REVIEW ARTICLE

Nanoscale Bioceramics In Bone Tissue Engineering- An Overview

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ABSTRACT

This review presents a general view on application of nanomaterials in the field of animal production and medicine especially of nanoceramics in bone tissue engineering and regenerative medicine (TERM). Nanotechnology as an emerging field provides new and better tools and technologies to biology, chemistry, engineering, and medicine for various applications. It has been widely employed in poultry and animal production, food safety, as well as diagnosis and treatment of animal and human diseases. Nanomaterials including nanoscale bioceramics are used to diagnose and treat diseases in the field of nanomedicine. TERM is continually attempting to find a reliable and efficient method to regenerate and heal tissue defects and bone defects in particular. Due to appropriate surface features and superior biomechanical properties to biopolymers, nanoscale bioceramics have gained more attention in bone tissue engineering. These nanomaterials provide many advantages that are essential for bone regeneration including high bioactivity, biocompatibility, biodegradability, suitable biomechanical strength, osteoconductivity, and osteoinductivity. However, some concerns such as cytotoxicity potentials are associated with nanomaterials.

Keywords: Bone tissue engineering, Nanocomposites, Nanomedicine, Nanoscale bioceramic, Nanotechnology.

Ind J Vet Sci and Biotech (2020): 10.21887/ijvsbt.16.(2,3,&4).2

INTRODUCTION

One of the main concerns of orthopedic surgeons is to treat large and critical-sized bone defects and to promote bone regeneration (Oryan *et al.,* 2014^{a}). For this purpose, many researchers have made massive and continuing efforts to find an appropriate methodology with minimal side effects. The final goal of any attempt in bone tissue engineering is to find and fabricate an appropriate scaffold causing faster bone regeneration and restoration of normal bone structure and function. The discovery and design of the ideal scaffolds to accelerate bone regeneration and to overcome serious problems with mostly unfavorable treatments has been an area of interest.

An ideal biomaterial should exhibit some properties such as bioactivity, biocompatibility, controlled biodegradability, osteoinductivity as well as osteoconductivity to establish a bond between the host bone tissue and the implant material (Oryan *et al.,* 2014^{a}; Oryan *et al.,* 2017^{a}). Moreover, it should have adequate biomechanical properties especially in the case of the force-bearing tissues like bones (Oryan *et al.,* 2017^{a}). A scaffold will be desirable in the field of bone tissue engineering and regeneration medicine (TERM) if it is processed into a three-dimensional (3-D) porous structure mimicking the bone composition, is highly conducive for cell adhesion and matrix deposition, has passages for nutrients and oxygen, and is well vascularized (Oryan *et al.,* 2014^{a}; Oryan *et al.,* 2017^{b}). The most commonly used materials for fabrication of scaffolds employed in bone TERM include natural or synthetic polymers, ceramics and composites (Oryan *et al.,* 2014^{a}; Oryan *et al.,* 2017^{a}). Despite the obvious advantages of biopolymers, some issues should also be considered including their minimal biomechanical properties and lack of a controlled degradation rate in accordance with new bone formation and deposition (Oryan *et al.,* 2017^{a}). On the other hand, bioactive and biodegradable ceramics such as hydroxyapatite (HA), tricalcium phosphate (TCP), dicalcium phosphate (DCP), and bioactive glasses are widely used for biomedical applications because of their high compressive strength and hardness along with good biocompatibility (Gao *et al.,* 2014; Oryan *et al.,* 2017$^{a;}$ Oryan *et al.,* 2017^{b}). They can create strong bonds to bone tissue by dissolving in physiological fluids and degradation and, thereby improve bone graft incorporation and osseointegration. The degradation and solubility of these materials highly depend on their crystallinity and particle size and their degradation is controllable during the manufacturing process (Lv *et al.,* 2013; Pachauri *et al.,* 2014). However, they are brittle and fragile.

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How to cite this article: Alidadi, S. (2020). Nanoscale bioceramics in bone tissue engineering- An Overview. Ind J Vet Sci and Biotech, 16(2,3,&4): 7-11.

Source of support: Nil

Conflict of interest: None.

Submitted: 08/08/2020 **Accepted:** 27/11/2020 **Published:** 25/12/2020

Bone has a hierarchical structure ranging from nano- to macroscale and it is composed of organic (collagen fibrils) and inorganic (nanocrystalline HA) phases (Vieira *et al.,* 2017). Conventional tissue engineering usually neglects the nanoscale structures and properties of the bone tissue and the biomaterials implanted into the bone defects mostly have micron scales and restore the macro- and microscale properties of the native bone tissue. Unfortunately, the micro-scale scaffolds have failed to satisfactorily reconstruct the bone defects as a result of insufficient bone regeneration resulting from some complications including undesirable local cell and tissue responses (Mohammadi *et al.,* 2018). Given the organized nanoscale hierarchical structure of the bone, there is no doubt that nanotechnology and implementation of the nanoscale structure of scaffolds is central to bone healing. One of the candidates for this regard is to fabricate scaffolds or composites containing nanoscale structures by deriving benefit from tissue engineering and nanotechnology. Indeed, to mimic the hierarchical organization of the natural extracellular matrix (ECM), one strategy is to incorporate nanoscale features in the 3-D scaffold design. The current review focusses on the role of nanotechnology in animal health with emphasis on bone tissue engineering. Nanoscale bioceramics are specifically highlighted for the fabrication of novel bone-graft substitutes to improve bone regeneration in this review.

Nanotechnology in Animal Production and Health

Nanotechnology is defined as the study of materials at the nanoscales ranging from 1 to 100 nanometers. This field of science helps researchers and scientists to make functionally promising products in imaging, printing, material synthesis, and medicine as well as other fields on the basis on the fact that most of biological processes occur at the nanoscale (Mostafavi *et al.,* 2019). Nanoscale materials have higher reactivity and solubility associated with their chemically and physically structural changes at a size-dependent manner compared with microscale counterparts (Tatli Seven *et al.,* 2018). Nanoscale materials have a larger surface areas and thus they can establish efficiently direct contact with cells, biological factors, matrix ingredients, and surrounding materials (Mostafavi *et al.,* 2019). Nanotechnology has shown the potential for alleviating problems and shortages of various aspects associated with animal health, production and reproduction (Sekhon, 2014; Tatli Seven *et al.,* 2018; Fesseha *et al.,* 2020). The implementation of nanotechnology in the field of animal production, breeding and reproduction, nutrient delivery, food safety, biocides, veterinary medicine, medical diagnostics as well as drug delivery is becoming attractive and increasing since the past few years (Kuzma, 2010; Hill and Li, 2017; Tatli Seven *et al.,* 2018). Other applications of nanotechnology in animal nutrition and production include food hygiene and biosafety, food packaging, feed supplements using nanocapsulation, delivery of growth hormone, feed quality control, and digestion and absorption improvement by the use of nanoparticles (Kuzma, 2010; Hill and Li, 2017).

Biotechnology can be used to make nanosensors for detecting, binding, and removing pathogens in the food production chain. Form this point of view, nanotechnology might provide alternatives to antibiotics in animal production and; therefore, the crucial issues concerning antibiotic resistance in foodborne pathogens might be partly avoided (Kuzma, 2010; Hill and Li, 2017). Indeed, nanosensors could detect antibiotic or hormone residuals in meat and poultry and improve meat quality (Sekhon, 2014; Hill and Li, 2017). Nanomaterials due to their small size exhibit considerable properties including greater surface area, quantum, reactivity, and penetrability in comparison to micro- or macroscale materials (Kuzma, 2010; Fesseha *et al.,* 2020).

Nanotechnology and Regenerative Medicine

In addition to poultry and livestock feed, nanotechnology can also be employed in different areas of medicine, namely nanomedicine, including cancer diagnosis and therapy, drug delivery, tissue engineering, and regenerative medicine for diagnostic and therapeutic purposes (Ghorbani *et al.,* 2015; Mostafavi *et al.,* 2019; Rabiei *et al.,* 2020). Regenerative medicine is defined as an interdisciplinary field of medicine that develops methodologies to replace or regenerate damaged tissues or organs to re-establish and restore their normal structure and function (Oryan *et al.,* 2014[a]; Mostafavi *et al.,* 2019). This branch of medicine with the help of tissue engineering, known as TERM, has made tremendous advances in wound dressing and healing, engineering of various soft and hard tissues such as cardiac and neural tissues, tendon, cartilage, and bone (Mostafavi *et al.,* 2019).

Nanotechnology can be employed to deliver biological agents including hormones, growth factors, genes and other factors required for tissue regeneration (Mohammadi *et al.,* 2018; Tatli Seven *et al.,* 2018; Rabiei *et al.,* 2020). Concerning hard tissue, some common orthopedic diseases such as osteoarthritis and osteoporosis can be diagnosed and treated by nanotechnology (Rabiei *et al.,* 2020). Orthopedic applications of nanotechnology include drug delivery, surface modification of implants, tissue engineering for regeneration of soft and hard tissues (Mohammadi *et al.,* 2018; Rabiei *et al.,* 2020). Regeneration of large and critical-size bone defects is one of serious challenges to orthopedics. In other words, bone healing and regeneration is one of the areas in which designing a model that mimic all tissue properties remains still challenging (Oryan *et al.,* 2014[a]). Efforts for healing bone injuries especially large bone defects have been greatly hampered by hierarchical structure and high vascularization and metabolic requirements of bone (Vieira *et al.,* 2017).

Nanotechnology seems to open up a new era for bone tissue engineering to create nanostructures comparable to natural bone in size (Mohammadi *et al.,* 2018). Inflammatory response, macrophage infiltration, and foreign body reaction occurred after biomaterial implantation interfere with and delay the healing process (Khang *et al.,* 2008). This detrimental impact of such persistent inflammatory responses can be diminished by using nanoscale surfaces of biomaterials (Khang *et al.,* 2008; Mostafavi *et al.,* 2019). Nanomaterials include metals, polymers, organic materials, ceramics, and composites (Mostafavi *et al.,* 2019). Bioceramics at the micro- and nanoscale have been frequently used in bone tissue engineering to accelerate bone formation and regeneration (Ghorbani *et al.,* 2015; Oryan *et al.,* 2017^{a}; Singh *et al.,* 2020).

Nanoscale Bioceramics in Bone Tissue Engineering

Compared with their micro-sized conventional bioceramics, nano-sized bioceramics can mimic nanocomposition and nanoscale features of the bone tissue, bind to bone tissue, improve osseointegration and bone growth in the bone-implant interface, and osteoconductive and osteoinductive capacities of the implant (Zhou and Lee, 2011; Lyons *et al.,* 2020). They have a strong adsorption feature of bioactive molecules and cells and roughness. Nano-sized bioceramics enhance protein adsorption, and cell adhesion, proliferation, and differentiation by providing optimal chemistry for cell interactions and bone growth (Zhu *et al.,* 2017; Mondal *et al.,* 2020). These biomaterials can diminish osteoclast activity and inhibit fibrous callus formation while stimulating osteoblasts and encourage new bone formation (Pang *et al.,* 2015). They have attracted the attention of the researchers in the field of TERM because of their unique properties including improved surface topography and chemistry, fine grain size, enhanced hydrophilicity, large surface area, and strong interfacial interactions (Stassi *et al.,* 2012; Mohammadi *et al.,* 2018; Mondal *et al.,* 2020). These characteristics are key factors positively affecting biomechanical properties such as torsion and tensile modulus. Therefore, nano-sized bioceramics seem promising to effectively solve intrinsic brittleness and low bending strength of conventional bioactive ceramics particularly for the load-bearing bone sites (Hasnain and Kumar Nayak, 2019). Moreover, it is well established that nanoceramics have superior strength, toughness, stability, and hardness to conventional microscale bioceramics (Gao *et al.,* 2014; Lyons *et al.,* 2020).

Besides, the scaffolds made from nanoceramics also has superior properties in other respects. For instance, the degradation rate of scaffolds could match the formation and growth rate of new bone by controlling the size of the crystalline grain (Gao *et al.,* 2014). These merits of nanoceramics are highly important for the researchers to improve the bone graft healing rate and reduce complications. In addition, the size of the cell surface receptors is nanoscale, and also the interactions among cells, the components of ECM, and biological molecules all occur on the nanoscale which affects directly the cell behavior such as proliferation and gene expression (Pereira *et al.,* 2005; Chun *et al.,* 2013). In fact, nano-sized bioceramics provide a higher surface area that positively influences on protein adhesion and promotes cell adhesion mediated by integrins through an increased expression of integrins in osteoblasts (Gao *et al.,* 2014; Venkatesan *et al.,* 2016).

HA resembles the inorganic phase of the natural bone resulting in osteoconductive properties and the formation of tight bonds with surrounding tissues (Oryan *et al.,* 2014^{a}; Oryan *et al.,* 2017^{b}). It has been consistently demonstrated that nanophase HA (nHA) could improve cytocompatibility and bone formation in comparison to conventional micro-scale HA scaffolds (Pang *et al.,* 2015; Mondal *et al.,* 2020). The low strength and brittleness of conventional HA can be attributed to its low fracture toughness that limits its use to non-load-bearing applications (Gao *et al.,* 2014; Hu *et al.,* 2014; Venkatesan *et al.,* 2016; Mondal *et al.,* 2020; Singh *et al.,* 2020). Some authors have suggested that the nHA coating has great impacts on the bone regeneration at earlier healing periods, while the influence of microtopography is more efficient at completely healed stages (Kalita *et al.,* 2007; Bryington *et al.,* 2013; Hu *et al.,* 2014). Bioceramic-based nanomaterials have been also used to cover implant surfaces. For instance, the experimental studies on rats demonstrated that nHA-coated titanium scaffolds accelerate new bone formation when used as osteoconductive coatings compared to the uncoated or conventional micro-sized HAp coated scaffold (Jiang *et al.,* 2015; Pang *et al.,* 2015). Additionally, porous biphasic calcium phosphate (BCP) scaffolds coated with nHA have been shown to be more conducive for cell adhesion, proliferation, and osteogenic differentiation than conventional uncoated BCP scaffolds (Hu *et al.,* 2014). These findings confirm that the nHA coating and a nanostructured surface can enhance the osteoinductive potential of BCP ceramics, making it more suitable to be used in bone tissue engineering (Hu *et al.,* 2014; Zhu *et al.,* 2017). Many techniques have been used to synthesize nano-sized TCP and nHA including sol-gel synthesis, mechanochemical synthesis, solid-state reactions, co-precipitation, wet chemistry, micro-emulsion synthesis, and hydrothermal reaction. The most applicable and affordable fabrication method is still unknown; however, the sol-gel method has gained more popularity because it improves purity and reduces synthesis temperature in comparison to other methods (Kalita. *et al.,* 2007; McMahon *et al.,* 2013; Singh, 2018).

Nano-sized bioglasses have also shown a capacity for inducing cell proliferation and differentiation into bone-forming cells (*i.e.* osteoblasts) with a great bioactivity and developing strong bonds between the implant and bone compared with micro-sized conventional bioglasses (Wu and Chang, 2012). Nano-sized mesoporous bioglasses (MBGs), a new generation of bioactive glass, have shown the stimulatory effect on new bone formation compared with conventional bioactive glasses (Wu and Chang, 2012; Baino and Fiume, 2020).

Reinforcement of Bioactive Ceramics

Nanoscale bioceramics and biopolymers can be used together to fabricate nanocomposites so that their properties are combined and augmented toward accelerating bone healing and regeneration (Gao *et al.,* 2014; Ghorbani *et al.,* 2015; Hasnain *et al.,* 2019). Employment of nanoceramics for fabrication of inorganic-inorganic or organic-inorganic hybrid nanocomposites can add sufficient mechanical strength to the implant (Hasnain *et al.,* 2019).

Moreover, blends of scaffolds, cells, and growth factors can mimic the components of native bone and provide exhibit functional properties (Oryan *et al.,* 2014[a]). Nanoceramics like nHA can serve as an appropriate osteoinductive element for biological factors and stem cells to differentiate the cells (Xia *et al.,* 2014; Ghorbani *et al.,* 2015; Venkatesan *et al.,* 2016). Besides, neovascularization adds the fourth dimension in the biomimetic effort for bone graft engineering (Gao *et al.,* 2014; Xia *et al.* 2014). Bone morphogenetic proteins (BMPs) are important osteoinductive growth factors and they have tremendous potential for inducing bone growth (Oryan *et al.,* 2014[b]). BMP delivery systems based on nanostructured systems are likely to sustain BMP controlled release at the defect site to enhance repair efficacy (Oryan *et al.,* 2014[b]; Mostafavi *et al.,* 2019). Nanoscale bioceramics could also be applied for this purpose and for delivering angiogenic growth factors such as platelet-derived growth factor and vascular endothelial growth factor to enhance vascularization that is important and key in bone repair (Farokhi *et al.,* 2013). Xia *et al.* (2014) have suggested that HA-based scaffolds with a micro-nano hybrid surface can act as a cell carrier for adipose stem cells and construct a vascularized tissue-engineered bone. These highly interconnected macroporous scaffolds could promote cell attachment, migration, proliferation, and osteogenic differentiation and enhance the expression of angiogenic factors. Nonetheless, nanotechnology alone may not be the answer to improving angiogenecity and the mechanical properties of scaffolds. Nanotechnology with the help of gene therapy might become a potential tool to apply osteoinductive growth factors and/or angiogenic growth factors in bone regeneration by inducing long-term osteoinductive stimuli and promoting angiogenesis for new bone formation (Oryan *et al.,* 2014[a]; Mohammadi *et al.,* 2018; Mostafavi *et al.,* 2019).

Concerns about Nanoscale Bioceramcis Toxicity

Despite all the mentioned advantages and superiority of nanomaterials and specifically nanoscale bioceramics over microscale ones, there are some main concerns about their health because of their probable effects on biological systems and cytotoxicity or genotoxicity (Hill and Li, 2017; Mostafavi *et al.,* 2019). The materials at the nanoscale level might interact with components of extracellular matrix, bind to receptors on the cell surface, and interfere with cell signaling, behavior, and activities (Hill and Li, 2017). It is worth noting that the toxic effects of nanomaterials are largely determined by their size, shape, and charge, so that the risk of toxicity increases with decreasing size, fiber-like shape, and positive charge (Engine *et al.,* 2017). A key technology and new generation of material engineering namely picotechnology, employment of materials at the atomic levels, have been suggested to modify surfaces without potential cytotoxicity concerns associated with nanomaterials (Mostafavi *et al.,* 2019).

CONCLUSION

Despite all the mentioned advantages of nanomaterials including nano-sized bioceramics, they have some challenges, and their potential toxicity because of the nano-size is regarded as one of the main concerns. In addition to the biochemical damage, they can cause cell destruction by physical contact with biological molecules. Moreover, nanomaterials can lead to an increase in the production of reactive oxygen species and therefore, their toxicological risks remain challenging. Future studies are needed for establishing the standard protocols to diminish the cytotoxicity of nanomaterials.

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