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Prospects of chitosan-based scaffolds for growth factor release in tissue engineering

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ABSTRACT

Tissue engineering is concerned about the rejuvenation and restoration of diseased and damages tissues/organs using man-made scaffolds that mimic the native environment of the cells. In recent years, a variety of biocompatible and biodegradable natural materials is employed for the fabrication of such scaffolds. Of these natural materials, chitosan is the most preferred one as it imitates the extracellular matrix (ECM) of the cells. Moreover, chitosan-based materials are pro-angiogenic and have antibacterial activity. These materials can be easily fabricated into the desired shape of the scaffolds that are suitable for tissue support and regeneration. Growth factors are small proteins/peptides that support and enhance the growth and differentiation of cells into a specific lineage. It has been observed that scaffolds capable of delivering growth factor promote tissue repair and regeneration at a faster rate when compared to scaffolds without growth factor. The present review focuses on the recent developments on chitosan-based scaffolds for the delivery of growth factors thereby improving and enhancing tissue regeneration.

1. Introduction

Tissue engineering is the field that deals with restoration of diseased or damaged tissues and organs by controlling the biological microenvironment [1,2]. Tissue engineering consists of a complex cascade of events which includes cell proliferation, differentiation, and synthesis of extracellular matrix [3]. A perfect tissue engineering scaffold should be a template for three-dimensional (3D) growths of tissues by providing porous structure for the growth of tissues, diffusion of oxygen, and delivery of nutrients and it should also mimic the natural microenvironment of the tissue. It should also interact with the surrounding cells and maintain the phenotype of the regenerated tissue. An ideal scaffold should be biocompatible, biodegradable, promote cell adhesion, proliferation and maintains the metabolic activity of the cells [4,5]. Moreover, the scaffolds with suitable pluripotent stem cells, angiogenic potential and prolonged nutrient supply will support the repair and regeneration of various tissues [6].

Chitosan, poly(β-(1–4)-linked-2-amino-2-deoxy-*O*-glucose), is a biocompatible and biodegradable natural polymer. It is obtained by the partial deacetylation of chitin that is found in the exoskeleton of many crustaceans and has many desirable properties in order to use as a biomaterial for tissue engineering and regenerative medicine [7]. Due to the presence of positively charged amino groups, chitosan is mucoadhesive, hemostatic, and capable of binding with cell membranes [8–10]. Chitosan has an ability to make the scaffolds with well interconnected porosity and desired shapes such as hydrogels, sponges, two-dimensional fibers/sheets and 3D porous structures for the improved cell viability by providing the supply of enough oxygen and nutrients [11–13]. Chitosan-based scaffold materials can also exhibit a controlled delivery of loaded therapeutic molecules and growth factors, which makes them a suitable candidate for tissue engineering and regenerative applications. In addition, due to the presence of primary amine and hydroxyl groups, chitosan can be chemically modified to obtain the verities of its derivatives with desired functionalities and properties.

During the tissue regeneration, growth factors play a vital role as the essential signaling molecules to initiate the cells for carrying out specific cellular response in the biological environment and proceeding towards the specific lineage [14]. Hence, tissue regeneration can be successfully achieved by controlling the local delivery of growth factors. Different types of growth factors such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and transforming growth factor (TGF) have been considered for the

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process of vascularization in bones [15]. It was found that basic fibroblast growth factor (bFGF) promotes angiogenesis, osteogenesis and nerve regeneration [16]. In addition, bFGF and TGF-β was found to play an important role in the initiation and progression of tissue repair [17]. In recent years, various types of scaffolds based on chitosan alone or chitosan combined with other biomaterials have been developed for the loading and delivery of growth factors. These scaffolds have shown their ability to accelerate the regeneration of tissues at a higher rate. The aim of this review is to discuss the recent developments of chitosan-based scaffolds designed for the delivery of growth factors in bone, periodontal, nerve, cartilage, and skin tissue engineering and regeneration.

2. Delivery of growth factors

2.1. In bone tissue engineering

Bone regeneration and repair is an intricate and challenging process, which involves the role of various hormones, growth factors, and cytokines. A complex cascade of molecular events governs the bone regeneration process. Among the various growth factors involved in bone regeneration, bone morphogenetic proteins (BMPs) such as BMP-2, BMP-6 and BMP-7 that belongs to TGF-β family are the most important and influence bone repair to a great extent. It is proved that BMPs are capable of inducing *in vitro* osteoblast differentiation, *in vivo* bone formation and increased alkaline phosphatase (ALP) expression which is the early indicator of cellular differentiation towards osteoblast phenotype [18]. Although BMPs are highly required for bone formation, their structural complicity, systemic side effects, short half-life and rapid clearance from the system are some of the hurdles in current usage of these growth factors. An ideal carrier for the delivery of BMPs should exhibit overall increased total release amount as well as a sustained release for the prolonged time. Moreover, the carrier material should protect the protein from denaturation. In this context, chitosan-based materials can be ideal for the immobilization and delivery of BMP-2 since BMP-2 maintains its intact structure at the isoelectric point between 5 and 6 that is close to the isoelectric point of chitosan [19]. BMP-2-loaded polyelectrolyte complex made up of chitosan and hyaluronic acid exhibited controlled release of the growth factor in pre-osteoblastic cells *in vitro* [20]. Real-Time PCR analysis revealed the increased expression of pre-osteogenic genes in the cells treated with BMP-2-loaded chitosan-hyaluronic acid microspheres. It is found that negatively charged heparin forms a complex with the basic amino acids of BMP-2 and thus confers good stability and controlled release to the growth factor. Since sulfated chitosan mimics the structure of heparin, it was proved to be a better candidate for the delivery of BMP-2 *in vitro*. Cao et al. developed gelatin hydrogels loaded with recombinant human BMP-2 (rhBMP-2) encapsulated 2-*N*, 6-*O*-sulfated chitosan nanoparticles for bone regeneration [21]. These systems protected and enhanced the bioactivity of encapsulated rhBMP-2 due to the formation of electrostatic assemblies of sulfated chitosan nanoparticles in the hydrogel network. Due to the presence of rhBMP-2, the scaffold systems enhanced the ALP activity and mineralization in cultured human mesenchymal stem cells (hMSCs). The rhBMP-2 loaded scaffolds were also found to exhibit the complete regeneration of critical size defect induced in rabbit femur and an increased bone mineral content and bone mineral density when compared to the control groups. The gelatin hydrogels loaded with sulfated chitosan nanoparticles presented a two-phase release of the encapsulated rhBMP-2 as shown in Fig. 1. First, an initial burst from the partially swollen hydrogels was observed. Thereafter, they showed a sustained release as the hydrogel degraded over a period of time. Niu et al. fabricated chitosan microspheres loaded with BMP-2 derived synthetic peptide by emulsion method using sodium tripolyphosphate (TPP) as an ionic cross-linking agent [22]. These microspheres were found to be possessed 80% encapsulation efficiency and exhibited slow release up to 7 days at pH 7.4. The peptide released from the microspheres was found to be retained its biological activity *in vitro*.

For *in vivo* tissue engineering application, 3D scaffolds are advantageous as they possess appreciable mechanical strength and provide a suitable microenvironment for the growth of cells. In this respect, a porous composite scaffold made up of nano-hydroxyapatite, collagen and poly (L-lactic acid) (PLA) loaded with growth factor encapsulated chitosan microparticles was developed as a promising material for tissue engineering [23]. In this work, chitosan microspheres loaded with bovine serum albumin (BSA) and BMP-2 were prepared by the emulsion method using TPP as a cross-linking agent. The microspheres were found to be spherical in shape with the sizes in the range of 10–60 μm as shown in Fig. 2. It was found that the mechanical property and degradation rate of the 3D composite scaffold was increased due to the presence of encapsulated chitosan microspheres. This composite scaffold was found to mimic the natural bone microenvironment and showed an appreciable *in vitro* bioactivity. Ferrand et al. developed an improved method for the delivery of BMP-2 for bone regeneration using electrospun poly(ε-caprolactone) nanofibers [24]. In this study, BMP-2 combined with chitosan was coated onto the surface of poly(ε-caprolactone) nanofibers by the layer-by-layer (LBL) method. The nanofibers prepared using this technique were found to mimic the fibrillar nature of the bone matrix. Moreover, they exhibited an improved *in vitro* and *in vivo* osteopontin gene expression and calcium phosphate biomineralization. As the small amount of BMP-2 protein is used, LBL method is more economic and leads to reduced side effects due to BMP-2 overdosing. Freeze-dried chitosan scaffolds loaded with BMP-6 were developed by embedding technique [25]. The loading of BMP-6 was determined as 100 ng/3 mg of dry chitosan scaffolds. When compared to the control, BMP-6-loaded scaffolds exhibited an increase in the expression of osteocalcin, alkaline phosphatase as well as mineralization, which infers that BMP-6-loaded chitosan scaffolds support and enhance the osteogenesis *in vitro*. Macroporous chitosan scaffolds loaded with either BMP-2 or insulin-like growth factor (IGF-1) was studied for their bone healing property *in vivo* [26]. In this study, chitosan scaffold with varying pore size from 70 to 900 μm was prepared by liquid hardening method. The absorption efficiency of BMP-2 and IGF-1 was found to be 87 ± 2% and 90 ± 2%, respectively. In the *in vivo* rabbit models, chitosan scaffolds loaded with IGF-1 exhibited good osteoblastic differentiation than BMP-2-loaded chitosan scaffolds. Sintered porous scaffolds based on chitosan-poly(lactide-*co*-glycolide) (PLGA) microspheres loaded with heparin and rhBMP-2 were developed and studied their bone regeneration ability *in vivo* in a rabbit model [27]. The rhBMP-2 loaded scaffolds showed an improved mechanical strength than growth factor free scaffolds. Moreover, they found to accelerate bone formation more efficiently.

A simultaneous or subsequent release of two or more growth factors can accelerate bone regeneration more efficiently when compared to single growth factor release. Yilgor et al. prepared chitosan-poly(ethylene oxide) blended fibers by the wet spinning method using acetic acid as a solvent [28]. These blended fibers showed an improved stability and fiber thickness due to the increased total polymer concentration. It was observed that the concentration of acetic acid had an effect on the surface morphology of the fibers. The fibers prepared in 2% and 5% acetic acid showed smooth and rough surfaces, respectively (Fig. 3). In this study, BMP-2 encapsulated PLGA and BMP-7 encapsulated poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) nanocapsules were loaded within or surface of the chitosan-poly(ethylene oxide)

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