

Advancing with Ceramic Biocomposites for Bone Graft Implants

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Abstract: Bone grafting has come a very long way since a Dutch surgeon used pieces of a dog's skull to repair a soldier's cranium in the 17th Century. Current technology aims to deliver a scaffold that combines the unique osteogenic properties of ceramic biocomposite materials to make the best mimic of physiologic conditions. To do so, a scaffold must provide: i) A three-dimensional platform allowing for osteogenic cellular attachment and growth and vascular formation, ii) Structural integrity while the damaged tissue heals, and iii) Non-toxic integration, degradation or resorption into the host over an appropriate time. The combination of inorganic, ceramic materials with cells, polymers and growth factors has come very close to creating a bone graft capable of meeting each of these requirements. Recent patents describe new methods to forming an ideal osteogenic matrix for both large and small bone repair. Many new technologies have been introduced that are very potent in their ability to heal small bone wounds and induce new bone formation, such as porous calcium phosphate pastes and hydroxyapatite cements. However, there is still a lack of quality and proven materials for load bearing purposes. This is a reminder of how much there still is to improve upon and that we are still a long way from creating bone products that are identical to the natural product. Despite these shortcomings, ceramic biocomposites represent one of the most promising materials in the bone graft field and their development and improvement will surely lead to a more natural bone replacement.

Keywords: Bioactive, bioceramic, biocomposite, ceramic, orthopedic implants, stem cell, tissue engineering.

INTRODUCTION

Both the natural science and engineering communities have contributed to the rapidly advancing field of bone repair. Bone grafts require particular mechanical properties as well as an enormous degree of biocompatibility. Thanks to this collaborative effort, many new products are able to offer top degrees of both design aspects. In this short review, we will present a brief run-up to the current state of bone graft technology and analyze patents which we find especially exciting for the future of the field.

Bone grafting and bone repair have changed dramatically since a Dutch surgeon used pieces of a dog's skull to repair a soldier's cranium in the 17th Century [1]. Autografts, the gold standard of tissue transplantation and bone repair, provide an almost perfect scaffold for bone grafting, as there is little to no chance of rejection by the host and an already developed structural and vascular network. However, autografts usually require a second surgery, meaning additional wound, prolonged recovery time, more risk of infection, as well as being severely limited in supply [1-4]. Allografts also provide an excellent material for bone grafting but have the added risks of rejection and disease transmission. To overcome the difficulties with autografts and allografts, scaffolds designed to repair and permit regeneration of bone have been produced from an array of materials each having a unique set

of mechanical and biologically relevant properties. These materials include natural polymers (collagen, chitin, alginate), synthetic polymers (Polyglycolic acid (PGA), Poly (lactic-co-glycolic acid) (PLGA), Poly (lactic acid) (PLA)), metals (titanium, nitinol), and ceramics such as calcium phosphates (hydroxyapatite, tricalcium phosphate), calcium sulphates, and biological glass Fig. (1). It should be noted that there is great diversity between materials even belonging to the same product group. For instance, hydroxyapatite is rather inert whereas other ceramics are resorbed over a large range of times.

Current technology aims to deliver a scaffold that combines the unique osteogenic properties of these materials to make the best biomimic of physiologic conditions. To do so, a scaffold must provide: i) A three-dimensional platform allowing for vascular formation and osteogenic cellular attachment and growth, ii) Structural integrity while the damaged tissue heals, and iii) Non-toxic integration, degradation or resorption into the host over an appropriate time [5-7].

The first requirement can be met by using biocompatible materials that allow for cell growth (an osteoconductive material) [1, 5, 8, 9] or by the attachment of signaling molecules to the material, which themselves promote the attachment and proliferation of relevant cell populations. Adult stem cells including bone marrow mesenchymal stem cells (MSCs), MSC from adipose tissue, cord blood stem cells, as well as differentiated embryonic stem cells (ESCs), when combined with an appropriate 3D scaffold, have been shown to be capable of producing bone *in vivo* Fig. (1) [10-16]. MSCs can provide a large amount of potential osteogenic cells for use in tissue engineering and offer great excitement

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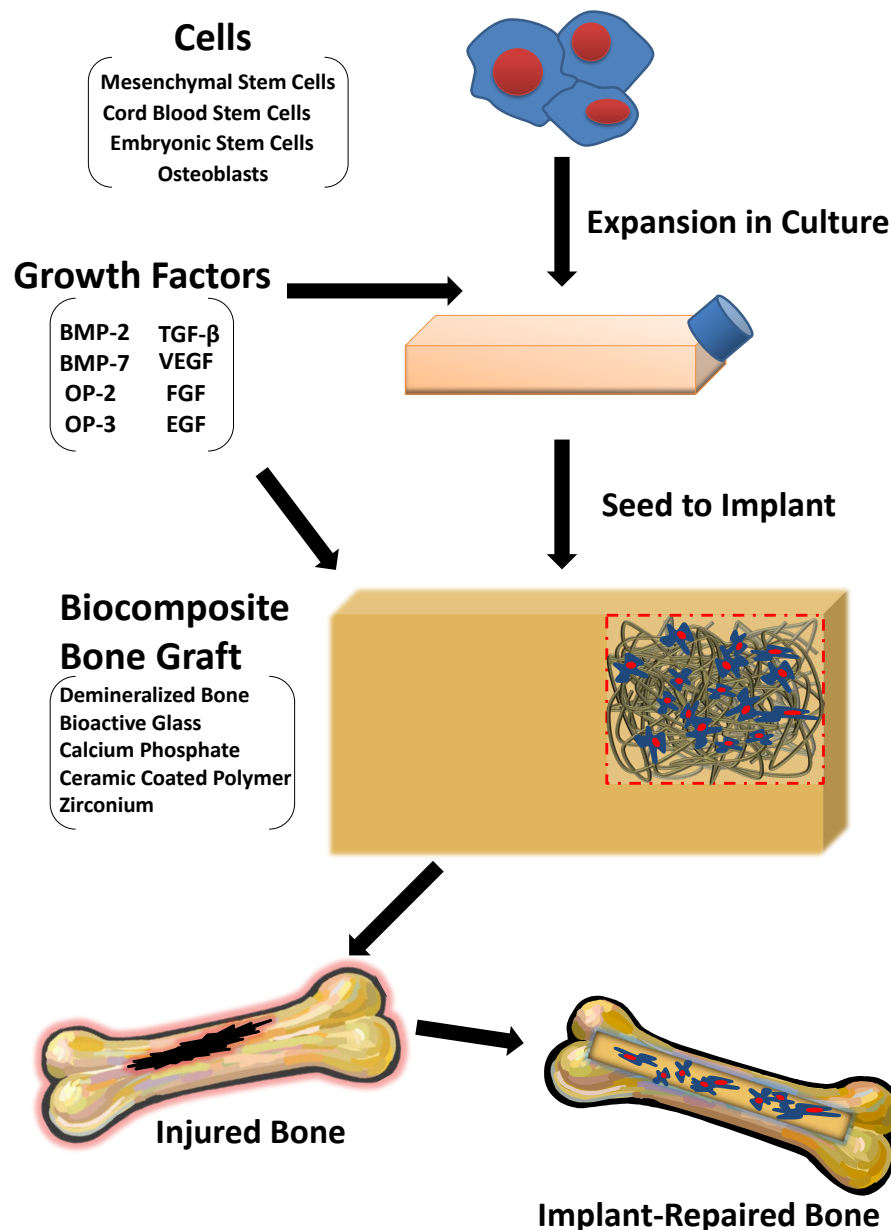


Fig. (1). Steps for creating a cell-seeded biocomposite bone implant. Scheme showing the process of preparing a biocomposite bone material for implantation using a cell-seeding technique as well as growth factors to promote osteogenic lineage. First, osteogenic cells are expanded in culture to produce sufficient cell number. The cells may be derived from adult stem cells such as bone marrow mesenchymal stem cells (MSCs), MSC from adipose, cord blood stem cells, as well as differentiated embryonic stem cells. Each of these cell types has been shown to produce bone *in vivo*. Next, the addition of growth factors may or may not be necessary to drive cellular differentiation, depending on the material. For example, bioactive glass is capable of producing ossified tissue without the assistance of exogenous growth factors. The cells are then seeded onto the biocomposite bone graft and allowed to expand on and into the material. The material (demineralized cadaveric bone, bioactive glass, zirconium, calcium phosphate, etc.) is to be made porous to allow new bone formation in all three-dimensions. The implant is then placed at the site of injury and, over time, completely integrates into the host tissue.

for the future of bone grafting. Signaling molecules such as bone morphogenic proteins (BMP-2, BMP-7/OP-1, OP-2, OP-3), transforming growth factor beta (TGF-beta), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF) among many others are known osteogenic proteins that are capable of inducing a progenitor cell to form bone and/or cartilage tissue Fig. (1) [1, 4, 17, 18]. Furthermore, we and others showed that extracellular matrix independent from other factors can also

induce differentiation of bone marrow MSC [19-21]. The combination of cells, molecules and materials is essential to providing a scaffold with osteoconducting and osteoinducting properties.

Equally important to the proliferation of osteogenic cells is the development of a functioning vascular supply to the growing bone graft, particularly very large ones that become necrotic in their centers when wholly dependent on diffusive transport of oxygen [22]. Strategies to produce a vascular

network include using a mix of MSCs and umbilical cord-derived endothelial cells to seed a scaffold [23], targeting proximal vasculature to integrate and expand into the graft using cytokines [24], or simply implanting a vascular bundle to the injury and graft site [25]. The construction of a complete vascular system is the single most important factor in determining the success of an orthopedic implant.

A major concern of bone grafts is the ability to bear significant load while the damaged tissue heals [5]. Furthermore, a proper implant should not overtake the loads of proximal tissue as this may cause the healthy bone to weaken, called stress shielding [26]. Additional to these very specific mechanical properties, a scaffold must be porous enough to allow osteoblasts or other progenitor cells to populate the entire scaffold and osseointegrate with surrounding tissue [5, 27]. The need for vascular networks also requires much larger pores in the bone graft material than would otherwise be needed, adding yet another challenge to scaffold design [28].

The third requirement is possibly the most difficult to provide, since bone in-growth occurs differently at all injury sites and must be finely tuned so as not to cause more harm [2, 3]. A proper scaffold should be completely and totally replaced by regenerated tissue, that is, the patient should not depend on the mechanics of the scaffold long after implantation. Rates of degradation of materials vary greatly, as well as the location at which they begin to erode (surface, bulk, both). Materials that are biodegradable, bioresorbable, bioerodible and bioabsorbable must also retain the properties to meet the first two requirements [2, 5, 18, 27, 29]. Therefore, the search for the perfect bone tissue scaffold will require a great deal of arrangement to generate a whole greater than the sum of the parts.

These materials, which combine structural and biologic properties from different materials, are known as biocomposites. Containing cells, signaling molecules, or a combination of the two within a structural matrix, biocomposites are biologically active materials with great promise for tissue engineering. Here, we give a brief review of the most current technology pertaining to the use of ceramic biocomposites in bone grafts.

RECENTLY ISSUED PATENTS ON CERAMIC BONE GRAFT MATERIALS

Natural Ceramics, Endochondral Ossification and Bioactive Glass

The most obvious means of recreating bone is to simply replace the tissue with already formed bone. A recent patent published May 2010 by inventor Shi titled *Allografts Combined with Tissue Derived Stem Cells for Bone Healing*, describes a method of using donor bone for use as a scaffold for bone formation from adipose-derived mesenchymal stem cells (Table 1) [30]. The bone substrate, which is to be retrieved from a cadaveric donor, may be seeded with adipose tissue either from the same cadaveric donor or the implant recipient. The patent also describes a demineralization process to remove blood, lipids and other cellular products that

may initiate an immune response, leaving just a matrix for the MSCs to adhere to. The bone may then be formed into any number of necessary shapes, such as strips and dowels. Once the bone is properly prepared, the author recommends *in vitro* culturing of the cells until they have adhered to the bone substrate before implantation. This process takes advantage of a scaffold produced in nature, requiring no outside engineering, as well as the advantages of using patient MSCs for osteogenesis. The porosity of the bone substrate is not well manipulated however, and may not be well suited for small repair operations. This method is probably best served when relatively large implants are required or when a matrix is called for with *in vitro* study.

Another method that uses an equally natural approach is presented by Jukes *et al.*, published in PNAS May 2008 [13]. Following the model of endochondral ossification, the authors use ESCs to first produce a cartilage layer atop a ceramic scaffold before implanting the product. Since ESCs having up-regulated osteogenic genes do not form bone when implanted, as opposed to osteogenic MSCs, it is necessary to follow the course nature uses by differentiating the ESCs into chondrocytes. The ESCs are therefore differentiated *in vitro* towards the chondrogenic lineage and seeded onto the ceramic material. Upon implantation, these cartilage tissue-engineered constructs do indeed form bone, in stark contrast to constructs seeded with ESCs differentiated towards an osteogenic lineage. While this method produces bone having a much more natural derivation, the issues surrounding ESCs implantation, such as tissue rejection, persist, once again pointing towards the benefit of MSCs use.

Synthetically produced materials amass the vast majority of biocomposite materials. One such ceramic that offers a great deal of excitement is bioactive glass. Discovered in 1969 and first used in clinical settings in 1985, bioactive glasses have a very unique property that promotes osteoblasts maturation into osteocytes [31]. The mechanism believed to be responsible for this is the constant leeching of ions from the dissolution products of the glass that results in the up-regulation of certain genes that are implicated in osteogenesis (insulin like growth factor II (IGF-II), CD44, c-Myc, activator protein 1 (AP-1)) [4, 31]. In a patent published in January, 2002 by Hench *et al.* titled *Use of Bioactive Glass Compositions to Stimulate Osteoblast Production* the authors illustrate a more patch like method of applying the bioactive ceramic (Table 1). The patent describes the use of bioactive glass powder in an aqueous solution combined with osteoblasts for the formation of bone tissue. As published, the bioactive glass is to contain a majority silicon dioxide by weight, as well as sodium oxide, calcium oxide and phosphorous oxide and is to be produced as a particulate rather than a “fused matrix of particles or a mesh or fabric of glass fibers.” This allows for extreme customization in the hands of the surgeon or researcher. However, the product is only to be used as an initiator to bone formation, not as a structural implant due to the lack of strong mechanical properties. Therefore, the use of bioactive glass in this manner is best used for small repairs, tissue culture, or combined with some other scaffold material having much better mechanical strength.

Table 1. Natural Ceramic and Bioactive Glass Patents.

Patent # (Year)	Inventor(s)	Description
US20100124776 (2010)	Shi Y	A method for seeding a bone substrate with mesenchymal stem cells (MSCs). The inventor describes the digestion of a whole tissue, seeding the cell mixture on a bone substrate, culturing on the bone substrate and rinsing to remove unwanted cells.
US20040009598 (2004)	Hench LL Polak JM Buttery LDK Xynos ID Maroothernaden J	Bioactive glass powder combined with osteoblasts in an aqueous solution used as a cement.

Ceramic-Polymer Composite Materials

A good example of a scaffold that is coated with a bioactive material is presented in a patent published March 2009 by Johnson and Tavakley (Table 2) [18]. Titled *Composite Scaffold Structure*, the authors describe a scaffold design having a polymeric core that is discretely coated with calcium phosphate, resulting in a porous scaffold with a high amount of surface area. The use of a polymeric core ensures the flexibility of the scaffold and allows for a compressive strength similar to cartilage, for which the invention was designed. The authors outline a variety of materials that may be used as the core structure including Poly(lactic acid) (PLA), Polyglycolic acid (PGA), Poly(lactic-co-glycolic acid) (PLGA), polycaprolactone, and polyanhydrides as well as natural materials like collagen, albumin, fibrin and alginate, all of which are bioresorbable, that is, they will be completely removed through degradation from the final scaffold. The surface coating of calcium phosphate, which facilitates the attachment and growth of cells on the scaffold, is said to be discrete so as to allow the polymeric core to maintain its lower elastic modulus. An entire surface coat would render the structure much more brittle, such that it could crack or break under normal body stresses.

This idea of using mechanical properties of one material along with the bioactive properties of another is very similar to the actual structure of bone. Bone itself contains a precise mixture of collagen fibers weaving through a harder hydroxyapatite (HA) phase. This combination allows bone to possess both bioactive and mechanical properties. Biomimics containing this very make up have been shown to support the growth and proliferation of MSCs both *in vitro* and *in vivo* [6]. In his 2007, manuscript published in *Biomaterials*, Dr. Huanan Wang described a method of designing a porous hydroxyapatite-polyamide structure having very similar mechanical strengths as bone, including load-bearing bone. His groups' scaffolds were able to produce bone with or without seeding of MSCs when implanted into mice.

Two patents published in 2007 and 2008 by inventors Erbe and Clineff, respectively, present another embodiment of a composite containing a polymeric phase (Table 2) [32, 33]. Both patents describe a resorbable graft having improved flexibility and compressive strength. The bone graft material is comprised of an oxidation-reduction reaction

product of "one metal cation, one oxidizing agent, and one oxidizable precursor anion," such as calcium phosphate, combined with a biocompatible, resorbable polymer, such as collagen or another structural protein. Another embodiment of the material contains a bioactive glass, as well, to promote osteoblast growth. The 70/30 volumetric ratio of reaction product to polymer creates a unique material having macro, meso and micropores due to the reaction product as well as beneficial handling and flexibility from the polymer. The porosity leads to outstanding imbibition and protein adsorption, while the flexibility of the material allows a great deal of customization in the hands of the surgeon.

A patent published in 2004 from inventor Wang introduces another variation on the combination of polymer and ceramic calcium phosphate (Table 2) [34]. Here, the polymer is used simply to create pores in the ceramic medium. One embodiment of the invention calls for a composite mixture of calcium phosphate cement, an aqueous medium and a biodegradable polymer as a porogen. The porogen can be finely tuned to degrade at the exact rate of osteoblast reseeding of the calcium phosphate matrix and the inventor even describes a porogen filled with osteoinductive growth factors. This material is once again best suited for small repair jobs or coupled with another bone graft system to increase the osteoconductiveness of the implant.

More recently, a patent published in February 2011 from inventor Yayon describes yet another bone graft material comprised of an inorganic mineral phase as well as a bioactive polymer (Table 2) [35]. The invention goes one step further in the formation of bone to include an anti-resorptive agent, such as bisphosphonates. These agents inhibit the recruitment or activity of osteoclasts, the bone resorbing cells responsible for the subtractive side of bone remodeling. Rather than simply promoting osteoblast activity, the inventors have taken an approach that aims to protect the newly formed, delicate bone from the natural process all bone experiences *in vivo*. The composite material is formed by coprecipitation of calcium, phosphate, and carbonate ions, one amino acid molecule either as a monomer or polymer, a bioactive agent such as heparin, hyaluronic acid, starch, collagen, chitin or the like, and the anti-resorptive agent. The final product is deemed a synthetic apatite having poor crystallinity that highly mimics the structure of natural bone. The inventors specifically avoid hydroxyapatite due to its high de-

Table 2. Ceramic-Polymer Composite Material Patents.

Patent # (Year)	Inventor(s)	Description
US20090062821 (2009)	Johnson JR Tavakley A	Polymer scaffold having a discrete, non-continuous coating of calcium phosphate on the surface.
US7189263 (2007)	Erbe EM Clineff TD Bagga CS Nagvajara G M Koblish A	Bioactive ceramic phase such as calcium phosphate, mixed with a biocompatible, resorbable polymer, such as collagen. The porous material is greatly flexible and displays high adsorptive properties.
US20080187571 (2008)	Clineff TD Koblish A Bagga CS Erbe EM Nagvajara G M Darmoc MM	Same as above, except with the addition of bioactive glass.
US20040137032 (2004)	Wang FW	Calcium phosphate mixed with a biodegradable polymer acting as a porogen. Best suited as a cement material.
US7887831 (2011)	Yayon A	Composite of synthetic apatite, a bioactive polymer and an anti-resorptive agent, limiting osteoclast activity.

gree of crystallinity, claiming less organized structures improve solubility *in vivo* and thus allow the material to be used as a powder or paste.

Osteoinductive Calcium-Phosphate Grafts without Polymers

Scaffolds produced purely from calcium phosphate ceramics, without a polymeric phase, are themselves capable of supporting MSCs and initiating bone formation [36]. The clear advantage of producing a biocomposite containing both polymeric and ceramic materials is an elastic modulus much lower than the pure ceramic. However, if a ceramic material can be made to be as dynamic as a polymer, one could remove the polymeric material all together, as it is not the bioactive phase. A recent patent published in June 2010, describes such an invention [2]. Authors Day and Erbe reveal a dynamic, nonrigid, nanofiber scaffold produced from bioactive materials to be combined with progenitor cells (Table 3). These materials may include but are not limited to calcium phosphate, hydroxyapatite, and bioactive glass. Used alone or in combination with others, the material is spun or blown into an interwoven network of 1 to 6 micron diameter fibers, depending on desired porosity. Since the scaffold is not fused to itself the individual fibers are free to deform and shift due to environmental changes. The scaffold is designed to be highly dynamic before and during osseointegration. This is a very important trait as the injury site will change drastically as it heals, possibly over the course of many months. The invention may also be coupled with previous devices, such as the selectively-expandable bone scaffold

described in a November 2006 patent published by Edidin, which uses an actuator to maximize the dynamic nature of the scaffold Table 3 [37]. The flexibility of the authors' invention, as well as its bioactivity, represents an exciting leap with ceramic scaffolds.

Another patent which focuses solely on calcium phosphate was published May 2011 by inventor Yuan (Table 3) [38]. The patent introduces a very porous calcium phosphate material made using grains of the raw material ranging from 0.1 to 1.5 microns in diameter. When sintered with a porogen, the final material contains mainly micropores ranging from 0.1 to 1.5 microns in diameter and having a surface area percent micropores of 40%. The invention stems from the idea that small grain sizes, higher surface area percentages of micropores and higher protein adsorption all increase the osteoconductiveness of the material. Here the author aims to simply produce a material that can act much quicker than less porous calcium phosphate graft materials. Due to the sand-like nature of the material, the inventor envisions the product being injected into wound sites and rapidly enhancing new bone formation.

Load Bearing Zirconia Grafts

Although dynamic, shape-changing ceramic scaffolds give highly customizable results, they may not always be best suited for major bone grafts, requiring intense load-bearing properties. One material that is able to withstand these stresses is zirconia (zirconium dioxide). Zirconia has been extensively studied for orthopedic applications due to its high elastic modulus [39]. However, the material itself

Table 3. Osteoinductive Calcium-Phosphate Grafts Without Polymer Patents.

Patent # (Year)	Inventor(s)	Description
US20100136086 (2010)	Day TE Erbe EM	Dynamic, non-rigid nanofiber scaffold made from bioactive ceramic, such as calcium phosphate, bioactive glass, or hydroxyapatite.
US20060264945 (2006)	Edidin AA Ferdinand AE Ico CA Markworth AD	Dynamic ceramic scaffold surrounding a mechanical actuator. The actuator allows the scaffold to expand into the wound and shrink as it heals.
US7942934 (2011)	Yuan H Bruijn JDD	Porous calcium phosphate scaffold made from granules of calcium phosphate. The material's flow properties resemble those of sand.

has a very inert surface, as most ceramics do, which limits interaction with surrounding tissue. More specifically, the surface does not form biochemical bonds, thus leading to *in situ* slippage [26, 40]. Therefore, to fully take advantage of the mechanical strength of zirconia it must first be coated with a biologically active material. One interesting approach was published in January 2010 in the journal *Biomaterials* by Zhou *et al.* [41]. The authors present a method of coating zirconia with a sheet of cultured MSCs. The process requires a monolayer of cells to be rolled up such that it completely encapsulates a zirconia implant. The wrapped implants were shown to contribute heavily to *in vivo* bone formation and osseointegration when implanted subcutaneously in mice following histological analysis. This approach represents a novel technique in priming ceramic surfaces for implantation, but is still far from perfect.

In a patent published in May 2007 by inventor Sugaya, the author describes a porous, ceria stabilized zirconia for use as an osteoinductive scaffold (Table 4) [17]. The use of cerium oxide to stabilize zirconia produces an oxygen rich layer at the surface of the zirconia about 100 to 300 nm thick. This produces a highly biocompatible product, suitable for MSC or osteoblast seeding, along with high strength and fracture toughness. Another advantage of the dynamic and customizable material is that it may be shaped into any number of appropriate forms. The relatively easy preparation of the ceria-stabilized zirconia (plasma spray) allows the material to be attractive for both research and tissue repair applications. The clear drawback of zirconia is that it lacks any bioresorbable characteristics, resulting in a permanent scaffold structure. However, due to the porosity of the implant, osseointegration may completely annul any complications due to implant-slippage overtime.

Another recent invention that takes advantage of the mechanical strength of zirconia as well as the bioactive properties of calcium phosphate is presented in a patent published in May 2010 by Zhang *et al.* (Table 4) [26]. The inventors describe a material having a strong zirconia core and a bioactive, but mechanically weaker, glass surface connected through a graded glass-zirconia layer. The material is fabricated in such a way by using a glass-ceramic infiltration technique. This method produces a material having an

increasing elastic modulus from the surface to the interior, which may then be spun or molded into any number of porous scaffold structures. An advantage of this type of graded material is that it provides the scaffold with excellent resistance to contact damage. In fact, when the authors subject a sample of their graded material to loading tests they find that it is able to resist over three times the load it takes to crack a sample of monolithic zirconia. The production of a material that has a low modulus, bioactive surface while still having high flexural strength is quite advantageous and has numerous applications in the field.

CURRENT & FUTURE DEVELOPMENTS

Biocomposites represent the future of material science in the medical setting, especially in orthopedic implantation. Porous ceramic scaffolds have already been noted as the most suitable material for reproducing the structural integrity of ossified tissues. When combined with the bioactive attributes of calcium phosphate, hydroxyapatite, bioactive glass, or other similar ceramics, the composite material is able to support progenitor cells and mimic the natural characteristics of bone. Whether it is applying new technologies to old problems, as Shi was able to accomplish with donor bone, or conquering the boundary between polymers and ceramics, like Day and Erbe demonstrated with their hydroxyapatite "cotton ball", biocomposites of this nature encompass a broad spectrum of regenerative scaffolds and bone grafting needs. However, though current technology is able to produce accurate biomimics of natural bone, there is still much to be gained; none of these presented materials is quite able to meet the standard of an autograft implant and all would require a certain degree of invasiveness to deploy. Furthermore, there is still a large gap to be covered with vascularized implants. That said, overcoming boundaries such as immune responses, osseointegration and material resorption are all feats to be proud of and to build off of for future generations of bone grafts.

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Table 4. Load Bearing Zirconia Graft Patents.

Patent # (Year)	Inventor(s)	Description
US20070123996 (2007)	Sugaya K, Merchant S Seal S, Georgieva P Vrotsos M	Ceria stabilized zirconia scaffold. Mechanically strong and bioactive, due to an oxygen rich layer at the zirconia surface.
US20090118114 (2009)	Zhang Y, Regeros R Kim JW	Graded glass-zirconia graft material, having a strong zirconia core and a bioactive surface containing bioglass.

CONFLICT OF INTEREST

The authors do not know of any conflicts of interest.

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