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# Bone tissue engineering: state of the union

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**The quest to surpass the clinical efficacy of the allogeneic bone graft has had limited success, an outcome that is symbolic of tissue engineering as a whole. In this 'State of the Union'-type review, we highlight recent advances in the design of bone regenerative therapeutics using the primary elements of stem cells, growth factors and scaffolds, and identify major obstacles in their paths to the clinic. We underscore the need for rigorous performance criteria in the design of holistic tissue regenerative therapeutics, and an increased emphasis on the product production, storage and handling issues that will ultimately influence clinical success.**

## Introduction

Tissue engineering and regenerative medicine focus on the restoration of form and function to tissue insufficiencies. In the context of bone, a clinical osseous insufficiency is defined as a discontinuity in bone integrity resulting from trauma, congenital malformation or surgical resection [1]. Of particular importance is the critically sized osseous deficiency (i.e. defect), which is one that will not regenerate spontaneously over the patient's lifetime and, therefore, requires surgical intervention. The quest for regeneration in critically sized bone defects has inspired a search for clinically efficacious tissue-engineering therapeutics that has spanned over two decades.

Bone has a remarkable intrinsic ability to remodel and spontaneously regenerate. Bone tissue exists in a dynamic state of homeostasis mediated by bone cells: namely, osteoblasts, osteoclasts and osteocytes. As a consequence of osseous trauma (a common example being a class of trauma clinically recognized as 'fracture'), a powerful cascade of osteogenic events occurs. Included in this cascade are myriad cell phenotypes, biological signaling molecules and matrix proteins that are structured spatially and temporally to produce the desired outcome: fracture repair. Therefore, we can categorize a spontaneously healing fracture as a subcritically sized defect, for which the typical outcome is the restoration of form and function to the locale of the defect. However, this intrinsic fracture healing (i.e. spontaneous bone regeneration) does not extend to bone insufficiencies greater than a critical size. The question then follows: how do we extend intrinsic bone regeneration of a fracture to discontinuities that are critically sized?

Here, we attempt to answer this daunting question. We highlight current clinical practices for bone regeneration and underscore key components of tissue engineering that may have an impact on clinical care for patients. We discuss progress in designing bone regenerative therapeutics along with the challenges encountered, and provide potential solutions founded on biological wisdom.

## Performance criteria for bone regeneration

A bone regenerative therapeutic to treat patients must fulfill basic, fundamental criteria in the clinic that include safety, predictability and reproducibility in providing the clinical outcome of regenerating bone tissue. A tissue regenerative therapeutic should, in particular, also include the properties of osteogenicity, osteoconductivity and osteoinductivity. Osteogenesis is the process during which osteoprogenitor cells mature into osteoblasts, which subsequently mineralize and form bone tissue [2]. Osteoconduction refers to the process by which bone forms on a surface. With respect to biomaterials, osteoconduction is measured by the ability of an implant to support the growth of bone in a 3D manner at a defect site [3]. Finally, osteoinduction, as defined by Marshall Urist, is the process of recruitment of immature osteoprogenitor cells to a wound site and the subsequent differentiation of these cells into osteoblasts under the influence of a diffusible bone morphogenetic protein [4].

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## Current clinical practices

Autogenous bone grafts and allogeneic bank bone are the contemporary therapies that fulfill the majority of the stated performance criteria. Autogenous bone grafts (i.e. autografts), in particular, are clinically approved therapies that excel in demonstrating the biological properties of osteogenesis, osteoconduction and osteoinduction. They are secured from a donor site on the patient and are surgically implanted to the recipient site; that is, the bone-deficient locale [5]. Clinical successes with autografting are notable; however, the trauma associated with donor site surgeries can be problematic [6]. Consequently, allogeneic bank bone is the alternative of choice for most surgeons. Allogeneic tissues (i.e. allografts) obtained from human donors are processed in a highly regulated, standardized manner at a tissue bank and are used to treat patients. Through typical allograft processing, the osteoinductive property of the allograft is neutralized, although the characteristics of osteogenesis and osteoconductivity remain [7,8]. Furthermore, allogeneic tissues avoid the need for an additional surgical donor site on patients, although there remain concerns regarding disease transmission, immunogenicity and sufficient donor tissue availability [9]. The concerns regarding bone tissue transplantation necessitate the search for synthetic tissue-engineered alternatives.

## The tissue-engineering approach

The building blocks to assemble tissue-engineered therapies that fulfill the demanding biological performance standards required for bone regeneration include cells, signaling molecules and scaffolds. The conceptual simplicity of this tissue-engineering triad is misleadingly seductive; transforming this concept to reality remains an elusive vision. These unanticipated challenges have obstructed progress and dampened optimism. A major impediment to the development of successful tissue-engineering products is the nebulous performance criteria for therapeutic design. Simple statements championing the amalgamation of cells, growth factors and matrix elements have not yet translated into direct clinical success. Rather, *clinically* specific questions relevant to this triad must be asked and answered; our answers must include rigor and quantifiable performance standards. Important questions include: (i) what combinations of cells, biologicals and matrix elements are necessary? (ii) What are the required physiological and therapeutic doses for cells and biologicals? (iii) What temporal and spatial distribution of triad elements is required for tissue regeneration, and what are the physiological dynamics and kinetics associated with these distributions? (iv) Does each clinical application have its own customized suite of unique, performance-specific design criteria? Finally, (v) How do we navigate the necessary regulatory pathways caused by the requirement for increased sophistication in tissue-engineering therapeutics?

To answer these questions, engineers, biologists, chemists and material scientists must engage along a common front, coalescing multidisciplinary principles into tissue-engineering design and development code. We must push beyond philosophical generalities of the tissue-engineering triad into a clinically guided reality for therapeutics. Specifically, we must achieve the precise, predictable coordination of cell responses with matrix scaffolds and biological signaling molecules to match the dynamics of physiological fracture repair.

## Stem cell approaches

During fracture healing, undifferentiated cells are recruited on site and cued by biomechanical and biochemical signals to differentiate and rebuild the wound site. The essence of this cascade is the recruitment of phenotypically specialized cells to re-establish tissue continuity at the wound site and restore osseous form and function. The involvement of exogenous progenitor cells in these processes is pivotal. The logic for their inclusion in regenerative therapeutics is founded on bypassing the progenitor cell recruitment stage and segueing directly to phenotypic differentiation.

The implantation of exogenous pluripotent stem cells has been central to modern regenerative medicine approaches. The isolation of human embryonic stem cells (ESC) from blastocytes by James Thompson in 1998 [10] demonstrated the potential for stem cells to differentiate into specialized tissue phenotypes. In addition to their potential to form cells from all three germ layers, these immunopriviledged ESC provided an exciting prospective therapeutic for tissue-engineering applications. Subsequently, the ethical debate sparked by stem cell isolation from fertilized embryos tempered the initial enthusiasm; however, the landmark discovery of induced pluripotent stem cells (iPSC) in 2006 by Yamanaka [11] rekindled the passion for stem cells in tissue engineering. Yamanaka demonstrated that somatic, terminally differentiated cells could be reprogrammed into an ESC-like state by the overexpression of major ESC markers. This process results in the production of cells that are capable of self-renewal and differentiation into specialized cell phenotypes, including neural cells [12], cardiomyocytes [13] and hematopoietic cells [14].

The iPSC revolution continues to inspire contemporary tissue engineers. Initially, the genes required for the reprogramming of adult cells to an ESC-like state included the oncogenes Kruppel-like factor 4 (*KLF4*) and *C-MYC*, in addition to octamer-binding transcription factor 4 (*OCT4*) and SRY (sex determining region Y)-box 2 (*SOX2*) [11]. In 2007, studies showed that *C-MYC* was not essential to achieve reprogramming of somatic cells and that the reduced efficiency of omitting it was offset by the elimination of an oncogene [15]. Further progress has been made regarding the methods of reprogramming. Initial iPSC efforts relied on the use of retroviral vectors to integrate exogenous ESC genes into the genome of target cells. The modification of the genome raised concerns regarding an increased potential for host oncogenesis. Recent efforts have achieved reprogramming of adult somatic cells by transient expression of DNA delivered by plasmid [16], lentiviral [17] and adenoviral [18] vectors, as well as the delivery of mRNAs [19] and recombinant proteins [20]. These alternative strategies have assuaged fears regarding the tumorigenic potential of iPSC and have begun to address a significant hurdle to their success in the clinic. The true significance of iPSC is the possibility of patient-specific pluripotent cell libraries for clinical regenerative applications while avoiding the ethical debates that plagued ESC [21].

However, questions regarding iPS cells persist, obstructing their path to the clinic. Chief among them is the inefficiency of current cellular reprogramming efforts. Typically, millions of cells are isolated and induced to express key ESC markers, with fewer than 2% (at best) achieving an ESC-like state. Furthermore, upon reprogramming of somatic cells, there appears to be an epigenetic

'memory' associated with iPSC that might bias cells towards the original cell phenotype despite triggers to induce alternate pathways of differentiation. Finally, because of our inability to control precisely the *in vivo* environment, the potential for tumorigenesis owing to implanted iPSC remains a cause for concern.

Although the prospect of patient-specific disease treatments and tissue regeneration is appealing, we question the feasibility of this approach and the unresolved dangers associated with their use. At this stage, the field of iPSC research has not matured sufficiently to transition successfully to the clinic for any manner of tissue regeneration. Instead, we must turn our attention to the calibration of the soluble signals and matrix components that might be required to facilitate the use of stem cell therapeutics in patients.

## Growth factors

Growth factors and scaffolds provide essential biological roles for the augmentation of stem cell-based therapeutics. They might also offer stand-alone therapeutic options. Growth factors are signaling molecules that instruct cells during developmental and regenerative processes. For consistency, we refer to growth factors as all protein-based agents used to regulate cell response and behavior. Examples of growth factors for bone regenerative purposes include the fibroblast growth factor (FGF), bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and platelet-rich plasma (PRP). Rather than summarize independent efforts to achieve bone regeneration using these growth factors (reviewed in [22]), we provide guidance to further advance growth factor delivery for bone regeneration.

The delivery of exogenous growth factors to specific anatomical sites has been hampered by poor tissue penetration, uncontrolled migration to desired sites and inefficient cellular internalization because of extracellular enzymatic degradation [23,24]. Furthermore, growth factors delivered to injury sites during the destructive phase of wound healing are exposed to inflammatory environments that are hypoxic, acidotic and populated by neutrophils and macrophages; as a result, unprotected growth factors have an *in vivo* half-life on the order of minutes [25]. A consequence of the rapid depletion of administered growth factors is the frequent usage of supraphysiological doses of growth factors to achieve a therapeutic outcome. The contemporary delivery methods of growth factors are unsatisfactory and, consequently, the library of delivery systems available for controlled release must be improved.

To improve growth factor delivery for bone regeneration, we must obtain our inspiration from embryogenic bone formation and the fracture-healing mechanisms that occur naturally in subcritically sized osseous defects [26]. Growth factor-based approaches have traditionally focused on the delivery of a single agent, at a single dose, at a single time to elicit a multifaceted biological outcome. This concept defies the complexity of biology and trivializes the intricacies of tissue regeneration. Rather, we must mimic the expression profiles of growth factors that temporally and dynamically interplay during the ideal osteoregenerative cascade. Although multiple growth factors have a role in bone tissue regeneration, the major factors (as previously stated) are PDGF, FGF, VEGF and, of course, the BMPs. The precise interactions between each of these growth factors have yet to be fully elucidated, although evidence exists to suggest synergy among these growth factors for bone regeneration. For example, BMP-2 and VEGF have been reported to function synergistically in bone regeneration applications when delivered sequentially via a poly(-lactic-co-glycolic acid) PLGA scaffold [27]. VEGF has no osteoinductive properties, although it is angiogenic, which fosters the vasculature required to support bone. The administration of recombinant human BMP-7 (rhBMP-7) in conjunction with rhPDGF-BB has been demonstrated to promote more bone formation than each factor alone in critically sized defects in osteoporotic mice [28]. Similarly, dual delivery of rhVEGF and rhPDGF-BB suggests a greater angiogenic outcome than the delivery of either factor alone [29]. The combination of rhVEGF and rhFGF-2 has also been investigated for increasing angiogenesis [30].

It has become clear that the delivery of multiple growth factors with biologically inspired temporal, spatial and dosing parameters is crucial for the development of successful growth factor-based regenerative therapeutics. The sequential delivery of these four growth factors at the appropriate doses might be the key to recreating the bone regenerative processes that occur naturally during embryogenesis and fracture healing. However, such growth factor-based tissue-engineering approaches rely on the development of scaffolds and biomaterials capable of multiphase, tunable release properties for biologics. For this reason, the path towards clinical success with growth factor regenerative therapeutics is intertwined with the continued development and maturation of biomaterials for drug delivery.

## Scaffolds and biomaterials

A primary purpose of biomaterials engineered for tissue regeneration is to support and facilitate the requisite physiological functions at the injury site. Broadly, this includes providing an extended framework for regenerative cell population migration and specialization, as well as the sequestration of extracellular matrix (ECM) components and growth factors. We have already detailed the desired capabilities for growth factor binding and release; however, in the context of cellular content, support is multidimensional and can be defined as providing the capability for cell attachment, anchorage, differentiation, proliferation and function. The physiological role of bone tissue also demands that biomaterials at defect sites be capable of withstanding loads associated with compressive loading of bone. These functional properties are paramount in the function of biomaterials for bone tissue regeneration that are, first and foremost, determined to be biocompatible and patient safe.

The contemporary biomaterials for bone tissue regeneration can be classified broadly into inorganic and organic materials, which include both naturally derived and synthetic components. Inorganic materials, such as beta tricalcium phosphate (β-TCP), hydroxyapatite (HA) and bioactive glasses, have long been used for bone tissue-engineering purposes because of their similarities in structure and composition to the inorganic elements of bone itself [31]. Among the benefits of inorganic biomaterials are their compressive strength (which is often equal to, or greater than bone tissue) and potential for osteoconductivity [32]. The main deficiency in these materials is their brittle nature, which poses a concern in high load-bearing biological applications.

The alternatives to inorganic materials are organic polymers, which can be either naturally occurring or chemically synthesized. These materials provide an alternate set of characteristics that

encourage their use for tissue-engineering applications. We begin with biomaterials derived from natural sources, such as collagen [30], hyaluronic acid [33], cellulose [34], silk, alginate [35] and chitosan. Generally, naturally derived biomaterials are characterized by biocompatibility, enabling the adhesion and migration of cells within their structures. Collagen sponges, in particular, have long been used to deliver growth factors to promote bone regeneration. Products such as InFuse™ from Medtronic are approved by the US Food and Drug Administration (FDA) for the delivery of rhBMP-2 through a purified collagen matrix for interbody fusion in the anterior lumbar [36].

The major limitations of naturally derived polymers include difficulties in processing and purification as well as concerns regarding immunogenicity. The potential also exists for batch-to-batch variability in materials, diminishing the predictability of results in the clinic. Finally, no naturally derived organic biomaterial is capable of matching the mechanical properties of bone tissue, which contains both organic and inorganic components.

The field of organic polymer synthesis for tissue engineering has grown considerably as a consequence of the limitations associated with naturally derived polymeric materials. Through advances in polymer synthesis technologies, particularly with regards to controlled radical polymerization and scaffolding techniques, synthetic biomaterials with tunable micro- and macroscale features are being developed. Microscale features include composition, architecture and binding groups, whereas macroscale features include porosity, stiffness and elasticity. With respect to polymer composition, polymers frequently used for bone tissue regeneration include polylactic acid (PLA), polyglycolic acid (PGA), PLGA, polycaprolactone (PCL), polyethylene (PE), polyethylene glycol (PEG) and poly(methyl methacrylate) (PMMA), among others. Biologically inspired synthetic polymers derived from amino acids, such as tyrosine-derived polycarbonates, polyethers and, to a lesser extent polyarylates, have also been investigated for tissue repair and regeneration [37,38]. Despite the high degree of versatility available with synthetic polymer synthesis, they too have shortcomings as platforms for tissue engineering. Their lack of bioactivity restricts positive biomaterial–host interactions, particularly in comparison to naturally derived polymers that have ECM-binding domains. Additionally, the degradation products of synthetic polymers often include acidic byproducts (*e.g.* PLA or PGA) that might hinder regenerative processes.

The clinical success of scaffold-based approaches for bone regeneration relies on overcoming the limitations associated with single-phase biomaterials by developing synergistic combinations of inorganic and organic biomaterials. The field of bone tissue regeneration has already made progress on this quest for hybrid biomaterials. Intelligent scaffold design is achieved by combining organic and inorganic materials, enabling the creation of biocompatible scaffolds with the compressive strength required in osseous defect sites. Combining electrospun collagen nanofibers with PCL microstrands, for example, has been achieved without compromising the cell adhesive properties of collagen or the mechanical strength of PCL [39]. The blending of chitosan and hydroxyapatite in scaffolds has resulted in materials with mechanical properties, porosity and bioactivity to support ingrowth of cells and new bone formation [40]. Other examples of recent ingenuity with combinatorial biomaterial platforms include collagen and HA [41], PGA and βTCP [42], as well as a particularly novel combination of PEG, PCL, collagen and nano-HA [43].

Scaffolds, in particular, might be instrumental to success of both growth factor- and stem cell-based tissue-engineering therapeutics. In fact, the quest to produce a comprehensive tissue-engineering approach to bone regeneration in the clinic has given rise to several novel approaches that might be clinically impactful. There exist several commercially available materials, both inorganic and organic, that enable bone ingrowth in clinical scenarios. Furthermore, the successful combination of scaffold and growth factor approaches has been demonstrated with products such as InFuse™. Although these approaches currently lack the integration of cells, they are nevertheless byproducts of the development of tissue-engineering solutions for bone regeneration.

These successes to date, although notable, have not surpassed the autograft and allograft in their abilities to treat critically sized bone defects. The next generation of biomaterials for bone regeneration must physically support osseous defects, as well as chemically and biologically sustain growth factors and stem cells. The convergence of organic and inorganic materials for bone tissue engineering has already begun. Further advancements in technologies that combine scaffolding with growth factors and stem cells might ultimately dictate whether tissue-engineering approaches to bone regeneration will be clinically impactful.

## Emerging technologies

We have described the roles that stem cells, growth factors and scaffolds might have in the tissue-engineering theatre. Moreover, we have summarized the significant research advances in each area and provided global tissue-engineering context in which to view these accomplishments. Here, we discuss the progression of these concepts to the clinic, with a focus on technologies that might significantly enhance the current cast of tissue engineering.

To date, the field of tissue engineering has failed to produce a compelling therapy. The emergence of technologies such as bioreactors and freeform fabrication might create an ecosystem that facilitates the successful unity of triad elements. Since the advent of bioreactor technology over 15 years ago, it has been viewed as the logical environment for the combination of stem cells, growth factors and scaffolds. Recent advances in the controlled manipulation of the bioreactor environment have made the creation of *ex vivo* tissues a real possibility. Stimuli provided in 3D cultures might be able to direct cellular differentiation and behavior, producing specialized tissue-engineered constructs for implantation *in vivo*. One of the major challenges faced in tissue creation is the generation of vasculature, although incremental progress is being made towards the creation of synthetic vascularized bone grafts. For a comprehensive review of recent advances in bioreactor technology for bone regeneration, see [44]. Overall, the pathway towards clinical relevance for this technology still faces challenges of operator dependency and quality control. These barriers obstructing the clinical use of bioreactors have been extensively detailed elsewhere [45]. Although the advancement of bioreactors towards clinical use still faces challenges, the benefits provided by this technology might be the key to creating tissue-engineered bone grafts [44].

Looking beyond bioreactor technology, the next generation of *ex vivo* tissue production might be via advancements in solid

freeform fabrication (SFF) technology. SFF material synthesis has traditionally been used for rapid prototyping, although recently it has been explored for the fabrication of biomaterials [45]. Conventional methods of scaffold synthesis might be hampered by limited scaffold interconnectivity, porosity and their reliance on organic solvents [46]. The translation of computer-generated models into tissue-engineering products via SFF can overcome some of these obstacles. SFF techniques can combine both natural and synthetic polymers, as well as inorganic materials to produce biomaterials including thermoplastics [47,48], hydrogels [49] and composite scaffolds [50,51]. Looking ahead, SFF technology might enable precise control of internal scaffold architecture and composition, limited only by the resolution of the dispensing technology. This could open doors for the production of scaffolds and tissues with patient-specific geometrical parameters, a feat unattainable with the current methods of scaffold synthesis. For an extensive review of current SFF technology, we recommend a recent review by Li *et al.* [52]. As we strive towards the synergistic fusion of tissue-engineering triad elements, bioreactor and SFF technologies could emerge as cornerstones of *ex vivo* tissue production.

## Concluding remarks

Bone regeneration is a complex process encompassing both spatial and temporal dimensions, orchestrated to rebuild bone that is indistinguishable from its original, undamaged state. When the innate ability of the body to harness this regenerative machinery is compromised, therapeutic intervention is needed to facilitate bone regeneration. However, over the past 25 years, most tissue-engineering efforts to surpass the efficacy of autografts and allografts have been unsuccessful. Although this time period has seen several successful manifestations of tissue-engineering approaches in the clinic, we, as tissue engineers, have overpromised and underdelivered. The current shortages in the supply of bone grafts have intensified the need for clinically viable alternatives, heightening anticipation for regenerative medicine approaches in the clinic. The challenges we face are further heightened by the time- and cost-intensive processes required to regulate combination device and biologic approaches by the federal sector. Although these processes are expected to be streamlined in the future, achieving clinical success through tissue-engineering approaches nevertheless requires a paradigm shift. Scientific ingenuity and rigor will drive success in the laboratory, but challenges such as production, storage and ease of use of therapeutics will ultimately dictate their use in the clinic. Bone- and tissue-regenerative therapeutics must be designed and conceptualized with these parameters in mind. Over the next decade, emerging technologies will enable the production of regenerative therapeutics with increased sophistication to match the biology of the human body. As tissue-engineering technologies mature, we underscore the need for pragmatism in the design of tissue-regenerative therapeutics and the profound need for collaboration among scientists, engineers, clinicians and the federal regulatory sector.

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