DEVELOPMENT OF COMPOSITE SCAFFOLDS FOR LOAD BEARING SEGMENTAL BONE DEFECTS

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Abstract

The need for a suitable tissue engineered scaffold that can be used to heal load bearing segmental bone defects (SBD) is both immediate and increasing. During the past 30 years various ceramic and polymer scaffolds have been investigated for this application. More recently, while composite scaffolds built using a combination of ceramics and polymeric materials are being investigated in greater number, very few products have progressed from laboratory benchtop studies to preclinical testing in animals. This review is based on an exhaustive literature search of various composite scaffolds designed to serve as bone regenerative therapies. We analyzed the benefits and drawbacks of different composite scaffold manufacturing techniques, the properties of commonly used ceramics and polymers and the properties of currently investigated synthetic composite grafts. To follow, a comprehensive review of *in vivo* models used to test composite scaffolds in SBDs is detailed to serve as a guide to design appropriate translational studies and to identify the challenges that need to be overcome in scaffold design for successful translation. This includes selecting the animal type, the anatomical location within the animals, choosing the correct study duration, and finally an overview of scaffold performance assessment.

Keywords: Bone grafts, scaffolds, composite, regeneration, in vivo

Introduction

Orthopedic injuries have been a major area of concern in medicine. In the late 1990's, it was estimated that 7 million fractures occurred each year in the US alone [1, 2], and the total medical costs associated with all musculoskeletal conditions added up to nearly $215 billion/year [1-3]. About 800,000 bone grafting procedures conducted annually in the US contributed to these costs [4]. Currently however, the bone fractures due to trauma related injuries account for more than 1.3 million procedures in the United States alone [5, 6]. The aging of the U.S. population as well as the increase in both frequency and severity [7] of these injuries among the elderly have resulted in a significant increase of orthopedic needs and are expected to increase in the near future[1, 4, 8-10]. In the military field, the combat wars in Afghanistan and Iraq have increased the number of trauma procedures performed on a daily basis [11]. These conflicts demonstrated the highest number of debilitating extremity wounds (54%) [11]. Indeed, out of more than 40,000 injuries and casualties sustained in these 2 wars, 82% of them resulted in at least one musculoskeletal extremity wound [12]. The extent of these incapacitating injuries includes soft tissue wounds (53%) and fractures (26%), most of which (82%) were severe open fractures [13].

Segmental Bone Defects

Natural bone has the ability to repair itself through a very well-studied healing cascade (Table 1). However, in the case of segmental bone defects (SBDs), the body cannot heal on its own and therefore they represent a significant challenge in the orthopedic community. SBDs are defined as injuries in which a section of bone is completely shattered and/or absent. Usually the size of the missing section is large enough that bone either cannot regenerate on its own (critical sized defects) or results in the formation of pseudoarthrosis (non-unions), mal-unions and loss of

function, making corrective surgery or additional fixation using metallic fixators a common complication [14-23].

SBDs can be brought on by on trauma, disease and age. Trauma can be related to fractures, sport and blast injuries. Diseases include bone cancer (osteosarcoma), tumor resection and reconstruction, osteoporosis, osteoarthritis[24], generic infections, congenital deformity corrections [25], pathological degenerative bone destruction and other degenerative diseases [20, 26, 27]. Table 2 summarizes the most common causes of SBDs and lists the risks associated with leaving these injuries untreated.

Bone Tissue Engineered Scaffolds for the Treatment of SBDs

Autologous bone grafting has long been considered the gold standard for treating SBDs, but synthetic alternatives are being increasingly investigated to overcome the problems of limited availability of secondary graft sites and associated donor site morbidity [28]. Within the past two decades the advent of tissue engineering has brought new ideas and the discovery and/or development of innovative biomaterials, for bone tissue engineering purposes [8]. The implantation of these tissue-engineered biological constructs, also known as scaffolds, has been a major advancement in the field of orthopedics [22, 29-36]. Before understanding what the ideal bone scaffold requirements are, it is first necessary to determine the material and biological properties of these constructs. A schematic of the relationship between material and biological tissue engineering scaffolds is seen in Figure 2; Tables 3 and 4 describes the ultimate biological and material requirements that a bone tissue engineered scaffold should possess.

Bioceramics for SBD Repair

As mentioned earlier, the inorganic component of bone is a carbonated Calcium Phosphate (CaP). One phase of the CaP ceramic is hydroxyapatite (HAp). Having a chemical

formula ($Ca_{10}(PO_4)_6(OH)_2$), stoichiometric HAp has a structure very similar to that of natural bone [24, 26, 34, 37-39]. For this reason, synthetic CaP materials including HAp are FDA approved and are among the most investigated materials for scaffold composition for over three decades [40-45]. CaPs differ from one another in origin, composition, morphology, and physicochemical properties. Most CaP ceramic materials are biocompatible, osteoconductive, have a bioactive surface, and can be biodegradable [18, 46], making them very well suited for bone repair, augmentation or substitution [21, 24, 47-49]. The osteoconductive properties of CaPs support tissue ingrowth, osteoprogenitor cell growth, and the development of bone formation[50] by promoting the attachment, proliferation, differentiation, and migration of bone cells [18, 26, 43, 49]. Their surfaces also allow for a direct, adherent, and strong bond with the bone tissue that can mediate an exchange of Ca^{2+} and P ions between cell matrix and substrate [51-53]. The structural or chemical aspect of CaP ceramics can be modified. CaP coating on metals enhance osteoconductivity by stimulating rapid bone growth onto its surface [54].

Current Bioceramics Being Investigated

There are many different types of CaP currently being investigated for tissue engineering purposes. HAp [23, 38, 40, 45, 55-58] is the most commonly used ceramic biomaterial in orthopedic applications because of its biocompatibility [59-61]. HAp scaffolds stimulate cell attachment, growth and differentiation [23, 42, 62], even though they degrade at a very slow rate [63]. β-Tricalcium Phosphate (β-TCP) is the second most widely used CaP ceramic in bioengineering, and its alkaline nature makes it a good candidate for hybrid scaffolds to counteract the acidity resulting from polymer breakdown [8, 27, 40, 58, 64]. β-TCP degrades much faster than HAp and is known for its excellent biocompatibility and osteoconductivity that stimulates the proliferation and differentiation of cells [40]. Because HAp and β-TCP are so well

suited for this application, scaffolds composed of a mixture of HAp and β-TCP (biphasic CaP - BCaP) are commonly reported in the literature. This combination is known for its osteoconductivity, bioactivity, biocompatibility and degradability [48] and has drawn the attention of researchers [40]. BCaP can have a controllable degradation rate [43] specific ceramic properties can be found in Table 5.

The other generic classes of ceramic materials commonly used for orthopedic fixation are the Bioglasses (BGs) and the CaP Cements (CPC). BGs are silicon-based materials that are known for promoting bioactivity, by being able to bond to bone by developing a bone-like apatite layers on its surface *in vivo* [49], promoting osteoblast differentiation and have similar degradation properties than HAp and β-TCP [25, 65]. A different material, Mesoporous BG (MBG), has been reported to have greater bioactivity than BG alone[59]. Nevertheless, being a ceramic, all BGs are very brittle [49, 59]. CPCs are combinations of soft form Dicalcium Phosphate (DCP) and Tetracalcium Phosphate (TTCP) that hardens when the two are combined [39, 66]. An advantage of self-hardening CPC is that it allows for surgeons to fill in the gap between the two bone endings and conform to the shape of the defects rather than matching the gap with standard size scaffold [1, 39, 66]. Bone cements are also readily available and don't cause any major issues with either immunogenicity or disease transmission [20]. However this material lacks a 3-dimensional porous structure and does not support bone growth well [20].

Polymer Properties

Polymeric materials are classified as natural and synthetic polymers. The former are further divided into proteins and polysaccharides. Natural polymers have weak mechanical properties but have hydrophilic surfaces that favor cell attachment and differentiation. Silk fibroin, collagen, and chitosan belong to this family. Silk is known for its biocompatibility, mechanical

properties, can be handled in many different ways and has relatively slow degradation rates [59]. Interconnected porosity can be achieved when the silk is prepared with organic solvent and salt leeching [62]. Collagen-I (Col-I) is the organic component of bone ECM. This makes it biocompatible and biodegradable [56] yet it lacks compressive mechanical strength and stiffness [55]. Col-I is sometimes associated with immunogenic responses from the host as well as pathogen transmission. However, the major problems associated with collagen are its cost, solubility and lack of commercial sources [58]. Gelatin collagen is the denatured version of collagen and does not have the same drawbacks as the naturally occurring counterpart [58]. It is inexpensive, widely available, and mass-producible [58]. Chitosan, a partial derivative of chitin, has received much attention because of its excellent biocompatibility and biodegradability [23]. It is only soluble at acidic pH (usually below 6.0), and when degraded it breaks down into non-toxic compounds [67].

In contrast, synthetic biodegradable polymers, such as polylactic acid (PLA), polyglycolic acid (PGA), or poly (lactic-co-glycolic acid) (PLGA), have a common disadvantage of possessing a hydrophobic surface that does not facilitate cell attachment after cell seeding [40, 59, 60]. However, these biodegradable polymers have attracted significant attention from the scientific community because of the ease with which these materials can be fabricated, and because of their relative high elasticity. In general polymers and organic scaffolds can be shaped into many different structures, creating a well suited architecture. In doing so, the mechanical properties of the material are usually lost, often resulting in very low or no compressive strength [58, 62]. These polymeric materials (PLA, PGA and PLGA) have been used as temporary extracellular matrices in bone tissue engineering scaffolds [60] as well as sutures, thereby providing evidence of safety [27]. Nonetheless, before PLGA polymers are metabolized into

their final product, they can release by-products such as lactic acid and glycolic acid. Being acidic these byproducts are known to cause bacteria-free inflammation or a foreign body reaction [64, 68]. There are however ways to neutralize these acids. One such way is by adding CaP ceramics, an alkaline material to the polymeric scaffold [64]. One other disadvantage for using the PLA, PGA, or PLGA is their degradation rates. Although it has been reported that degradation of these polymers can be adjusted in the laboratory by influencing molecular mass, co-monomer ratios, specimen size, configuration, and environmental conditions [8], degradation *in vivo* is difficult to control and remains among the major disadvantages for the use of these polymers [38].

Polycaprolactone (PCL) has been shown to have good mechanical properties, and have fully interconnected pores that increase biocompatibility *in vitro* [5, 6, 48]. PCL also has a slower degradation rate than other polymers [48, 49], exists in an elastic state at room temperature and has a low melting point of 60º, making it a good candidate for fabrication using fused deposition modeling [69]. Moreover when implanted, PCL scaffolds don't interfere with imaging techniques [6]. However, PCL's surface is still hydrophobic and presents low affinity towards cellular attachment. This makes it necessary to find a surface modification technique to make the surface more osteoconductive [69]. Regardless, the mechanical properties of these polymeric materials alone are not similar to those of native bone [38]. PU has been shown to be polymerized in a specific manner that favors biocompatibility with human cells and tissues. It has also been shown to have adequate mechanical properties due to its hydrogen bonds within the macroparticles that hold the structure together. However, after Pierschbacher and Ruoslahti found the protein sequence needed for the cell to attach to the surface [70], cell attachment and affinity increased [68].

Hybrid Bone Scaffolds

Even though there are many advantages to CaPs, the drawbacks are significant and include mechanical instability, difficulty at shaping and forming it into a specific architecture, its long degradation rate and possible bioactivity issues. The greatest concern with HAp specifically, and with ceramics in general, is that they cannot be used alone for load-bearing applications due to the brittleness (failure due to lack of plastic deformation) of these materials and the overall poor mechanical properties [20, 21, 23, 24, 26, 38, 48, 49, 55, 57, 59, 61-63, 66, 71-73]. In addition, CaP ceramics degrade very slowly [23, 26, 56, 72, 74, 75]. In contrast, fast CaP degradation is also not necessarily beneficial for tissue regeneration. It is known that β-TCP degrades at a much faster rate than HAp. However, faster degradation often equates to a higher level of Ca^{2+} being released, and any localized Ca^{2+} released higher than 10mMol [76] is detrimental, including being cytotoxic at the site. It is also known that the sintering process makes CaP ceramics stronger, but compromises the surface bioactivity of the scaffold due to the overall increase in crystal size and crystallinity [48]. It has been reported that β-TCP is mechanically weaker than HAp and when released the body cannot break it down into CaP, preventing strong bonding between the scaffold and the newly secreted bone [75]. In addition some researchers believe that CaPs are difficult to process [61] and are not porous enough [62]. Moreover, since it is difficult to shape sintered scaffolds as a result of their brittleness, surgeons are often forced to create an incision in the injured bone to match the scaffold when using ceramic scaffolds, thereby leading to more bone loss, trauma, and increased surgical time [1]. CPCs also cannot be used in load bearing applications because of their low mechanical strength, and lack of porous architecture that makes it difficult to integrate with adjacent bones.

By developing a hybrid scaffold composed of CaP and other polymeric materials, researchers believe that the original structure of bone could be recreated by taking advantage of the CaP's osteoconductivity as well as eliminating the brittleness of the scaffold. The combination of the osteoconductivity and the strength of CaP, in conjunction with the good workability and elasticity of bio-polymers, make hybrid composite scaffolds a very good candidate for bone tissue engineering [27, 57, 62]. Hybrid scaffolds are currently being studied and developed to try to simulate the organic (Col-I) and the inorganic (HAp) portion of natural bone [38, 61]. Hybrid scaffolds consist of polymeric matrices that are paired with ceramics [65]. However, there is a wide range of polymeric materials with different properties available (Table 6). Currently the challenge in developing a tissue engineered scaffold with ideal properties is to find a way to combine these completely different materials together, while maintaining a porous architecture and adequate mechanical properties that favor bone formation. Even though material selection for the scaffold has a direct impact on the biological and physical properties of the construct, there are some factors contributing to the low mechanical properties that are not related to the material used. The more porous the architecture of the scaffold is, the weaker its compression strength.

Properties of Currently Investigated Hybrid Scaffolds

Scaffold fabrication techniques currently used include casting and particulate leaching [5, 40, 68, 77-79], gas foaming [5, 77], freeze-drying [5, 25, 55, 77], electrospinning [9, 45, 77], thermally induced phase separation (TIPS) [5, 61, 77, 78], microsphere sintering [77], supercritical CO_2 technology [80], fused deposition modeling (FDM) [5, 6], 3D printing [5], in situ precipitation [57], thermally induced phase inversion [38], selective laser sintering [81], low temperature deposition manufacturing (LDM) [64] ceramic or polymeric coating with either

polymers or ceramic slurry respectively [27], solid-liquid phase separation (SLPS) [49], and a combination of these [8, 82].

As mentioned previously, mechanical strength is considered one of the most important properties and requirements of load bearing scaffolds. When designing a bone scaffold for tissue engineering, its mechanical properties should match that of natural bone. However, only a small percentage of investigated scaffolds in the literature are being tested for mechanical properties. Of the scaffolds tested compression strength and/or bending and elastic modulus strength were usually investigated. However, in bone, failure due to compression is very rarely seen; most fractures are due to torsion or bending forces [83]. In contrast, the elastic modulus property determines the slope of the stress-strain curve. Ceramics have been reported to have high elastic modulus and low ductility, whereas polymers, on the contrary, have lower hardness and modulus.

Although the concept of hybrid bone scaffold is a relatively new one, the literature has already been inundated with journal articles describing the technique and the properties of such scaffolds. A wide range of scaffolds with different properties can be found in the literature. It has been demonstrated that hybrid designs increase the overall mechanical properties of the existing scaffolds. There was a wide range of scaffolds fabricated and mechanically tested to determine their similarities to natural trabecular bone. Some implants exhibited very low compressive strength properties, 2-12MPa, which did correlates to the natural compressive strength of trabecular bone [23, 48, 57, 60, 65, 73, 82, 84]. These scaffolds should be considered for non-load bearing applications because they also exhibited lower modulus values (up to 25 MPa), which is well below the natural range of trabecular bone (50-500 MPa). These scaffolds

were generally very porous with ranges between 80 and 87%, which is essential for bone ingrowth.

Scaffolds investigated for load bearing applications generally had a compressive strength that ranged between 1 and 80 MPa [6, 21, 27, 38, 77, 78]. However, these samples with higher compression strength had porosities lower than 70%. The scaffold with the highest compression strength was investigated by Zhang *et al.* using an *in situ* precipitation technique. This HAp/PLLA composite scaffold reported compression values of 110-155MPa [57], although no information was provided on the porosity/pore size. Nevertheless, the lack of scaffold characterization for porosity or pore size renders inconclusive whether this scaffold has tissue regenerative properties. Overall scaffolds with lower compressive strength and modulus had a higher porosity which helped with bone growth, but scaffolds with a higher compressive strength needed for stabilization of SBDs had a lower porosity.

In Vivo Load Bearing Application Techniques

With many different *in vivo* animal studies used to investigate bone regeneration, deciding on the appropriate model and application can be challenging. First researchers need to determine an appropriate animal model to recreate a SBD large enough that cannot self-heal. Then proper anatomical location of the investigated scaffold in the animal should be decided, in order to recreate active loading on to the construct. At this point an estimate length of study to see perceptible results should be determined as well as valid control groups and an adequate analysis technique.

To date only a few hybrid design scaffolds have been investigated *in vivo*. However the literature is overwhelmed with the *in vivo* investigations of ceramic scaffolds [85]. This is because the concept of hybrid composite scaffolds is still relatively new, and to date insufficient

animal validation testing has been performed. Table 7 shows the summary of the reviewed studies and differentiates them between animal choice, anatomical location, length of study, control, and analysis of results.

Animal Choice

With many different animals used for research, choosing an adequate model platform for the scaffolds is essential. The most important consideration is that the animal chosen should have anatomical, physiological and pathophysiological analogies with humans. Previous models that have been used in the field vary in size. They include mice, rats, rabbits, dogs, sheep, and pigs. Once the similarities with humans are determined, it is important to keep in consideration the ability to physically provide care for many animals both during and after the testing. Other factors to keep in mind, yet not as critical, are the costs of acquisition and maintenance of the animals vs statistical size, tolerance to captivity and ease of housing [86]. Mice and rats are used to test basic cytotoxic properties of the scaffolds, and also they are used to implant scaffolds subdermally to initiate vascularization within the construct or test osteogenecity away from an existing bone source. These are the smallest animals on which bone regeneration is tested. Rabbits, also a small animal species, show easier, faster and more consistent bone healing. Unfortunately this model is limited by the size and maturity of the bones, and the weight of the animals [87]. Dogs have similar bone density to humans but have a series of disadvantages. Canines have faster solid bony fusion than humans, low non-union rates, high variation between breeds, and negative perception from the public. Sheep are also very similar to humans in body weight and bone dimensions (especially long bones). The drawbacks are age-dependent remodeling of bone around 7 to 9 years of age. The pig is the largest animal model used for SBD regeneration. They have a very similar bone density, anatomy, and microstructure to

humans. The main drawbacks, especially in load bearing scaffolds, are that the animals are extremely large and heavy, and have an accelerated rate of bone growth , which makes it difficult to differentiate between early and late remodeling [86].

Of the studies reviewed, the animal that was most often chosen was the rat [25, 40, 82, 88, 89]. Other studies also used rabbits [71, 90] and sheep [91]. Since hybrid scaffolds are still a relatively new concept in the bone regenerative field, it is understandable why researchers chose to test the construct on a small animal first to determine initial performance and change it accordingly. It is foreseen that this area of research will yield noticeable results in the near future to help fill the knowledge gap.

Anatomical Choice for Load-Bearing SBDs

Once the animal model is chosen, the next step requires determining an appropriate surgical site that will accurately test the behavior of the bone scaffold *in vivo*. For load-bearing applications it is necessary to use a limb or vertebral bone. This criterion allows focusing on the following bone choices: the femur or the tibia in the hind leg, and the ulna/radius or humerus in the fore leg, in addition to the lumbar vertebrae. In most of the studies reviewed, the femur was the preferred anatomical choice [25, 40, 71, 82, 88-90]. However, in the only large animal model reviewed (the sheep) the tibia was chosen with a triangular defect on the medial tibia plateau [91]. The femur is the largest long bone in most animals. Since humans stand up right, the femur directly supports the body's entire load, but in animals that use four limbs for stabilization, both the humerus and the femur distribute the animal's weight. Because of these characteristics the femur is preferred for testing load-bearing SBD model.

After choosing the animal and the bone to test, the specific location is selected. One model involves creating a midshaft defect and placing a matching size scaffold within it [88, 89].

This model requires fixation devices to be implanted around the surgical site to maintain the two endings of the scaffold from crushing the scaffold and from moving. This model tests the mechanical properties of the scaffold and the ability to regenerate large amounts of bone. Also this surgical site most closely resembles what occurs in clinical practice, as it gives an accurate representation of how well the scaffold will perform in a segmental setting. Extremity injuries from trauma affect all of the bone, not just parts of it. Another model involves creating only a partial defect. This is the case of rabbit radius/ulna, where only the radius is removed and the scaffold is placed next to the ulna [92]. This model might avoid the requirement of utilizing fixation devices to stabilize the animal; however, in the rabbit, this model is not truly weight bearing.

A third model involves drilling longitudinally through the cortical bone into the cancellous bone at the medial epicondyles of femur [25, 40, 71, 82]. The advantages of the medial epicondyle of the femur model is that no surgical fixation devices are required, and since the defects span both the cortical and the trabecular bone, histological evaluation *ex vivo* can determine how the bone regenerated and whether remodeling occurred. However, if the scaffold being investigated is made to be loaded unidirectionally then this model will likely fail *in vivo*. When implanted in the epicondyle, the scaffold is exposed mostly to circumferential forces and therefore, will sustain damages before it can regenerate. Overall this model is load protected and is seen more as a non-load bearing model. Thus, based on the scaffold properties and architecture the femur epicondyle might not be the best anatomical choice for this application.

Length of Study – Modeling Vs. Remodeling

The reviewed *in vivo* studies were analyzed for as little as 2 weeks [82], and for as long as 48 weeks [71]. When selecting the length of study for the *in vivo* study, there are times during

which different stages of healing occur depending on the animal model which was utilized for the study. While animal models do have species-dependent bone formation rates, typically the first 4 weeks of most animal studies are used to assess the host's response to the implant (biocompatibility). The first two months are then used for assessment of healing (modeling). Anything beyond that will be testing the remodeling process, during which the newly healed bone will gain strength. Of the reviewed studies two rat femur models were investigated for less than 4 weeks [82, 89]. This step is of critical importance for hybrid biomaterials, since they have a high risk of causing acute inflammatory reactions depending on what polymeric material was used when constructing the scaffolds. Two other studies reviewed were performed for 1 to 2 months to assess bone healing (modeling) [25, 91], and 4 other studies instead investigated long-term remodeling of bone [40, 71, 88, 90]. Perhaps if the researcher has early *in vitro* data showing biocompatibility of the scaffold, the animal study will have different time intervals that demonstrate the healing process through early response, modeling and remodeling processes.

Analysis of the SBD Models

After developing, characterizing, and implanting the scaffold in an animal, the test subjects are sacrificed so that the ability to regenerate bone can be assessed. There are a number of techniques used by researchers to investigate bone growth: histology, micro-Computed Tomography (μ-CT) analysis and bone density scans. Load bearing scaffold should also be investigated for their ability to gain strength while implanted. This is accomplished through the use of mechanical testing. Using histology, the tissue where the bone scaffold was implanted is processed and stained on thin sections that can be observed by microscopy.

Most researchers use histology (either decalcified or undecalcified) to analyze new bone volume, new soft tissue formation, and area of scaffold resorbed. In hybrid scaffolds, the foreign

body reaction should also be analyzed. The other common characterization performed on explanted tissues is μ-CT. This technique is used to analyze new bone formation, regeneration patterns, and bone density. However, because μ-CT reconstructions are very subjective to the operator, there is always the need for histology to support the data. The last test that should be required after investigating a load-bearing scaffold is mechanical analysis of the construct post implantation. The excised tissues should be tested for ultimate compression, tensile strength and elastic modulus. Surprisingly, only two long term studies performed mechanical analysis of the defect after the sacrifice of the animals. Without this test it is difficult to determine whether the implant was successful at recreating a load-bearing structure.

When analyzing the performance of the scaffold it is usually compared to positive and negative controls and preferably both. In hybrid scaffolds a control is usually the ceramic or polymer alone, and often an autograft from the same donor. It has been previously shown that there is no need to compare scaffold performance to a defect only control since it has been shown repeatedly that an empty critical sized defect does not heal on its own [88].

Summary and Perspectives

The clinical issues surrounding the treatment of load bearing SBDs need a multi-pronged approach for treatment. Current strategies focus on a combination of osteoconductive substrates delivering osteoinductive growth factors and osteogenic cell sources. This review focuses on the development of composite hybrid scaffolds composed of ceramic and polymeric materials that provide the mechanical stability, structure and calcium source required to serve as a suitable osteoconductive surface in healing SBDs. This information can be used to develop and implement *in vivo* testing of the investigated implants by selecting the appropriate animal model,

the most accurate anatomical positioning in the bone, determining the length of the study and finally the analysis of the samples.

Clearly there is a need for a hybrid scaffold technology with the strength of the ceramics and the elasticity of polymers that will move the field closer to a functional load-bearing scaffold. When developing the scaffolds it is important to take into consideration the requirements of scaffolds as well as the drawbacks of the materials being used, so that the researcher can then test accordingly. This includes testing for cytotoxicity, and mechanical properties. Moreover, when investigating a load-bearing scaffold, the experimental mechanical properties should always be reported. The true difference between a bone scaffold and the load-bearing scaffold resides in the mechanical properties. Surprisingly this was not the trend seen in the literature, where many load-bearing scaffolds should have been considered safe to handle, but not to load. There are many journal articles that investigate *in vitro* hybrid bone scaffolds, but very few that have moved on to *in vivo* testing. Of those few, the majority have used small animal models. It is expected that as more suitable composite scaffolds perform well *in vitro*, *in vivo* characterization will significantly increase.

After years of focusing on purely ceramic or purely polymeric scaffolds, researchers have started to consider hybrids, and despite the fact they have high potential, the field is still far from having a scaffold that can be fully loaded that supports viable bone regeneration in a reasonable time after the implant. In the best case scenario these revolutionary scaffolds would eliminate the need of fixation devices, or at least they could minimize their need. They could also be used to deliver growth factors to accelerate healing from the scaffold surface and to deliver appropriate stem cells into the wound environment to tackle critical sized defects. The overall

cost of surgeries would decrease, the healing time would be decreased significantly, and there would not be a need for multiple revision surgeries once these technologies are optimized.

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LIST OF TABLES

Table 1
Stages of the bone healing cascade. Adapted into a table form from [93]

Early Inflammatory Stage	***Hematoma development*:** After injury blood permeates into the wound site and a hematoma forms within hours. ***Granulation tissue formation*:** Inflammatory cells including macrophages, monocytes, lymphocytes and polymorphonuclear cells infiltrate the wound through blood. Fibroblasts also infiltrate the bone through the mediation of prostaglandin. This mixture results in the formation of granulation tissue. ***Ingrowth of vascular tissue*:** Inflammatory cells forming the granulation tissue will also stimulate vasculogenesis. ***Migration of mesenchymal cells*:** This is the third and final process that is stimulated by the inflammatory cells found in the wound.
Repair Stage	***Stroma formation to support vasculature*:** During the first stage of the repair process fibroblasts deposit stroma . This is aimed at supporting vasculature ingrowth. ***Vascular ingrowth progress*:** After the stroma is formed, vascular tissue can continue to grow and distribute nutrients to all areas of the wound. ***Mineralization of the osteoid*:** The collagen matrix is deposited by osteoblasts, and subsequently is mineralized. ***Soft callus formation*:** Mineralization of the osteoid leads to the formation of a soft callus around the wound area. The soft callus is a very weak structure that hardens as the callus ossifies. Any movement between bone during this stage will result in macrotrauma potentially disrupting the healing cascade. ***Woven bone formation*:** As the callus ossifies, the opposite bone extremities are bridged by woven bone formation.
Remodeling Stage	***Bone returns to original structure*:** The last stage of bone healing is also the longest, as it takes a minimum of 3 months and continues for the life of the bone. A factor that affects bone remodeling time is adequate mechanical loading. This allows for the organic matrix to mineralize where needed and for bone to be resorbed where it is not.

Table 2
Different causes of SBDs and consequences of untreated SBDs.

Causes of SBDs	
Trauma Injuries	***Blast injuries:*** These types of injuries are very common amongst military personnel. The injury that results from bomb or explosive detonation is of significant nature because it not only affects the skeletal structure, but musculature, blood supply, and nervous system. ***Fractures and sport injuries:*** These types of fractures are often segmental and are characterized by shattered, fragmented pieces of bone. After the broken pieces are surgically removed, a gap is left between the opposite extremities.
Diseases	These include bone cancer (requiring tumor resection and reconstruction), osteoporosis, osteoarthritis, generic infections, congenital deformity corrections, pathological degenerative bone destruction and more. The commonality among these diseases is that the bone is either abnormally weak or needs to be removed to prevent spreading of the disease. As a result, large segments of bone are missing or are surgically removed and need to be replaced.
Complications from untreated SBDs	
Mal-union	The two fractured bone ends are able to bridge, although they are not symmetrically aligned. As a result the new bone is still susceptible to fracture. This is commonly seen in undiagnosed/untreated fractures and leads to loss of bone function.
Non-union (Pseudoarthrosis)	The two fractured bone ends are not able to heal and no bridging occurs between them. This is seen in critical defects. Another case of non-union is observed when there is too much movement between the bone ends (insufficient surgical fixation) and the callus is never able to ossify and harden. In many cases surgical intervention is needed to resolve the problem and avoid further loss of bone function.

Table 3
Bone tissue engineered scaffold requirements

Biological Properties	***Osteogenicity:*** Ability of a bone scaffold to allow bone cells to induce differentiation from uncommitted mesenchymal cells to preosteoblast lineage, and to secrete and mineralize extracellular matrix. ***Osteoconductivity:*** Quality of a bone scaffold having a surface that is bioactive and promotes cell attachment and migration, as well as penetration within the construct. ***Ostoinductivity:*** Ability of a bone scaffold to not only support but to initiate bone growth through growth factor or hormone release. ***Biocompatibility:*** Ability of a bone scaffold to not cause an immune reaction or rejection when interacting with the body. ***Promotes Vasculogenesis:*** Ability of a bone scaffold to promote and/or easily allow for vasculogenesis to occur within the construct.
Material Properties	***Mechanical Stability:*** Quality of a bone scaffold to have an ultimate compression strength that is similar to bone, while maintaining the appropriate architecture. ***Biodegradability:*** The quality of a bone scaffold to degrade naturally without creating toxic byproducts while being resorbed. The rate of degradation should match the rate of new bone formation, to avoid possible gaps in regeneration. ***Architecture:*** The quality of a bone scaffold to have a very open porous structure that is interconnected throughout the construct. This allows for greater attachment surface area, higher cell density, and easier nutrient/growth factor flow within the construct. ***Pore size/ Porosity:*** Quality of a bone scaffold to have pore size and porosity percent similar to established guidelines. Ideal pore size ranges from 300 to 900um in diameter, whereas overall porosity ranges from 60 to 99%.

Table 4
Importance of mechanical stability in Segmental Bone Defects.

Mechanical Properties	Ultimate strength of cortical bone ranges between 100-230MPa. Any scaffold that does not match this strength requires surgical fixation to prevent crushing/failure of the implant. Because bone is a mechanosensor organ, it is believed that a scaffold that is loaded cyclically will benefit from faster healing time.
Surgical Fixation	Current bone tissue engineered scaffolds require surgical fixation of the fractured extremities to prevent movement between the bone endings. This will allow for callus formation and ossification to occur. Surgical fixation devices include screws, hardware, intramedullary nails and are often made of metals, specifically titanium or titanium alloys.
Stress Shielding	Condition caused by the use of surgical fixation devices in load bearing bones. Because metals have higher modulus and compression strength, they support nearly all of the weight. In return, the fractured bone does not sense a significant change in mechanical activity, leading to a loss in bone density over time.

Table5
Significant physical properties of several of the most common bioceramics used as biomaterials [94]

Material	Density (g/cm^3)	Tensile Strength (MPa)	Compressive Strength (MPa)	Modulus (GPa)	Fracture Toughness K_{1c} (MPa $m^{1/2}$)	Hardness (Knoop)	Mass Fraction α (ppm/°C)	Fracture Surface Energy (J/m^2)	Poisson's Ratio	Thermal Conductivity k ($Wm^{-1}K^{-1}$)
Hydroxyapatite	3.1	40-300	300-900	80-120	0.6-1.0	400-4500	11	2.3-20	0.28	N/A
Tricalcium Phosphate	3.14	40-120	450-650	90-120	1.2	N/A	14-15	6.3-8.1	N/A	N/A
Bioglasses	1.8-2.9	20-350	800-1200	40-140	~2	4000-5000	0-14	14-50	0.21-0.24	1.5-3.6
Wollastonite glass ceramic	3.07	215	1080	118	2	N/A	N/A	N/A	N/A	N/A
SiO2 glass	2.2	70-120	N/A	~70	0.7-0.8	7000-7500	0.6	3.5-4.6	0.17	1.5
Al2O3	3.85-3.99	270-500	3000-5000	380-410	6-Mar	15000-20000	6-9	7.6-30	0.27	30
Zirconia Ceramics	5.6-5.89	500-650	1850	195-210	8-May	~17000	9.8	160-350	0.27	4.11
Si3N4	3.18	600-850	500-2500	300-320	3.5-8.0	~22000	3.2	20-100	0.27	25-Oct
Silicon Carbide	3.10-3.21	250-600	~650	350-450	6-Mar	~27000	4.3-5.5	22-40	0.24	100-150
Graphite	1.5-2.25	5.6-25	35-80	3.5-12	1.9-3.5	N/A	1-3	~500	0.3	120-180
MULTI ceramics	1.5-2.2	200-700	330-360	25-40	N/A	N/A	1-10	N/A	0.3	2.5-420
Carbon fiber	1.5-1.8	400-5000	330-360	200-700	N/A	N/A	N/A	N/A	N/A	N/A
Glassy carbon	1.4-1.6	150-250	~690	25-40	N/A	8200	2.2-3.2	N/A	N/A	N/A

Table 6
Significant physical properties of several of the most common polymers [95].

Polymer	Glass Melting Point Tm ©	Glass Transition Point	Biodegradation time (months)	Compressive Strength	Tensile Strength	Modulus
PE				0.1-1.0	0.4-4.0	170
PMMA				3.5	1.5	160
PDLLA	Amorphous	55–60	12–16	Pellet: 35–150*	Film or disk: 29–35	Film or disk: 1.9–2.4
PLLA	173–178	60–65	>24	Pellet: 40–120*	Film or disk: 28–50 Fiber: 870–2300	Film or disk: 1.2–3.0 Fiber: 10–16
PGA	225–230	35–40	6–12		Fiber: 340–920	Fiber: 7–14
PLGA	Amorphous	45–55	Adjustable: 1–12		41.4–55.2	1.4–2.8
PPF		N/A	Bulk	2–30*		
PCL	58	−72	>24			
PHA and blends	120–177	−2 to 4	Bulk		20–43	
Poly(anhydrides)	150–200		Surface	30–40*	25–27	0.14–1.4
Poly(ortho-esters)	30–100		Surface	4–16*		2.5–4.4
Polyphosphazene	−66 to 50	242	Surface			

Table 7
Summary of the in vivo preclinical studies reviewed in which hybrid scaffolds were tested

Author, Year	Ceramic Material	Polymeric Material	Animal Choice	Anatomical Choice	Length of study	Time points	Sample Size	Defect Size	Scaffold Size	Control
Cao 2010	β-TCP	PGA	Sprague-Dawley Rats	Femur; Medial Epicondyle	12 wks	0, 14, 30, 90 days	5/timepoints /group	3mm diameter; 2mm depth	n/a	(+) HAp (-) no implant
Chu 2007	TCP	PPF	Long Evans Rats	Femur	15 wks	6 and 15 wks	4 or 7/timepoint/ group	5mm	OD: 4mm - ID: 2mm; Height 5mm	no BMP
Jegoux 2007	BCaP	Collagen	New Zealand White Rabbit & Beagle Dogs	Femur	18 wks	18wk	6 rabbits, 6 dogs	20mm	5x5x5mm	
Guda 2011	HAp		New Zealand White Rabbit	Radial Diaphysis	8 wks	4 and 8 wk	12/timepoint /group	10mm		(+) autograft (-) no implant
Ignatius 2001	β-TCP	PLA	Merino Sheep	Tibia	8 wks	6, 12, 24 months	6/timepoint /group	n/a	24mm length, 14mm wide, 6 mm thick	(+) TCP (-) Autograft
Jayabalan 2010	HAp	HT-PPFhm	Rabbit	Femur	48 wks	12, 24, 48 wks	2/timepoint	4mm diameter; 2mm depth	n/a	(-) no implant
Lickorish 2007	TTCP and DCPA	PLGA	Winstar Rats	Femur	2 wks	2 wks	n/a	2.3mm diameter	2mm diameter	PLGA Scaffold
Rai 2010	TCP	PCL	CBH/Rnu Rats	Femur	3 wks	3wks	6/timepoint	8mm	8mm high, 4 mm diameter	(-) non-seeded
Xu 2011	Bioglass	Collagen-Posphatidyl serine	Sprague-Dawley rats	Femur	6 wks	3day, 3 and 6 wks	3/timepoint /group	3.5mm diameter 4.5mm diameter	n/a	no Phosphatidyl Serine

Table 7(Continued)

Author, Year	Type of Testing	Type of Histology	Histological Parameters Analyzed	μ-CT Parameters Analyzed	Mechanical Testing
Cao 2010	μ-CT, Bone Mineral Density (New Bone Quantity), Histology, Biodegradation	Decalcified Histology	Area of Material in Defect, New Bone Volume/Total Volume; Percent Material Biodegradation	Bone Reformation	No
Chu 2007	Radiograph, μ-CT, Histology	MMC Histology	New Bone Formation	Callus and Scaffold Volumetric Bone Mineral Density	4 Point Bending
Jegoux 2007	Polarized Light; μ-CT, SEM	Gkycol Methacrylate	Used Thick Histology Sections for Observation Under Poler	Bone at the Center, Superior and Inferior Quarter of the Implant	No
Guda 2011	Radiograph, μ-CT, Histology	MMC Histology	Mineralized Bone, Fibrous Tissue	Bone Regeneration Pattern, Bone Density, Bone Growth Profiles, Overall Bone Volume;	4 Point Bending
Ignatius 2001	Mechanical, Histology	Undecalcified Histology	New Bone Formation, New Soft Tissue Formation, Remaining Implant Components	No	Compression of 5X5x3mm Cubes
Jayabalan 2010	Histology	Resin Histology	Foreign Body Giant Cell, Bone Growth,	No	No
Lickorish 2007	Histology	Decalcified Histology	Fibrous Tissue Formation, Bone Ingrowth, Foreign Body Reaction	No	No
Rai 2010	Radiograph, μ-CT, Histology	Decalcified Histology	Presence Of Fibroblasts, Chondrocytes, Woven Bone	New Bone Formation	No
Xu 2011	Histology; Radiography	Decalcified Histology	Inflammatory Reaction, New Bone Formation, Scaffold Resorption	No	No

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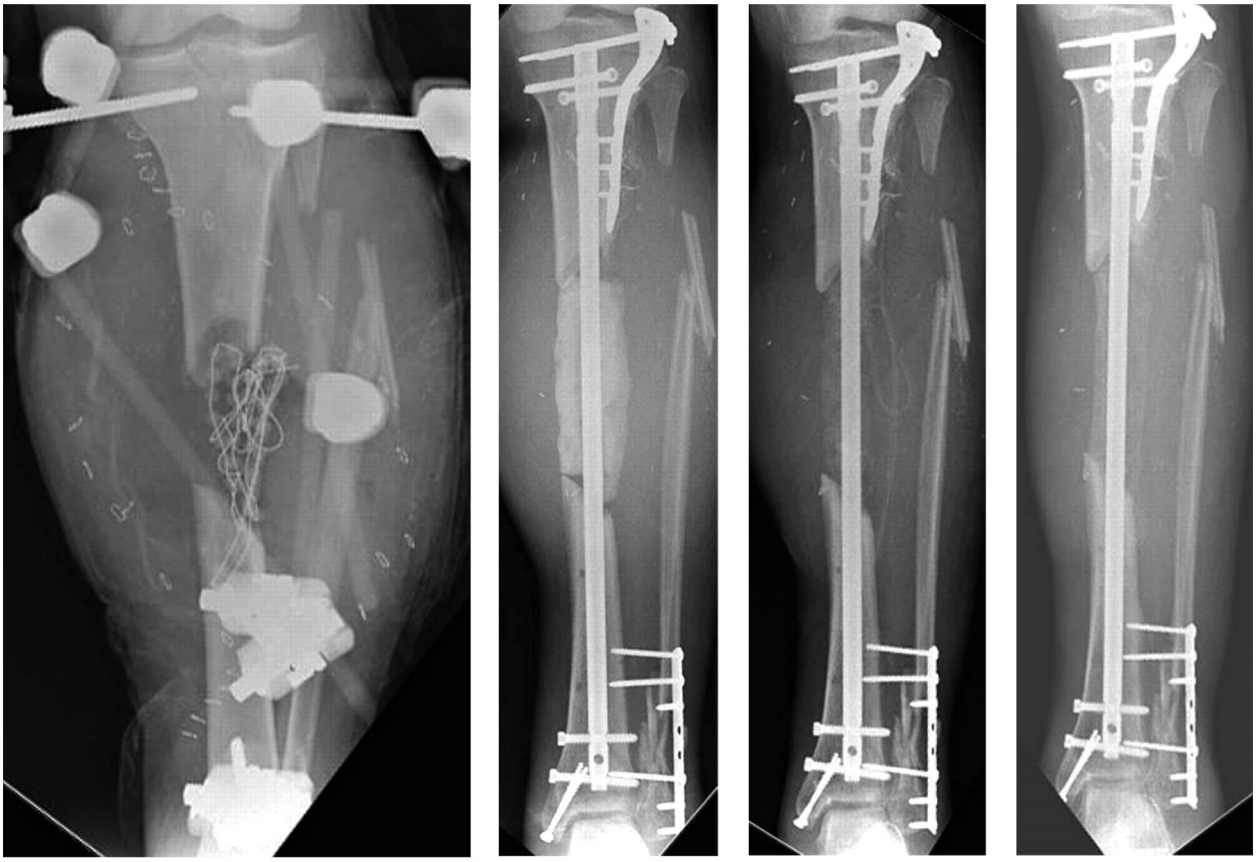

Figure 1

A) radiograph of open tibial fracture with segmental bone loss as a result of trauma injury; *B)* radiograph of the damaged tibia after intramedullary nail and internal fixation at the extremities. The defect is filled with cement spacer that had been previously impregnated with antibiotic; *C)* radiograph of the defect after 3 and *D)* 4 months. Bone healing never occurred, and the fracture is considered a non-union. Printed with permission from Dr. Steve Morgan [96].

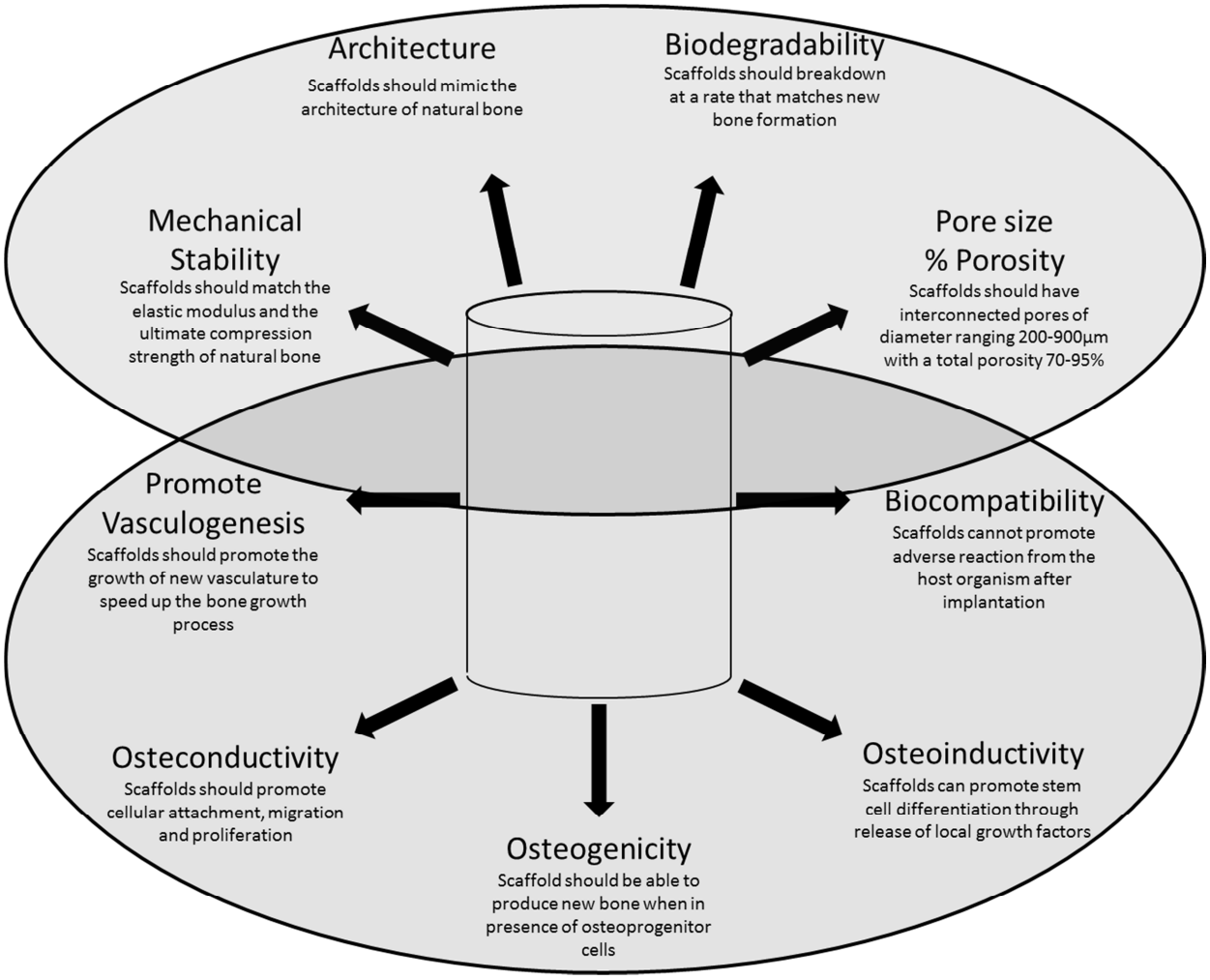

Figure 2

Diagram showing material (top) and biological (bottom) properties of ultimate regenerative bone scaffolds. It is necessary for engineered scaffolds to have both of these properties to promote bone growth. One class of properties alone is not sufficient to promote bone growth in a timely manner. Data from [21, 23, 24, 28, 48, 49, 81, 97, 98]

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