REVIEW

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Hard tissue regeneration using bone substitutes: an update on innovations in materials

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Bone is a unique organ composed of mineralized hard tissue, unlike any other body part. The unique manner in which bone can constantly undergo self-remodeling has created interesting clinical approaches to the healing of damaged bone. Healing of large bone defects is achieved using implant materials that gradually integrate with the body after healing is completed. Such strategies require a multidisciplinary approach by material scientists, biological scientists, and clinicians. Development of materials for bone healing and exploration of the interactions thereof with the body are active research areas. In this review, we explore ongoing developments in the creation of materials for regenerating hard tissues.

Keywords: Bone substitutes; Bone tissue engineering; Bioceramics; Hydrogel; Biopolymers

INTRODUCTION

Repair of bone defects using implanted material commenced millennia ago; ancient Peruvian and Egyptian societies used implants to heal bone defects [1-4]. The modern era of bone substitutes commenced with the attempt of the Dutch surgeon Job van Meekeren to repair a soldier's broken skull using a skull fragment from a dog [5]. Fred Albee first described autologous bone grafting, using part of the tibia to achieve spinal fusion. The Swedish surgeon Levander showed that osteoinduction could be used to induce regeneration of hard tissue [6]. Urist [7] first reported, in 1965, that bone morphogenetic proteins (BMPs) exhibited osteoinductive potential. Hard tissue repair, and regeneration science and technology, have advanced rapidly in the modern era. An in-depth understanding of the underlying principles has been attained, new methods and materials developed, and a multidisciplinary approach used to achieve successful hard tissue regeneration. Many scaffold systems have been proposed for bone tissue engineering. Innovation have been made in all of scaffold design, material selection, incorporation of drugs and growth factors, mechanical stability, and bone regeneration efficiency. However, autografts are still considered to be the best bone graft option for hard tissue repair; synthetic bone graft substitutes do not exhibit equivalent osteogenic or osteoinductive performance. However, autografting does not meet the overall medical demand for orthopedic implants. Harvesting of adequate quantities of bone is difficult and postoperative complications occur at harvest sites. Allografts and xenografts are both good alternatives, but are associated with risks of disease transmission and immunorejection. Thus, synthetic bone graft substitutes are the logical option when it is sought to meet the rapidly increasing demand for orthopedic implants, even though synthetic bone substitutes have some in-

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herent limitations in terms of strength, osteoconduction, osteoinduction, osseointegration, and biodegradation. Current studies on bone substitutes are focused on improving various features of scaffolds; and include the development of new biomaterials, modification of mechanical and structural-morphological features, enhancement of biocompatibility by chemically modifying the surfaces of materials, improvement of osteoinductive capabilities and the ability to incorporate growth factors, and loading of stem cells onto scaffolds to induce self-initiated tissue regeneration. These remarkable advances have helped reduce the gap between autografts and synthetic bone graft substitutes.

Key issues in successful implantation are the initial and long-term immune reactions of the body to the implant. The immune system recognizes the implant, and may reject it, initiating many physiological responses involving immune cells. Thus, the chemical nature of the implant material is key to its biocompatibility. Consequently, cell-material interactions within the defect zone determine the overall success of healing. Hard tissue repair also requires high-level mechanical stability; this is not the case for other injured tissue. Load-bearing capacity and structural rigidity is afforded by the skeletal system, and repaired hard tissue is directly subjected to or is expected to tolerate significant mechanical loading, which limits the choice of bone substitute materials. Thus, only a few materials are presently considered useful. Hard tissue is composed of carbonated hydroxyapatite (HAp) crystals and collagen (the principal building blocks) with cellular and systemic components. Thus, calcium phosphate ceramics and collagen are natural choices of bone substitutes. Positive cell-material interactions are also observed with several other inorganic materials like bioglasses, phosphates of magnesium; sulfate, carbonate, and silicate of calcium. Some very inert inorganic materials, including alumina, zirconia, titanium alloy, and cobalt-chromium alloy, find specific hard tissue applications, but these materials are nonresorbable and osseointegration is absent at the bone-implant interface. Synthetic biodegradable polymers, including polylactic-co-glycolic acid (PLGA), polycaprolactone (PCL), and polyethylene glycol (PEG), interact positively with cells, and are used as substitute bone scaffolds [8,9]. These materials are degradable in the physiological environment and the degradation products have no harmful effects. Moreover, degradation rate, hydrophilicity, and mechanical strength can be controlled by manipulating the chemical composition. Many natural biopolymers are also available, and are very suitable bone substitutes in terms of cell-material interactions. Chitosan, alginate, cellulose, gelatin, collagen, keratin, and hyaluronic acid are inherently recognizable by cells and exhibit favorable cell-material interactions. These are large polymers of very high molecular weight. Biodegradation of such molecules is very rapid, and the degradation products may stimulate the physiological mechanisms of healing. Bone substitute materials are currently selected based on an ability to impart additional biocompatibility to a structurally stable scaffold [10,11].

Healing of bone defects in adults closely resembles bone formation during organogenesis. Most fractures heal by indirect or secondary fracture healing, via formation of an intermediate callus [12]. An inflammatory response occurs soon after a fracture of bone, or surgical intervention, and extravascular blood cells form a blood clot. After this initial immune reaction, collagen fibers and mineralized osteoids combine to form a soft callus around the injury site. This soft (or fracture) callus ossifies to form a disorganized structure termed woven bone. During a later phase of bone formation, this woven bone is replaced gradually with highly organized lamellar bone, which begins to form soon after the collagen matrix of either tissue becomes mineralized. Osteoblastic cells penetrate the mineralized matrix and angiogenesis begins with creation of microvessels. Osteoblasts deposit new lamellar bone on the surface of the mineralized matrix. Eventually, all woven bone and the fracture callus are replaced by lamellar or trabecular bone. This remodeling process transforms trabecular bone into natural compact bone Said so, the whole process should occur inside a bone substitute scaffold without jeopardizing the series of events and occasionally complementing the process by its morphological and chemical attributes.

Bone substitute scaffolds must meet stringent requirements; they must be nontoxic, mechanically sound, have a three-dimensional (3D) porous structure, exhibit optimum biodegradation, allow new bone formation at an acceptable rate, be economical to make,

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and allow easy fabrication into the final preforms [13-15]. Scaffold architecture is critical to optimize the microenvironment for the synthesis of new tissue, and to allow flow or diffusion of nutrients between cells and the surrounding environment. Scaffold properties depend primarily on the biomaterial used and the fabrication process. Several ceramic and glass materials have superior biocompatibility but poor mechanical strength and stability, rendering them unsuitable as porous scaffolds for bone tissue regeneration. Apart from their lower intrinsic strength, processing defects (such as irregularly shaped pores), surface defects, and residual stress, all lower the mechanical strength of the scaffold systems made of these materials. Thus significant research is devoted to come up with stronger and more biocompatible systems.

Recent advances in bone substitutes have made significant progress regarding these challenges. Advances in materials design; chemical modification; fabrication techniques creating stronger, more porous, and more biocompatible scaffolds; combinations of various strategies to enhance cell-material interactions; and stimulation of cells to ensure rapid but controlled bone regeneration, are continuously reported. Tissue engineering has opened a new dimension in bone substitute technology.

The aim of this review is to explore modern frontiers of bone substitute technologies. We will explore how the technology is shaping its current form. Our discussion broadly covers innovations in materials development and fine-tuning, together with structural and functional improvisations.

INNOVATIONS IN MATERIALS

As discussed earlier, the chemical nature of the scaffold material is fundamental for successful implantation. Cell-material interactions govern the adaptation and systemic integration of the foreign body into the physiological environment. Many choices of material are available; each has advantages and disadvantages. Thus, ever-higher performance bone substitute systems (in terms of both mechanical and biological properties) are under continuous development. Some innovations are discussed below under different categories of material.

Bioceramics

Bioceramics are the best-studied bone substitute materials because they are chemically similar to bone. Fabrication of bioceramic porous scaffolds is achieved using various techniques to create pores, including salt leaching [16], sponge replica and gas foaming [17-20], porogen-based method [21], 3D printing [22], etc. Fig. 1 shows a scaffold fabricated by sponge replica method.

Both the microstructure and pore size and porosity significantly influence the mechanical properties and osseointegration of a scaffold [23]. Newer fabrication techniques have been proposed which allow greater control of pore size, porosity, scaffold shape, ease of fabrication, and reliability of physicomechanical properties. Recent fabrication techniques include 3D printing [24], stereolithography [25], *in situ* synthesis using a reactive phase [26], and laser cladding [27]. These methods are particularly useful for creating customized scaffolds with predictable mechanical performance. Moreover, these methods afford new opportunities for development and customization of scaffolds, allowing achieve greater control over cell-material interactions in the biological environment.

The bioactive concept seeks to balance mechanical strength and bioresorbability using biphasic calcium phosphate (combination of hydroxyapatite and tricalcium phosphate). This material is under intense study in terms of chemical modifications, for use as a base material allowing further functionalization via surface treatment, as a component of hybrids, and in terms of loading of bioactive secondary phases. Calcium phosphates have been chemically modified

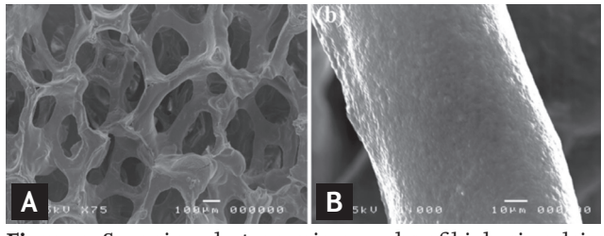

Figure 1. Scanning electron micrographs of biphasic calcium phosphate scaffolds fabricated using the sponge replica method. Low magnification image (A) and high magnification image of the scaffold strut (B).

by incorporating Si, Sr, and Zn [28-35]. These chemical modifications enhanced osteoblastic proliferation and material performance; dissolution rate, densification behavior, mechanical strength, and biocompatibility. The ions promote bone formation whern the ions are present in dissolution products adjacent to the cell-material interface.

Nanophase bioceramics have unique advantages; size effects and nanoscale surface phenomena are in play. These materials may be used as fillers in polymeric scaffold systems, to improving both mechanical and biological properties, and to coat on metallic implants [36]. Various synthetic processes are used to prepare nanophase calcium phosphate, modify process chemistry and powder characteristics (in terms of morphology and biocompatibility). Hydrothermal, sol-gel, wet chemical, and biomimetic deposition methods have been investigated [37].

A key issue with bioceramic bone substitutes is the fact that load-bearing is limited during healing. However, ceramics are historically regarded as nonload-bearing materials. Bioceramics, such as HAp, tricalcium phosphate (TCP), and other calcium phosphate materials, including calcium sulfate, are brittle. Introduction of pores significantly decreases bulk strength. However, high-level porosity is indispensable for sound osteointegration, and efforts are being made to modify the microstructures and structural features of porous bioceramics, to achieve higher strength. Processing conditions and methods drastically affect the surface characteristics of fabricated bioceramics scaffolds; paving the way for construction of high-strength scaffolds. Bone substitutes of block, cylindrical granule, and spherical granule types have been developed using sponge replica, fibrous monolithic, and slurry drip processes [38-41] Fig. 2 shows a multichannel granular bone substitute and its internal microstructure. Bone formation with angeogenesis using this type bone substitutes are described in the schematic model.

The use of ceramic-polymer hybrid systems to fabricate scaffolds has attracted much attention. However, the choice of polymeric materials is increasing only gradually. The use of polymers alone may not be optimal when it is sought to create biocompatible bone substitutes with adequate mechanical strength.

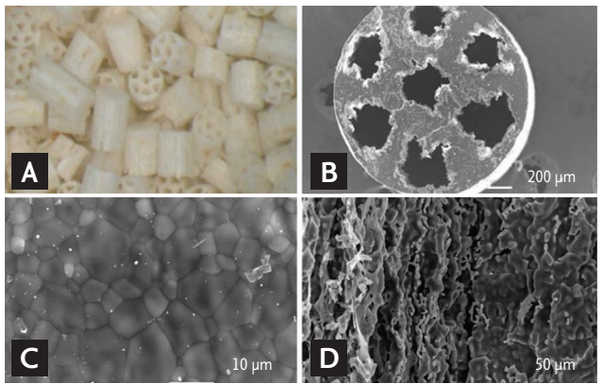

Figure 2. Optical microscopic (A) and SEM images (B) of granular bone substitutes. Frame (C) and pore (D) surface of the granular bone substitute. Adapted from Byun et al., with permisson from Springer [39].

Calcium phosphates, and (in some cases) bioglass and glass ceramics, combined with polymers, afford good mechanical properties and high-level biocompatibility. Bioceramics may be added to reinforce the matrix, improve both mechanical characteristics and biocompatibility of synthetic biopolymers that do not exhibit adequate levels of cell- material interaction [42-44].

Ceramic-polymer hybrid composite systems enhance the morphological and functional properties of scaffolds. Usually, ceramic-only scaffolds are prepared via high temperature sintering to ensure strength and stability. This prohibits *in situ* functionalization by biochemical agents, such as drugs and/or growth factors, and hampers replication of any biomimetic process, such as co-deposition and co-precipitation, that occurs in the physiological environment during natural bone regeneration. HAp nanocrystals serve as the chief building blocks of natural bone. Thus, thermally prepared ceramic scaffolds are entirely different from those prepared in a low-temperature environment. Biomimetic scaffold fabrication has been investigated in the context of ceramic-polymer hybrid systems [45,46]. Incorporation of active biomolecules, including growth factors, drugs, and even genes, is of great interest (please see below).

Glass and glass ceramics

Bioactive glass and glass-ceramics exhibit superb biocompatibility and can directly bond to living tissue [47,48]. Bioactive glass is amorphous, whereas glass-ce-

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ramic is a crystallized glass (the crystalline phase is created during thermal treatment) with a residual glass phase. Both materials trigger specific biological responses that enhance cell-material interactions. The products of rapidly degrading bioglass materials up-regulate gene expression to directly promote cellular activity, accelerating bone regeneration and formation of natural bonds with existing bone. The bioactive and bone-bonding mechanisms of 45S5 glass (developed by Professor Hench) have been widely studied and are described in detail elsewhere [49,50]. The best bioglass occupies a narrow range in the ternary phase diagram of Na_2O-CaO-SiO_2, with a constant P_2O_5 level. Several modifications (via addition of B_2O_3, TiO_2, Li_2O, FeO, and/or SrO) have been proposed [51-57]. All materials are prepared by melt quenching; the molten phase is quenched to stabilize the glass structure at room temperature. Fabrication of a bioglass scaffold bone substitute requires thermal reprocessing, triggering crystallinity and disruption of the glass structure. The glass phase is the key to biocompatibility; disrupting the phase has adverse effects. Thus bioglass scaffolds made via thermal reprocessing exhibit decreased biocompatible. Either a glassy or crystalline phase may form, depending on the nature of Si-O bonding in the glass structure. Nonbridging Si-O (compared to the bridging Si-O bond of SiO_2) allows bioglass to dissolve in aqueous environments; bioactivity follows. Sintering of bioglass changes nonbridging Si-O bonds to bridging Si-O bonds. Addition of K_2O, MgO, B_2O_3, and/or Al_2O_3 allows bridging Si-O bonds to be retained at higher sintering temperatures [58]. Several modified systems are already available, including 13-93, ICIE16, and BioK [59-61]. In a recent attempt bioactive glass system has been synthesized using conventional SiO_2-CaO-Na_2O-P_2O_5 composition, employing an ultrasound-assisted hydrothermal method [62].

Bioglass systems were prepared by sol-gel processing to avoid thermal treatment. This process allows creation of nanophase and nanoporous systems. This system invites entirely new applications in drug and growth factor delivery; the scaffolds are rapidly biodegraded, and thus offer enhanced biological responses [63]. Compositional variation has been reported during sol-gel processing [64-67]. Sol-gel bioglass of high silica content can be prepared in the absence of network modifier cations. Bioglass of similar composition to the melt-derived counterpart is also available. As stabilization of glass via conventional heat treatment alters glass properties, including particle size and density, stabilization of sol-gel-derived glass at room temperature would aid in biocompatibility. Such properties are also significant in the field of composites; bioactive glass powder is used to reinforce polymeric matrices of a low elastic modulus [68,69]. Textural features, including particle size distribution, specific surface area, and porosity, strongly influence bioactivity. Thus, the rate of formation of the interfacial hydroxyl carbonate apatite layer, which is structurally and chemically equivalent to the mineral phase of bone, is influenced by particle size range and the powder volume fraction during bone-bonding.

Bioglass of excellent bioactivity has been used to coat the surfaces of less biocompatible or bioinert materials, such as titanium or steel. Metallic materials are the first choice when mechanical stability is desired. However, the inherent lack of any direct bond with natural bone poses a significant postoperative risk of implant loosening and friction damage to surrounding tissue. Various methods have been used to modify metallic implant surfaces via coating with bioglass. Surface modification can also be achieved using calcium phosphate materials. Plasma spraying, electrophoretic deposition, and dip coating methods have been used to coat metallic scaffolds [70,71].

Biopolymers and hydrogels

The most diverse range of materials for hard tissue regeneration are biopolymers. Many such polymers are naturally derived and thus, are inherently safe. Many natural polymers have been used for bone tissue engineering; these include chitosan, hyaluronic acid, alginate, oxidized alginate, gelatin, pectin, starch; and proteins including soy, collagen, fibrin gels, and silk [72]. Synthetic polymers, including polyglycolic acid, polylactic acid, their copolymer PLGA, polyanhydrides, polycarbonates, polyphosphazenes, polyfumarates, and poly(butylene terephthalate)/poly(ethylene oxide), have also been used extensively among others [73-75].

Biopolymers are degradable under physiological conditions and the degradation products are metabolically discarded. Natural biopolymers have been used to

manufacture porous scaffolds. All natural polymer systems exhibit poor mechanical stability; and must thus be coupled with natural polymers and/orsynthetic biodegradable polymers to create stable systems. Composites have been used to physically or chemically crosslink and stabilize scaffolds. New fabrication techniques, (particularly those applicable at room temperature) including electrospinning and 3D printing, have rendered such approaches feasible. Various polymer scaffolds have been created via electrospinning of collagen, gelatin, PLGA, PEG, and PCL. The inherent nanoporous structure of an electrospun mat enables easy diffusion of nutrients through the scaffold. The high surface area of the mat improves tissue-material interactions. These features have been exploited to deliver growth factors and drugs enhancing bone tissue regeneration [76,77]. Modifications of basic electrospinning technology have allowed the creation of patterned mats, composite scaffolds, and complex interlayers in artificial biomimetic scaffolds [78-80]. Fig. 3 depicts a hybrid ceramic-polymer scaffold for artificial small bone with electrospun mat forming a bone conducive porous scaffold assembled around a porous zirconia core. Electrospun mats have been used to deliver drugs; the stability and biocompatibility of such mats are enhanced by the use of both synthetic and natural polymers, and HAp nanoparticles [81]. Fig. 4 showed polymer-ceramic hybrid electrospun scaffold as a career for bone morphogenetic growth factor and depicted the enhanced bone regeneration ability of the drug loaded scaffold using a rat calvarial defect model.

Natural polymers, including hyaluronic acid, collagen, gelatin, alginate, and chitosan, are obtained from animal and plants wherein they play physiologically important roles. Thus, these biopolymers are inherently favor cell-material interactions and have been widely used in bone tissue engineering [82]. These polymers undergo extensive hydration to form hydrogels under physiological conditions. Hydrogels serve as matrices

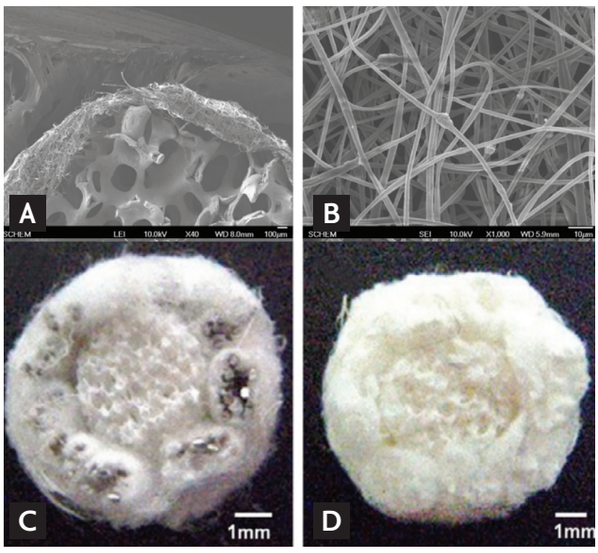

Figure 3. Scanning electron microscopic images of a ZrO2/biphasic calcium phosphate scaffold wrapped with poly(methylmethacrylate)-poly-ε-caprolactone-hydroxyapetite (PMMA-PCL-Hap) fibers (A), and the fibers themselves (B). (C) Bundles surrounded by PMMA/PCL/Hap; (D) the bundles after removal of the steel wires. Adapted from Kim et al. [80].

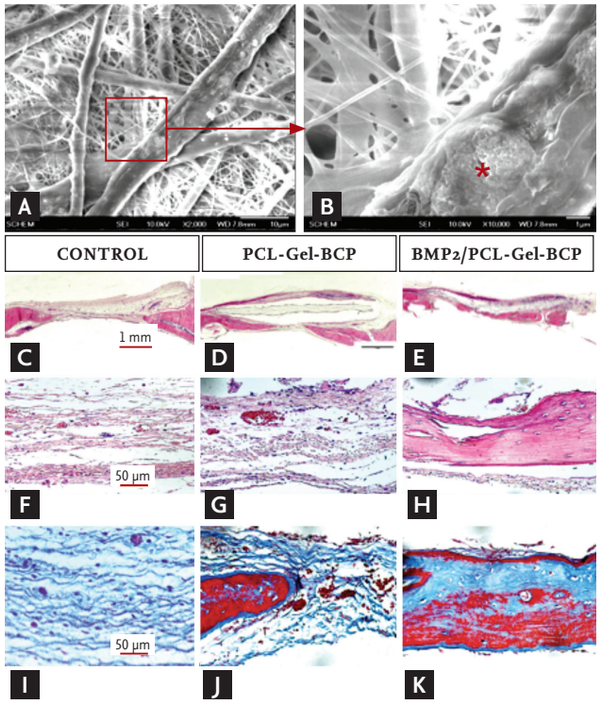

Figure 4. Scanning electron micrographs of polycaprolactone (PCL)-gelatin (Gel)-biphasic calcium phosphate (BCP) (A, B) electrospun scaffolds. Histological cross-sections of rat skull implanted with PCL-Gel-BCP and bone morphogenetic protein-2 (BMP-2)/PCL-Gel-BCP scaffolds, and a negative control (defect only) stained with H&E (C-H) and Masson's trichrome (I-K) 4 weeks after implantation. Adapted from Kim et al., with permission from Mary Ann Liebert, Inc. [81].

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for tissue engineering, mimicing the topology of the extracellular matrix, thus improving cellular adhesion and proliferation [83]. However, hydrogels lack the mechanical strength needed for weight-bearing, which is a serious disadvantage, rendering it impossible to use them alone as bone regeneration systems *in vivo*. Hybrid scaffold systems in which ceramic scaffolds are loaded with hydrogels impart mechanical stability [84]. Hydrogels have been admixed with growth factors and drugs to enhance bone regeneration [85]. Various types of hydrogels, containing drugs or growth factors, are being actively developed, drug encapsulation and subsequent release in the required zone are goals of such work.

Bone tissue engineering will potentially overcome many drawbacks of bone substitute scaffolds, including the lack of osteoinduction, poor vascularization, and delayed healing. It is possible to create tissues (including bone) on preformed scaffolds loaded with stem cells, which can differentiate into any desired cell type. Bone marrow- or adipose tissue-derived mesenchymal stem cells (MSCs) have high proliferative capacities and can differentiate into osteoblasts; thus, they have been used in bone tissue engineering [86,87]. Tissue engineering using adipose tissue-derived MSCs is attractive, such cells are abundant and donor site morbidity is minimal. Hydrogel scaffolds are indispensable in such work. Highly swollen hydrogels can suspend cells 3D and support nutrient diffusion to the cells. Additional modifications of hydrogels seek to enhance cell homing, by improving adhesion and attachment behaviors [88-90]; thesefeatures may enhance the biomimetic environment for the encapsulated cells. Hybrid system of hydrogel loaded into β-TCP/HAp ceramic scaffold was shown to facilitate delivery and distribution of cells in a mechanically stable manner [91]. Several hydrogels, including those made of alginate, fibrin glue, hyaluronic acid, chitosan, pluronic F12, thiol-norbornene, and PEG-poly (l-alanine) thermogel, promoted bone formation induced by MSCs and osteoblasts [92-95].

Often, use of a single polymeric hydrogel system is inadequate to overcome the drawbacks of rapid degradation and lack of stability in the physiological environment. Polyelectrolyte complexes (PECs) are formed by reaction between oppositely charged polymers [96]. Both cationic and anionic polymers are biocompatible and biodegradable, and form a PEC via weak bonding between the anionic and cationic groups enhancing the stability of the scaffold. PECs prepared from natural polymers, such as polysaccharides, have the additional advantages non-toxicity and bioabsorbability. Three-dimentional PEC scaffolds, fabricated via gas foaming, phase separation, electrospinning, or freeze-drying, have been used for cartilage repair and to reconstruct oral/maxillofacial defects [97,98]. Complexation can be enhanced by chemically modifying the functional groups via oxidization; this enhances interaction during polyelectrolyte complexation. Such scaffolds exhibit excellent degradation characteristics and form bone *in vitro* and *in vivo*. PEC scaffolds immobilize growth factors, allowing their controlled release to improve the functionality and performance of hydrogel scaffolds [96]. PEC microspheres, membranes, nanotubes, nanoparticles, fibers, and coarcevates have been developed, using different polysaccharides and polyamines [99-103]. These materials have been used for core encapsulation, surface adsorption, and matrix entrapment of various biomolecules and cells, including proteins, enzymes, and stem cells [104-107]. In particular, this approach has been successfully used to immobilize and deliver nanosized biomolecules, such as peptides and DNA plasmids [108,109].

Hydrogel systems have also been used as ECM-like materials in conjunction with HAp/TCP hybrid composites. Hydrogels can be modified in many ways to alter hydrophilicity/hydrophobicity and enhance biological activity. Freeze drying of hydrogel systems creates a macroporous microstructure enhancing cellular proliferation and growth. Composite scaffolds featuring stabilized macroporous hydrogels loaded into a macroporous biphasic calcium phosphate scaffold have been used to impart ECM-like attributes [110] affording superior bone regeneration and hydrogel stability Such a system is shown in Fig. 5 where Hyaluronic acid-Gelatin/BCP hybrid scaffold, where the biopolymer were loaded as hydro-gel, showed excellent bone formation potential.

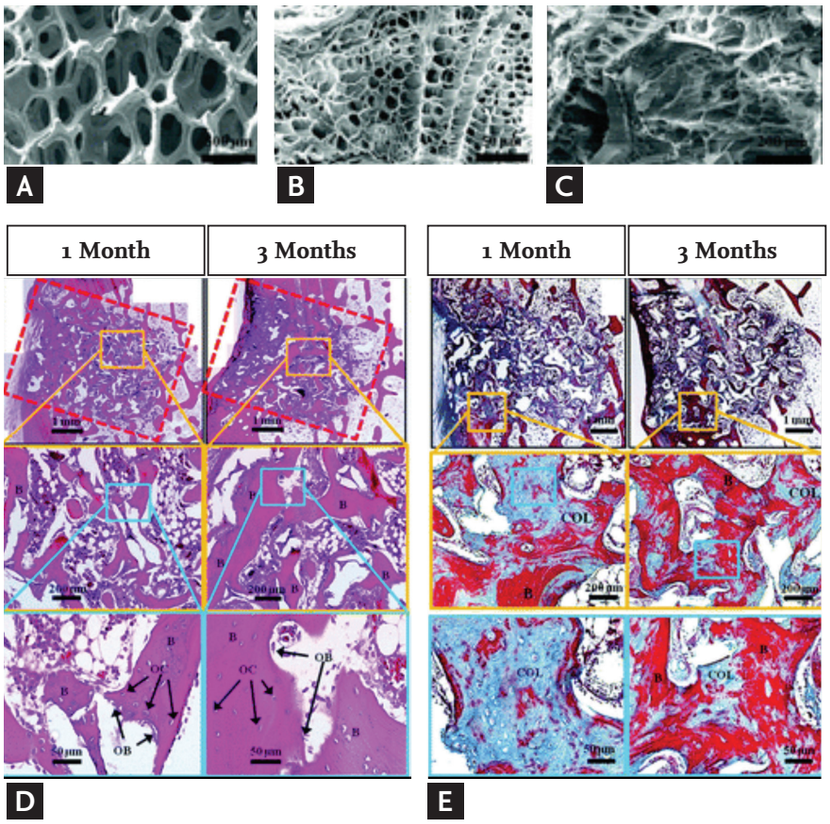

Figure 5. Morphologies of a sponge biphasic calcium phosphate (BCP) scaffold (A), a hyaluronic acid (HyA)-gel hydrogel (B), and a HyA-Gel/BCP scaffold (C), as revealed by scanning electron microscopy. (D) Histological sections of a rabbit femur implanted with HyA-Gel/BCP; H&E staining reveals new bone formation (B), osteocytes (OC), and osteoblasts (OB). Most pores are filled with new bone after 1 month; bone growth continued for 3 months. (E) Histological sections of a rabbit femur implanted with HyA-Gel/BCP; Masson's trichrome staining shows collagen (COL) deposited within the scaffold site (blue) and new bone formation (B) (red). Adapted from Nguyen et al., with permission from Mary Ann Liebert, Inc. [110].

OPTIMIZING THE MICROSTRUCTURE AND MORPHOLOGY OF BIOCERAMIC-BASED BONE SUBSTITUTES

Scaffold mechanical properties, such as compressive and bending strength, sharply decrease as porosity increases. Porous TCP ceramic scaffolds also lack fracture toughness [111]. Reinforcing with particles, fibers, and whiskers can improve the mechanical properties of ceramic scaffold [112-114]. *In situ* formation of nano-HAp whisker-reinforced porous TCP scaffolds has been reported [115]. Enhanced mechanical (load-bearing) and biological performance of PLGA-coated TCP composite scaffolds has been reported [116]. Porous HAp scaffolds with functionally graded core/shell structures exhibit improved mechanical properties [117]. Calcium silicate ceramic scaffolds toughened with HAp whiskers have been used in bone tissue engineering [118]. Silk has been used as a biocohesive sacrificial binder during fabrication of HAp load-bearing scaffolds [119]. Additive manufacturing (AM) techniques have been developed to enable production of free-form porous scaffolds with custom-tailored architectures [120]. Commercially available AM techniques include selective laser sintering, stereolithography, fused deposition modeling, precision extrusion deposition, and 3D printing. Detailed descriptions of the working principles, recent trends, and current limitations of these techniques are provided in several review articles [121-123].

Porous bioactive glass scaffolds with oriented microstructures have been prepared by unidirectional freezing of organic (camphene)-based suspensions [124]. Porous material (based on spongy titanium granules) has been used in bone tissue engineering [125]. Granular bone substitutes with unidirectional channels have been fabricated using a fibrous monolithic process; the pore geometry was regular and the mechanical strength increased [126]. Granular scaffolds have been prepared by electrospraying, microemulsion, and phase-separation methods.

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CLINICALLY AVAILABLE BONE SUBSTITUTES AND CLINICAL INVESTIGATIONS

Many commercial products are available, with specific applications, such as filling of bone voids, craniofacial bone voids; and facilitation of spinal fusion. These fillers have either been submitted to the US Food and Drug Administration for premarket approval, or have such approval. HAp ceramic grafts include Cerabone (Botiss biomaterials GmbH, Zossen, Germany), Endobon (Biomet Inc., Wilrijk, Belgium), Ostim (Heraeus Kulzer, Hanau, Germany), and Pro Osteon 500 (Interpore Cross, Irvine, CA, USA). ChronOS (Synthes, West Chester, PA, USA) and Vitoss (Orthovita, Malvern, PA, USA) are both made of TCP. Composite HAp and TCP ceramic grafts include BoneSave (Stryker, Hopkinton, MA, USA) and Mastergraft (Medtronic, Minneapolis, MN, USA). Calcibon (Biomet Inc.), ChronOS Inject, HydroSet (Stryker, Hopkinton, MA, USA), and Norian SRS (Synthes) are calcium phosphate cements. Bone Plast (Biomet Inc.), MIIG X3 (Wright Medical Technology Inc., Memphis, TN, USA), OsteoSet (Wright Medical Technology Inc.), and Stimulan (Biocomposites, Staffordshire, UK) are calcium sulfate-based systems. NovaBone (NovaBone, Jacksonville, FL, USA) and Vetros (Biomedtrix, Boonton, NJ, USA) are bioactive glass-based bone substitute. Many clinical evaluations have been performed using these systems. Maxillary sinus floor augmentation, reconstruction of periodontal osseous defects and the alveolar ridge, hip replacement, and anterior cervical fusion have been performed, and the results documented. Clinical trials, clinical series, and case reports have canvassed possible applications of tissue engineering using MSCs. Clinical trials on non-unions or delayed unions treated via cell therapy have been reported [127-130]. Several studies used cells and scaffolds [131,132]. These trials and studies have established the feasibility and (reasonable) safety of cell therapy-based approaches, and provide measures of the efficacy of bone healing.

CURRENT CHALLENGES AND FUTURE DIRECTIONS

The mechanical stability and osteointegrity of scaffolds that must bear loads long-term are critical problems. Insufficient vascularization of the interiors of thick bone substitutes, limiting cell ingrowth and survivability, is associated with poor osseointegration. Mechanical strength is heavily dependent on porosity and geometry of the scaffold, and pore and strut dimensions. These features are primary dependent on the type of fabrication process, specially for ceramics. In this case it is difficult to guarantee microstructural integrity and the required surface characteristics when the pore geometry is intricate and irregular. However, new manufacturing techniques like additive manufacturing, are promising; pore dimensions can now be precisely controlled, reducing surface irregularities on porous ceramics scaffolds. Other tissues, such as vascular and nerve tissue, must also grow to allow maturation of new bone within the porous structure. Vascular infiltration, nutrient transport, and cell migration must be optimal in any scaffold. Angiogenic growth factors and vasculogenic cell sources are being actively researched to resolve the poor vascularization of large bone graft substitutes. Bone formation involves a complex cascade of signaling pathways triggering a range of cellular and biochemical processes. Use of BMP-2 has been widely considered as highly effective to facilitate this process. However, bone tissue regeneration involves many growth factors and chemokines; the optimal mix of such materials and their synergies with other growth factors in terms of release kinetics and dosage requires further work. A controlled drug delivery system treating or preventing infection, in combination with a bone graft substitute, may allow optimal bone regeneration.

Successful clinical application of bone substitutes requires interplay among cells, biological signals, and biomaterials. Many unanswered questions and unexplored frontiers remain for the optimal use of nanostructured materials. Fundamental advances in in life and materials sciences are required.

CONCLUSIONS

Bone substitute development is a multidisciplinary research field, and significant improvements in current options, and new developments, are likely to increase

our basic understanding of the underlying principles. New biomaterials are dramatically broadening the options for advanced therapeutic remedies. Various material systems are being modified to elicit better biological and systemic responses. However, a perfect treatment option for bone defects remains elusive; the multidisciplinary approach seeks to overcome existing problems.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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