Chitosan-Based Biomimetically Mineralized Composite Materials

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Contributor: Die Hu , Qian Ren , Zhongcheng Li , Linglin Zhang

Chitosan-Based Biomimetically Mineralized Composite Materials is a kind of organic-inorganic composite materials fabricated by biomimetic mineralization technology using chitosan as a organic scaffold or template.

chitosan biomimetic mineralization template composite materials

1. Introduction

Bone and teeth are the two main hard tissues in the human body. Bone is a mineralized inorganic-organic composite, which is mainly composed of carbonated hydroxyapatite (HAP) and type I collagen[1]. Dentin, cementum, and enamel are hard structures of teeth. Dentin and cementum are both collagenous composites similar to bone, with apatite as the mineral phase[2][3]. However, enamel is quite special, as it is acellular, non-collagenous, and composed of 95–97% mineral by weight, with less than 1% organic material[4]. While there are differences in the composition and structure of these hard tissues, they are all organic-inorganic composites formed through biomineralization processes regulated by a series of cells and organic matrices (proteins, polysaccharides, etc.)[5][6].

Bone tissue engineering is now a popular and promising method for repairing bone defects due to the large-scale destruction or loss of bone tissue caused by events, such as trauma, infection, and tumor [7]. It is a technique involving the provision of three-dimensional scaffolds that act as artificial extracellular matrices, allowing cells to proliferate and maintain their specific functions, and serve as a template for new bone formations [8]. Numerous biomimetic scaffolds of different biomaterials have been applied in bone tissue engineering[9][10]. Similarly, when dental caries, trauma, or erosion cause defects of dental hard tissue, current clinical treatments cannot restore the original structure and properties of teeth. Some biomimetic materials and strategies that have appeared in recent years may be promising ways to fabricate enamel-like or dentin-like structures[11][12][13].

Chitosan, a natural cationic polysaccharide, has a similar chemical structure and biological behaviors to the components of the extracellular matrix (ECM) of bone and teeth. Chitosan has many biological properties, such as biocompatibility, biodegradability, polyelectrolyte action, etc.[14], which make it a suitable organic scaffold or template for the fabrication of organic-inorganic composites. Unlike simply mixing chitosan and inorganic minerals to construct composite materials, biomimetic mineralization technology, which is inspired by the biomineralization process, can deposit minerals in situ on chitosan organic templates, thereby constructing composite materials with closer structures and functions to those of bone or teeth. In recent years, chitosan-based composite materials

fabricated by the biomimetic mineralization technique have been widely used in the field of bone tissue engineering and enamel or dentin biomimetic repair. Comparing with the artificial materials currently used to repair human hard tissues in clinic, such as ceramics, alloys, etc., chitosan-based materials have reduced costs and improved biocompatibility, with low possibility of causing allergic and inflammatory reactions in human body[15]. Besides, the bioactivities and mechanical properties of chitosan-based materials can also be improved with the addition of inorganic minerals[16].

2. Structure, Properties and Applications of Chitosan

Chitosan is a natural cationic polysaccharide that is obtained by the N-deacetylation of chitin, which is the second most ubiquitous polymer-after cellulose-on earth[17]. It is a linear copolymer composed of D-glucosamine (GlcN) and N-acetyl-d-glucosamine (GlcNAc), which are linked by the β-1, 4-glycosidic bond, with molecular weight ranges from 10 to over 1000 kDa [17][18]. The chemical structure of chitosan is similar to that of glycosaminoglycan, the main component of the extracellular matrix (ECM)[19]. Deacetylation degree (DD), molecular mass, solubility, viscosity, crystallinity, flexibility, porosity, tensile strength, and conductivity are frequently evaluated physicochemical properties of chitosan[20][21]. Among them, DD and molecular mass are two of the most important physical characteristics that affect both the chemical and biological properties of chitosan[22][23][24]. In recent years, chitosan preparations with various DDs, molecular masses, and molecular derivatization patterns have attracted much attention because of their potentially beneficial biological properties. Chitosan has various outstanding biological properties, including a good polyelectrolyte action, biodegradability, biocompatibility, bioactivity, antimicrobial property, anticancer property, antioxidant property, cell adhesion properties, non-toxicity, and high flexibility for chemical functionalization[25][26][27]. However, as chitosan is only highly soluble in most diluted acidic solutions at a pH below 6.5 and has a poor solubility in water or most organic solvents, its application field is severely limited[15]. Therefore, improving the solubility of chitosan is a crucial step in extending its scope of application. Deacetylation, chemical modification by adding hydrophilic biomolecules to amino or hydroxyl groups (acylation, carboxylation, alkylation, quaternization, sulfonation, and phosphorylation), crosslinking and chemical or enzymatical depolymerization or degradation are available methods for improving the solubility of chitosan and also optimizing its biological properties[28][29]. Because of the diverse properties of chitosan and its derivatives, they have been extensively applied in the medical and pharmaceutical fields, for example, they have been used in drug delivery[30][31], tissue engineering[19][32], wound management[33][34], gene and cancer therapy[35][36][37], antibiofilm drugs[38], etc.

3. Applications in Bone Tissue Engineering

Natural bone exhibits a hierarchical structure, mainly consisting of multilayered collagen fibers and the inorganic component, HAP[39]. In consideration of events such as trauma, infection, and tumor, which cause the large-scale destruction or loss of bone tissue, exploring materials that can replace or even reconstruct bone structure is an urgent challenge in orthopedic clinical practice. Bone tissue engineering is currently a hot research field and aims to realize bone reconstruction and regeneration, focusing on scaffolds, cells, growth factors, and their interrelation

in a microenvironment[40]. For a bone tissue engineering scaffold to be successful, it must be highly porous, osteoconductive, biodegradable, biocompatible, mechanically strong, and capable of efficiently guiding new bone formation in the defect[41][42]. Preparing organic-inorganic composite nanofibers to simulate the composition of the ECM is an effective strategy for providing bone tissue engineering scaffolds. According to the structure and composition of natural bone, natural macromolecule/HAP composite scaffolds synthesized by biomimetic mineralization with natural bioactive macromolecules are currently key research focuses in this field [43][44][45]. Among these macromolecules, chitosan is a popular alternative because of its excellent biological properties[46]

Different chitosan-based organic-inorganic composite materials using the biomimetic mineralization technique and their important properties in the field of bone tissue engineering are presented in Table 1. Using a wet chemical method, Doan et al. prepared chitosan/hydroxyapatite (CS/HAP) nanofibers with a homogeneous HAP deposit. The composite nanofibrous scaffold promoted osteogenic differentiation by inducing ossification and enhanced the expressions of collagen type I, alkaline phosphatase, osteocalcin, bone sialoprotein, and osterix, thus showing that it has considerable potential in bone tissue engineering applications. Compared with ordinary chitosan, carboxymethyl chitosan (CMCS) has a better water-solubility, biodegradability, and bioactivity, which allows CMCS to chelate Ca^{2+}[47] and induce the deposition of apatite[48][49]. H[42]AP-coated electro-spun CMCS nanofibers prepared by biomimetic mineralization using 5 times simulated body fluid increased the ALP activity and the gene expression level of Runx2 and ALP and promoted new bone formation and maturation[42]. In order to fabricate a hybrid nanostructured HAP-CS composite scaffold with HAP nanorods perpendicularly-oriented to CS fibers, Guo and his co-workers[50] applied a two-stage preparation process using brushite (DCPD, $CaHPO_4{\cdot}2H_2O$) as transitory precursors and mimicked the biomineralization process of the apatite in bone tissue. The process included the deposition of DCPD on the CS fiber porous scaffold using a dip-coating method and the formation of a hybrid nanostructured HAP-CS composite scaffold through the in-situ conversion of DCPD into HAP using a bioinspired mineralization process. The composite scaffold exhibited good mechanical properties and could support the adhesion and proliferation of hBMSCs. Moreover, it could promote the formation of new bone in rat calvarial defects. To further improve the mechanical and biological properties of chitosan-HAP composite scaffolds, graphene was also introduced into the composite scaffolds. Graphene and its derivatives, such as graphene oxide (GO) and reduced graphene oxide (RGO), are highly biocompatible and can easily be functionalized by various organic and inorganic compounds due to the presence of various functional side groups (hydroxyl, carboxyl, and epoxides) on its surface[51][52]. It was found that extensive mineralization occurred in the CS-GO conjugate system because of strong electrostatic interactions between the functional groups (carboxyl groups of GO and amino groups) of CS and calcium ions in an SBF solution. The combination of a chitosan–graphene oxide conjugate and biomimetic mineralization was advantageous in favorably modulating cellular activity. It induced homogeneous spatial osteoblastic cell growth and increased mineralization [53]. Another study showed that chitosan acted as an interfacial soft polymeric template on the surface of RGO, promoting an ordered growth of the hydroxyapatite particles. The three-component composite mineralized scaffold mimicked the structure and composition of natural bone and exhibited a relatively higher rate of cell proliferation, osteogenic differentiation, and osteoid matrix formation[54].

Table 1. Applications of chitosan-based biomimetically mineralized composite materials in bone tissue engineering.

Chitosan or Its Derivatives	Composite Forms	Minerals	Other Organic/Inorganic Components	Preparation Techniques of Chitosan Template	Methods of Biomimetic Mineralization	Important Properties	Reference
Chitosan	Nanofibers	HAP	-	Electrospinning	Alternate soaking of WCM	Promoted osteogenic differentiation by inducing ossification	[55]
Carboxymethyl chitosan	Nanofibers	HAP	-	Electrospinning	Soaking in 5 times SBF solution	Increased the ALP activity, promoted the gene expression level of Runx2 and ALP, promoted new bone formation and maturation	[47]
Chitosan	Porous scaffolds	DCPD, HAP	-	Needle-punching process	dip-coating method and in situ precipitation by WCM	Excellent biocompatibility, osteoinductivity and mechanical properties	[50]
Chitosan	Membranes	HAP	GO	Chemical conjugation with GO	Soaking in 5 times SBF solution	Influenced osteoblastic cell differentiation, mineralization, and cell growth	[53]
Chitosan	Aerogel networks	HAP	RGO	Functionalize RGO	Soaking in 1.5 times SBF solution	Exhibited relatively higher rate of cell proliferation, osteogenic differentiation and osteoid matrix formation	[54]
Carboxymethyl chitosan	Nanocomplexes	ACP	Collagen	Dissolved in water	PILP method	Promoted the proliferation and differentiation of mouse preosteoblasts, accelerated the regeneration of bone in the defects of rat calvaria bone	[56]

Chitosan or Its Derivatives	Composite Forms	Minerals	Other Organic/Inorganic Components	Preparation Techniques of Chitosan Template	Methods of Biomimetic Mineralization	Important Properties	Reference
Chitosan	Porous scaffolds	nHAP	Collagen, PLA	Emulsion-crosslinking	WCM	Improved the mechanical properties and the formation of crystals in SBF, had good biocompatibility, maintained the cell growth	[57]
Chitosan	Core-shell structured nanofibers	HAP	Gelatin	Coaxial electrospinning technique	WCM	Enhanced osteoblast cell proliferation	[58]
Chitosan	Fibers	HAP	Gelatin	Net-Shape-Nonwoven (NSN) technique	Double migration technique	Improved attachment, proliferation, and differentiation of hBMSC	[59]
Chitosan	Nanofibers	HAP	Cellulose, phosvitin	LBL self-assembly technique	Soaking in 1.5 times SBF solution	excellent cytocompatibility, as well as good performance of cell adhesion and spreading	[60]
Chitosan	Fibers	HAP	PLA	Modification on electrospun PLA nanofiber	Soaking in 10 times SBF solution	Mimicked structural, compositional, and biological functions of native bone	[61]
Chitosan	Hydrogel	HAP, DCPD	PEG	Chemical crosslinking with PEG	Alternate soaking of WCM	Induced excellent cell adhesion ability	[62]
Chitosan	Porous scaffolds	HAP	Silk fibroin	Freeze drying	Alternate soaking of WCM	Good mechanical property, promoted early cell attachment and enhanced osteogenic differentiation	[63]
Chitosan	Porous scaffolds	nHAP	ALP	Freeze drying	ALP enzyme-induced	promoted the osteogenic	[64]

Hydroxyapatite (HAP); wet chemical method (WCM); simulated body fluid (SBF); alkaline phosphatase (ALP); dicalcium phosphate dihydrate (DCPD); graphene oxide (GO); reduced graphene oxide (RGO); amorphous calcium phosphate (ACP); polymer-induced liquid precursor (PILP); nanohydroxyapatite (HAP); layer-by-layer (LBL); poly(lactic acid) (PLA); poly(ethylene glycol) (PEG), calcium phosphate (CaP).

Chitosan or Its Derivatives	Composite Forms	Minerals	Other Organic/Inorganic Components	Preparation Techniques of Chitosan Template	Methods of Biomimetic Mineralization	Important Properties	Reference
					mineralization method	differentiation of pre-osteoblasts in vitro and demonstrated excellent tissue integration in vivo	
Chitosan	Thermosensitive hydrogels	CaP	ALP	Gelation [56] [68]	ALP enzyme-induced mineralization method	Promoted mineralization, may be suitable materials for bone replacement.	[65]
Chitosan	Hybrid scaffolds	Silica	-	Freeze drying	Sol-gel process	No cytotoxicity, excellent in vitro bone bioactivity [57]	[66]
N-guanidinium-chitosan acetate	Hybrid scaffolds	Silica	-	Freeze drying	Sol-gel process	Acted as versatile templates for biomineralization, inducing the formation of HAP	[67] [58][59][70]

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coaxial electrospinning technique. An arginine-glycine-aspartic acid (RGD)-like structure was formed to mimic the organic component of the natural bone extracellular matrix, and then homogeneous HAP was deposited on its surface using a wet chemical method. The biomimetic composite scaffolds could further enhance osteoblast cell proliferation[58]. Heinemann et al. prepared organically modified hydroxyapatite (ormoHAP) in gelatin gels using the double migration technique and mineralized chitosan porous scaffolds created using the Net-Shape-Nonwoven (NSN) technique. The mineralized NSN-scaffolds improved the attachment, proliferation, and differentiation of hBMSC, presenting a remarkable application potential for bone tissue engineering[59]. In addition to collagen and gelatin, other biodegradable polymers or proteins, such as cellulose[60], PLA[61], PEG[71], and silk fibroin[63], were also effective organic additives, acting as crosslinking agents or scaffolds of the chitosan-based composite materials. While various chitosan/calcium phosphates are the most common biomimetically mineralized composite materials used in bone tissue engineering, some chitosan/silica biomimetically mineralized scaffolds have also been applied due to their capability in inducing the formation of apatite and good potential for promoting new bone regeneration[66][67].

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