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Nanotechnology in bone tissue engineering

Graham G. Walmsley, BA[a,b,1], Adrian McArdle, MB, BCh, BAO, MRCSI[a,b,1], Ruth Tevlin, MB, BCh, BAO, MRCSI[a], Arash Momeni, MD[a], David Atashroo, MD[a], Michael S. Hu, MD[a], Abdullah H. Feroze, BA[a], Victor W. Wong, MD[a], Peter H. Lorenz, MD[a], Michael T. Longaker, MD, MBA[a,b], Derrick C. Wan, MD[a,*]

[a]*Hagey Laboratory for Pediatric Regenerative Medicine, Department of Surgery, Plastic and Reconstructive Surgery, Stanford University School of Medicine, Stanford, CA, USA*

[b]*Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, USA*

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Abstract

Nanotechnology represents a major frontier with potential to significantly advance the field of bone tissue engineering. Current limitations in regenerative strategies include impaired cellular proliferation and differentiation, insufficient mechanical strength of scaffolds, and inadequate production of extrinsic factors necessary for efficient osteogenesis. Here we review several major areas of research in nanotechnology with potential implications in bone regeneration: 1) nanoparticle-based methods for delivery of bioactive molecules, growth factors, and genetic material, 2) nanoparticle-mediated cell labeling and targeting, and 3) nano-based scaffold construction and modification to enhance physicochemical interactions, biocompatibility, mechanical stability, and cellular attachment/survival. As these technologies continue to evolve, ultimate translation to the clinical environment may allow for improved therapeutic outcomes in patients with large bone deficits and osteodegenerative diseases.

Key words: Bone; SPIONs; Nanoparticle; Osteogenesis; Scaffold; Nanotechnology

Introduction

Bone grafts represent one of the most common tissue transplants, with over 2.2 million performed annually worldwide.[1] While autologous bone grafting for the reconstruction of skeletal defects is the current gold standard, this technique is hindered by variable resorption, limited supply, donor site morbidity, and high failure rates (up to 50%) in certain sites.[2–4] These limitations lead to the development of synthetic biomaterials for the replacement of bone tissue. However, these synthetic materials are hindered/limited by their potential for both foreign-body reactions and infection. In recent years, nano-engineered particles and porous 3D scaffolds that facilitate growth of new bone have garnered significant attention.

There are several critical considerations which must be made to successfully guide bone regeneration. Importantly, natural bone is comprised of 30% w/v organic collagen fibrils and 70% inorganic calcium phosphate crystals. This composition has served as a model to mimic bone structure on a macro- and nanoscale level.[5,6] Polymeric matrices combining calcium phosphates with materials such as chitosan have been studied to treat various bone defects.[7] Advances in nanotherapeutic approaches, however, have allowed for further manipulation of the extracellular matrix to provide a more appropriate surface chemistry and interconnected porosity for cellular proliferation and angiogenesis. Another important factor is the need for

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*Corresponding author at: Department of Surgery, Hagey Laboratory for Pediatric and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA 94305-5418.

E-mail address: dwan@stanford.edu (D.C. Wan).

[1] These authors contributed equally to this work.

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controlled spatial and temporal delivery of signaling molecules to guide cellular survival and differentiation. Finally, biocompatibility is key, as synthetic nanomaterials should remain inert or ideally resorb in a predictable and controlled manner to allow for remodeling.

Nanoparticles exist in the nanosize range, usually <100 nm, and due to their size and surface area, they can be exploited as vectors for delivery of drugs, growth factors, and genetic material.[8] Importantly, the size of nanoparticles can determine their half-life and distribution. While particles <10 nm are cleared by the kidney, those larger than 200 nm are typically phagocytosed and removed by the spleen.[9–11] Most therapeutic nanoparticles therefore range from 10 to 100 nm where they can be distributed throughout the circulatory system and penetrate through small capillaries.[11] Surface properties may also affect stability and localization in the body, and charge has been shown to be a large determinant impacting internalization of nanoparticles into various target cells.[8] For example, superparamagnetic iron oxide nanoparticles (SPIONs) have been employed to convey drugs or genetic material to target sites/cells in the body under the influence of a magnetic field. Similarly, hydrophobic surfaces have been found to promote engulfment by circulating macrophages whereas surface-engineered hydrophilic polymers (e.g. polyethylene glycol with hydroxyl or amino functional groups) allows for escape of nanoparticles from reticuloendothelial cells.[12,13] Importantly, the physical properties of nanovectors should allow for loading that does not compromise functionality of the package, distribution to desired sites, and finally release at a desired rate.

In this review, we discuss past and current advances in nanoparticle-based therapies for bone tissue engineering. These include developments in nanotherapeutic strategies to deliver drugs and growth factors promoting bone formation, as well as gene therapy reagents (i.e. siRNAs or plasmid DNA). Nanomaterials have also allowed for significant advances in imaging and stem cell targeting and these applications will be elaborated. Lastly, recent discoveries in nano-composite designs and scaffold modifications will be highlighted aiding mechanical stability, biocompatibility, and cellular survival for implanted constructs.

Nanoparticle-based delivery

In general, nanoparticles can be applied locally in bone tissue engineering (BTE) to augment tissue regeneration, enhance osseointegration of implants, and to prevent infections.[14] Given unsatisfactory outcomes with many contemporary biomaterials alone for bone replacement, increasing interest has thus developed in the use of bioactive molecules aimed to promote bone formation. Direct administration of therapeutic agents suffers from the intrinsic limitations of these small molecules including poor physiological stability, non-specific targeting and low cell membrane permeability.[15] In many cases, supraphysiological doses are necessary to combat the poor pharmacokinetics of these compounds, thereby increasing the potential risk of adverse effects.[16] Nanomaterial carriers can overcome these limitations by stabilizing the bioactive molecules through encapsulation or surface attachment,[16] facilitating entry into cells, targeting cellular delivery,[17] and providing controlled drug release at the designated target[18] (Figure 1).

Nanospheres have been widely accepted as a useful tool for controlled drug delivery due to their inherently small size and corresponding large specific surface area, a high drug loading efficiency, a high reactivity towards surrounding tissues *in vivo*, and an ease of diffusion of drug-loaded particles.[19] A goal of modern clinical therapeutics is the targeted delivery of drugs. To this end, the small size of nanospheres allows them to quickly respond to stimuli from the surrounding environment (for example pH, magnetic fields, ultrasounds, and irradiation) and thus, these spheres can serve as stimulus-driven delivery for biologically or chemically active agents, and subsequently, establish triggered release by responding to external stimulation.[19–23]

Delivery of drugs, growth factors, or genetic material may be accomplished following encapsulation in, either, degradable or non-degradable nano-spheres. Examples of non-degradable nanoparticles include hydroxyapatite, gold, dendrimer, and silica[24–27]; while degradable nanoparticles include poly(L-lactide) (PLA) or poly(L-lactide-*co*-glycolic) (PLGA).[28,29] The selection of the base biomaterial for nanosphere construction depends on the desired end application criteria. It depends on many factors such as (i) size of the desired nanoparticles, (ii) properties of the drug (aqueous solubility, stability, etc.) to be encapsulated in the polymer, (iii) surface characteristics and functionality, (iv) degree of biodegradability and biocompatibility, and (v) drug release profile of the final product.[30] Frequently, nanoparticles can be combined with scaffolds such as proteinaceous hydrogels or biodegradable polymeric matrices to facilitate application in bone. Osteoblasts and osteoclasts have an intricate relationship and their respective activity is key to bone homeostasis.[14] Osteoblasts can be supported by nanoparticle-based drug/growth factor (GF) delivery or alternatively osteoclasts can be modulated by nanoparticles locally releasing specific inhibitors.[14]

Biodegradable nanospheres can be prepared from a variety of materials such as natural polymers (proteins and polysaccharides) and synthetic polymers. In contrast to injected proteins, which are usually rapidly cleared from the body, locally adsorbed proteins are released by desorption or diffusion and thus, can be retained longer.[31] Towards this end, nanospheres are being explored as finely adjustable delivery systems with regard to the location and time period of drug release. Local drug delivery is favorable to systemic application to minimize adverse effects. Moreover, adequate tuning of the nanoparticles allows for a temporally-controlled, sustained delivery according to requirements. Furthermore, unstable biological activity of growth factors, genes and drugs can result in inefficient delivery of these bioactive molecules.[32] Compared to direct adsorption of a bioactive molecule on the surface of an implanted scaffold, a carrier delivery system provides controlled, long-term release with adequate efficacy.[33] Delivery vectors require materials that are biocompatible, biodegradable as well as suitable for encapsulation of bioactive molecules. In particular, encapsulated growth factors may be released as the polymer degrades following a controlled and predetermined profile, a key factor of biodegradable nanosphere design. Thus, nanospheres are being increasingly explored as finely adjustable delivery systems with regard to the location and time period of drug release, while