

Prospects of Bioactive Chitosan-Based Scaffolds in Tissue Engineering and Regenerative Medicine

M. Prabakaran and P.R. Sivashankari

Abstract Chitosan, a natural-based polymer obtained by alkaline deacetylation of chitin, is non-toxic, biocompatible, and biodegradable. Due to its desired properties, chitosan-based materials are widely considered to fabricate scaffolds for tissue engineering and regenerative medicine. These scaffolds provide characteristic advantages, such as preservation of cellular phenotype, binding and enhancement of bioactive factors, control of gene expression, and synthesis and deposition of tissue-specific extracellular matrix (ECM), to tissue regeneration. Therefore, the scaffolds based on chitosan and its composites have potential to be used in bone, cartilage, liver, nerve, and musculoskeletal tissue engineering.

Keywords Chitosan · Scaffolds · Tissue engineering · Bioactivity · Regenerative medicine

1 Introduction

The objective of tissue engineering is to regenerate and repair damaged tissues and organs of the human and animal body. In tissue engineering, three-dimensional (3-D) porous scaffolds are used for cell adhesion, proliferation, and differentiation and development of an extracellular matrix (ECM) [1]. The tissue scaffolds may have an ability to load bioactive/therapeutic substances and to release them at a controlled manner in the defected sites. This phenomenon can be used to improve the bioactivity of the scaffolds and therapeutic effects in the injured tissues [2]. For the betterment of tissue engineering approaches, more appropriate materials with the suitable cells and bioactive molecules need to be considered for

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the fabrication of scaffolds. In this regard, chitosan, a biopolymer consisting of glucosamine and *N*-acetylglucosamine repeating units, has received much attention to develop the scaffolds due to its desired properties such as low cost, large-scale availability, antimicrobial activity, low toxicity, and biodegradability. Using chitosan, a range of scaffolds with different microstructures (hydrogels, open-pore structures, fibrous matrices, etc.) have been prepared for the specific tissue engineering application. These scaffolds permit the development of normal tissue regeneration due to their minimal foreign body reaction and ability to attain hemostasis [3, 4].

In recent years, a variety of hybrid scaffolds based on chitosan and other biodegradable and/or biocompatible materials have been developed. These hybrid scaffolds have an improved microstructure, swelling ability, mechanical strength, compression modulus, and biocompatibility due to their counter part of chitosan. The materials used for the preparation of hybrid scaffolds play an important role on the properties and applications of hybrid scaffolds in tissue engineering. The objective of this chapter is to consolidate and discuss the recent advancements of chitosan-based materials as scaffolds for bone, cartilage, liver, nerve, and musculoskeletal tissue regeneration.

2 In Bone Tissue Engineering

Bone tissue engineering is an important field of research for developing new three-dimensional (3D) scaffolds with highly interconnected porous structure. These scaffolds should match the characteristics of the tissue that is to be replaced. The tissue engineering scaffolds need to be biocompatible, osteoinductive, osteoconductive, and mechanically well-suited in order to restore bones which have been lost or damaged. In this respect, artificial scaffolds based on chitosan, inorganic materials, and/or synthetics polymers have received much attention in recent years due to their desirable properties for bone tissue engineering application.

2.1 Chitosan–Synthetic Polymer Hybrid Scaffolds

Poly(L-lactic acid) (PLLA)/chitosan hybrid scaffolds were developed using chitosan solution and previously prepared PLLA scaffolds for bone tissue engineering as shown in Fig. 1 [5]. The shape and size of microstructure of the hybrid scaffolds were found to be depended on the concentration of chitosan solution used to soak the PLLA scaffolds. It was found that PLLA/chitosan hybrid scaffolds can produce calcium phosphate precursors on its structure on dipping them into alternate phosphorous and calcium solutions. The formation of apatite layers within the hybrid scaffolds was also observed by bioactivity tests. Santo et al. [6] prepared a hybrid scaffolds based on poly (D, L-lactic acid) (PDLLA) impregnated with

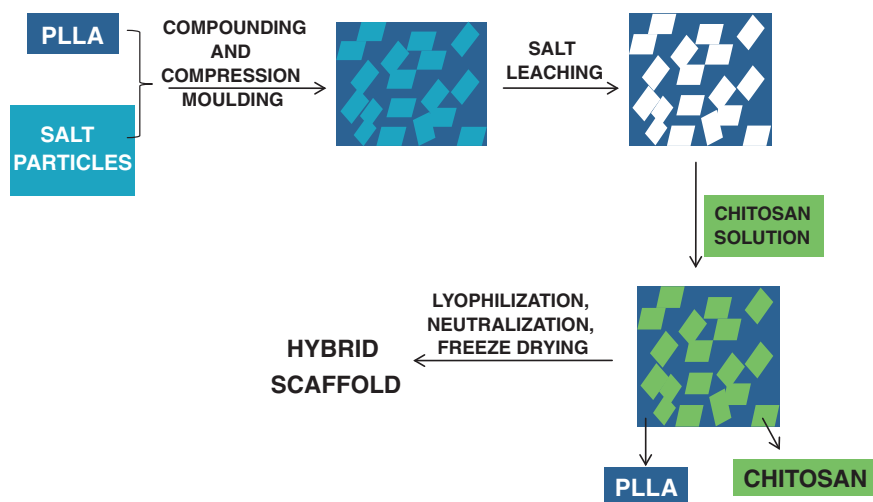


Fig. 1 Schematic representation for the preparation of PLLA/chitosan hybrid scaffolds

chitosan/chondroitin sulfate nanoparticles (NPs) with drug release capabilities. Due to the presence of chitosan/chondroitin sulfate NPs, these hybrid scaffolds showed higher swelling characters and adequate mechanical properties for cell adhesion and support for bone tissue engineering. Using thermally induced phase separation and lyophilization techniques, hybrid scaffolds based on chitosan and PDLLA-*co*-glycolide were developed for bone tissue engineering applications [7]. FT-IR and field-emission SEM studies confirmed the formation of apatite layers on the hybrid scaffolds after impregnated with stimulated body fluid (SBF). These studies revealed the potential of chitosan-based hybrid scaffolds in bone tissue engineering.

Niu et al. [8] reported the properties of chitosan microsphere-loaded porous PLLA scaffolds as a carrier for BMP-2-derived synthetic peptide. There were strong hydrogen bonds between the PLLA and chitosan component observed by FT-IR. When the chitosan microspheres' contents increased from 0 to 50 %, the compressive strength of the PLLA scaffolds was increased from 0.48 to 0.66 MPa, while the compressive modulus increased from 7.29 to 8.23 MPa. The insertion of chitosan microspheres into the PLLA scaffolds was found to neutralize the acidity of PLLA degradation products. Release studies showed that PLLA /chitosan hybrid scaffolds presented a controlled release of loaded peptide when compared with control chitosan microspheres. The release pattern was found to be depending on the degradation of PLLA matrix. This result indicates that PLLA/chitosan scaffolds can be used to deliver bioactive factors for a range of non-loaded bone regeneration.

Santo et al. [9] developed a hybrid scaffolds based on PDLLA containing chitosan-chondroitin sulfate NPs loaded with Platelet Lysate (PL) by supercritical fluid foaming technique as shown in Fig. 2. Release studies demonstrated that PL was released in a sustained manner from the hybrid scaffolds. Due to the presence of PL and hASCs, this hybrid scaffolds can be used as multi-functional materials for bone tissue

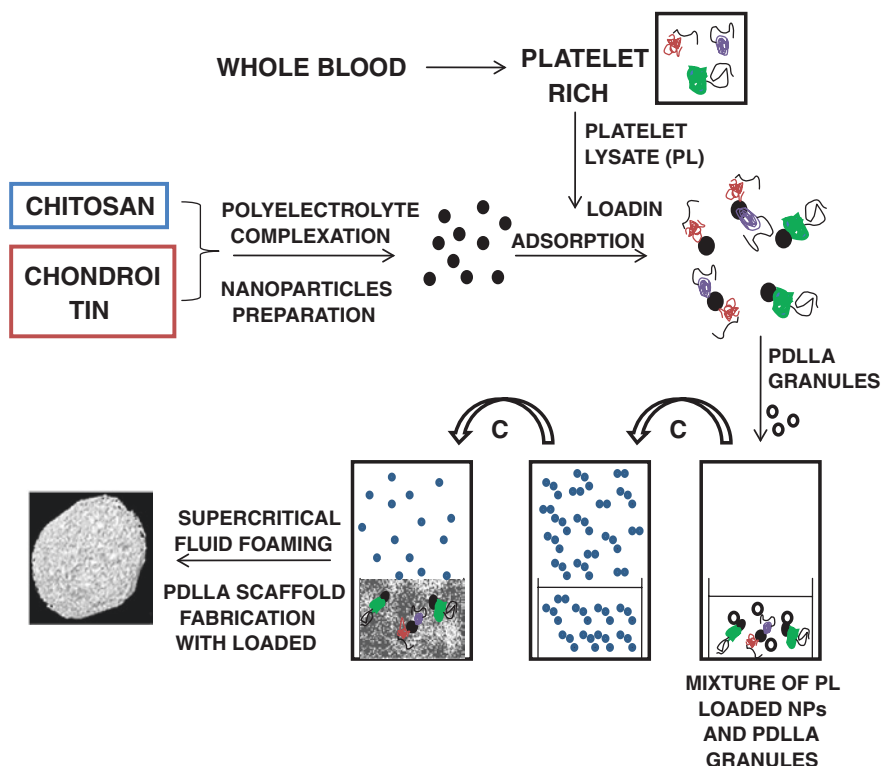


Fig. 2 Schematic representation of experimental procedure for the preparation of PDLLA scaffolds incorporating the PL-loaded chitosan–chondroitin sulfate NPs

engineering applications. Recently, L-lactide-methoxy PEG–tetrandrine nanosphere-loaded chitosan–gelatin hybrid scaffolds were prepared using freeze-drying method for bone tissue engineering [10]. Tetrandrine is a plant derivative, which can be used as a modifier to hybrid scaffolds to promote chondrocyte differentiation and secrete type-II collagen. Since tetrandrine-loaded nanospheres implanted within chitosan–gelatin scaffolds, sustained release of tetrandrine was observed from the hybrid scaffolds.

2.2 Chitosan–Calcium Phosphate Hybrid Scaffolds

Hybrid scaffolds based on calcium phosphate cements (CPCs) and chitosan have been widely used as bone graft substitutes due to their in situ-setting ability and bioactivity. Zhao et al. [11] studied human umbilical cord mesenchymal stem cells (hUCMSCs) delivery of CPC–chitosan–polyglactin fiber scaffolds for bone tissue engineering. The fatigue resistance of CPC–chitosan–polyglactin fiber

scaffolds was found to be increased due to the presence of chitosan and polyglactin fibers. In addition, it was found that the CPC–chitosan–polyglactin fiber scaffolds supported hUCMSCs attachment and proliferation. hUCMSCs showed well distribution and anchored on the polyglactin fibers in scaffolds via cytoplasmic extensions. These results propose that CPC–chitosan–polyglactin fiber scaffold may be appropriate for stem cell delivery and bone tissue engineering. Wen et al. [12] fabricated an iron foam coated with calcium phosphate/chitosan using electrophoretic deposition method for bone tissue engineering. The deposition of calcium phosphate/chitosan on iron foam improved the interfacial bonding strength and the in vitro bone-forming bioactivity. Moreover, it was observed that the bioactivity of the implant was not affected by the iron foam coated with calcium phosphate/chitosan. Recently, Meng et al. [13] prepared bioactive cement based on CPC containing chitosan microspheres as an injectable material for the bone regeneration. The bioactive cement containing 10 % (w/w) chitosan microspheres had a compressive strength of 14.78 ± 0.67 MPa. In this study, CPC/chitosan microsphere and α -TCP/CPC (control group) were implanted into the bone defects in both femoral condylar regions of New Zealand white rabbits. SEM and histological examination after implantation showed the formation of more new bones and degradation of the bioactive cement in the bone defects. These studies show the potential application of CPC/chitosan hybrid scaffolds in bone regeneration.

2.3 Chitosan–Bioactive Glass Hybrid Scaffolds

Bioactive glasses are used for bone regeneration due to their osteoconductive and biodegradable properties. Degradation products of bioactive glasses could stimulate the production of growth factors and cell proliferation, and activate the gene expression of osteoblast [14]. In recent years, bioactive glasses combined with chitosan have been extensively considered to fabricate hybrid macroporous scaffolds for the improved bone repair. Mansur and Costa studied the physical, mechanical, and biological properties of hybrid scaffolds consisting of poly(vinyl alcohol), chitosan, and bioactive glass [15]. The results revealed that these hybrid scaffolds can be used for bone tissue engineering applications due to their appropriate mechanical, morphological, and cell viability properties. Couto et al. [16] developed injectable hybrid scaffolds based on chitosan– β -glycerophosphate and bioactive glass NPs for orthopedic reconstructive and regenerative medicine applications. The formation of apatite layers on the hybrid scaffold was observed after soaking them with SBF solution due to the presence of bioactive glass NPs in the scaffolds. The thickness of the apatite layer formed on the scaffold was found to be increased with increasing bioactive glass content and soaking time in SBF.

A hybrid scaffold composed of chitosan and bioactive glass ceramic NPs (nBGC) was fabricated by blending nBGC with chitosan solution using freeze-drying technique [17]. This hybrid scaffold showed adequate swelling and degradation properties due to the presence of hydrophilic chitosan and nBGC. The

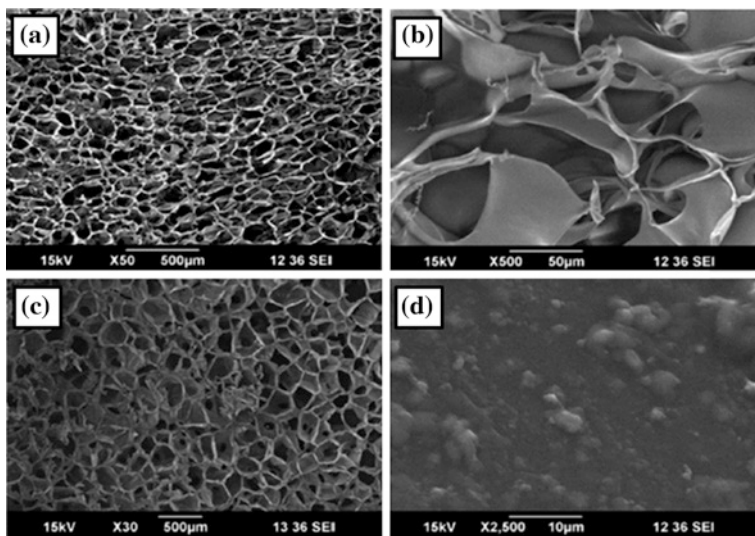


Fig. 3 SEM images of chitosan-gelatin (a, b) and chitosan-gelatin/nBGC composite scaffolds (c, d)

bioactivity of chitosan/nBGC hybrid scaffold was confirmed by the biomineralization studies. Peter et al. [18] developed composite scaffolds with pore size ranging from 150 to 300 μm based on chitosan-gelatin/nBGC for alveolar bone tissue engineering (Fig. 3). In this study, the scaffold properties and biocompatibility were analyzed in order to understand the role nBGC in the scaffold matrix. The results showed that the degradation and swelling behavior of the composite scaffolds were decreased with the addition of nBGC. Biomineralization studies showed that mineral deposits on the nano-composite scaffold were increased with the increase in time of incubation. In vitro studies demonstrated that the composite scaffolds are capable to provide a healthier environment for cell attachment and spreading. Yang et al. [19] fabricated alginate and chitosan-reinforced bioactive glass scaffolds with superior mechanical properties and structural stability. In this study, the bioactive glass scaffolds were developed using microsphere replication method. Alginate- and chitosan-reinforced bioactive glass scaffolds showed an enormous improvement in compressive strength and nearly 30 % of shrinkage in wet state when compared to blank bioactive glass scaffold. This biopolymer-reinforced bioactive glass scaffolds presented an excellent strain tolerance during the prolonged immersion in simulated body fluid. These results indicated that biopolymer-reinforced bioactive glass scaffolds can be used for bone repair.

Multi-functional hybrid scaffolds with drug loading and releasing abilities have received considerable interest for bone tissue engineering due to their efficacy to improve drug delivery, healing, and regeneration. A scaffold with controlled drug release ability can be developed by integrating biomolecules within biodegradable carriers and further inclusion of such carriers into tissue-engineered scaffolds. Nazemi et al. [20] developed chitosan-bioactive glass scaffolds loaded

with poly(lactic-*co*-glycolic acid) NPs by freeze-drying method. In this study, the mechanical properties of the hybrid scaffolds were found to be improved due to the presence of poly(lactic-*co*-glycolic acid) NPs in the scaffolds. It was observed that the presence of poly(lactic-*co*-glycolic acid) NPs did not affect the morphology of the hybrid scaffolds. Recently, porous poly(caprolactone) and vancomycin-loaded chitosan-coated hybrid scaffolds have been developed by the replication technique using 45S5 Bioglass® (BG) powder [21]. The mechanical properties and compressive strength of the poly(caprolactone) and vancomycin-loaded chitosan-coated scaffolds were found to be improved when compared with uncoated scaffolds. The coated scaffolds presented a sustained release of encapsulated drug for a period of 11 days. This result suggests the potential of the caprolactone and vancomycin-loaded chitosan-coated scaffolds as bone tissue scaffolds. Soundrapandian et al. [22] formulated porous scaffolds based on BGZ and MBG bioactive glasses. In this study, these scaffolds were loaded with the model drug, gatifloxacin, by vacuum infiltration technique. Thereafter, the drug-loaded scaffolds were coated with 0.5–1 % chitosan solution. The results demonstrated that 63–66 % porous and 5–50 μm porous MBG and BGZ bioactive glass scaffolds were capable of releasing drugs effectively for prolonged periods. In addition, the coating of chitosan on the scaffolds decreased the release of drug. The scaffolds based on MBG bioactive glass were found to be bioactive, biocompatible, non-cytotoxic, and exhibited excellent wound healing potential.

Pon-On et al. [23] fabricated poly(vinyl alcohol)–chitosan–collagen hybrid scaffolds loaded with bioactive glass by three mechanical freeze–thaw followed by freeze-drying methods. The porosity and compressive strength of the hybrid scaffolds were found to be controlled by the weight ratio of poly(vinyl alcohol) and mixtures of chitosan–collagen. Formation of apatite layers on the scaffold surface was observed after seven days of incubation in SBF. MTT assay revealed that there is no cytotoxicity of hybrid scaffolds on UMR-106 cells. The drug release studies showed that poly(vinyl alcohol)–chitosan–collagen hybrid scaffolds loaded with bioactive glass hybrid scaffolds presented a controlled release for about a month. The effect of poly(lactic-*co*-glycolic) acid NPs on a chitosan–bioactive glass scaffold was reported recently [24]. In this work, two types of chitosan–bioactive glass scaffolds, with and without poly(lactic-*co*-glycolic) acid NPs, were prepared. The mechanical strength of the hybrid scaffolds was found to be improved while adding the poly(lactic-*co*-glycolic) acid NPs. However, the swelling behavior of the hybrid scaffolds was marginally decreased as a result of adding NPs. The results demonstrated that these hybrid scaffolds can be used as a controlled-release platform of model drugs to the bone regeneration.

2.4 Chitosan–Hydroxyapatite Hybrid Scaffolds

The ECM present in bone tissue contains a porous composite of interpenetrating phases of type-I and type-III collagen and hydroxyapatite. In recent years,

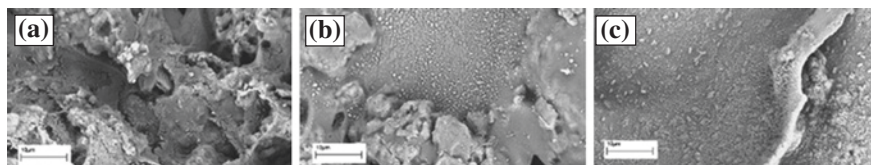


Fig. 4 SEM images for showing the mineralization of **a** chitosan–gelatin, **b** chitosan–gelatin/nanohydroxyapatite and **c** chitosan–gelatin/nanohydroxyapatite–montmorillonite composite scaffolds immersed in SBF after 14 days of incubation

chitosan, gelatin, and hydroxyapatite in different combinations have been developed as hybrid scaffolds for bone regeneration because of their chemical similarity to natural bone ECM. Oliveira et al. [25] prepared hybrid scaffolds based on dexamethasone-loaded carboxymethyl chitosan/poly(amidoamine) dendrimer NPs and the mixture of hydroxyapatite and starch–poly(caprolactone). In this work, the effect of these hybrid scaffolds on the proliferation and osteogenic differentiation of rat bone marrow stromal cells (RBMSCs) was studied. It was observed that RBMSCs seeded onto the surface of both hydroxyapatite and starch–poly(caprolactone) scaffolds differentiate into osteoblasts when cultured in the presence of 0.01 mg ml^{-1} dexamethasone-loaded carboxymethyl chitosan/poly(amidoamine) dendrimer NPs. The dexamethasone-loaded carboxymethyl chitosan/poly(amidoamine) dendrimer NPs combined with the hydroxyapatite was also found to enhance osteogenesis by increasing alkaline phosphatase activity and mineralization of the ECM.

Olad and Azhar developed highly porous chitosan–gelatin/nanohydroxyapatite–montmorillonite hybrid scaffolds with the pore size of $100\text{--}350 \text{ }\mu\text{m}$ using freeze-drying method for use in bone tissue engineering [3]. Bioactivity study conducted with the SBF showed a decreased degradation rate and increased biomineralization of the hybrid scaffolds due to the presence of nanohydroxyapatite and montmorillonite as shown in Fig. 4. Recently, Zhu et al. [26] developed a biomimetic hybrid scaffolds which consists of hydroxyapatite and human bone marrow mesenchymal stem cells (BMSCs)-loaded chitosan hydrogel. The breast cancer adhesion and proliferation of the hybrid scaffolds were found to be influenced by the amount of nanohydroxyapatite present in the scaffolds. The maximum breast cancer adhesion and proliferation was found on 10 % nanohydroxyapatite–chitosan scaffold.

3 In Cartilage Tissue Engineering

In recent years, a wide variety of chitosan-based biomaterials have been developed as scaffolds for cartilage tissue engineering due to their desirable properties such as biocompatible, biodegradable, highly porous, suitable for cell attachment, proliferation and differentiation, osteoconductive, non-cytotoxic, flexible and elastic, and nonantigenic.

3.1 Chitosan-Based Fibrous Scaffolds

Iwasaki et al. [27] fabricated alginate–chitosan hybrid fibrous scaffolds for cartilage tissue engineering. These scaffolds presented superior adhesion ability with chondrocytes when compared with alginate fiber. SEM studies showed that the presence of the distinctive round morphology of the chondrocyte and the formation of type-II collagen fibers by the chondrocytes in the hybrid scaffolds. A stratified composite scaffold based on chitosan nanofibrous layer on a porous 45S5 bioactive glass was developed for osteochondral segment regeneration by Liverani et al. [28]. In this study, chitosan and alginate were used for constructing the interface between the scaffold and the soft cartilage. A chitosan-based electrospun nanofibrous membrane was used for constructing the upper layer of the scaffold. This composite scaffold had good resistance to layer delamination, preservation of the bioactivity, and improvement of the mechanical properties.

3.2 Chitosan-Based Scaffolds

Chitosan-based scaffolds can deliver ECM components such as type-II collagen and chondroitin sulfate in a controlled fashion, which endorses the in-growth and biosynthetic capability of chondrocytes. Choi et al. [29] developed chitosan hydrogel containing type-II collagen and chondroitin sulfate for the management of cartilage defects. The addition of type-II collagen and chondroitin sulfate into chitosan hydrogels was found to increase chondrogenesis. In particular, type-II collagen was found to be responsible for the improved chondrogenesis. Kim et al. [30] stabilized transforming growth factor (TGF- β 1) signaling in the chitosan hydrogel for use in cartilage regeneration. In this study, TGF- β 1 was linked to chitosan with preserving type-II collagen in order to reduce the burst release of protein in a complex biological environment of serum and cells. The ability of TGF- β 1 linked chitosan to promote cartilage regeneration in a rat partial-thickness chondral defect model was confirmed.

3.3 Chitosan-Based Composite Scaffolds

Bi et al. [31] prepared a biphasic scaffold based on collagen–chitosan and bioactive glass collagen by combination of sol–gel, freeze-drying, and cross-linking techniques. In this scaffold, cross-linking agents were used to connect the collagen–chitosan and bioactive glass collagen phases. This scaffold presented interconnected porous structures and precipitation of hydroxyapatite grains after being immersed into SBF. As shown in Fig. 5, BMSCs were found to be anchored on this scaffold with healthy spreading. This result shows that this

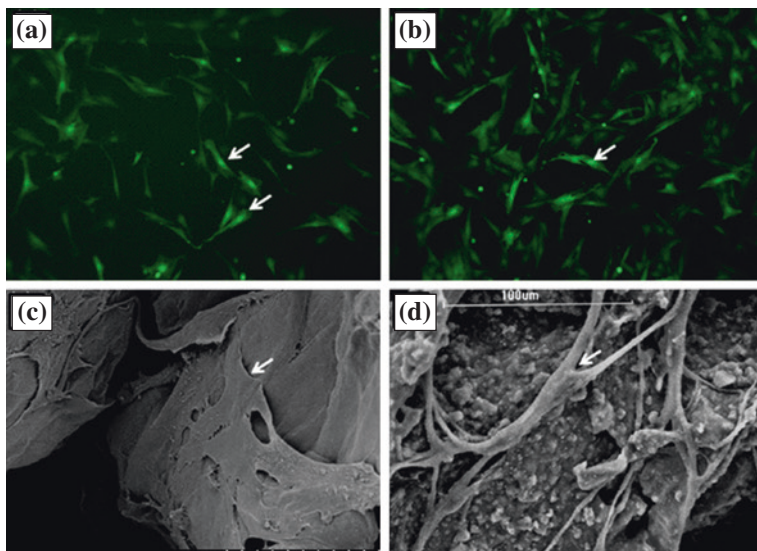


Fig. 5 Fluorescence and SEM images of BMSCs on the biphasic scaffold **a** chondral phase (fluorescence of 7th day), **b** osseous phase (fluorescence of 7th day), **c** chondral phase (SEM of 7th day), and **d** osseous phase (SEM of 7th day)

biphasic scaffold can be used for osteochondral tissue engineering. Silva et al. [32] developed chitosan and chondroitin sulfate 3-D nanostructures to support the attachment and proliferation of bovine chondrocytes. The obtained 3-D nanostructure had a high porosity and water uptake capacity of about 300 %. The results of this study showed that cells were attached, proliferated, and metabolically active over the entire 3-D chitosan and chondroitin sulfate nanostructure scaffold.

Porous scaffolds composed of poly(L-glutamic acid) and chitosan was prepared for the repair of articular cartilage defects using a freeze-drying method [33]. The scaffolds fabricated from 2 % poly(L-glutamic acid)/chitosan content and at a freezing temperature of -20°C exhibited an interconnected porous structure with average pore size between 150 and 200 μm , the contact angle of less than 75° , and high swelling ratio about 700 %. In vitro culture of rabbit adipose-derived stem cells indicated that poly(l-glutamic acid)/chitosan porous scaffolds supported cell attachment and growth. Lee et al. [34] developed macroporous poly(vinyl alcohol)-carboxymethyl chitosan-poly(ethylene glycol) hybrid scaffolds for cartilage tissue engineering. The MTT, immunohistochemistry, SEM, and TEM analyses confirmed that these scaffolds promoted cell attachment and proliferation in vitro. It was observed that the chondrocyte-poly(vinyl alcohol)-carboxymethyl chitosan-poly(ethylene glycol) scaffolds secreted glycosaminoglycan (GAG) and collagen type-II. Moreover, these scaffolds were not shown any adverse effects on the host tissue.

Chen et al. [35] prepared histidine-*graft*-chitosan/PLLA scaffolds using a dual-phase separation technique by changing the weight ratio of histidine-*graft*-chitosan and PLLA. In this study, the chemical structure, morphology, and mechanical properties of NHCS/PLLA hybrid scaffolds were characterized through FT-IR, WXR, thermal gravimetric analyzer, and field-emission SEM. The results showed that the pore size of histidine-*graft*-chitosan/PLLA hybrid scaffolds decreased with the decrease of the weight ratio of histidine-*graft*-chitosan and PLLA. The pore size and porosity of the scaffolds were found to be about 12–25 μm and >92 %, respectively. The comprehensive strength and the comprehensive modulus were found to be 0.33–0.78 MPa and 1.75–5.28 MPa, respectively, which indicates the potential application of histidine-*graft*-chitosan/PLLA hybrid scaffolds in cartilage tissue engineering. Kamoun et al. [36] developed an injectable hydrogel based on *N*-succinyl chitosan cross-linked with water-soluble dialdehyde starch (DAS) for tissue engineering and cartilage repair. In this study, *N*-succinyl chitosan content was found to have an important role for the formation of highly cross-linked hybrid hydrogels. However, the strength of the cross-linked hybrid hydrogels was found to be decreased when increasing the concentration of DAS. It was found that the content of *N*-succinyl chitosan in the hybrid hydrogels influenced the model drug, curcumin, release profile, and adherence of HGF cells on the hydrogels.

4 In Liver Tissue Engineering

The objective of liver tissue engineering is to construct an artificial liver tissue for the replacement of the failure liver function in patients. Liver tissue engineering strategies can be used to overcome the drawbacks of liver transplantation such as requirement of immunosuppressive drugs, donor organ and its storage and high cost, etc. Using chitosan-based materials, a variety of scaffold systems were developed for liver tissue engineering applications.

4.1 Chitosan–Collagen Matrices

Chitosan–collagen composite system was synthesized using coupling agents 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and *N*-hydroxysuccinimide as implantable artificial livers by Wang et al. [37]. The platelet deposition and hepatocyte culture studies revealed that chitosan–collagen composite had adequate mechanical properties, outstanding blood, and cell compatibility. The highly porous hybrid scaffolds with adequate blood compatibility based on collagen/chitosan/heparin were also fabricated by Wang et al. [38]. Hepatocytes cultured on these hybrid scaffolds presented maximum urea and triglyceride discharge after 25 days from their seeding, which indicates the potential of collagen/chitosan/heparin hybrid scaffolds in liver tissue engineering.

4.2 Alginate–Chitosan Composite Matrices

Yang et al. [39] fabricated porous alginate/galactosylated chitosan hybrid scaffolds for liver tissue engineering. The hybrid scaffolds seeded with primary hepatocytes presented greater cell attachment and viability due to the definite interactions between the asialoglycoprotein receptors on hepatocyte and galactose ligands on hybrid scaffolds. Recently, Chen et al. [40] reported a scaffold with average pore size of 50–150 μm and interconnected pore structure based on galactosylated chitosan cross-linked with oxidized alginate for liver tissue engineering. The porosity and compressive modulus of the scaffolds was determined as about 70 % and 4.2–6.3 kPa, respectively. The equilibrium swelling and in vitro degradation rate of the scaffolds were found to be decreased with the increase of the oxidized alginate content. The hepatocytes seeded on the scaffolds showed multi-cellular aggregates with a characteristic spheroidal morphology and great interactions with the scaffolds.

4.3 Chitosan-Based Microfibers

Lee et al. [41] reported the chemical, mechanical, and diffusion properties of microfluidic chitosan microfibers for liver tissue engineering applications. In order to assess the potential of the chitosan microfibers as scaffolds for liver tissue regeneration, hepatoma HepG2 cells were seeded onto microfibers. These microfibers presented albumin secretion and urea synthesis. Fan et al. [42] fabricated highly porous hybrid scaffolds which consist of galactosylated hyaluronic acid and chitosan using freeze-drying method for the enhanced function of hepatocytes in vitro. Due to the presence of hyaluronic acid, the hybrid scaffolds had an improved hydrophilicity and mechanical strength. In this study, rat primary hepatocytes seeded in the hybrid scaffolds demonstrated the multi-cellular spheroid morphologies.

5 In Nerve Tissue Engineering

In recent years, a wide variety of biocompatible materials have been considered for the construction of artificial tubes for nerve repair. Among these, chitosan-based materials are more promising for nerve regeneration due to their desired properties such as biocompatibility and biodegradability, and ability to provide a cellular and molecular framework for Schwann cells and neurite migration across the nerve gap.

5.1 Chitosan-Based Membranes

Yang et al. [43] studied the effects of chitosan–collagen hybrid membrane on the behavior of rat neural stem cells. The hybrid membranes were found to be more appropriate for the co-culture with rat neural stem cells because of their low

cytotoxicity and supporting ability for the cell survival. Recently, tissue engineering scaffolds based on conductive polymers combined with electrical stimulation are considered as prospective materials for the treatment of neural injuries. Huang et al. [44] reported a conductive hybrid membrane based on polypyrrole (2.5 %) and chitosan (97.5 %) in order to electrically stimulate Schwann cells. These membranes showed superior cell adhesion, spreading, and proliferation with or without electrical stimulation. Due to the electrical stimulation, these hybrid membranes expressed the emission of NGF and BDNF when compared with control cells. Wrobel et al. [45] developed chitosan film for nerve tissue engineering application. To study the biocompatibility of film, various types of Schwann cells were seeded onto chitosan film and found that all cell types were viable on the chitosan film. Moreover, different types of metabolic activities and proliferation behavior were observed on the of Schwann cell-seeded chitosan films. Recently, Morelli et al. [46] prepared hybrid membranes which consist of chitosan, poly(caprolactone), and poly(urethane) using phase-inversion techniques. The efficacy of these membranes to enhance the adhesion and differentiation of neuronal cells was determined. The results showed that neural cell responses of the hybrid membrane were found to be depending on the type and properties of the polymers used for the fabrication of membranes.

5.2 Chitosan-Based Hydrogels

Hydrogels based on chitosan can be suitable for neural tissue engineering due to their physicochemical and mechanical properties to hold neurite extension and assist transplantation of cells. Freier et al. [47] developed chitin and chitosan hydrogel tubes using mold casting method. Both chitin and chitosan hydrogel tubes facilitated adhesion and differentiation of primary chick dorsal root ganglion neurons in vitro. However, chitosan hydrogel tube showed improved nerve cell adhesion and neurite outgrowth, which represents the potential of this material in nerve tissue engineering. Valmikinathan et al. [48] prepared a photo cross-linked chitosan hydrogel as shown in Fig. 6. This hydrogel showed less cytotoxicity against hMSCs. An improved neurite differentiation from primary cortical neurons and neurite extension from dorsal root ganglia was observed on the chitosan hydrogel when compared to the control agarose hydrogen under similar conditions. Moreover, neural stem cells seeded on the chitosan hydrogels assisted differentiation into tubulin-positive neurons and astrocytes.

6 In Musculoskeletal Tissue Engineering

In the recent years, chitosan-based functional materials are widely considered to fabricate the scaffolds for regeneration of severely damaged tissues. Masuko et al. [49] prepared chitosan-RGDSGGC peptide complex by reacting thiolated chitosan with RGDSGGC peptide containing RGDS groups as shown in Fig. 7. In this

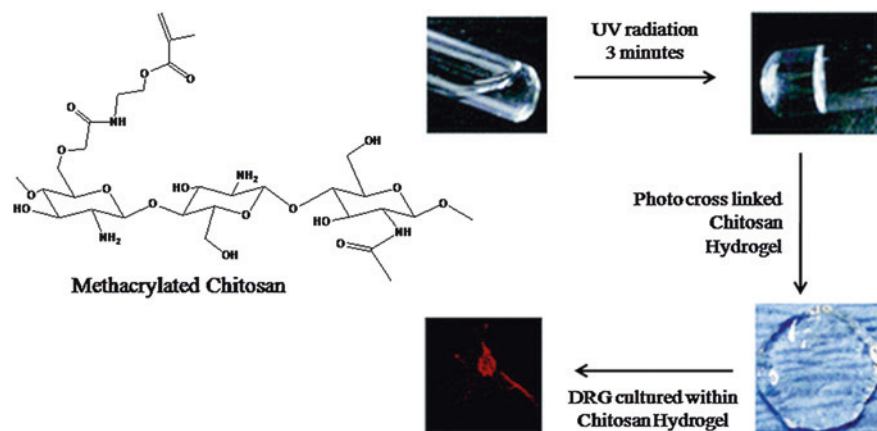


Fig. 6 Synthesis of photo cross-linkable hydrogel

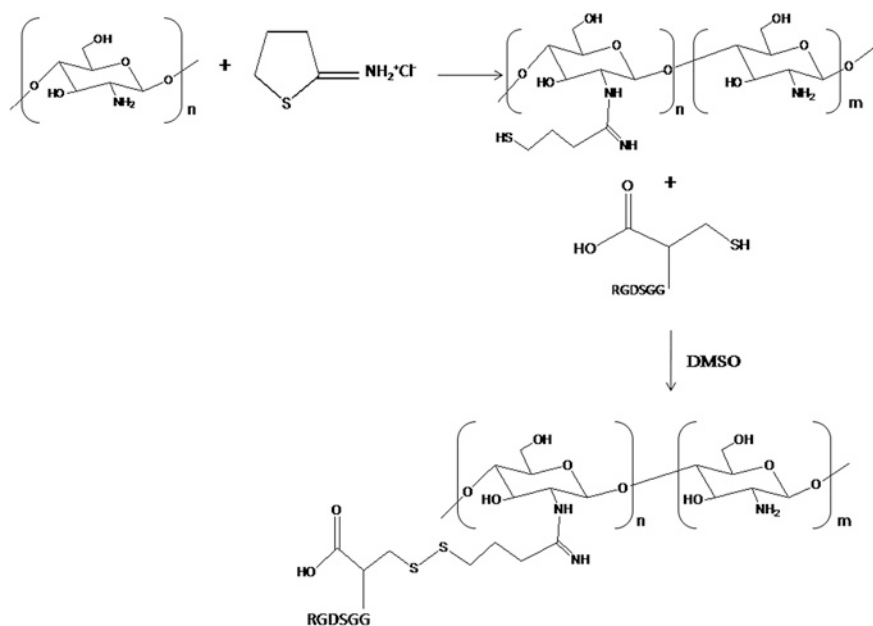


Fig. 7 Synthesis of chitosan-RGDSGGC conjugates

study, the effects of RGDSGGC peptide to thiolated chitosan on cell adhesion and proliferation activity of chondrocytes and fibroblasts were determined. The results showed that chitosan-RGDSGGC peptide complex can improve both cell adhesion and cell proliferation of chondrocytes and fibroblasts.

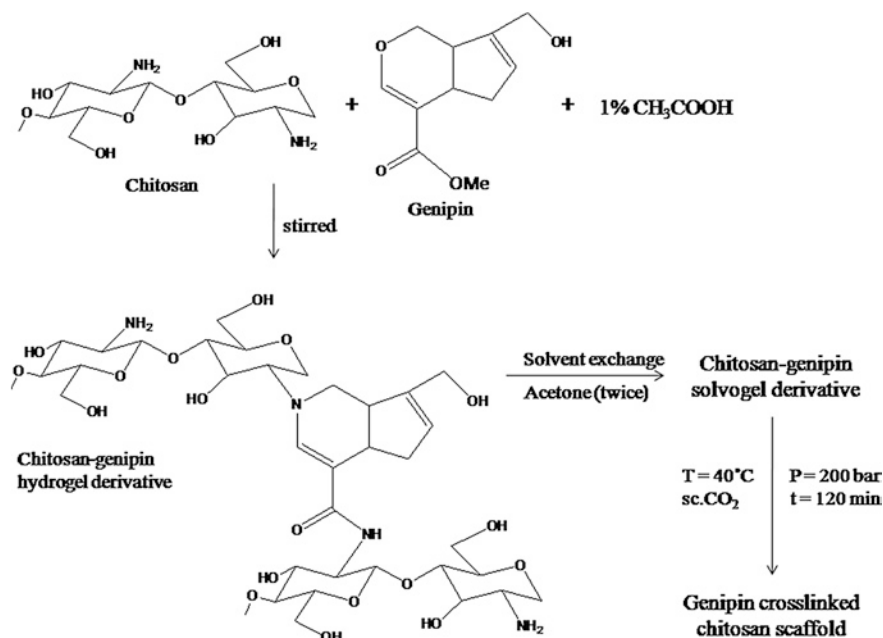


Fig. 8 Preparation of chitosan–genipin scaffold

Rinki and Dutta fabricated supercritical carbon dioxide-assisted porous chitosan scaffolds using chitosan in aqueous acetic acid and genipin by as a cross-linking agent (Fig. 8) [50]. These scaffolds showed an improved bioactivity in SBF and cellular attachment with MG63 osteoblastic cells. These results indicate that supercritical carbon dioxide-assisted porous chitosan scaffolds can be used for musculoskeletal tissue engineering application. Zhang et al. [51] recently prepared conducting glutaraldehyde cross-linked hydrogels based on carboxymethyl chitosan and aniline. These hydrogels presented a controlled release of encapsulated model drug diclofenac sodium. Live/Dead assay and Alamar blue assay proved the biocompatibility of the conducting hydrogels by C2C12 myoblast cells.

Adipose tissue engineering is considered as a promising technique for reconstructive and cosmetic applications in plastic surgery. In this context, Cheung et al. [52] developed photo cross-linked methacrylated glycol chitosan and methacrylated chondroitin sulfate scaffolds integrated with bioactive decellularized adipose tissue. The results showed that these scaffolds improved hASCs viability due to the presence of adipose tissue as a cell-supportive matrix. In addition, methacrylated chondroitin sulfate-based scaffolds presented a better implant integration and adipogenesis, with allogenic hASCs promoting cell infiltration, angiogenesis and eventually, fat formation. Recently, Martel-Estrada et al. [53] prepared porous chitosan/mimosa tenuiflora hybrid scaffolds by thermally induced

phase separation and lyophilization methods. Due to the existence of more amine groups, apatite layer was formed efficiently on the hybrid scaffolds, indicating the potential application of these hybrid scaffolds in tissue regeneration.

7 Concluding Remarks

Using chitosan and its composites with inorganic and/or polymeric materials, a variety of porous scaffolds have been fabricated for tissue engineering and regenerative medicine. These chitosan-based scaffolds are non-toxic, biocompatible, and biodegradable. Due to the desirable physicochemical and biological properties, chitosan-based scaffolds are widely considered as potential biomaterials for bone, cartilage, liver, nerve, and musculoskeletal tissue regeneration. However, for the efficient bone and cartilage tissue engineering applications, chitosan-based scaffolds still require adequate stability and mechanical strength. To fulfill these requirements, different types of hybrid scaffolds based on chitosan and other biocompatible materials have been developed. These hybrid scaffolds have highly porous structure with inter-connectivity and enough mechanical properties for cell adhesion and support for bone and cartilage tissue engineering. Due to the presence of bioactive and functional materials, chitosan-based hybrid scaffolds are bioactive and can be used to deliver bioactive materials for exciting cell differentiation and proliferation or drug molecules to provoke therapeutic effects in tissue engineering approaches.

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