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Perspectives on the Role of Nanotechnology in Bone Tissue Engineering

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Abstract

Objective—This review surveys new developments in bone tissue engineering, specifically focusing on the promising role of nanotechnology and describes future avenues of research.

Methods—The review first reinforces the need to fabricate scaffolds with multi-dimensional hierarchies for improved mechanical integrity. Next, new advances to promote bioactivity by manipulating the nano-level internal surfaces of scaffolds are examined followed by an evaluation of techniques to using scaffolds as a vehicle for local drug delivery to promote bone regeneration/integration and methods of seeding cells into the scaffold.

Results—Through a review of the state of the field, critical questions are posed to guide future research towards producing materials and therapies to bring state-of-the-art technology to clinical settings.

Significance—The development of scaffolds for bone regeneration requires a material able to promote rapid bone formation while possessing sufficient strength to prevent fracture under physiological loads. Success in simultaneously achieving mechanical integrity and sufficient bioactivity with a single material has been limited. However, the use of new tools to manipulate and characterize matter down to the nano-scale may enable a new generation of bone scaffolds that will surpass the performance of autologous bone implants.

Keywords

bone; nanotechnology; bone scaffolds; composites; drug delivery; cell seeding

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Introduction

Bone is one of the most commonly transplanted tissue with 2.2 million bone grafts performed annually worldwide [1]. Indeed, surgeons face a diverse spectrum of clinical challenges in bone reconstruction reflecting the variety of anatomic sites, defect sizes, mechanical stresses, and available soft tissue cover (see Table 1). Autologous bone grafting remains the gold standard for reconstruction of skeletal defects [2]; however, this technique does have some drawbacks including limited supply, bone graft loss/resorption, short-term instability in large defects, complications associated with a second surgical site, and autograft failure rates exceeding 50% in difficult healing environments [2-5]. Bone allografts (i.e., bone transplanted from a donor) present another option, accounting for about one-third of bone grafts performed in the United States [6]. However, their clinical success does not approach that of autologous bone, with higher failure rates (30-60% over 10 years in vivo with significant decreases in strength) [7] and prevalence of late rejection [8].

As a result of these limitations, the use of synthetic implants to replace damaged bone is growing exponentially. However, current synthetic biomaterials were developed originally for other engineering applications and often do not integrate well with host tissue resulting in possible infection, foreign-body reactions, and extrusion/loss of the implanted material. While current biomaterials result in a time-limited and unpredictable outcome [9-11], an alternative that has attracted widespread attention in recent years is the engineering of new bone to replace the damaged or diseased tissue. A critical component of this tissue engineering approach is the development of porous 3D structures—scaffolds—that will provide cell support and guide bone formation. Numerous porous materials have been investigated, but despite substantial progress in the field, the development of synthetic structures able to fully harness the bone's capability to regenerate and remodel itself still present challenges.

Autologous bone grafts owe their success to the presence of endogenous bioactive molecules and cells able to respond to the signals in the graft and the surrounding microenvironment. A successful bone engineering therapy must recapitulate, and ideally accelerate, the process of bone regeneration, which will only be possible if we understand the complex process of bone healing and identify the critical steps. Following the example of autologous grafts, most bone engineering approaches are based on the combination of four factors: a matrix (i.e., the scaffold), cells to “build” the new tissue, cell signaling (BMPs and growth factors) to guide cell differentiation and tissue formation, as well as an adequate blood supply (i.e., vascularization) (see Figure 1). Thus, there are multiple physical and biological requirements that an ideal bone scaffold should address: *i*- supply a porous matrix with interconnected porosity and tailored surface chemistry for cell growth, proliferation, and transport of nutrients and metabolic waste; *ii*- resorb/remodel in a predictable way with controlled osteogenic activity and produce only metabolically acceptable substances; *iii*- deliver a controlled cascade of signaling (both in time and space) to guide cell differentiation and promote tissue regeneration; *iv*- match the mechanical properties of the host tissues with a strong, stable material-tissue interface persisting through the implant resorption process; *v*-eliminate the risk of rejection or foreign-body reaction; and *vi*-achieve good adaptation and coverage by the surrounding soft tissue. By meeting these requirements, the implant can substitute, at least temporarily, for natural tissue, providing sufficient strength and stiffness to prevent fracture under physiological loads and provide a framework for the body to create new bone tissue.

Although the field of tissue engineering emerged almost 20 years ago [12], progress has been slow. The physical and biological requirements of an ideal scaffold require it to balance a combination of complex material designs incorporating pore gradients and

different material combinations with sophisticated nanoscale functional capabilities through surface engineering, cell encapsulation, and controlled chemical release [13-15]. Indeed, nanotechnology has impacted multiple areas of medicine from the development of diagnostic image-based techniques to the formulation of synthetic targeted nanoscale therapeutic agents for drug and gene delivery to treat inflammation related to cancer (e.g., Doxil for treatment of ovarian cancer), cardiovascular, rheumatological, and other diseases [16]. The key question is: how can nanotechnology open new opportunities in bone tissue engineering?

Nanocomposite approaches for scaffolds

While the mechanical design requirements for bioresorbable scaffolds vary greatly depending on the functional requirements of the bone it is replacing (see Table 1), an emphasis has been given to the development of strong and tough scaffolds able to sustain loading in vivo for moderate and high load-bearing applications [8, 13-15, 17-20]. Indeed, natural bone derives its unique combination of mechanical properties from an architectural design that spans nanoscale to macroscopic dimensions, with precisely and carefully engineered interfaces (see Figure 2). The bone's fracture resistance originates from toughening mechanisms at each of these dimensions; specifically, the smallest length-scales govern bone's intrinsic fracture resistance by promoting plasticity and influencing bone strength, while the larger length-scales extrinsically toughen bone by shielding the growing crack and affecting its toughness [21]. To date, there are no synthetic biomaterials with such a hierarchical structure, yet the message from nature is clear – unique mechanical properties can be achieved through the combination of mechanisms acting at multiple length scales.

In this light, many different groups have tried to manipulate the mechanical properties (e.g., stiffness, strength and toughness) of scaffolds through the design of nanostructures (e.g., the inclusion of nanoparticles or nanofiber reinforcements in polymer matrices) to mimic bone's natural nanocomposite architecture. However, as the incorporation of nanoscale reinforcement increases the strength, the toughness drops dramatically. Indeed, strength, the resistance to non-recoverable deformation, and toughness, a measure of damage tolerance or resistance to fracture, may seem similar to many, but changes in material microstructure often affect the strength and toughness in very different ways; a combination of high strength and high toughness tend to be mutually exclusive [22]. In ceramic-based materials, toughness is derived mainly at larger length-scales that are able to shield the crack by deflecting it or by sustaining uncracked material bridging the crack wake; indeed, nanocomposites with no structural design at larger length scales have disappointing properties to date [23, 24]. In this way, the design of stronger and tougher scaffold materials requires incorporation of a hierarchical design encompassing many length-scales from the nanolevel to generate strength (i.e., to mimic composite deformation of nanocrystals of HA and collagen) as well as micro-level structures to influence the crack path and generate toughness (e.g., to mimic osteons and cement lines) [25]. Thus, nanotechnology alone may not be the answer to improving mechanical properties of scaffolds.

Progress in the development of new scaffolds has been hampered, in part, by the limitations in processing techniques to form structures with a multi-dimensional architecture. For the fabrication of macroporous materials, abundant established methods can be adopted, including solvent casting/particle leaching [26], phase separation [27], solvent evaporation [28], fiber bonding to form a polymer mesh [29], freeze-drying [29] and foaming [30]. These techniques could be adapted for nanocomposites or polymer matrices for further mineralization but offer relatively modest control over the shape and distribution of the macroporosity. In recent years, new solid free form fabrication techniques (see Figure 3) that allow the implementation of complex computer-designed architectures mixing different

materials have been used to create scaffolds on-demand following computer designs [13, 31-41]. Other techniques such as ice-templating (Figure 3) [42, 43] can be used to fabricate materials with complex architectures controlled at multiple length scales from the nano- to the macro levels. The challenge is to use these technologies in combination with nanomaterials. It is possible that at the end an optimum scaffold should combine several materials and techniques (e.g., a complex polymer structure can be created by ice-templating or computer-assisted fabrication that can subsequently be mineralized to achieve the desired mechanical and biodegradation responses).

It is important to note that the structural design of hierarchical scaffolds for mechanical property requirements contrasts with other biological requirements. For instance, while decreasing the grain size to the nanolevel without hierarchically incorporating larger structures shows no benefit to the toughness, several studies on ceramic materials, such as calcium phosphates, indicate that grain size may have an effect on cell response [11, 44, 45]. As will be discussed in later sections, studies suggest that smaller grains favor cell attachment, proliferation and differentiation towards osteoblastic lineages although the causes are unclear. Additionally, the strength requirement is counterbalanced by the need for large porosity to allow cell seeding, to transport gases, nutrients and metabolic waste, and to promote angiogenesis [17]. Thus, further ideas must be taken from bone's natural structure to fulfill more than just the mechanical requisites.

Additional key issues in making organic/inorganic bone-like nanocomposites are control of the nanoparticles' spatial distribution in the matrix and the manipulation of adhesion at the organic/inorganic interface. Several techniques have been used to control particle distribution with a varying degree of success [46-49]. Conventional approaches, such as mixing particles in dissolved or molten polymer, have shortcomings, such as agglomeration and difficulties associated with the handling of highly concentrated, viscous suspensions. Another issue is adhesive degradation at organic/inorganic interfaces in vivo, which can significantly deteriorate the mechanical strength and toughness [50, 51]. This problem can be addressed at the molecular level either by treating the particle surface (e.g., adding silanol groups as in conventional dental composites) or by modifying the polymer with functional groups promoting adhesion to the mineral. For example, synthetic mimics of L-3,4-dihydroxyphenylalanine (DOPA), an amino acid that is believed to be responsible for both adhesive and crosslinking characteristics of mussel proteins can be used to promote adhesion at the organic/inorganic interface [52]. However, these alternatives may present some drawbacks as the biological implications of chemical manipulation need to be assessed, for example DOPA is bioresorbable in vivo. In many cases, adhesion could also degrade after going through wet and dry cycles during preparation and implantation.

An alternative path for the fabrication of nanocomposites is the mineralization of organic matrices in a process designed to mimic the natural mineralization of collagen scaffolds during bone formation. These techniques have in common the immersion of the polymer in a concentrated Ca^{2+}, $(PO_4)^{-}$ solution, such as simulated body fluid, to promote the heterogeneous nucleation of apatite often by increasing pH to decrease apatite solubility [53-57].

The mineralization process should result in the templated growth of nanocrystals with good adhesion to the organic matrix (Figure 4). Here the polymer chemistry plays a defining role. The presence of specific sites for Ca^{2+} binding seems necessary to template the growth of nanocomposite, bone-like, materials. In their absence, uncontrolled growth of micron-sized particles occurs with poor integration into the organic matrix. Also, the immersion in simulated body fluid often results in the uncontrolled precipitation of apatite on the materials surface.

3D polymer networks with the ability to efficiently mineralize across their volume serve as an approach to provide bulk nanocomposites with practical dimensions. Synthetic hydrogels are an appealing candidate, as their intrinsic elasticity and water retention resemble those of natural hydrogels, such as collagen. However, quite often the mineral layer forms on the polymer surfaces and not in the interior. Thus, hydrogels present an appealing approach to coat the internal surfaces of macroporous scaffolds but not to prepare nanocomposites. This is a particular problem when working with nanoporous, elastic hydrogels with limited water incorporation. The use of electrical assisted diffusion seems to be a path to promote ion diffusion and true 3D mineralization (Figure 5) [58, 59]. It has been observed that phase separation during the process can result in the formation of microscopic liquid vesicles in the organic matrix that will play a similar role to the vesicles secreted by osteoblasts during bone formation [59]. However, there is still much work to do in the design of polymer matrices and processing approaches before biomimetic mineralization becomes a feasible technique to build practical 3D structures.

Design of scaffolds internal surfaces

The surfaces of scaffolds can be designed internally to promote cell attachment and guide cell differentiation, while the external surfaces secure integration with the surrounding tissue to avoid extrusion and movement. Scaffold fixation and integration with the surrounding tissue attachment is an issue that has not been extensively explored. However, adequate fixation and limiting micromotion are critical for the scaffold's success. Some studies in large animals have resorted to the use of external hardware such as metallic plates or screws to enhance fixation [60]. However this may not be the most practical approach in the long run. In this respect, it could be fruitful to look at the large existing literature on orthopedic implant fixation in search for useful clues for the design and fabrication of scaffold surfaces. Most practical developments have manipulated the external surfaces by changing their roughness at the micro- and nanolevels to improve osseointegration [61-64] or have implemented coatings (for example of bioactive glasses) [65].

Recent studies have also manipulated the internal surface roughness of scaffolds down to the nanolevel to assess in vitro topological effects on cell response. Indeed, cell attachment, proliferation and differentiation have been repeatedly shown to be responsive to nano-scale features such as pillars or grooves prepared, for example, using nanolithography [66-70]. In this respect, not only the size of the feature but also its distribution (ordered vs. random) can play a role. Nanopatterned surfaces may also provide better adhesion of the fibrin clot that forms right after implantation, facilitating the migration of ostegenic cells to the material surface [71]. Additionally, measurements of expression of early osteoblast markers (Runx2, Osterix) to late osteoblast markers (osteocalcin and osteopontin) have shown clear differences [72-75]. However, we lack systematic studies and predictive models that will relate these in vitro results to scaffold performance in vivo. And, in the case of dental endosseous implants, osteoblasts have a way of sensing the stiffness and topography of scaffold materials, although they may never actually be in contact with implant or scaffold surfaces in vivo [76] and therefore the usefulness of surface topographic features in vivo is often questionable.

Grafting surfaces with different molecules has also been shown to enhance cell adhesion, promote mineralization, and create or produce matrix and marker proteins [77]. Early studies focused on the proteins present in the extracellular matrix and diverse biomaterial surfaces have been functionalized with proteins such as fibronectin (FN), vitronectin (VN), and laminin (LN) [78, 79]. Lately the focus has shifted to the use of signaling domains, composed of several amino acids. These domains are present along the chain of the extracellular matrix proteins and are the ones that interact with cell membrane receptors.

Perhaps the best-known example is Arg-Gly-Asp (RGD), the signalling domain derived from fibronectin (FN) and laminin (LN). However, other sequences such as Tyr-Ile-Gly-Ser-Arg (YIGSR) or Arg-Glu-Asp-Val (REDV) have also been used [80-82]. The use of a short peptide is more convenient because the long molecules can be folded and as a result the binding domains may not be available. Here two important aspects are the development of techniques to bind the molecules to the biomaterial surface and the control of their spatial distribution.

Both covalent and non-covalent bonds have been used to promote molecular adhesion to the biomaterials. For example, covalent bonds with linker molecules promote stronger adhesion but may limit functionality if they impose a particular molecular spatial orientation. Regarding the spatial distribution, it has been shown that a way to enhance the function of osteoblastic cell lines is to match the surface density of the selected molecules to the distribution of the corresponding receptor in the cell membrane. This can be achieved, for example, by using functionalized gold nanoparticles whose density on the surface can be manipulated [83, 84].

At this time, most studies use simplified 2D models of scaffold surfaces, which utilize materials that were specifically developed for surface nanoengineering tools (e.g., silicon or PMMA). [61-64, 85-87]. While sophisticated surface engineering can be performed on flat surfaces, the degree of internal surface manipulation in 3D scaffolds that are composed of biopolymers and ceramics has been much more limited with a marked lack in systematic studies incorporating surface nanoengineering. The key is to develop the nanotechnology tools needed to implement lessons from basic 2D studies into practical 3D scaffolds. Manipulation of the scaffold's internal surface topography down to the nanolevel is extremely challenging. Some studies have incorporated microscopic features and several approaches (e.g., electrospinning) have been developed for the creation of nanofibrous scaffolds to mimic the extracellular matrix's structure. However, the mechanical response of these nanofibrous materials has not been well characterized and may prove insufficient for any load-bearing situation. Recent studies have also manipulated the internal surfaces of scaffolds down to the molecular level to assess in vitro the effect of surface chemistry on cell response. For example, in order to avoid excessive cell colonization in the periphery of the scaffolds that could hamper mass and waste transport to and from the center, cell-adhesive and non-adhesive surface coatings can be distributed through the scaffold structure [88]. However, much work is still needed as overall it is not clear whether nanopatterning will be substantially better than patterning at the micron scale or what the interplay between surface topography and chemistry is.

Signals and drug delivery

In addition to providing temporary structural support, scaffolds should serve as carriers for drugs and chemical cues promoting angiogenesis and differentiation towards osteoblastic lineages. For example, implants cause an inflammatory response in the body; however, the typical approach to suppress inflammation with medication is insufficient or entirely ineffective in many cases [89-91]. Applying a coating of anti-inflammation agents to the scaffold's surfaces could provide a means to gradually release a local dose of medication [92, 93] (Figure 6). Direct delivery to the implant site would require less medication, which in turn would reduce both toxicity and side effects. Similarly, via chemical delivery mechanisms, scaffolds could play a more active role in the regeneration process, which requires a cascade of biological events in which growth factors provide signals to initiate healing. Specifically, bone formation in vivo requires the release of chemicals (e.g., growth factors) at critical time points to stimulate osteoinduction and bone regeneration can be substantially accelerated by the localized delivery of appropriate growth factors, such as

TGF-β, BMPs, IGF, or FGF [94-98]. Other factors such as vascular endothelial growth factor (VEGF) are also being considered to promote vascularization [99]. If the signaling for osteoinduction can be further specified, drug release spatio-temporal profiles can be designed to mimic signaling in vivo [18, 100]. The four basic questions that should guide the design of chemical release tools are: what to release, when, where, and how much.

A variety of techniques have been developed to incorporate drug delivery mechanisms into the scaffold: microparticles (e.g., microspheres) of releasing agents embedded into the scaffold, chemicals coatings on the scaffold's internal surfaces, and incorporation of the drugs into the scaffold material (i.e., either in the microporosity of ceramic scaffolds or in the matrix of polymer-based structures). Microparticles can be prepared from polymer solutions containing the chemical by means of solvent evaporation techniques [101] or spraying [102]. Chemical modifications of the microparticles' surface may be needed to robustly and stably immobilize them, requiring further engineering of the surface to improve integration with the surrounding matrix [103]. The use of surface treatments, such as coating the microparticles with a second layer, can also prevent “burst” release [104]. The same polymer suspensions used in the fabrication of microspheres can also be applied directly to scaffold surfaces (e.g., by immersion) to fabricate thin coatings or to infiltrate the microporosity of ceramic scaffolds. To further control the release profiles, graded coatings obtained by sequential immersion should also be considered.

Another drug delivery option is to use polymer-based scaffolds with chemicals incorporated into the polymer. An interesting variation of this approach is the use of glass-based scaffolds (e.g., Bioglass®) for the controlled release of ions. Bioactive glasses can bond chemically to bone, while they degrade in the body in a controlled manner. Because the glass composition can be easily modified and many different ions can be incorporated, numerous bioactive glasses have been developed with different degradation rates and targeting very diverse applications: bone fillers, implant coatings, and scaffold fabrication. Indeed, the release of Si from bioactive glasses or calcium phosphates can have a beneficial effect, while the liberation of other ions from the glass in vivo can be used to promote angiogenesis (e.g., Cu^{2+}) or differentiation towards osteoblastic lineages (e.g., Sr) [105-111]. The release rates could be manipulated by using different glass compositions. However, the exact role of the different ions, the ideal release rates, the effect of pH changes triggered by glass degradation, and the role of glass surface roughness have not been studied systematically [112, 113].

Currently, the burst release of drugs as well as the inappropriate initiation of host wound healing response due to the degradation of polymer carriers or the interaction between growth factors and solid scaffold materials present problems in terms of release mechanisms. Models based on the mechanisms of polymer erosion, swelling, and diffusion [114-119] can be used to manipulate polymer and drug compositions to study drug release kinetics, which in turn may prevent effects such as the “burst release” [120, 121] of large drug quantities right after implantation and achieve controlled release of drugs for times spanning hours to weeks or months. The use of growth factors in scaffolds presents additional challenges due to their short biological half-life, limited stability, tissue specificity, and potential dose-dependent carcinogenicity [99]. The development of a systematic approach towards effective chemical delivery is an extremely complex problem that has not been fully addressed. Clear guidelines coming from the combination of in vitro and in vivo studies are critical to define effective release sequences with the needed spatial and temporal accuracy.

Cells seeding

Cultured osteoprogenitor cells, such as bone marrow stromal cells (BMSCs), are usually "seeded" onto the scaffold when it is transplanted to promote successful tissue regeneration throughout the entire cross-section of the scaffold. Multiple seeding techniques have been developed to distribute cells evenly for bone and cartilage regeneration [14, 122-128] and generally can be divided into static and dynamic methods [129-131]. The more commonly used static methods involve directly injecting cells into the construct and onto the surface, while dynamic methods introduce cell solutions into a scaffold by either gently shaking or centrifuging the cell solution with the scaffold.

Through imaging methods, such as iron-oxide labeling combined with MRI or microCT analysis as well as fluorescein staining and cryosectioning with 3D imaging, dynamic seeding has been shown to improve cell coverage and distribution in comparison with static methods. However, the biggest obstacle to seeding is that bone formation fails in the interior of large cell-seeded scaffolds. While imaging techniques demonstrate cell infiltration, distribution, and survival, it is unclear whether migration occurs and whether the cell distribution continues to favor generalized tissue formation throughout the scaffold. Indeed, as dense scaffolds typically do not allow light transmission, live imaging of cell migration is currently not possible. Accordingly, most studies examine very small scaffolds with little clinical application.

Analytical techniques need to be developed to characterize cell infiltration and distribution in scaffolds with a clinically relevant size. Then, it can be understood whether insufficient bone/tissue formation or inadequate vascularization and ultimately cell survival limit bone formation within the core of larger scaffolds. This can only be answered by carefully assessing three key biological processes: BMSC survivability, differentiation, and vascularization.

Summary and Future Challenges

New design concepts and fabrication techniques are urgently needed to develop novel scaffolds for bone regeneration. In particular, more research is required to uncover the relationships linking composition and materials architecture at multiple-length scales with macroscopic mechanical behavior and the capability for osteogenesis. Once basic strategies are defined, the knowledge could be used to design new systems capable of manipulating chemistry and cellular responses. These materials will also serve to perform much needed systematic studies on the effect of parameters such as surface roughness or drug delivery profiles. The possibilities opened by the growing emphasis on nanotechnology now permit the tailoring of scaffold chemistry and structure with an unprecedented degree of control. For the first time, we have tools that could be used to monitor and manipulate the physicochemical environment and to monitor key cellular events at the molecular level. Yet, it is unclear what the final role of nanotechnology will be and how it will be integrated in the design and fabrication of an ideal bone scaffold. To reach this goal, answers are needed to numerous scientific questions:

- What is the optimum scaffold permeability (pore size, shape, and interconnectivity)? Does it depend on the composition and specific application?
- What are the biodegradation rates of different polymers, calcium phosphate materials, and composites in vivo and are they appropriate for their respective applications?
- Is it possible to design hierarchical architectures combining good mechanical behavior with optimum biological response?

- Angiogenesis and nutrient transport seem to be critical problems that hamper the development of scaffolds for large bone defects. What are the best structures to promote vascularization? How can they be combined with controlled growth factor release?
- What is the best scaffold design from mechanical and biological standpoints (a porous material or a dense one able to generate porosity in vivo)?
- What is the best approach to integrate scaffolds with the surrounding tissue?
- How do surface topography and chemistry control cell response, in particular the differentiation of stem cells towards osteoblastic lineages?
- What hurdles must be overcome to go from generating small amounts of bone to treating clinically sized defects?
- Define and overcome problems associated with the long-term stability of new bone that forms in grafts or scaffolds.
- Identify methodologies for recruiting and guiding the differentiation of endogenous osteoblastic and vascular cells.
- How does the scaffold structure (from the nano- to the macro-level) direct bone formation and what are the synergistic effects of structural features at multiple length scales?

Answers to these questions will yield information needed to build a library of bone scaffolds for treating diverse skeletal defects. To do so requires a coupling of surgical insight with the integration of principles drawn from materials science, biology, computational and quantitative science, and tissue engineering. Novel ideas that bridge the disciplines can result in the formulation of new and unexpected design paradigms for superior scaffold materials. The goal is to fabricate “active” scaffolds and structures specifically designed for bone regeneration that will temporarily substitute for natural tissues while interacting with their surroundings, respond to environmental changes, and actively direct cellular events. These adaptive scaffolds will integrate with bone tissue while they are actively resorbed or remodeled in a predictable way, with controlled osteogenic activity, taking advantage of the biological principles of bone repair that have been developed over millions of years of evolution. These capabilities will result in faster bone formation, reduced healing time, and rapid recovery to function.

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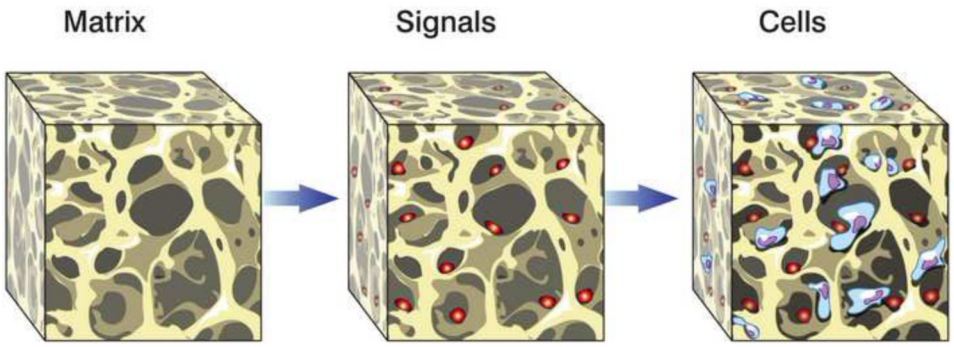

Figure 1.

Bone regeneration requires three essential elements: osteoconductive matrix (scaffold), osteoconductive signals, osteogenic cells that can respond to these signals and an adequate blood supply [2]. The first step, fabrication of strong and porous scaffolds remains the Achilles' heel of the whole process. Natural composites or hybrid structures, such as bone and teeth, display properties that are invariably far superior to their individual constituent phases. The understanding of the mechanisms to achieve these remarkable properties has become far clearer in recent years, and consequently the notion of biomimicry has received much interest in the materials communities; however, resulting advances in new bone materials have been few if any, primarily due to the fact that such materials are difficult to fabricate. Fabrication alone, however, will not be enough to create an optimum scaffold. In this respect, nanotechnology provides new and useful tools to engineer the scaffold's internal surfaces and to create devices for drug delivery with carefully controlled spatial and temporal release patterns. Synthetic scaffolds can also serve as a vehicle for the delivery of cells to build new tissue. Different techniques have been proposed to successfully seed scaffolds with cells. They can be roughly divided into two main groups: attaching the cells to the internal scaffold surface, or distributing them in the scaffold porosity using a gel-like vehicle [14]. Injectable gels containing cells could also be used directly in non-load bearing applications. Seeding with skeletal stem cells has attracted much attention, but it is critical to develop the adequate chemical and physical extracellular milieu to promote differentiation towards the osteoblastic lineage. For example, it has been observed that the presence of calcium within the matrix favors osteogenic differentiation of the appropriate progenitor cell population [86].

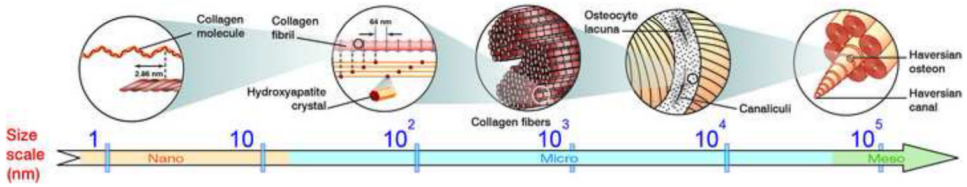

Figure 2.

Natural composites or hybrid structures, such as bone and teeth, display properties that are invariably far superior to their individual constituent phases. The origin of these remarkable properties derives from the deformation and fracture of its hierarchical structure, spanning molecular to macroscopic length-scales. The macro-scale arrangements of bones are either compact/cortical (dense material found at the surface of all bones) or spongy/cancellous (foam-like material whose struts are some 100-μm thick). Compact bone is composed of osteons surrounding and protecting blood vessels. Osteons have a lamellar structure, with each individual lamella composed of fibers arranged in geometrical patterns. These fibers are the result of several collagen fibrils, each linked by an organic phase to form fibril arrays. These mineralized collagen fibrils are the basic building blocks of bone, which are composed of collagen protein molecules (tropocollagen) formed from three chains of amino acids and nanocrystals of hydroxyapatite. Adapted from Reference [132], copyright © 1993, with permission from Macmillan Publishers Ltd.

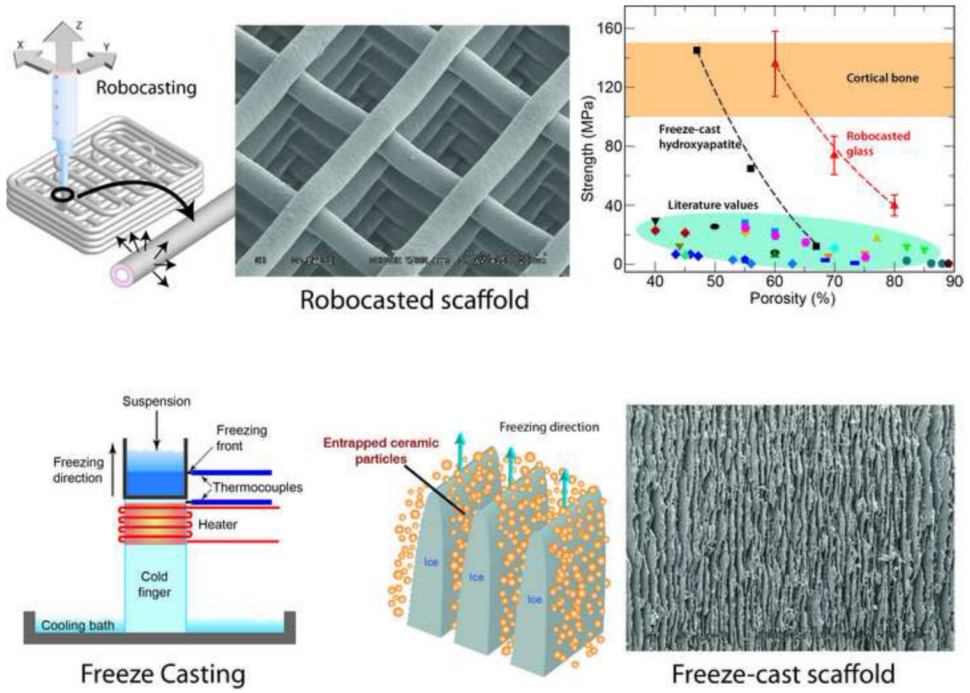

Figure 3.

Solid freeform fabrication techniques that are precise and reproducible, such as direct ink-jet printing, robotic-assisted deposition or robocasting (top of the figure), and hot-melt printing —which usually involve “building” structures layer-by-layer following a computer design, or image sources such as MRI—can be used to fabricate custom-designed scaffolds with complex architectures. Freeze casting, a technique that uses the microstructure of ice to template the architecture of ceramic scaffolds, can be used to produce porous lamellar materials that replicate the structure of the inorganic component of nacre at multiple-length scales [42, 133]. These materials can be much stronger than others with similar porosity described in the literature.

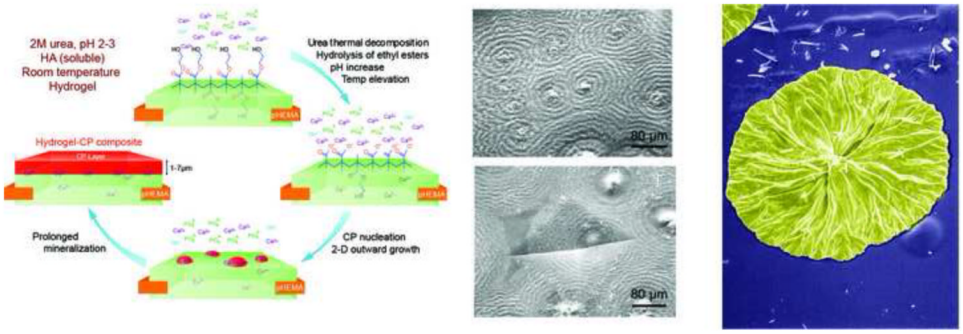

Figure 4.

Urea-mediated solution mineralization of hydroxyapatite (HA) onto pHEMA hydrogel scaffolds. Thermo-decomposition of urea produces a gradual increase in pH, resulting in the hydrolysis of surface 2-hydroxyethyl esters and the precipitation of HA from the aqueous solution. The *in situ* generated surface carboxylates strongly interact with calcium ions and facilitate the heterogeneous nucleation and 2D growth of a high-affinity calcium-phosphate (CP) layer on the pHEMA surface. Prolonged mineralization allows for the growth of a thicker CP layer that covers the entire hydrogel surface. The SEM micrograph on the right shows the 2D circular outward growth of a calcium apatite layer from multiple nucleation sites on the acidic surface of pHEMA. The calcium phosphate layer did not delaminate even after Vickers indentation with a load of 5 N (bottom micrograph). Further functionalization of the hydrogel with either carboxylate or hydroxy ligands can be used to manipulate organic/inorganic interactions [55, 134]. Reprinted with permission from Reference [55], copyright © 2005, American Chemical Society.

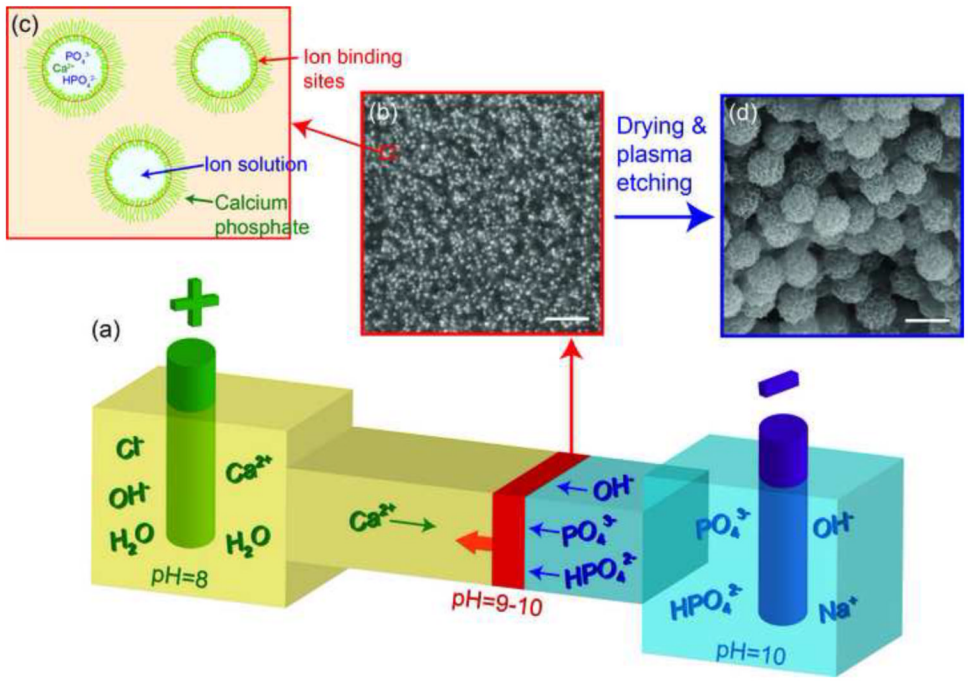

Figure 5. Biomineralization by diffusion

The interdiffusion process can be used to nucleate and grow inorganic CPs on the hydrogel matrix. In this process, the hydrogel is placed between two ion solution reservoirs (Ca^{2+} and HPO_4^{2-}-PO_4^{3-}). (a) When a current flows, the ions are forced to diffuse accordingly through the hydrogel. (b) *In situ* SEM shows the homogeneous distribution of the small (< 1 μm) mineralized spherical vesicles (white dots) formed inside the polymer through phase separation. The pH in the hydrogel changes according to the movement of the OH^- solution front. (c-d) The ions meet at a certain position in the hydrogel, where minerals precipitate due to the pH change and the associated decrease in solubility. The precipitation also creates a local ion-concentration deficiency. The Ca^{2+} and PO_4^{3-} will keep precipitating in the gel along the OH^- path, creating local concentration gradients that promote ion transport to the mineral-forming location. The mineral concentration in the gel can be controlled by the movement of the OH^- front. Reprinted with permission from Reference [59], copyright © 2009, American Chemical Society.

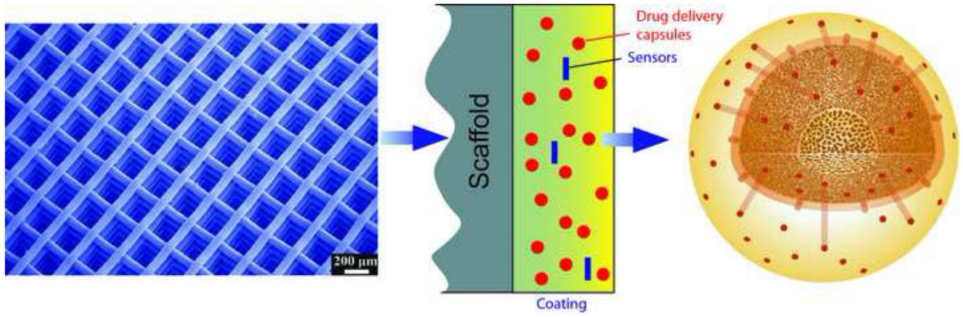

Figure 6.

A robocasted scaffold like the glass one shown in the scanning electron micrograph in the left could be coated with polymer layers containing drug delivery spheres to manipulate the spatio-temporal release of chemicals. The inside of the sphere will be made from porous hydrogel, or gelatin (sponge) into which the drug (BMP-2, VEGF, etc.) will be infiltrated. The outside will be made from degradable PLGA, or polycaprolactone, to protect the hydrogel and prevent burst release of the drug. The micro-capillaries will be introduced with a laser. The optimum amount and diameter of the micro-capillaries could be determined using diffusion calculations to project the release of the drug for the desired time, also assuming the thinning of the PLGA capsule. The coating composition can also be tailored to manipulate drug release and control its biodegradation, while sensors embedded in the coating monitor the biochemical environment.

Table 1

Scaffold design criteria. The strength and modulus for materials designed for load-bearing applications are based on the mechanical properties of bone, while for low-load and medium-load-bearing situations, the main requirements are to combine flexibility and strength to survive handling and placement while maintaining shape in vivo. Initiation toughness should be equal or superior to ceramics currently used in load-bearing situations and similar to the initiation fracture toughness in bone (>5 MPa·$m^{1/2}$). Bone regeneration and return-to-function times should match or improve those of current treatments.

Functional Requirement ***potential clinical application***	**Mechanical Requirements**		**Bone Formation**	**Candidates**		**Benefits of Appropriate Scaffolds**
	Strength	**Young's modulus**		**Material**	**Form**	
Low load-bearing • Sinus *augmentation* • Extraction sites	Minimal	0.05-1 GPa	4 months	• Polymers • Polymer/ceramic hybrid composites	• Injectable • Flexible	• Eliminates risks of bone grafting • Eliminates xenogeneic materials and potential inflammatory response • Ease of handling
Medium load-bearing • Alveolar cleft repair • Alveolar ridge augmentation • Calvarian repair	> 50 MPa	1 GPa	4-5 months	• Polymers • Polymer/ceramic hybrid composites	• Some density to support soft tissue or limit soft tissue collapse • Porous matrix	• Eliminates risks of bone grafting • Ease of handling • Shortens surgical time
High load-bearing • Segmental mandibular defect repair • Segmental tibial defect repair • Cervical disk	> 150 MPa	10-30 GPa	> 6 months	• Inorganic • Hybrid composites with high mineral content	• Porous matrix • Dense:able to create • porosity in vivo	• Eliminates risks of bone grafting or flap surgery • Shortens surgical time • Rapid return of function