of bone graft substitutes through



**Figure 5 – Demineralized bone matrix:** Demineralized bone matrix (DBM) is used as a bone graft substitute or extender for other types of bone graft substitutes. Made from ground bone, DBM is a form of allograft that can have both osteoconductive (structural) and osteoinductive (promoting bone growth) properties. It is available to surgeons in a variety of forms, including as a powder, paste, or putty, so that it can be molded to the shape necessary to replace the missing bone or bone fragment. Image courtesy of the Musculoskeletal Transplant Foundation.

creation of biomaterials. One such alternative, called demineralized bone matrix, is an improvement of the bone allograft. It was discovered by serendipity in the mid-1960s by Marshall Urist at the University of California, Los Angeles (Figure 4). Urist’s discovery was able to explain an observation that had long perplexed scientists: scrapings from healthy bone could help speed healing of fractures.

Urist had been trying to find the mechanism by which abnormal calcium deposits develop in certain diseases, such as atherosclerosis, or hardening of the arteries. In one experiment, he excised bones from laboratory animals and removed the calcium from the bones with mild hydrochloric acid, a process called decalcification. He then soaked decalcified bone pieces in different concentrations of calcium solutions, while leaving some



**Figure 4 – Marshall Urist:** University of California researcher, Marshall Urist, discovered a group of growth factors known as bone morphogenetic proteins (BMPs) while investigating abnormal calcium deposits common in atherosclerosis or hardening of the arteries. In natural bone, BMPs stimulate bone cell growth, providing the osteoinductive property of bone. BMPs are now used clinically as part of the makeup of bone graft substitutes to encourage bone growth and healing.

samples untreated. The purpose of the experiment was to see what effect varying the calcium level had on a part of the body that normally has lots of calcium—namely, bone—in the hope of identifying factors that cause the formation of abnormal calcium deposits. (See sidebar, “Why is calcium good for bones?”)

Urist implanted the decalcified and untreated samples in the muscles of rabbits and rats. To his surprise, new bone started

## “Why is calcium good for bones?”

Calcium is an important mineral in the body, with functions ranging from heart health to activating nerves and muscles. It is also integral to the form and function of healthy bones and teeth. Around seventy percent of bone is made up of a calcium-phosphate compound called hydroxylapatite. This mineral forms the hardened, mineralized parts of the bone, needed for strength and structure.

One of the functions of bone is to help store calcium and release it when it’s needed by other parts of the body, through the process of bone remodeling. New bone formation stores calcium, in the form of hydroxylapatite, and resorption of bone releases calcium into the bloodstream. When you do not get enough calcium in your diet, the body withdraws the calcium it needs from where it is stored in the bones. If you do not have adequate calcium to replace what has been removed, over time this will weaken the bones and could increase the risk of breaks, a condition called osteoporosis. (See FASEB Breakthroughs in Bioscience article, “Bone Builders: The Discoveries Behind Preventing and Treating Osteoporosis.”) Vitamin D is also important in bone health, as it helps transport calcium to the bones.



forming within a few weeks in the untreated samples but took much longer in the samples that had been soaked in calcium solutions. Urist concluded that decalcifying, or demineralizing, bone uncovers a substance that induces rapid bone formation when implanted in a host. He later called this substance bone morphogenetic protein, or BMP. Subsequent work by Urist and others found that there are actually over 20 different bone morphogenetic proteins with different functions. Bone preparations that removed the calcium to expose these BMPs became known as demineralized bone matrix.

Demineralized bone matrix (DBM) remained a research material until the early 1990s, when it was developed as a solution to hospital power shortages that prevented the freezing required for preserving bone allografts. Demineralized bone is pulverized into a powder, which preserves the growth factors, like BMPs, that allow it to promote bone growth. The powder is then mixed with additional materials to provide the optimal handling characteristics desired by a surgeon. DBM is clinically available in gels, putties, pastes, and fabrics, all of which can be molded to fill the desired area (Figure 5).

Because demineralizing bone releases BMP and other growth factors, it has higher osteoinductivity (stimulating good bone cell growth) than traditional allografts. To increase its osteogenic-

ity (presence of bone-forming stem cells), DBM is sometimes mixed with bone marrow before use. A recent study looking at the use of bone graft options found that DBM was used in more than 80 percent of trauma cases and hand/foot surgeries involving bone grafts and it is often used for spinal fusion surgeries. However, because the matrix is made through grinding into a powder, it does not provide the structural support needed for many bone grafts. Because of this, DBM often does not fully substitute for a bone graft, but can be used to extend the limited material available for a bone autograph or with other bone graft substitutes, such as plaster of Paris.

Because of the limitations of autografts, allografts, and DBM, scientists continued to search for bone graft substitutes: biomateri-

als that could act in place of natural bone. The first synthetic material used for bone grafting was bioabsorbable ceramics. A ceramic is a hard brittle material produced by firing nonmetallic minerals at high temperatures, while the term “bioabsorbable” refers to the fact that the ceramic is absorbed by the body as new bone is formed. The first bioabsorbable ceramic used for bone grafting was plaster of Paris.

Bioabsorbable ceramics are made in such a way that they contain interconnected pores, a scaffold-like system that allows the growth of blood vessels and the influx of stem cells to form bone. The new bone forms at the junction between the ceramic and existing bone, often creating a tight bond. This bond serves as an attractive place for local bone growth factors, such as bone morphogenetic proteins (See

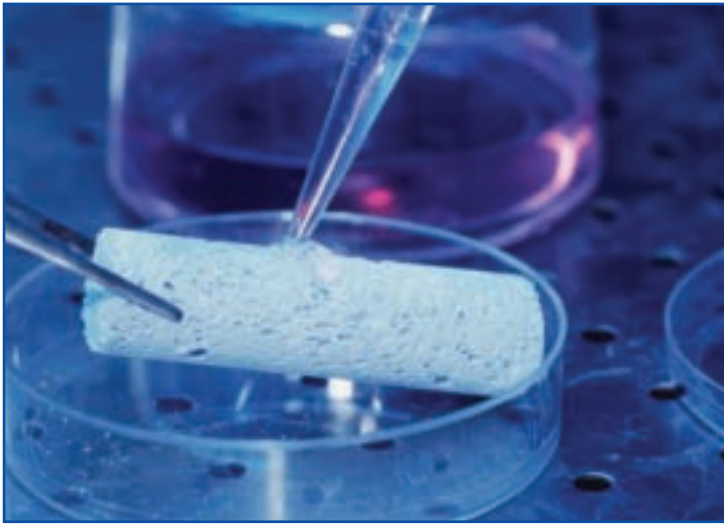
## *Ceramics: Not just for vases and pots*

Ceramics were among the first materials used to make artificial bone for use as bone graft substitutes. All ceramics used as bone graft substitutes share the characteristics necessary for biomaterials used in any medical application: they are non-toxic; elicit little to no immune response; do not cause blood clots; are non-allergenic; and non-carcinogenic (do not cause cancer).

The bioceramics currently used by surgeons for bone grafting can be divided into three main categories: bioabsorbable; bioactive; and bioinert. Bioabsorbable ceramics will dissolve over time and are replaced with new bone and/or surrounding tissue, whereas bioactive ceramics form bonds with the existing bone. Bioinert materials have greater strength and are used in more complicated bone or joint replacements, such as for hip or knee prosthetics. One of the advantages of bioabsorbable materials is that they can be used to carry doses of

growth factors, like the bone morphogenetic proteins (BMPs). As the material dissolves, the BMPs are released, helping the growth of new bone.

Two bioabsorbable ceramics commonly used as bone graft substitutes are tricalcium phosphate (TCP) and hydroxyapatite, a calcium-phosphate compound similar to the predominant mineral found in natural bone. Since TCP is absorbed as much as 20 times faster than hydroxyapatite, surgeons select TCP, hydroxyapatite, or combination of both based on how fast they want the implant to be absorbed. If the implant is absorbed too fast, bone may not have time to form, while if the implant is absorbed too slowly it may prevent some bone formation simply by occupying space that should be occupied by bone.



**Figure 6 – Bioceramic:** Bone bioceramic, for use in bone reconstruction, being treated with bone stem cells. The bioceramic is hydroxyapatite, a natural calcium phosphate mineral complex that is the crystalline component of bones and teeth. This synthetic bone mimics natural bone structure. Its porous structure allows a type of precursor (stem) cell to grow and develop into new bone tissue. The bioceramic can be shaped into implants that are treated with stem cells (obtained from the patient's bone marrow), and grafted into the body to replace missing bone or bone fragment. *Source: Science Photo Library*

sidebar, “Ceramics: Not just for vases and pots”). Bioabsorbable ceramics are readily available and can be shaped to fit the piece of bone that needs to be replaced (Figure 6). Unlike allografts, there is no concern over disease transmission, because these synthetic materials can be sterilized. They can also be engineered to release bone growth factors, which helps new bone to grow.

Although bioabsorbable ceramics had clear advantages over autografts and allografts, they are not without their own challenges. There is the chance that the patient’s immune system may react badly to the foreign substance. In addition, ceramics were originally chosen as a bone graft substitute because the pore structure was thought to be highly osteoconductive. Ideal bone graft substitutes have interconnected pores, which allow blood vessels to grow into the implant, thereby promoting new bone growth. However, there are limits in the porosity (number of pores) in ceramic. While a large number of pores were good for osteocon-

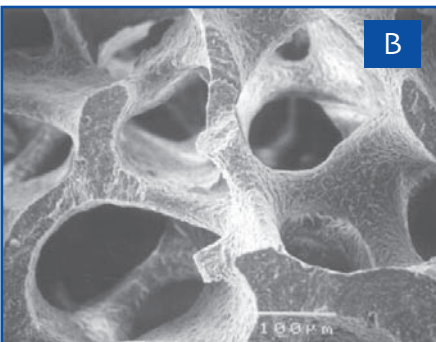
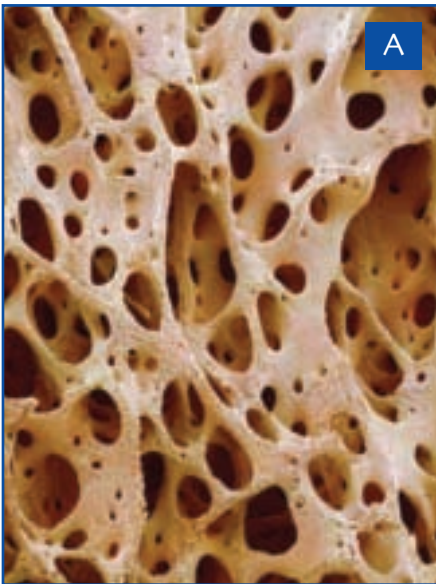
ductivity, the increase in the “holiness” of the ceramics make it weak and prone to breaking, a less than ideal quality for a bone substitute. The limitations of bioabsorbable ceramics led scientists and physicians to continue their hunt for a better bone graft substitute.

This focus on pore structure led researchers to an unexpected source: the coral found in tropical oceans. Like Urist’s discovery of bone morphogenetic proteins, the discovery of coralline ceramics was a case of serendipity. Unlike Urist’s discovery, it involved several researchers in different fields who just happened to be at the same university talking to each other about their research. In the 1960s, Jon Weber, a marine geologist at the Pennsylvania State University (PSU), was studying the chemical composition of corals and other marine animals with hard skeletons that he collected in the South Pacific. When he found out that Eugene White in the university’s Materials Research Lab was developing instruments for characterizing

materials using scanning electron microscopy (SEM), he asked White if he could examine the skeletons of his marine animals using the microscope.

As a scuba diver, White first became interested in coral while diving in the South Pacific and now found them equally exciting in the lab. When White looked at the micrographs, he became fascinated by the uniform and interconnected pores of some of the skeletons. “Having seen virtually every type of man-made material under the SEM, it was obvious that these marine animals were doing something humans had not been able to do,” he wrote later in a paper in *Materials Research Innovations*. “Nature had provided a whole range of architectures of 3-D solids. These could be ‘models’ or indeed templates.”

With no particular application in mind, White started making molds of the coral skeletons in ceramics, polymers, and metals. In the summer of 1971, White was joined in the lab by his nephew, medical student Rodney



**Figure 7 – Coral structure is similar to that of bone:** As seen in these micrographs, scientists at Penn State University noticed that the structure of coral (image A) under the microscope looked very similar to the structure of natural bone (image B) and the porous ceramics that were being developed to act as bone graft substitutes. Ceramics derived from sea corals are now approved for use as bone graft substitutes. *Source: Science Photo Library and Dr. Rinard, Penn State University.*

White, who pointed out the similarity of some coral skeletons to the porous ceramics and metals that were being developed for promoting bone ingrowth. When Rodney returned to medical school in Syracuse, NY that fall, he interested Richard Chiroff, an orthopedic surgeon, in conducting bone implant studies in rabbits using the substitutes he had

been developing from coral. The organization of pores in the coral was very similar to that of natural bone and had very high osteoconductivity (Figure 7).

One of the downsides of using coral skeletons as bone graft



**Figure 8 – Della Roy:** A significant breakthrough in the use of coralline bioabsorbable ceramics as bone graft substitutes was made by scientist, Della Roy, who developed a method of replacing the calcium carbonate in natural coral with hydroxyapatite, the material found in bone. Calcium carbonate was absorbed too quickly by the body, not allowing enough time for healing and bone growth to occur, making it ill suited as a bone graft substitute. *Source: American Ceramics Society*

substitutes was that they were made of calcium carbonate, a chemical compound that was quickly absorbed by the body, which didn't allow for enough time for new bone to grow on the coralline scaffolding. Another scientist at PSU, Della Roy, developed a method of replacing the calcium carbonate in the coral skeleton, which is absorbed quickly, with hydroxyapatite, which is absorbed more slowly

(Figure 8). This method is still used for making a family of coralline ceramics. Other coralline ceramics have been developed as bone graft substitutes, containing mixtures of hydroxyapatite and calcium carbonate, which allow variations in absorption times. Coralline ceramics have the advantage of being completely biocompatible, recognized by the patient's body as so similar to bone that they do not cause a negative immune reaction.

As the scientists at PSU were working on coralline ceramics as



**Figure 9 – Larry Hench:** - Illustrating the serendipity that often drives scientific discovery, scientist Larry Hench developed a bone graft substitute known as Bioglass following a chance conversation with a military officer about the need for better biomaterials. *Source: Science Museum/SSPL*

a bone graft substitute, a scientist at the University of Florida named Larry Hench was studying the electronic behavior of glass-ceramic semiconductors for the U.S. Department of Defense (Figure 9). He and his colleagues discovered certain glass-ceramics

that conducted electricity even when exposed to high radiation levels. This meant that these new materials might be used as electrical switches in satellites where they could survive high energy radiation, such as that produced by solar flares or certain types of weapons.

In 1967, Hench shared a bus ride with a colonel who had recently returned to the U.S. after a tour of duty with the Army Medical Corps in Vietnam. The colonel patiently listened as Hench enthusiastically described his semiconductor experiments and then asked, “If you can make a material that will survive exposure to high energy radiation, can you make a material that will survive exposure to the human body?” He explained that the body formed scar tissue around the metallic and plastic parts then available to surgeons. This often led to amputations, of which he had witnessed many during his tour of duty.

This got Hench thinking. He submitted a proposal to the U.S. Army Medical Research and Development Command to develop a material that would bond to bone without causing scar tissue. With the funding he received, he developed a glass-ceramic that later became known as Bioglass®. Bioglass is made of a type of glass that contains a high amount of calcium. Testing small rectangular Bioglass implants in rat thighbones showed that the implants bonded firmly to the bone. “These ceramic implants will not come out of the bone,”

remarked Ted Greenlee, who conducted the tests in the Florida Veterans Administration Hospital. “I can push on them, I can shove them, I can hit them, and they do not move.”

Subsequent research showed that Bioglass is able to form such a strong bond with bone because a layer of hydroxyapatite forms on the surface of the glass. The hydroxyapatite binds to the protein collagen, a key component of bone. Although Bioglass forms a strong bond with bone, the material is brittle, which keeps it from being used in load-bearing situations. It is currently used to make replacements for bones in the

middle ear, which corrects a type of hearing loss. It is also used to make a stable ridge for dentures following tooth extraction.

## Better Bone Repair Through Proteins

From the beginning, scientists knew that the ideal bone graft substitute had to have the same characteristics as an autograft – osteoconductivity, osteogenic potential, and osteoinductivity. In other words, an ideal bone substitute would have a porous structure that would allow fusion with existing bone and growth of supporting blood vessels while holding up under load-bearing

## BMPs Are Important for More Than Bone Grafts

Since Urist’s initial discovery, bone morphogenetic proteins (BMPs) have since been identified in a wide range of invertebrate and vertebrate species, including worms, flies, and frogs. In each of these species, BMPs play key roles in embryonic development. For instance, the fly counterpart of the human BMP-2 and -4 genes is required for specifying which parts of the body wind up on top and which on the bottom. BMPs are also important in embryonic development of mammalian species—and not just for skeletal development. For example, BMP-7 is crucial for the development of the eyes and kidneys. Mutations in the genes that produce BMPs or their receptors are associated with a number of human diseases and disorders affecting the skeleton and bone development. Fibrodysplasia ossificans progressive, or “stone man’s disease,” a rare and tragic disorder in which muscle and other soft tissue is gradually turned to bone, has recently been discovered to be caused by a mutation in a gene affecting BMPs. Further research on these critical proteins will help scientists develop treatments to this and other diseases.

pressure, the stem cells needed to regenerate the bone tissue itself, and growth factors to promote growth of the new bone tissue.

Neither DBM nor the synthetic bone graft substitutes met all of these requirements. For instance, none of them provide much strength and, as such, they are most appropriate for filling small holes in bone. When used to fill a large area of missing bone, surgeons must support the section with a variety of hardware until new bone is formed. Also, these materials provide little more than an osteoconductive matrix. DBM has some osteoinductive capacity, but it pales in comparison to bone autograft and growth factors may be reduced as it is processed. The ceramics and glass could provide the structure (osteoconductivity) and bone marrow could provide the stem cells (osteogenic potential), but researchers still needed to find a way to increase bone graft substitute's ability to promote regeneration of bone tissue: an increase in osteoinductivity.

The need for a better alternative led scientists to return to Urist's breakthrough discovery of bone morphogenetic protein (BMP). Twenty years were spent trying to purify what was then thought to be a single protein. Finally, a breakthrough came in 1988 with two studies by John Wozney and colleagues at the Genetics Institute, a biotechnology company in Cambridge, Massachusetts.

Starting with nearly 90 pounds of bone powder from cows, they

isolated about a millionth of an ounce of protein that caused bone to form when implanted under the skin of rats. To their surprise, the scientists discovered that what they thought was a single protein was actually a family of related proteins. The information they were able to gather from studying this family of proteins allowed the Genetic Institute researchers to use genetic engineering technology to synthesize human BMP-1 through -7. Finally, scientists held the key to the factors critical for encouraging bone to grow.

BMPs regulate bone formation by recruiting mesenchymal (bone-forming) stem cells to the place where new bone is needed, stimulating them to grow and reproduce, and then causing them to transform into mature bone cells called osteoblasts. Interestingly, only one type of BMP is needed for forming bone. Providing two or more at the same time does not have an additive effect. However, administering a higher dose of a BMP causes bone to form sooner and in greater amounts.

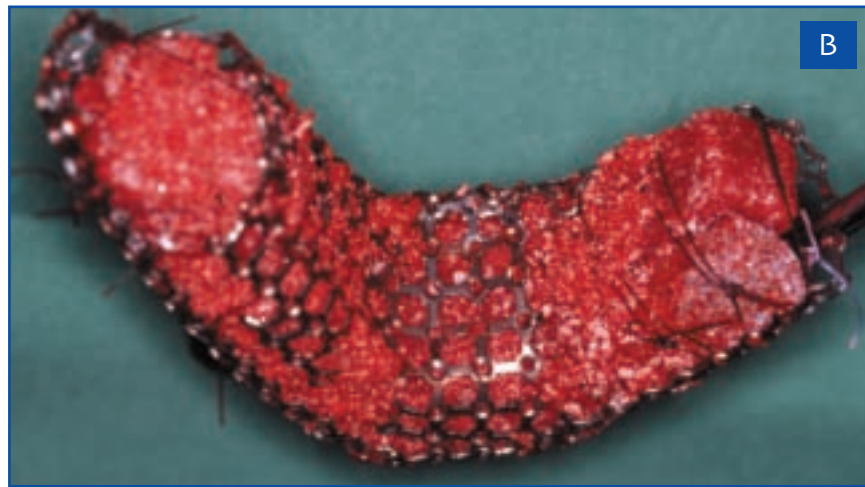
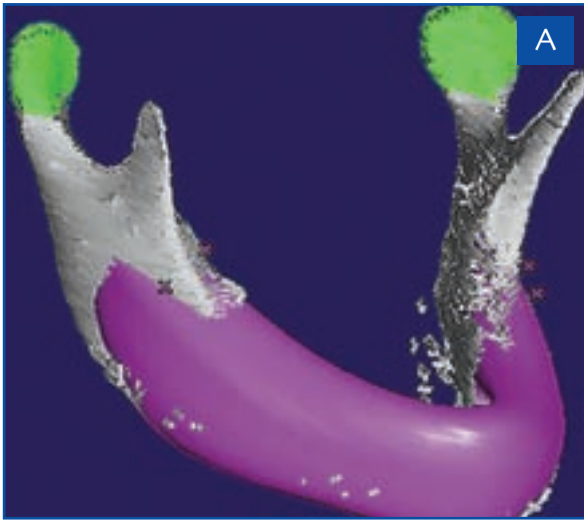
Besides being important in skeletal development in the embryo, BMPs stimulate bone formation during fracture repair. Two BMPs are currently available for clinical applications: human BMP-2 and -7. To keep the BMPs where they are needed, the proteins are added to a carrier, such as a collagen sponge that is absorbed by the body as new bone is formed. A large number of animal studies have shown that

both BMPs can be used to heal fractures and fuse spinal bones, often with results that were better than those obtained with bone autografts.

Currently, the Food and Drug Administration has approved the use of human BMP-2 for helping to treat fractures of the tibia, or shinbone, and for helping to fuse spinal bones in the lower back. Human BMP-7 has been approved for treating fractures of long bones in cases in which the fractures show no sign of healing, use of autograft is unfeasible, and alternative treatments have failed. It has also been approved for use in fusing spinal bones in the lower back for patients in whom standard treatments are unfeasible.

## Putting the pieces together: From Bone Grafts to Bone Engineering

When bone is damaged, the natural repair process involves the formation of a porous mineralized matrix, the growth of blood vessels through the pores, and the influx of cells to form bone on the surface of the matrix. The whole process is directed by signaling molecules, such as the BMPs. To repair large bone defects that will not heal ordinarily, scientists are trying a variety of approaches, but they all mimic the natural repair process. As such, they usually involve a porous mineralized matrix or scaffold to which is added bone-forming cells, signaling molecules, or both. Putting



**Figure 10 – Bone engineering:** The cutting edge of bone replacement is known as bone engineering, which puts the knowledge gained through years of studying bone biology and bone grafts to create new bones for transplant. Patrick Warnke, a German scientist, made an exciting breakthrough in bone engineering when he was able to replace a patient’s missing jaw bone or mandible with new bone. As shown in image A, a wire mold was built using a computer-aided design of the patient’s skull. The mold was filled with bone mineral taken from cows, growth factors, and stem cells from the patient’s bone marrow (image B). For the next 7 weeks, the mold was allowed to incubate inside the patient’s body, having been surgically implanted in the armpit, an area rich with blood vessels (image C). The new bone was removed and implanted in the patient’s jaw allowing him to eat solid food for the first time in more than 9 years. From Warnke *et al.* (2004) *The Lancet*, 364(9436):766-70. *Used with permission.*

together the pieces of breakthrough discoveries described above forms the foundation of the field known as tissue engineering of bone, or simply bone engineering. This is the cutting edge of creating replacement bone and an active area of research.

For scaffolds, many scientists utilize the same materials that are used as bone graft substitutes, including demineralized bone

matrix and the bioabsorbable ceramics. Also used are synthetic polymers, a polymer being a large molecule consisting of a linked series of repeating units, or monomers. The polymers used in bone engineering degrade in the body into carbon dioxide and water. Polymers are attractive chemicals for constructing scaffolds because bone growth factors can be incorporated into

the polymers and then released at a steady rate as the polymer degrades.

The cells used in these experiments are mesenchymal or bone forming stem cells, usually isolated from the bone marrow found in the cavities inside bones. From a small amount of bone marrow—10 to 20 milliliters—stem cells can be isolated and expanded in cell culture

into billions of cells. They can also be frozen and preserved without any loss of ability to form new bone cells. Instead of pure, isolated stem cells, some researchers just use bone marrow itself, which in addition to stem cells also contains bone growth factors and blood-forming cells.

An important question in bone engineering is whether it is more effective to use cells, bone growth factors, or both in constructs to heal bone defects. Using sheep, Frank den Boer and colleagues at VU University Medical Centre in the Netherlands compared BMP-7 with bone marrow for repairing bone and found that both produced about the same results. However, several studies have shown that combining bone growth factors with bone-forming cells yield better results than using either by themselves.

In these studies, as in most of the research discussed so far, scientists are using natural or synthetic materials to fill holes in long bones. The holes are too big to heal on their own, but they have a simple shape. In clinical practice, patients are often in need of large sections of bones with complicated shapes. For instance, a patient undergoing hip replacement therapy is in need of a new femoral head, which is shaped like a ball to fit into the socket of the hip bone.

Currently, surgeons replace the femoral head with a ball made of metal or a hard ceramic that is not absorbed by the body.

This unit is attached to a long metal stem that is inserted into the hollow canal inside the femur for support. Although this arrangement has worked well for many patients, replacing the entire top part of the femur with new bone may work better.

A bone engineering experiment conducted by Roger Khouri and colleagues at Washington University suggests that this idea may not be that far-fetched. The scientists created molds out of silicone in shapes of various bone parts. Into the molds the researchers placed muscles from rats, which served as the source of stem cells, similar to those found in bone marrow. The scientists also added demineralized bone matrix and one of the BMPs to promote bone growth. Finally, they placed the filled molds into the bellies of rats to incubate. After 10 days, the molds were removed and opened. Inside the molds they found well-defined new bone in the shape of the molds. Khouri and his colleagues had put together the pieces from decades of fundamental discovery to create new bone and a promising method for repairing large, bony defects.

Using a similar technique, Patrick Warnke and colleagues at the University of Kiel in Germany were able to replace a large section of a patient's lower jawbone, or mandible, with new bone. The patient had had about two-thirds of his mandible removed due to a bone tumor eight years previously, and the missing bone had been replaced

with a metal plate. He was only able to eat soft food and soup and had trouble pronouncing words. Due to his problems eating and speaking, he had isolated himself socially and was feeling depressed and suicidal.

Based on a three-dimensional scan of the patient's head, Warnke's team designed a virtual replacement of the missing bone using a computer program. Based on the design, a Teflon model was created and used as a mold to form a titanium mesh scaffold. After removing the model, the scaffold was filled with bone mineral, BMP-7 in a collagen carrier, and the patient's bone marrow. The titanium mesh scaffold with its cargo was then surgically implanted under a muscle in the patient's right armpit, an area rich in blood vessels (Figure 10).

After letting the ingredients incubate in the body for 7 weeks, the researchers removed the scaffold and the bone that had formed inside, along with an adjoining part of the muscle and the local artery and vein that had grown into the implant. The surgeons removed the metal plate that had been taking the place of the missing jaw bone and replaced it with the bone-muscle-blood vessel implant. Using microsurgical techniques, they connected the blood vessels in the implant to blood vessels in the head so that the new bone would continue to have a blood supply.

By the 4th week post-transplantation, the patient was able to eat

solid food for the first time in nine years. He was also able to speak more clearly and resumed socializing with family and friends. His mood improved and he gained weight.

Bone engineering in humans is still in the experimental stage and there is a great deal of work to be done. Even so, remarkable progress has been made since ven Meekeren reported on the Russian soldier and his dog skull patch. From the day that Marshall Urist first implanted demineralized bone matrix in the muscles of laboratory animals and saw bone form to White's first glimpse of coral's bone-like skeleton, incremental discoveries by scientists and physicians have brought us to the brink of creating new bone for transplant.

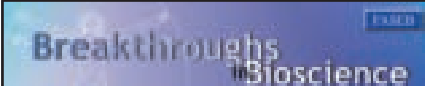
We are almost at the point where an oft-quoted childhood rhyme might be transformed to "Sticks and stones can break your bones, but scientists can replace them..."

## Biographies

*Steven Stocker* writes about biomedical research and health from the Philadelphia region. She has written for *Discover*, *Glamour*, *Physician's Weekly*, *Consumer Reports on Health*, *The Washington Post*, *Los Angeles Times*, *Dallas Morning News* and numerous other publications. She also writes frequently for the National Institutes of Health and the National Academy of Sciences. This is her seventh article in the *Breakthroughs in Bioscience* series.

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*Lynne Jones, Ph.D.*, is an Associate Professor and Director of the Center for Osteonecrosis Research and Education and Arthritis Surgery Bone Bank at the Johns Hopkins Medical Institutions. Her research interests include osteonecrosis pathogenesis and treatment, total joint arthroplasty successes and failures, evaluation of bone graft materials, and animal models of musculoskeletal disease. Dr. Jones currently serves as the President of the Society for Biomaterials and has also been selected as a Fellow, Biomaterials Science and Engineering, but the International Union of Societies for Biomaterials and Engineering for her work in the field. In addition, Dr. Jones serves as a Member-at-Large for the Orthopaedic Research Society.



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