Adv Polym Sci (2011) 244: 45–80
DOI: 10.1007/12_2011_112

Published online: 12 March 2011

Chitosan: A Promising Biomaterial for Tissue Engineering Scaffolds

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Abstract The contribution of chitosan as a scaffold material is quite significant in the field of tissue engineering, which is a multidisciplinary field of research and technology development requiring the involvement of chemists, physicists, chemical engineers, biologists, cell-biologists etc. to regenerate injured or damaged tissue. The advantages of using chitosan as a three-dimensional scaffold for tissue engineering applications are due to its versatile physicochemical and biological properties. Further, owing to its easy processability, it can be molded into the desired shape and size. Therefore, it is no exaggeration to say that chitosan is a promising biomaterial for tissue engineering scaffolds. There is an enormous body of work already published in various journals on chitosan as a tissue engineering scaffold but, to our knowledge, this work has not yet been brought together in one chapter. We have used our best efforts to accumulate the research work already done on chitosan in a single place so that chitosan researchers can easily find information and can therefore escalate their research activities. This chapter highlights different methods for the fabrication of scaffolds, the suitability of chitosan as a good scaffolding material, and its application as a scaffold for tissue engineering of bone, cartilage, skin, liver, corneal, vascular, nerve, and cardiac tissue.

Keywords Bone · Chitosan · Nerve · Scaffolds · Skin · Tissue engineering

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1 Introduction

A look at the world population reveals that the most common chronic problem associated with man is loss of tissue and organ damage. This can be cured by organ transplantation using tissue engineering techniques. The main problem associated with organ transplantation is shortage of suitable donors. This circumstance demands the need of a suitable scaffold wherein autologous cells can be grown under optimum conditions in vitro and subsequently transplanted back into the human body. This will obviate the need to wait for a donor and, on the other hand, will also increase the patients' comfort and compliance. The very fundamental of tissue engineering is the requirement for a scaffold material with specific characteristics that provides a temporary artificial matrix for cell seeding [1]. One of the most important characteristics of a scaffold material is that it should provide an ideal site for cell attachment and proliferation, leading to further tissue engineering. The extracellular matrix (ECM) not only provides the physical support for cells but also regulates their proliferation and differentiation. Therefore, scaffolds need to be developed for sustaining in vitro tissue reconstruction as well as for in vivo cell-mediated tissue regeneration. Repair of tissue defects can only be possible if the cells are supplied with such an ECM substitute [2]. A scaffold is a support, either natural or artificial, that maintains tissue contour. Substances that are frequently used for scaffold preparation are natural polymers, synthetic polymers, or ceramics with adsorbed proteins or immobilized functional groups [3]. Natural polymers have drawn the attention of various researchers because of their outstanding biocompatibility properties. Biodegradable materials have gained more attention because they have the advantage of allowing new tissue to take over their

load-bearing or other functions without creating any potential chronic problems associated with the presence of biostable implants [4]. The paradigm of tissue engineering consists of seeding cells on a scaffold made of either a synthetic or natural polymer blend, maturing the tissue in vitro, and finally implanting the construct at the desired site in the patient as an artificial prosthesis [5, 6]. Overall, the strategy of tissue engineering [7] generally involves the following steps:

1. Identify, isolate, and produce an appropriate cell source in sufficient amount
2. Synthesize a scaffold with the desired shape and dimension, which will subsequently be used as a cell carrier
3. Seed the cells uniformly onto or into the carrier and incubate for a predetermined time in a bioreactor
4. Implant the cell-seeded carrier in a proper animal model. Depending on the site and the structure, vascularization may be necessary

2 Scaffolds

Tissue scaffolds are synthetic bioresorbable polymers that act as functional substitutes for missing or malfunctioning human tissues and organ. The primary role of a scaffold is to provide a temporary substrate to which the transplanted cells can adhere [8]. The most important factors to be considered with respect to nutrient supply to transplanted and regenerated cells are porosity, pore size and pore structure for porous scaffolds with a large surface-area-to volume ratio, and void volume. Optimization of these parameters is desirable for attachment, growth, maximal cell seeding, ECM production, and vascularization. Pores of the same diameter are preferable in scaffolds in order to yield high surface area per volume, provided the pore size is greater than the diameter of a cell in suspension [1, 2].

Thus, scaffolds provide physical support to cells, and pores provide space for remodeling of tissue structures. The major challenge associated with the development of scaffolds is the organization of cells and tissue in a three-dimensional (3D) configuration so that molecular signals are presented in an appropriate spatial and temporal fashion in a common manner that promotes the individual cells to grow and form the desired tissue structures [9, 10].

2.1 Factors Governing the Design of Scaffolds

During design of a scaffold for real-life applications, we must pay attention in choosing the scaffold material, body acceptability, mechanical properties, surface chemistry, and porosity. The porosity, morphology, and mechanical strength of scaffolds are governed by various factors. Some of the factors governing the designing of scaffolds are discussed below.

2.1.1 Materials

During design of scaffolds for tissue engineering applications, one must emphasize the selection of suitable materials. The materials should be biocompatible and biodegradable (i.e., they can be degraded into harmless products, leaving the desired living tissue). Some of the materials used for fabricating scaffolds include natural polymers, synthetic polymers, ceramics, metals, and hybrids of these materials [11]. Metals and ceramics are not a good choice for tissue engineering applications because they are not biodegradable (except for bioceramics such as α-tricalcium phosphate and β-tricalcium phosphate) and because their processability is very limited. For these reasons, natural polymers have gained increased attention because they are biodegradable and biocompatible. One of the major drawbacks exhibited by scaffolds made up of natural polymers is their poor mechanical properties. These problems associated with natural polymers can be circumvented by using synthetic resorbable polymers such as poly(α-hydroxy esters), polyanhydrides, polyorthoesters, and polyphosphazens. Polyglycolic acid (PGA), polylactic acid (PLA), polydioxanone, and copolymers thereof are the only FDA-approved synthetic and degradable polymers.

2.1.2 Porosity and Surface Area

Scaffolds should be highly porous and the pores should be interconnected to favor tissue integration and vascularization. Scaffolds should have appropriate surface chemistry to provide the necessary initial support for the attachment and proliferation of cells, and for the retention of their differentiated functions [12]. The porosity and pore size of the scaffold play crucial roles in the regeneration of a specific tissue. For instance, scaffolds with pore size less than 150 μm have been successfully used for regeneration of skin in burn patients [13]. Angiogenesis is a requirement for some scaffold application scenarios and can be unpleasantly affected by material porosity. Pore morphology can also affect scaffold degradation kinetics and the mechanical properties of the developing tissue [14]. The degree of interconnectivity has a greater influence on osteoconduction than does the actual pore size [15]. Highly porous materials facilitate the easy diffusion of nutrients to, and waste products from, the implant. Similarly, the larger the surface area of the scaffold, the more it favors cell attachment and growth [16].

2.1.3 Mechanical Properties and Processability

The scaffold should possess good mechanical strength so that it can be used for the reconstruction of hard, load-bearing tissues such as bone and cartilage. The biomaterial should be easily processed so that it can be easily fabricated into different shapes and sizes to meet the needs of the desired tissue reconstruction. The scaffold's architecture plays a vital role in maintaining its dimensional stability [15].

The scaffolds should have sufficient structural integrity that matches the mechanical properties of native tissue [17]. The external shape of the scaffold is also extremely important from the clinical point of view because the final anatomical shape of a regenerated tissue is basically dependent on the shape of the associated scaffold [18]. The mechanical properties of scaffold in tissue-engineering applications are of great importance due to the necessity of the structural stability to withstand stress incurred during culturing in vitro and implanting in vivo. In addition, the mechanical properties can significantly affect the specific biological functions of cells within the engineered tissue [19].

2.2 Scaffold Fabrication Techniques

Scaffolds can be fabricated by using different types of methodologies such as fiber bonding, salt leaching, gas-induced foaming, phase separation, electrospinning, solid freeform fabrication, and molecular self assembly [15, 17]. Some of the fabrication techniques are discussed below.

2.2.1 Salt Leaching

Salt leaching is one of the simplest fabrication methods for producing scaffolds with controllable porosity and pore size using various biodegradable polymers. The process for the manufacture of solid polymer–porogen constructs consists of combination of a suitable porogen with a solution of polymer in an appropriate mold. The porogen is then leached out to form porous sponges [20]. The traditional methods generally employ a solid porogen within a 3D polymer matrix to create well-defined pore size, pore structure, and total scaffold porosity. Murphy et al. [21] has introduced a modified method for producing porous, biodegradable tissue engineering scaffolds with improved pore interconnectivity. They fabricated a 3D porous scaffold by using a copolymer of 85:15 poly(lactide-*co*-glycolide) (PLG) via a solvent casting and particulate leaching process. They partially fused the NaCl crystals via treatment in 95% humidity to create the interconnecting pores, prior to the formation of a 3D polymer scaffold. This technique allows scaffolds for tissue engineering to be formed with minimal laboratory equipment and polymer amounts. Several recent modifications to this method demonstrate the tremendous pace of improvement in the manufacture of scaffolds with precise chemical, physical, and biological properties.

2.2.2 Phase Separation

Phase separation is one of the most popular techniques for fabricating porous scaffolds for tissue engineering applications. In this process, phase separation is

induced by decreasing the temperature of a polymer solution, which results into two different phases, one having a high polymer concentration (polymer-rich phase) and one having a low polymer concentration (polymer-lean phase). The solvent from the polymer-lean phase is later removed by extraction, evaporation, or sublimation to leave behind open pores. The polymer in the polymer-rich phase solidifies into the skeleton of the polymer foam. This separation can be categorized into two types on the basis of the crystallization temperature of the solvent in the polymer solution. One type is solid–liquid phase separation and the other is liquid–liquid phase separation. When the solvent crystallization temperature is higher than the liquid–liquid phase separation temperature, then it can be separated by lowering the temperature and the process is known as solid–liquid phase separation. This process consists of crystallization of solvent, and the polymer is expelled from the solvent crystallization front. However, when the solvent crystallization temperature is much lower than the phase separation temperature, a liquid–liquid phase separation takes place on decreasing the temperature of the polymer solution. Phase separation is relatively a simple technique for the fabrication of scaffolds having highly organized structures [22].

2.2.3 Solid Freeform Fabrication

Control over internal architecture and interconnectivity is a tough task for researchers. These days, the solid freeform technique (SFF) has attracted the attention of researchers. SFF is a collective term for a group of techniques that can rapidly produce highly complex 3D physical objects using data generated by computer-aided design (CAD) systems, computer-based medical imaging modalities, digitizers, and other data makers. The technique involves in the manufacture of objects in a layer-by-layer fashion from the 3D computer design of the object [23]. Some of the advantages [24] of using SFF technique are listed below:

- In SFF scaffolds, the 3D interconnection of the scaffold can be maintained at a wide porosity level
- Using computerized tomography (CT) or magnetic resonance image (MRI) as the data source, scaffolds can be made with an external geometry conforming to the patients' anatomic structure, and thus the external geometry of the scaffolds can also be designed and customized to fulfill the need of the tissue engineer to construct scaffolds for specific tissues
- Scaffolds with a distinct material and design domain can be fabricated by using SFF techniques

Finite element analysis (FEA) and CAD can be combined with manufacturing technologies such as SFF to allow virtual design, characterization, and production of scaffold that is optimized for tissue replacement. This makes it possible to design and manufacture very complex tissue scaffold structures with functional components that are difficult to fabricate.

2.2.4 Supercritical Fluid Drying

Recently, various types of supercritical fluid processing methods have been developed for the production of microparticles, foams, fibers, and aerogels [25–27]. Fabrication of scaffolds using supercritical fluid has been reported recently by several researchers [28–30]. The rapid expansion of supercritical solutions (RESS) and the gas anti-solvent technique (GAS) are widely used for the formation of microparticles and fibers. In RESS, a supercritical solution is rapidly expanded, which leads to a rapid decrease of the polymer solubility in the supercritical fluid (SCF) and, finally, to the formation of microparticles or nanoparticles with narrow size distribution. In GAS, a polymer solution is expanded into a SCF, which acts as an anti-solvent since it is not miscible with polymer but is miscible with the organic solvent. Because the solvent is miscible with the SCF, it expands, resulting in the reduction of solvent capacity to support polymer dissolution [25]. The author's laboratory [29, 30] have prepared chitosan scaffolds using supercritical carbon dioxide ($scCO_2$). In the first step, the hydrogels were prepared and treated with organic solvent(s) and then placed in the chamber of a supercritical fluid reactor to undergo solvent exchange. Thereafter, the temperature and pressure were raised. Thus, the continuous flow of $scCO_2$ through the sample replaced all the organic solvent with CO_2 to obtain a porous chitosan scaffold.

2.2.5 Hydrothermal Preparation

The use of a hydrothermal bomb for preparation of a metal organic framework is a well-known technique in inorganic chemistry [31]. However, the use of a hydrothermal bomb for the preparation of scaffold is very rare. The final mixture with the appropriate composition for scaffold preparation is sealed in a PTFE-lined acid digestion bomb and heated at 40°C for 8 h under autogeneous pressure. After that, the bomb is kept at room temperature to cool the product, which is then frozen at −20°C. Finally, the product is vacuum dried to obtain the desired scaffolds [32–34] (Dutta PK et al., unpublished results).

2.2.6 Electrospinning

Another important scaffold fabrication technique is that of electrospun nanofibers. Electrospun nanofibers could be used to mimic the nanofibrous structure of the ECM in native tissue [35–37]. Electrospinning involves the ejection of a charged polymer fluid onto an oppositely charged surface. This technique is used to create polymeric fibers with diameters in the nanometer range. In electrospinning, a charge is applied to a polymer solution or melt, which is ejected toward an oppositely charged target. The body of the polymer solution or melt becomes charged, and electrostatic repulsion counteracts the surface tension so the droplets become stretched. At a critical point, a stream of liquid erupts from the surface. This point

of eruption is known as the "Taylor cone." When the applied voltage is increased beyond a threshold value, the electric forces in the droplet overcome the opposing surface tension forces and a narrow charged jet is ejected from the tip of the Taylor cone [38]. The commonly used polymers for the electrospinning method of fabrication are the aliphatic polyesters [39]. Preparation of chitosan scaffolds by electrospinning has been mentioned by various researchers [37, 40–42]. Duan et al. [43] developed a nanofibrous composite membrane of poly(lactide-*co*-glycolic acid) (PLGA)–chitosan/poly(vinyl alcohol) (PVA) by simultaneous electrospinning of PLGA and chitosan/PVA from two different syringes and mixing on a rotating drum to prepare a nanofibrous composite membrane, which was then crosslinked with glutaraldehyde (GA). The obtained composite membrane was cytocompatible for fibroblastic cells.

3 Chitosan as a Scaffolding Material

Among the naturally derived polymers such as gelatin, collagens, glycosaminoglycan (GAG), starch, and alginate, chitosan, a partially deacetylated derivative of chitin, is chemically similar to GAG and has many desirable properties that make it a suitable candidate for use as a tissue engineering scaffold. Fabricating the hybrid scaffolds by combining natural polymers with synthetic polymers and ceramics is the best method, because the hybrid scaffolds possess both the mechanical strength of synthetic polymers and the biodegradability of natural polymers [15].

The principal derivative of chitin, chitosan, has gained more attention as a scaffold in tissue regeneration due to: (1) the possibility of large scale production and low cost; (2) its positively charged and reactive functional groups that enable it to form complexes with anionic polymers, including proteins that help to regulate cellular activity, [44]; and (3) its antibacterial properties [45]. Apart from this, chitosan is hemocompatible and non-immunogenic, and is degradable into non-toxic oligosaccharides inside the body due to the action of lysozymes. But, chitosan lacks the tensile strength required to match that of several natural tissues [46, 47]. It has been reported that chitosan-based biomaterials do not lead to any inflammatory or allergic reaction following implantation, injection, topical application, or ingestion in the human body [48]. Chitosan possesses wound-healing properties and favors both soft and hard tissue regeneration [49, 50]. By contrast, many synthetic polymers exhibit physicochemical and mechanical properties comparable to those of the biological tissues that they are required to substitute, but are not sufficiently bioactive [51]. Polyesters such as PLA, PLGA, and polycaprolactone (PCL) can be reproduced with specific molecular weights, block structures, degradable linkages, and crosslinking modes, and have excellent mechanical strength [52, 53]. Thus, the lack of mechanical strength of chitosan scaffolds can be resolved by incorporation of inorganic materials so that the hybrid material possesses improved mechanical and biological properties. Many inorganic materials

such as calcium carbonate, calcium phosphate, and silica have been studied for the preparation of chitosan–inorganic composites [54].

3.1 Structural Analysis and Characterization of Chitosan

The structure of chitosan plays an important role if it is to serve as a scaffold material for application in tissue engineering. The biocompatibility of chitosan is attributed to its chemical properties. The polysaccharide unit of chitosan resembles the structure of GAGs, which are a major component of ECM of bone and cartilage and, hence, chitosan could be an attractive candidate for an ECM substitute [55]. The cationic nature of chitosan facilitates pH-dependent electrostatic interaction with anionic GAGs, proteoglycans, and other negatively charged molecules. This property is of particular interest in tissue engineering because it makes chitosan suitable in various shapes and sizes, i.e., porous scaffolds [14], planar membranes [56], and hydrogels [57], for specific interactions with growth factors, receptors, and adhesion proteins [58]. The cell adhesion, proliferation, and differentiation properties of chitosan are attributed to its hydrophilic nature, and its compact aggregated polymeric chains are helpful in providing stability to the scaffolds in terms of size and morphology during cell culture [59].

The physical and mechanical properties of chitosan can be ameliorated by using graft copolymerization and crosslinking. Chitosan forms aldimines and ketimines with aldehydes and ketones, respectively. Upon hydrogenation with simple aldehydes, chitosan produces *N*-alkyl chitosan [60]. The physicochemical and biological properties [61] as well as conformational structures [62] of chitosan are very effective for biomedical applications.

3.2 Role of Molecular Weight and Degree of Deacetylation

The molecular weight (Mw) and degree of deacetylation (DD) of chitosan play pivotal roles in dictating the biological properties of chitosan scaffolds. Notably, the DD itself influences many of the properties of chitosan, namely mechanical properties, biodegradability, immunological activity, wound-healing properties, and osteogenesis enhancement [63–71]. Chitosan scaffolds with higher DD showed higher cell proliferation, lower biodegradation rate, and higher mechanical strength. One of the studies in this direction was done by Hsu et al. [71]. They investigated the role of DD and Mw of chitosan in terms of hydrophilicity, degradation, mechanical properties, and biocompatibility by seeding fibroblastic cells and immortalized rat chondrocytes (IRC) on chitosan films of differing DD and Mw. They observed that in the chitosan films having similar Mw, the higher the DD of chitosan, the smaller was the elongation of chitosan films; with similar DD, a higher Mw led to higher tensile

strength. The results of degradation studies showed that for chitosan with the higher average Mw, higher DD led to a higher degradation rate. However, the result for chitosan films with the lower average Mw was found to be opposite, i.e., higher DD led to slower degradation. The average Mw has also some significant effect on degradation rate. For chitosan films having similar DDs, higher average Mw led to the higher degradation rate. The acetyl group, $–NHCOCH_3$ of chitosan plays an important role in deciding the degradation rate. Chitosan with lower DD have more $–NHCOCH_3$ groups and might be more amorphous and degrade faster. The results showed that with the lower average Mw, lower DD led to higher degradation rate of chitosan films. They got the inference from their study that hydrophilicity and biocompatibility of chitosan films were affected by DD. However, the rate of degradation and the mechanical properties were found to be affected by Mw.

Another study in this direction was performed by Chatelet et al. [72]. They investigated the effect of DD on the biological properties of chitosan films by culturing keratinocytes and fibroblasts on chitosan films having different DDs. They found that DD has no significant effect on the in vitro cytocompatibility of chitosan films towards keratinocytes and fibroblasts. They demonstrated that the lower the DD of chitosan, the lower was the cell adhesion on the films, and found that keratinocyte proliferation increases when the DD of chitosan films increases. They concluded from their study that the DD plays a key role in cell adhesion and proliferation, but does not change the cytocompatibility of chitosan.

4 Application of Chitosan for Regeneration of Various Types of Tissue

Chitosan scaffolds may find application in regeneration of skin tissue, liver tissue, bone and cartilage tissue, cardiac tissue, corneal tissue, and vascular tissue to mention a few [73]. A brief account of its application in various branches of tissue engineering is described in this section.

4.1 Skin Tissue

Dermal wounds are very widespread in man. The skin damage can be caused by heat, chemicals, electricity, ultraviolet, nuclear energy, or disease. In the case of wounds that extend entirely through the dermis such as full-thickness burns or deep ulcers, as a result of many skin substitutes such as xenografts, allografts, and autografts have been employed for wound healing. However, the disadvantages of these approaches include the limited availability of skin grafts in severely burned patients and the problems of disease transmission and immune response [52, 53]. One of the good

alternatives to skin grafts for curing skin damage is to develop a tissue-engineered skin equivalent. Polymeric tissue scaffolds made of PLGA, collagen, and chitosan are currently being employed for tissue reconstruction [74, 75]. An ideal scaffold for skin tissue engineering should possess the characteristics of excellent biocompatibility, suitable microstructure such as 100–200 μm mean pore size and porosity above 90%, controllable biodegradability, and suitable mechanical properties [76–78]. A brief account of work done by some researchers in the field of skin tissue engineering is given in Table 1.

There are complications in skin tissue engineering for cases of severe burn (third degree burns). Composite skin substitutes are applied to patients suffering from extensive burns, but slow cell ingrowths and insufficient vascularization has made it unreliable for curing the people suffering from third degree burns [81]. Consequently, some researchers are leaning towards the approach of tissue engineering, which utilizes both engineering and life science disciplines, to promote skin regeneration and to sustain and recover skin function [82].

In this direction, some good results-oriented data was reported by Liu et al. [81]. The work demonstrated the fabrication and effect of controlled-release of fibroblast growth factor (bFGF) from chitosan–gelatin microspheres (CGMSs) loaded with bFGF, where human fibroblasts were cultured on the chitosan–gelatin scaffold itself. The comparative study looked at cell morphology, cell proliferation, GAG synthesis, and gene expression with respect to loading of bFGF on the chitosan–gelatin scaffold. The DNA assay result indicated that the DNA content of human fibroblasts seeded on the scaffolds with and without bFGF-CGMS increased with culture time. The cell proliferation was 1.47 times higher over a period of 2 weeks on the scaffolds with bFGF-CGMS than on scaffolds without. GAG production was also higher on scaffolds with bFGF-CGMS than on the chitosan–gelatin scaffolds. Scanning electron microscopy (SEM) observations were also in accordance with the suitability of scaffolds containing bFGF for skin tissue engineering. They indicated that human fibroblasts attached and spread well on the scaffolds with bFGF-CGMS. Overall, the results indicated that chitosan–gelatin scaffolds with bFGF have a good potential as tissue engineering scaffolds to improve skin regeneration efficacy and to promote vascularization.

Very recently, Dhandayuthapani et al. [83] reported the development of novel chitosan–gelatin blend nanofiber systems for skin tissue engineering applications. In this study, they were able to electrospin defect-free chitosan, gelatin, and chitosan–gelatin blend nanofibers with smooth morphology and diameters of 120–200 nm, 100–150 nm, and 120–220 nm, respectively, by optimizing the process and solution parameters. Chitosan and gelatin formed completely miscible blends, as evidenced from differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy measurements. The tensile strength of the chitosan–gelatin blend nanofibers (37.91 ± 4.42 MPa) was significantly higher than that of the gelatin nanofibers (7.23 ± 1.15 MPa) ($p < 0.05$) and comparable with that of normal human skin.

Table 1 Work done in the field of skin tissue engineering

Matrix and its nature	Study conducted	Culture media	Observation	Conclusion	Name of researchers [Ref]
Nanofibrous composite membrane of PLGA–chitosan/PVA	Morphology, mechanical properties and cytocompatibility of the fibroblast cells	Isolated fibroblast dermal cells from rabbit back skin cultured into the composite membrane of PLGA–chitosan/PVA	Fibroblasts were attached on all the membranes and changed their original round shape to an elongated and spindle-like shape on all membranes. The crosslinked chitosan/PVA membrane showed a little activity	The electrospun PLGA–chitosan/PVA composite membranes combined the advantages of both PLGA and chitosan and would have a great potential for skin tissue engineering	Duan et al. [44]
Collagen/chitosan porous scaffolds prepared using a freeze-drying method	Morphology, the swelling capacity, as well as the in vitro and in vivo degradation of the scaffold crosslinked by different concentrations (0–0.25%) of GA	Human dermal fibroblasts seeded on GA-treated scaffold In vivo animal tests were performed by embedding the scaffolds subcutaneously on the dorsal surface of rabbit ear	The collagen/chitosan scaffolds can effectively support and accelerate fibroblast infiltration from the surrounding tissue	All the in vitro and in vivo results proved that the GA-crosslinked collagen/chitosan scaffold is suitable for skin tissue engineering	Ma et al. [75]
Chitosan-pectin-TiO_2 ternary nanocomposite film	Cytotoxicity	Two cell lines, L929 mouse fibroblast cells and NIH3T3 mouse fibroblast cells, using the MTT assay	The viability assay was measured at 24 h and 48 h after cell seeding. The cell viability was more than 97% for NIH3T3. For L929 it was 100% after 24 h and 97% after 48 h	The MTT assay indicated that the cells grew very well after 48 h of exposure to ternary nanocomposite film. Therefore, ternary film can be considered a biocompatible product for wound healing	Dutta et al. [79]
Chitosan crosslinked with dimethyl 3-3′, dithio bis (propionimidate) (CS-DTBP) or glutaraldehyde (CS-GA)	Cytotoxicity test	Human dermal fibroblast cells in the presence of leachate from different chitosan scaffolds (chitosan, CS-GA and CS-DTBP)	The number of cells that grew in the leachate from the CS-DTBP sample was significantly higher than the number of cells in the leachate from the CS-GA sample	DTBP-crosslinked chitosan is less toxic than CS-GA scaffolds	Adekogbe and Ghanem [80]

4.2 Bone and Cartilage Tissue

Scaffold serves as a temporary skeleton inserted into the sites of defective or lost bone to support and stimulate bone tissue regeneration while it gradually degrades and is replaced by new bone tissue [84–87]. Both bioactive ceramics and polymers are used as scaffolding materials. Bioceramics have chemical compositions resembling bone and they also allow osteogenesis. The major drawback of using bioceramics as scaffolding material are its brittle nature and low degradation rates. However, the biodegradation rates and mechanical properties of biopolymers can be tailored to a certain extent for specific applications. Biopolymers are particularly amenable for implantation and can be easily fabricated into desired shapes [15, 88]. Chitosan is widely used as scaffolding for the regeneration of bone tissue because of its osteocompatible and osteoconductive properties, and can enhance bone formation both in vitro and in vivo [51]. The scaffolds should possess excellent mechanical properties. The work done in the field of bone tissue engineering is outlined in Table 2.

As far as chitosan is concerned for cartilage tissue engineering applications, the rate of biodegradation of the scaffold (used to organize cells in vitro) plays a crucial role. The presence of non-biodegradable articles in soft tissue often causes acute foreign body reactions elicited by the body's immune system that can result in severe inflammation and soreness around the implant site. Many studies have reported that chitin and chitosan are biodegradable polymers and that they degrade in vivo mainly through their susceptibility to enzymatic hydrolysis mediated by lysozyme, which is ubiquitous in the human body. However, this action is dependent on factors such as pH, type of chitin or chitosan, and chitosan preparation method. The use of chitosan as scaffolding material for cartilage tissue has been reported by many researchers [2, 101, 102]. Composite chondroitin-6-sulfate/dermatan sulfate/chitosan scaffolds were reported to be used for articular cartilage regeneration [103]. A brief account of work done in cartilage tissue engineering is described in Table 3.

4.3 Liver Tissue

Liver is one of the most important and complex organs, serving several essential functions in the body. A biohybrid artificial liver using isolated hepatocytes and polymer scaffolds is expected to be an alternative method of treatment for liver failure because the shortage of suitable donors and costly surgical procedure has limited the use of liver transplantation. For this approach, various scaffolds have been used and it has been shown that the scaffolding material is crucial for control of cell adhesion, growth, and tissue reconstruction [107]. For the culture of anchorage-dependent cells such as hepatocytes, scaffolds require specific interaction with ECM components, growth factor, and the cell surface receptor to ensure cell survival, differentiation, and function [108]. This must be taken into account during

Table 2 Work done in the field of bone tissue engineering

Matrix and its nature	Study conducted	Culture media	Observation	Conclusion	Name of researchers [Ref]
Chitosan and alginate	Implantation of chitosan–alginate scaffolds into female Sprague-Dawley rats and cell viability test	MG63 osteoblast cells on chitosan–alginate scaffolds	In vitro study showed more calcium deposition after 28 days	The hybrid scaffold showed improved mechanical and biological properties	Li et al. [89]
Chitosan–hydroxyapatite (CS-HA) scaffold	Alkaline phosphate activity (ALP) and total protein content using commercially available kits	Primary human osteoblast (SAOS-2 cell line) incubated for 1 and 3 weeks	The CS-HA scaffold showed much faster cell growth. The cells seeded on the scaffold also expressed a distinct ALP activity	The CS-HA scaffold with excellent biocompatibility has the potential to serve for tissue engineering	Manjubala et al. [90]
Calcium phosphate–chitosan composite scaffold prepared by adding HA and calcium phosphate glass powder to chitosan and keeping it at −20°C for 4 days and then freeze-drying the frozen sample	Cell differentiation as assessed by total protein expression, ALP activity, and osteocalcin release measured spectro-photometrically	Human osteoblast-like MG63 cells on the composite scaffolds	The total protein content of the cells grown on the composite scaffolds increased faster with incubation time than that of the control	Calcium phosphate–chitosan composite scaffolds are suitable for bone tissue engineering	Zhang et al. [91]
3D chitosan/poly(lactic acid-glycolic acid) (PLAGA) composite porous scaffolds	Scaffold fabrication parameter and the cellular responses	Osteoblast-like MC3T3-E1 cells	Good proliferation, increased ALP activity of cells on the composite scaffolds and upregulated gene expression of ALP, osteopontin, and bone sialoprotein	Composite chitosan/PLAGA scaffolds showed excellent mechanical properties and bioactivity. Hence, they may serve as scaffolds for load-bearing bone tissue engineering	Jiang et al. [92]
Macroporous calcium phosphate cement (CPC)	The effect of chitosan on the mechanical properties of	Osteoblast mouse cells (MC3T3-E1)	Cell viability was quantified using an enzymatic assay	MC3T3-E1 cells were able to adhere, spread and	Xu et al. [93]

prepared by incorporating chitosan and mannitol with a series of powder-to-liquid ratios and a wide range of mannitol content	the scaffold and the composite mechanical properties, measured as a function of pore volume fraction up to 80% for the scaffold	on the CPC–chitosan scaffold		proliferate on CPC-chitosan	
Genipin-crosslinked chitin–chitosan scaffolds with hydroxyapatite (HA)	Mechanical properties, various fabrication variables and cellular behavior	Bovine knee chondrocytes (BKC)	Higher chitin content gives larger porosity and Young's modulus, but lower extensibility	More HA deposition has a beneficial effect on the cellular amount, GAG content, and collagen	Kou et al. [94]
Genipin-crosslinked collagen–chitosan biodegradable porous scaffolds	Influence of chitosan amount and genipin concentration on the physicochemical properties of the scaffolds	Culture of rabbit chondrocytes in vitro	Good viability of the chondrocytes seeded on the scaffold	The scaffold formulation may be promising for articular cartilage	Yan et al. [95]
HA incorporated into chitosan scaffold by an in situ method	Biomimetic studies leading to cell proliferation	MC 3T3-E1 cells on the apatite layer, as formed on the two kinds of scaffolds	MC3T3-E1 cells on apatite-coated chitosan–nano-HA scaffolds showed better proliferation than on apatite-coated chitosan scaffolds	The addition of nano-HA to the chitosan scaffold improved its bone bioactivity, which could develop the use of chitosan in bone tissue engineering	Kong et al. [96]
Composite scaffolds by incorporating 80 wt% HA in the polyelectrolyte complex matrix of chitosan and poly(acrylic acid) (PAA) in the ratio 40:60	Bioactivity study was performed by seeding	Human osteosarcoma (HOS) cells on the composite scaffolds. Cell viability was measured by MTT assay	–	Chitosan–PAA scaffolds incorporating HA showed better viability, cell attachment. and adhesion of HOS cells compared with the chitosan–PAA scaffold	Shailaja et al. [97]

(*continued*)

Table 2 (continued)

Matrix and its nature	Study conducted	Culture media	Observation	Conclusion	Name of researchers [Ref]
Chitosan sponges were prepared by freeze-drying, and then mineralized with calcium and phosphate solution using the double diffusion method, leading to the formation of HA nanocrystals on the surface of the scaffolds	Growth of osteoblast-like cells on biomimetic apatite-coated chitosan scaffolds and the influence of apatite nanocrystals on cells	Human osteoblast-like cell line (SaOS-2) for a period of 3 weeks on the mineralized scaffolds	The mineralized CS scaffolds and the pure CS scaffolds both showed a similar cell growth trend. The cells seeded on the mineralized scaffold showed a higher total protein content and higher ALP activity after 1 and 3 weeks of culture in comparison to those on the pure CS scaffolds	The presence of apatite nanocrystals in CS scaffolds has a good potential to serve for bone tissue engineering	Manjubala et al. [98]
Biphasic calcium phosphate (BCP) added to chitosan scaffolds	Influence of addition of BCP to porous chitosan scaffolds on the distribution, morphology, and phenotypic expression of osteoblastic cells	D1 ORL UVA mouse mesenchymal stem cells (MSCs) and MC3T3 E1 preosteoblastic cells on chitosan scaffold	Formation of more uniform and complete cells, ECM distribution on the BCP, significantly higher ALP activity and osteocalcin expression, and higher rate of migration for MC3T3 E1cells	Composite scaffolds for culture of MSCs and preosteoblasts enhance bone tissue development in vitro	Sendemir-Urkmez et al. [99]
Chitosan–silica hybrid membrane using a sol–gel process	In vitro cellular activity, in vivo bone regeneration ability, and the in vitro bone bioactivity test by immersing the hybrid and pure chitosan membranes in simulated body fluid	Osteoblastic cells, rat calvarial model to carry out in vivo study	Improved mechanical properties, excellent apatite-forming ability, good cellular responses of the hybrid membrane, and significantly higher rate of new bone regeneration	The enhanced properties of the hybrid membrane were attributed to incorporation of the rigid and bioactive silica xerogel into the hybrid membrane	Lee et al. [100]

Table 3 Work done in the field of cartilage tissue engineering

Matrix and its nature	Study conducted	Culture media	Observation	Conclusion	Name of researchers [Ref]
Porous poly(DL-lactide) (PDLLA)/chitosan scaffolds	Cell viability was measured using MTT assay	Rabbit chondrocytes seeded onto the PDLLA/chitosan scaffolds	Cells grown on PDLLA/chitosan scaffolds increased on increasing the weight ratio of the chitosan component, were able to preserve the phenotype of chondrocytes, and also supported the production of type II collagen	PDLLA/chitosan scaffolds are able to promote the attachment and proliferation of chondrocytes	Wu et al. [104]
Poly(L-lactide) (PLLA) microspheres using chitosan on the surface	Bioactivity study on control PLLA and the chitosan-coated PLLA microspheres by in vitro culture	Rabbit chondrocytes	Larger amount of chitosan-coated PLLA microspheres exhibited enhanced cell attachment and proliferation	Chitosan-coated PLLA microspheres may serve as injectable cell microcarriers for chondrogenesis in cartilage tissue	Lao et al. [105]
Composite scaffolds prepared by incorporating different amounts of PLGA microspheres into gelatin/chitosan/hyaluronan scaffolds by freeze-drying and crosslinking with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide	The effects of incorporation of PLGA microspheres on porosity, compressive modulus, PBS uptake ratio, and weight loss of the scaffolds	In vitro culture of chondrocytes	Composite scaffolds having 50 wt% PLGA microspheres exhibited larger compressive moduli, lower weight loss, and good porosity (>90%). In vitro, these scaffolds had good cell attachment, viability, proliferation, and GAG secretion	Scaffolds with 50 wt% PLGA microspheres have better physical performance and preserved biocompatibility and can be used for chondrogenesis	Tan et al. [106]

the design and selection of polymeric materials for liver tissue-engineering. Calcium alginate sponge has been used for hepatocyte culture [109]. However, the alginate sponge is mechanically unstable due to ion exchange of Ca^{2+} with monovalent cations, and it lacks the cell-adhesive signals that are necessary to preserve long-term hepatocyte function and to suppress apoptosis. Application of chitosan scaffolds in liver tissue engineering is described in Table 4.

4.4 Cardiac Tissue

Myocardial infarction is one of the major public health concerns and the leading cause of death all over the world [118]. Human myocardium lacks the possibility of regeneration after myocardial infarction [119]. This results in a progressive loss of functional myocardium and a successive enlargement of the left ventricular cavity, thus impairing cardiac function [120]. The loss of viable myocardium is irreversible and, if extensive, could result in heart failure. The only available treatment of end-stage heart failure is heart transplantation. Shortage of donor hearts and immunological rejection of the transplanted tissue has limited transplantation to certain patients only. One good alternative for the treatment of heart failure is the replacement of damaged tissue with a tissue-engineered graft generated using cells and biodegradable scaffolds [121]. An ideal scaffold for cardiac tissue engineering should be (1) highly porous with large interconnected pores, (2) hydrophilic, (3) structurally stable, (4) degradable, and (5) elastic (to enable transmission of contractile forces) [122, 123]. The main focus of cardiac tissue engineering is on the development of 3D heart muscle that can be utilized to augment the function of failing myocardium. Table 5 gives glimpses of work done on cardiac tissue engineering applications.

4.5 Vascular Tissue

Vascular diseases, such as blood vessel damage, atherosclerosis, and aneurysms, remain an obstacle for clinicians because of limited donor sites and the immune response to allograft and xenograft. Tissue-engineered blood vessel is an optimal alternative for blood vessel substitution. Vascular transplantation has been commonly used for the treatment of vascular diseases. An ideal scaffold for vascular tissue engineering should use a biocompatible polymer with suitable degradation rate and biological qualities that interact favorably with blood and cells. A variety of biodegradable polymers, like poly(glycerol-sebacate), PLA or PLGA, as well as collagen and chitosan have been evaluated as scaffolds to support the regeneration of tissue-engineered vascular graft. More detail work in this direction is presented in Table 6.

Table 4 Application of chitosan scaffolds to liver tissue engineering

Matrix and its nature	Study made	Culture media	Observation	Conclusion	Name of researchers [Ref]
Hepatocyte-specific porous scaffold by using alginate/galactosylated chitosan (ALG-GC) sponge	Behavior of hepatocytes in ALG-GC sponges	Primary hepatocytes from ICR mouse (5- to 7-week-old male) isolated and seeded in ALG-GC sponge	Albumin secretion and ammonia elimination were higher in ALG-GC sponge than those in the alginate sponge	The ALG-GC sponge may be used as an efficient 3D hepatocyte culture system for liver-tissue engineering	Yang et al. [110]
Porous 3D scaffold of ALG-GC	Viability of hepatocytes in ALG-GC hybrid sponge with respect to the ALG sponge	Primary hepatocytes isolated from ICR mouse (5- to 7-week-old male) into the ALG-GC sponge	High viability of the hepatocytes was obtained for the ALG-GC sponge. However, within the ALG sponge, only a few cells formed spheroids	Hepatocyte enhancement of the scaffold and the mechanical properties of ALG sponge increased	Chung et al. [111]
Hepatocyte attachment into chitosan-alone scaffold and fructose-modified chitosan	Albumin secretion and urea synthesis in both of the scaffolds	Isolated hepatocytes from the liver of male Wistar rats were prepared by two-step collagenase perfusion according to the method of Seglen [112] and cultured on chitosan alone and on fructose-modified chitosan scaffolds	Fructose modification caused an increase in cellular interaction that formed cellular aggregates similar to those in vivo, and was beneficial to cell attachment and the albumin secretion rate. Urea synthesis rate of cells was higher	Fructose-induced surface cellular aggregates enhance liver-specific metabolic activity and improve cell density to a satisfactory level, and so would be helpful in the development of an artificial liver system	Li et al. [113]

(*continued*)

Table 4 (continued)

Matrix and its nature	Study made	Culture media	Observation	Conclusion	Name of researchers [Ref]
Galactosylated chitosan derivative	Development of synthetic ECM, which could control spreading, adhesion, and proliferation of hepatocyteattachment into chitosan-containing polystyrene (PS) dishes, poly(*N*-*p*-vinylbenzyl-4-*o*-*β*-D-galactopyranosyl-D-gluconamide) (PVLA)-coated PS dishes, and GC-coated PS dishes	Isolated hepatocytes from ICR mouse (5- to 7-week-old male)	Hepatocyte adhesion in GC-coated PS and PVLA-coated PS was found to be similar (94.7%) after 120 min incubation. Hepatocyte adhesion on the PVLA was facilitated by the galactose-specific interactions between asialoglycoprotein receptors (ASGR) of the hepatocytes and galactose residues of the PVLA	GC showed excellent adhesion and spheroid formation of hepatocytes due to the galactose-specific recognition between GC molecules and ASGR of hepatocytes and provides a good alternative as a synthetic ECM for liver tissue engineering	Park et al. [114]
Matrix composed of collagen and chitosan prepared by using crosslinking agent EDC in *N*-hydroxysuccinimide (NHS) and a 2-morpholinoethane sulfonic acid (MES) buffer system	Cytotoxicity	–	The cytotoxicity of the urea derivative was found to be quite low compared to that of EDC	Water-soluble EDC is non-toxic and biocompatible. It is not incorporated directly into the crosslinked sponge structure, but is changed to water-soluble urea derivatives	Wang et al. [115]

Highly porous chitosan–gelatin scaffolds by combining three different fabrication techniques, i.e., rapid prototyping, microreplication, and freeze-drying	Hydrophilicity and biodegradability of the scaffolds by performing swelling and degradation studies	Cellular activity of the scaffolds was evaluated by seeding hepatocytes onto the scaffolds	The porous chitosan–gelatin scaffold reveals that the hepatocytes attached well to the matrix and that it has excellent biocompatibility	Albumin secretion and urea synthesis indicated that the well-organized scaffolds were suitable for hepatocyte culture	Jiankang et al. [116]
Natural nanofibrous scaffolds by electrospinning of GC into nanofibers	Mechanical cell culture	Hepatocyte culture studies of nanofibrous scaffolds	Nanofibrous scaffolds showed suitable mechanical properties and slow degradation	Superior cell bioactivity with higher levels of liver-specific functions like albumin secretion, urea synthesis, and cytochrome P-450 enzyme activity	Feng et al. [117]

Table 5 Work done on cardiac tissue engineering applications

Product developed	Procedure adopted	Evaluation	Conclusion drawn	Name of researchers [Ref]
Functional cell-based cardiac pressure generating construct (CPGC) using chitosan as scaffolding material	Isolated primary cardiac cells from rat heart were plated on the surface of fibrin gels cast in 35-mm tissue culture dishes	CPGC showed intraluminal pressure spikes of 0.08 mm Hg and 0.05 mm Hg without and with electrical stimulation, respectively	The model may provide a pathway towards developing cell-based cardiac pumps	Birla et al. [124]
Porous chitosan scaffolds were prepared using the freeze-drying method and fibrinogen added to the scaffold before cell seeding, resulting in the formation of contractile construct	Chitosan poured into a mold was frozen at −80°C and lyophilized. Fibrinogen was added to the scaffold to begin the gelling process	The lower cell seeding densities, in the range of 1–2 million cells, resulted in the formation of smart material integrated heart muscle	A scaffold thickness of 200 μm was optimal for cardiac cell functionality. Histological results showed a fairly uniform cell distribution throughout the thickness of the scaffold	Blan et al. [125]
Chitosan–collagen composite	Isolated aortic valve endothelial cell (VEC) cultures and preferential adhesion to fibronectin, collagen types IV and I over laminin and osteopontin. Chitosan–collagen type IV films act as protein precoatings	The composite showed improved VEC growth and morphology in comparison to chitosan alone	Certain alteration in the properties of chitosan can improve amenability to valve tissue engineering applications	Cuy et al. [126]

Table 6 Work done in the field of vascular tissue engineering

Product developed	Procedure adopted	Evaluation	Conclusion drawn	Name of researchers [Ref]
PCL membranes modified by deposition of chitosan/heparin multilayer via the electrostatic self-assembly method	A novel ternary polysaccharide derivate, chitosan-*g*-PCL-*b*-PEG was prepared in order to immobilize chitosan and provide positive charges onto PCL	Blood compatibility of the control PCL and chitosan-*g*-PCL-*b*-PEG/heparin multilayer-deposited PCL membrane was measured using static platelet adhesion and plasma recalcification time experiments	Chitosan/heparin deposition could reduce platelet adhesion and prolong the plasma recalcification. Platelet adhesion and aggregation are thought to be a major mechanism by which biomaterial thrombogenicity is transduced	Liu et al. [127]
A chitosan-based tubular scaffold with a sandwich-like structure for blood vessel tissue engineering	A combination of thermally induced phase separation and an industrial knitting technique was used to produce a porous chitosan–gelatin complex	Cytocompatibity of scaffolds with endothelial cells and vascular smooth muscle cells (vSMCs)	Cellular proliferation studies showed that vSMCs grew and proliferated rapidly on the chitosan-based tubular scaffolds	Zhang et al. [128]
Novel human-like collagen (HLC)/chitosan tubular scaffolds to mimic blood vessel morphologically and mechanically	Scaffold prepared using crosslinking and freeze-drying processes	Human venous fibroblasts onto the HLC/chitosan scaffolds. In vivo, scaffolds were implanted into rabbit liver	Scaffolds provided a more suitable cell environment for cell secretion, more ECM, and showed superior biocompatibility in vitro and in vivo	Zhu et al. [129]
Chitosan/heparin composite	Macroscopically homogeneous chitosan/heparin blended suspension fabricated into composite films and porous scaffolds by an optimized procedure	Immobilization of heparin in the composite matrices (i.e., films and porous scaffolds) showed improved blood compatibility, as well as good mechanical properties and endothelial cell compatibility	Chitosan/heparin composite matrices are promising candidates for blood-contacting tissue engineering	He et al. [130]

4.6 Corneal Tissue

In the human body, the eye is the most delicate and remarkable organ. The cornea is the transparent part of the eye that covers the iris, pupil, and anterior chamber. It has five distinct anatomical layers. From anterior to posterior, the five layers are corneal epithelium, Bowman's layer, corneal stroma, Descemet's membrane, and corneal endothelium. Due to some hereditary diseases, infection, or injury, the cornea becomes opacified and results in loss of vision. According to the World Health Organization, over 10 million individuals are blinded from corneal scarring. Corneal transplantation is the best way to overcome this kind of defect. In the USA, more than 40,000 corneas are transplanted successfully each year. Most patients receiving a corneal transplant suffer from corneal scarring or decompensation due to keratoconus, bullous keratopathy, corneal scars from trauma, Fuchs endothelial dystrophy, and stromal corneal dystrophies such as lattice, granular, or macular dystrophy. In addition to these, in much of the developing world, religious and cultural factors, lack of general education, and the absence of eye banking facilities prevent widespread cadaveric donation for corneal transplantation, leading to the need for an alternative to cadaveric corneal transplantation. Thus, shortage of donor corneal tissue has drawn the attention of researchers towards keratoprosthesis for the treatment of corneal blindness. The ideal keratoprosthesis would be inert and not rejected by the patient's immune system, inexpensive, and maintain long-term clarity. In addition, it would be quick to implant, easy to examine, and allow an excellent view of the retina [131]. Due to its good optical transmittance, chitosan is widely used in corneal tissue engineering scaffolds and corneal regeneration; its transparency is above 85% at 400 nm [132]. Some more details of work in this field are given in Table 7.

4.7 Nerve Tissue

The nervous system plays a vital role in maintaining body functions. Nervous tissue is composed of two main cell types: neurons, which transmit impulses, and the neuralgia, which assist propagation of nerve impulses as well as provide nutrients to the neurons. The nervous system is a complex, sophisticated system that regulates and coordinates the basic functions and activities of our body. Overall, it plays the role of headmaster in giving instructions to all parts of the body. Yet the nervous system is complex and is vulnerable to various disorders. Nervous system disorders cause many diseases such as Alzheimer's disease, brain cancer and brain tumors, Meningitis, Parkinson's disease etc. In recent years, researchers are devoting efforts to cure these diseases by regenerating nervous tissue. Some studies showed that chitosan promotes survival and neurite outgrowth of neural cells in vitro [137–141]. In most of the studies, a nerve guidance conduit is employed for peripheral nerve regeneration. In general, a suitable material for peripheral nerve regeneration should possess the following properties [142, 143]:

Table 7 Work done in the field of corneal tissue engineering

Product developed	Procedure adopted	Evaluation	Conclusion drawn	Name of researchers [Ref]
A chitosan-based membrane made of hydroxypropyl chitosan, gelatin, and chondroitin sulfate	Cellular activity was studied by seeding rabbit corneal endothelial cells onto the hybrid membrane and measuring the biodegradability and biocompatibility (in vivo) by its implantation into rat muscle	Higher glucose permeability than natural human cornea, and the optical transparency of the membrane is similar to that of natural human cornea	The membrane was suitable for corneal endothelial cells to attach and grow. The hybrid membrane also showed good bioabsorption in vivo	Gao et al. [133]
Membranes of poly-D,L-lactic acid (PDLLA), PDLLA modified with collagen (PDLLA/collagen) and PDLLA modified with chitosan (PDLLA/chitosan) were transplanted onto alkali-burned corneas	Reepithelialization of each cornea with fluorescein staining, and histological changes such as corneal wound healing, inflammation, and collagen synthesis	Effect of different biomedical membranes on alkali-burned cornea was studied	Wound healing rate of the PDLLA/chitosan group was higher than in the other groups. PDLLA/chitosan promoted wound healing of alkali-burned corneas in vivo and also decreased scar tissue formation	Du et al. [134]
Hyaluronic-acid-immobilized chitosan (CS-HYA) films crosslinked with EDC	Contact angle, cellular activity of human corneal epithelial cells (HCECs) on films, and cell viability	CS-HYA films had slightly increased transparency and good water absorption capacity for corneal regeneration	CS-HYA films have higher cell viability than the chitosan films. Hence, the CS-HYA films may serve as a potential candidate material for corneal regeneration	Wang et al. [132]
Collagen/chitosan/sodium-hyaluronate (Col-CS-NaHA) complexes	Biocompatibility for corneal tissues on these complexes was measured by cultivating rabbit corneal cells	Feasibility of using Col-CS-NaHA complexes for corneal tissues	Col-CS-NaHA complex may serve as a suitable substrate for cultivating corneal cells and as a scaffold of tissue engineered cornea	Chen et al. [135]
Collagen/chitosan composite hydrogels as corneal implants stabilized by EDC and NHS or a hybrid of poly (ethylene glycol) dibutyraldehyde (PEG-DBA)/EDC/NHS	In vitro, by seeding human corneal epithelial cells (HCECs) and dorsal root ganglia onto the composite hydrogels. In vivo, by implanting the composite hydrogels within the skin of rat and into the pig cornea for 12 months	Optical properties, optimum mechanical properties and suturability, and permeability to glucose and albumin were evaluated	Composite hyrogels had excellent biocompatibilty, with successful regeneration of host epithelium, stroma, and nerves	Rafat et al. [136]

1. It must be biocompatible
2. It must allow diffusion transport of nutrients while preventing external cells from entering the conduit
3. The material must be degraded slowly enough to maintain a stable support structure for the entire regeneration process, but it should not remain in the body much longer than needed to prevent later compression of the nerve
4. The material must be able to support cell adhesion and cell spreading on its surface

Chitosans have these biological properties and can serve as the main materials for artificial nerve conduits. Neural stem cells (NSCs) have drawn the interest of researchers because of their potential for neural regeneration [144]. Recently, Scanga et al. [145] reported that chitosan had the greatest surface amine content and the lowest equilibrium water content, which probably contributed to the greater viability of neural precursor cells (NPCs or stem and progenitor cells) as observed over 3 weeks in culture. Plating intact NPC colonies revealed greater cell migration on chitosan relative to the other hydrogels. Importantly, long-term cultures on chitosan showed no significant difference in total cell counts over time, suggesting no net cell growth. Together, these findings reveal chitosan as a promising material for the delivery of adult NPC cell-based therapies. Table 8 focuses on the development of chitosan-based biomaterials for neural tissue regeneration and neural stem cell implantation.

Table 8 Chitosan for neural tissue regeneration and neural stem cell implantation

Product developed	Procedure adopted	Evaluation	Conclusion drawn	Name of researchers [Ref]
Chitin and chitosan tubes	Chitin hydrogel tubes from chitosan solutions using acylation chemistry and mold cast techniques	Lumber dorsal root ganglion dissected from E9 White Rock chicks on chitin and chitosan films separately in a cell culture medium	Chitosan films showed more enhanced neutrite outgrowth than on chitin films	Frier et al. [146]
Porous chitosan scaffolds using different degrees of deacetylation	Buffalo embryonic stem-like (ES-like) cell culture	The polygonal buffalo ES-like cells proliferated well on 88% and 95% DD scaffolds	The scaffolds are promising for neural tissue regeneration and neural stem cell implantation	Thein-Han et al. [147]
Hybrid PCL/chitosan nanofibrous scaffolds	Culture of rat Schwann cells (RT4-D6PT2) on the PCL scaffolds and PCL/chitosan scaffolds	More cell proliferation on the PCL/chitosan scaffolds than on PCL-alone scaffolds	Hybrid scaffolds have more cell proliferation and are thus useful for peripheral nerve regeneration	Prabhakaran et al. [148]

(*continued*)

Table 8 (continued)

Product developed	Procedure adopted	Evaluation	Conclusion drawn	Name of researchers [Ref]
Porous tubular chitosan scaffolds	Novel method for synthesizing porous tubular chitosan scaffolds	Differentiated Neuro-2a cells grew along the oriented macrochannels and the inter-connected micropores for beneficial nutrient diffusion and cell ingrowth to the scaffold's interior	Porous chitosan scaffolds with well-defined architectural features may serve as a promising material for nerve tissue engineering	Wang et al. [149]
Chitosan films with different degrees of acetylation (DA)	Neural cell compatibility of chitosan and *N*-acetylated chitosan using primary chick dorsal root ganglion neurons	Dependency of neural cell compatibility on DA. 0.5% acetylated chitosan films showed the greatest cell viability	Cell compatibility can be adjusted by amine content for the nervous tissue system	Frier et al. [150]
Porous composite nerve conduit of collagen and chitosan prepared by freeze-drying steam extrusion	In vitro culture studies by seeding retinal pigment epithelial cells on composite scaffolds	Cell proliferation study	Composite scaffolds promote the adhesion and proliferation of cells. Increase of chitosan content decreases cell proliferation slightly	Xiangmein et al. [151]
Chitosan as a scaffold for transplantation of bone marrow stromal cell (BMSC)-derived Schwann cells	In vivo studies by transplanting the chitosan sponge containing BMSC-derived Schwann cells into Wistar rats	Faster regeneration of axons in chitosan gel	Chitosan gel sponges with BMSC-derived Schwann cells for regeneration of peripheral nerves	Ishikawa et al. [152]
Chitosan gel sponge	Axonal regeneration of the rat sciatic nerve	–	Sponge may serve as an effective scaffold for axonal regeneration of the rat sciatic nerve	Ishikawa et al. [153]

4.8 Some Other Applications

Apart from the above use of chitosan as scaffolding material in tissue engineering, it also finds application in periodontal tissue engineering [154, 155] and disc tissue engineering [156]. The other uses of chitosan as scaffolding material may find applications in esophageal tissue, dental tissue and breast tissue for organ-specific tissue regeneration.

5 Conclusions

Among other natural polysaccharides, chitosan is a very promising and versatile biomaterial due to the ease with which this material can be manipulated to fit certain circumstances. This discovery has opened several avenues of thought concerning chitosan as a biopolymer for tissue engineering applications. The fundamental principles of tissue engineering are based on living cells, signal molecules, and scaffold. Tissue engineering involves repair of injured body parts and restores their functions by using laboratory-grown tissues, materials, and artificial implants. In this chapter, we have mainly concentrated on the selection of chitosan as scaffolding material and looked at different types of scaffold fabrication techniques as well as the factors governing the design of scaffolds for various tissue engineering applications. It is hoped that this article will act as a research guide for beginners on the use of chitosan as a scaffold for the regeneration of various types of tissues and organs. The description of methods for producing tissue engineering scaffolds may also be useful for practitioners to understand the physicochemical and biological properties of chitosan scaffolds in their real-life application.

Acknowledgments Authors gratefully acknowledged the financial assistance to KR in the form of Institute Research Fellowship from MNNIT, Allahabad and research funding to PKD from UGC, New Delhi. The RSC (London) -Research Fund Grant Award-2009 to PKD is also gratefully acknowledged.

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