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Porous hydroxyapatite-gelatin nanoscaffolds for bone and teeth applications

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ABSTRACT

Tissue engineering as a novel promising means offers biological alternatives for implants and prostheses. Inorganic nanomaterials are widely used for bone and teeth applications. The nanomaterials porous structures are more bioresorbable as well as more potent bonding to the bone and give an improved mechanical interconnection resulting a stable fixation of the implant to the bone. Mimicking a real bone using porous scaffolds can reduce the stress-shielding effect. Also, using these constructions use the profit of combining portions with different porosities. Parts with a high porosity ease the transportation of body fluids that can improve the bone regeneration. Furthermore, less-porous areas support the mechanical load as load-bearing parts, that are vital for bone grafts. It has been testified that the biological response of porous HA nanoscaffolds can be further improved over the combination of collagen, gelatin, chitosan, etc.

Keywords: *Porous hydroxyapatite, gelatin, scaffold, nanomaterial*

1. INTRODUCTION

The scaffold is an important component in tissue engineering for bone regeneration that acts as a template for cell interactions and the formation of bone-extracellular bone matrix to offer structural support to the newly formed tissue [1-3]. Ceramic and inorganic materials are widely used for bone and teeth applications. They mainly include calcium derivatives like hydroxyapatite, tricalcium phosphate, tetra-calcium phosphate, or other materials such as alumina or silica based bioactive glasses and pyrolitic carbons. Hydroxyapatite (HA), also termed as hydroxylapatite, is a naturally occurring mineral form of calcium apatite with the formula $Ca_5 (PO_4)_3 (OH)$. It may be written as $Ca_{10} (PO_4)_6 (OH)_2$ to display that the crystal unit cell contains two entities. It is known as an osteoconductive material with enhanced bone curative ability. Reports have shown that there is a direct bonding of HA to bone [3, 4]. However, the exact mechanism of the bonding is still unclear with the need to more clinical tests. The HA with porous structures are more bioresorbable as well as more potent bonding to the bone and give an improved mechanical interconnection resulting a good fixation of the implant to bone [5, 6].

Nanoparticles as ultrafine sub-micron particles can valuably advance possessions of the materials compared to their similar bulk ones [7-12]. Nanotechnology has opened new views to prepare low-priced HA in nanosystems by different approaches. Osteoconductive nanoparticles like HA may induce a chemical bond with bone to advance the biological fixation when are used as coating of dental implants. Besides, bone regenerative possessions of these materials may offer a good condition for new bone formation [13].

The surface of porous HA nanoparticles includes higher surface area and therefore higher reactivity. It can offer outstanding possessions in different medicinal fields, especially in dentistry field. It has been reported that the biological response of porous HA nanoscaffolds can be further enhanced over the combination of collagen, gelatin, chitosan, etc [14, 15]. Based on the biomimetic reports, HA and collagen have been applied to prepare scaffolds as artificial bone substitutes. However, the main issues of collagen are the cost as well as the unknown availability of commercial sources. Gelatin as a degradable biopolymer derived from hydrolyzed collagen can be used as an efficient and low-cost alternative material. Similar amino acid sequencing makes gelatin a suitable material for the scaffold matrix. It also eliminates the immunogenicity and pathogen transmission problems of collagen.

2. POROUS HA AS SCAFFOLD

A perfect scaffold should possess biodegradability in reasonable time frame and lower toxicity as well as suitable strength, porosity and microstructure. A porous scaffold should provide cellular activities such as adhesion, growth, and differentiation, and also permit osteocytes to seed into interconnected pores to integrate with the surrounding bone histologically. Microporosity is required to gain a fine capillary network and cell-scaffold interactions. Macroporosity also allows osteocytes seeding into scaffolds. In dental surgical trials, porous three dimensional scaffolds are often applied for regeneration and repair of damaged bones. Such scaffolds involve in endorsing cell-biomaterial interaction, cell adhesion, transporting vital elements, bone cell proliferation and differentiation [16, 17].

HA seems to be attractive material for orthopedics and dentistry due to its similarity to the in-organic constituent of teeth and bone. The porous HA is more bio-resorbable and forms stronger bonding to the bone. Furthermore, the pores give an improved mechanical interconnection resulting a good implant fixation. The ideal physical and biological possessions of HA convert it to a unique option for the scaffold application. Indeed, a three dimensional interconnected porous HA is necessary for cell attachment, proliferation, differentiation as well as maintaining circulation of biofluids at the damaged bone areas. Various approaches to yield porous HA have been examined, including polymer replication, solid freeform fabrication, rapid prototyping, and freeze-casting [18, 19].

3. POROUS HYDROXYAPATITE-GELATIN NANOSCAFFOLDS FOR BONE AND TEETH APPLICATIONS

The pores of porous ceramics provide rough surfaces and they are preferred by bone cells to grow into the interconnected pores. This can improve the adhesion between the bone and implant. Besides, required possessions for bone scaffolds can be obtained by regulating pore size and porosity to further mimic the properties of a human bone [19, 20].

It has been reported that the biological response of porous HA nanoscaffolds can be further enhanced over the combination of collagen, gelatin, chitosan, etc. HA gelatin or collagen based scaffolds seem to be more appropriate in bone tissue engineering applications due to their chemical and structural similarity to native bone. Both of them are readily assimilated by the body. However, the main issues of collagen are the cost as well as the unknown availability of commercial sources [20-25]. Then gelatin can be used as an efficient and low cost alternative material. A scaffold of HA-gelatin reported showing improved osteoconductivity and biodegradation beside enough mechanical strength [26]. For example, in a recent report by Jun et al. HA-gelatin nanoscaffolds enhanced the fibroblast iPSC (induced pluripotent stem cells) osteogenesis as compared with rod shaped HA nanoscaffold for *in vivo* and *in vitro* conditions [27].

Despite the mentioned advantages, gelatin shows poor mechanical possessions as well as a rapid degradation because of high degree of solubility. A hopeful method to overcome these issues, and also meet the biomimetic suggestion is to support it with bioactive ceramics to attain an optimal balance between bioactive and chemical behaviors, and physiomechanical possessions. Besides, studies exhibited that the effect of gelatin in bioactivity over the diffusion of calcium ions that lead to improving biomineralization especially when impregnated by various inorganic agents.

Kazemzadeh et al prepared HA-gelatin scaffolds by solvent-casting method joint with freeze drying process. The results showed that the organized structures show an open, interconnected porous construction with a pore size of 80-400 μm, with the ability to osteoblast cell proliferation. The obtained results also showed that adding of HA can decrease the water absorption as well as the porosity. Based on cell culture results, fibroblast cells partly proliferated and covered on scaffold, 48h after seeding. The authors concluded that, the manufactured scaffolds are an appropriate example for trabecular bone tissue engineering [27].

Lee et al reported a simple technique for preparation of porous HA scaffolds with homogeneous porous structures and graded porosity using sequential freeze-casting. Their results showed that as porosity decreased the compressive strengths were improved. Furthermore, the mechanical and structural characteristics could be adjusted while maintaining the bone-like structure, by changing the proportions of the porous and dense parts. In vitro biocompatibility test showed confirmed the biocompatibility of the scaffolds. Besides, preosteoblast cells exhibited well spread on the scaffolds. Then, the cell viability increased meaningfully after 3-5 days of culturing [28].

Azami et al prepare a nano-structured scaffold for bone repair using HA and gelatin using freeze-drying and lamination techniques. They seeded osteoblast-like cells on the fabricated scaffolds and their proliferation rate, intracellular alkaline phosphatase (ALP) activity and ability to develop mineralized bone nodules to compare with the osteoblasts grown on cell culture plastic surfaces. The implantation of the scaffolds is also tested in a serious bone defect shaped on rat calvarium. The results showed that the scaffold had a 3D dimensional interconnected homogenous porous structure pore sizes ranging from 300-500 μm. In vitro cell culture results exhibited that the scaffold had no negative effect on osteoblasts proliferation rate and progressed osteoblasts function by growing the ALP activity and calcium deposition and development of mineralized bone nodules. Also, the scaffold showed healing of critical size calvarial bone defect in rats. They concluded that this scaffold justifies all the key necessities to be measured as a bone substitute [29].

Khaled et al tested the in vitro possessions of nano-hydroxyapatite/chitosan–gelatin composites. To confirm the development of apatite layer on the composite surfaces, they tested in vitro behavior of the structures in simulated body fluid (SBF). The results showed the deposition of calcium and phosphorus ions onto hydroxyapatite /polymeric composite surfaces particularly the composites with high concentrations of polymer content. The results of scanning electron microscope (SEM) and Fourier transformed infrared reflectance (FT-IR) presented the creation of bone-like apatite layer on the composite surfaces. They concluded that such a hopeful composite can be appropriate for bone grafting as well as tissue engineering utilization in the future [30].

Raucci et al reported the preparation of a HA-gelatin scaffold by merging a sol-gel technique and freeze-drying approach [31]. The sol-gel transition of calcium phosphates approves the particles dispersion into the gelatin matrix. It is also shows a direct control of interaction among COOH gelatin /Ca2+ ions. Microscopic results confirmed that the control of the three-dimensional distribution of nanoparticles around the gelatin helix is probably based on the amount of inorganic constituent and the procedure situations. The results signified improved cell attachment and proliferation. Beside, the increased levels of alkaline phosphatase was shown that indicated osteoblastic differentiation of human mesenchymal stem cells toward the osteogenic lineage, representing the impact of bioactive solid signals on cellular actions. The authors suggested that the prepared scaffolds have good potential application for example as filler to in use for bone defects repairing [31-33].

4. CONCLUSIONS

Biomedical approaches of the porous nanomaterials in bone and supporting tissues are highly valued in the modern therapeutics and surgery. Mimicking the ordered structure of a real bone using porous 3D scaffolds as functionally graded structures, can reduce the stress-shielding effect. Besides, using of these structures use the benefit of combining parts with different porosities. Areas with a high porosity simplify the transportation of body fluids that leads to enhanced bone regeneration. Furthermore, less-porous areas support the mechanical load as load-bearing parts, that are vital for bone grafts.

5. REFERENCES

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