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Research review paper

Chitosan and its derivatives for tissue engineering applications

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Abstract

Tissue engineering is an important therapeutic strategy for present and future medicine. Recently, functional biomaterial researches have been directed towards the development of improved scaffolds for regenerative medicine. Chitosan is a natural polymer from renewable resources, obtained from shell of shellfish, and the wastes of the seafood industry. It has novel properties such as biocompatibility, biodegradability, antibacterial, and wound-healing activity. Furthermore, recent studies suggested that chitosan and its derivatives are promising candidates as a supporting material for tissue engineering applications owing to their porous structure, gel forming properties, ease of chemical modification, high affinity to in vivo macromolecules, and so on. In this review, we focus on the various types of chitosan derivatives and their use in various tissue engineering applications namely, skin, bone, cartilage, liver, nerve and blood vessel.

Keywords: Chitin; Chitosan; Chitosan derivatives; Scaffold; Tissue engineering

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1. Introduction

Tissue engineering consists of a multidisciplinary science, including fundamental principles from materials engineering and molecular biology in efforts to develop biological substitutes for failing tissues and organs. In the most general sense, tissue engineering seeks to fabricate living replacement parts for the body. Langer and Vacanti (1993) reported that the most common approach for engineering biological substitutes is based on living cells, signal molecules, and polymer scaffolds. The cells synthesize matrices of new tissue as well as function on behalf of the diseased or damaged tissues, while the scaffold provides the suitable environment for the cells to be able to effectively accomplish their missions such as adherence, proliferation and differentiation. The function of the signal molecules is to facilitate and promote the cells to regenerate new tissue. In this regenerative program, the scaffolds provide not only temporary three-dimensional frameworks to form the designed tissues, but also space filling and controlled release of signal molecules. To perform these varied functions in tissue engineering, the scaffold should meet the following requirements: (1) biocompatibility with the tissues, (2) biodegradability at the ideal rate corresponding to the rate of new tissue formation, (3) nontoxicity and nonimmunogenicity, (4) optimal mechanical property, and (5) adequate porosity and morphology for transporting of cells, gases, metabolites, nutrients and signal molecules both within the scaffold and between the scaffold and the local environment.

Recent biological achievements regarding cell culture using signal molecules are promising techniques with polymer scaffolds to regenerate tissues and organs. Functional biomaterial research has been directed toward the development of improved scaffolds for tissue engineering (Watanabe et al., 2003). A number of biodegradable polymers have been exhaustively explored as scaffolds for tissue engineering applications. The materials include synthetic polymers like polycaprolactone (Sarasam and Madihally, 2005; Williams et al., 2005), poly (lactic-co-glycolic acid) (Wu et al., 2006), poly(ethylene glycol) (Wozney and Seeherman, 2004; Leach and Schmidt, 2005), poly(vinyl alcohol) (Schmedlen et al., 2002; Oh et al., 2003) and polyurethane (Santerre et al., 2005) and natural polymers such as alginate (Li et al., 2005), gelatin (Li et al., 2006), collagen (Ignatius et al., 2005), starch (Gomes et al., 2002) and chitosan (Seo et al., 2006; Adekogbe and Ghanem, 2005; Huang et al., 2005). Among them naturally derived polymers are of special interest due to, as natural components of living structures, their biological and chemical similarities to natural tissues (Krajewska, 2005). In this context, chitosan has been found a fascinating candidate in a broad spectrum of applications along with unique biological properties including biocompatibility, biodegradability to harmless products, nontoxicity, physiological inertness, remarkable affinity to proteins, antibacterial, haemostatic, fungistatic, antitumoral and anticholesteremic properties (Nishimura et al., 1984; Tanigawa et al., 1992; Okamoto et al., 1993; Khnor and Lim, 1993; Mori et al., 1997; Tokura et al., 1997; Singla and Chawla, 2001). The choice of chitosan as a tissue support material is governed among others by multiple ways by which its biological, physical and chemical properties can be controlled and engineered under mild conditions (Krajewska, 2005).

The history of chitosan dates back to the 19th century, when Rouget discussed the deacetylated form of chitosan in 1859 (Valérie and Vinod, 1998). Studies on chitosan have been intensified as biomaterials for tissue engineering applications during the past 25 years. Chitin, the source material for chitosan, is one of the most abundant organic materials, being second only to cellulose in the amount produced annually by biosynthesis. It is an important constituent of the exoskeleton in animals, especially in crustacean, molluscs and insects. It is also the principal fibrillar polymer in the cell wall of certain fungi (Eugene and Lee, 2003). Chitosan is a linear polysaccharide, composed of glucosamine and *N*-acetyl glucosamine units linked by β (1–4) glycosidic bonds. The content of glucosamine is called as the degree of deacetylation (DD). Depending on the source and preparation procedure, its molecular weight may range from 300 to over 1000 kD with a DD from 30% to 95% (Dornish et al., 2001; VandeVord et al., 2002). In its crystalline form, chitosan is normally insoluble in aqueous solution above pH 7, however, in dilute acids ($pH < 6.0$), the protonated free amino groups on glucosamine facilitate solubility of the molecule (Madihally and Matthew, 1999). Generally, chitosan has three types of reactive functional groups, an amino group as well as both primary and secondary hydroxyl groups at the C(2), C(3), and C(6) positions, respectively. These groups allow modification of chitosan like graft copolymerization for specific applications, which can produce various useful scaffolds for tissue engineering applications. The chemical nature of chitosan in turn provides many possibilities for covalent and ionic modifications which allow extensive adjustment of mechanical and biological properties.

For the breakthrough in tissue engineering applications, this review will focus on the properties of chitosan as a tissue supporting material, its modification to introduce various functional groups and recently, their

applications in various artificial organs such as skin, bone, cartilage, liver, nerve and blood vessel will be explained.

2. Chitosan as tissue supporting material

Chitosan-based scaffolds possess some special properties for use in tissue engineering. First, Chitosan can be formed as interconnected-porous structures by freezing and lyophilizing of chitosan solution or by processes such as an "internal bubbling process (IBP)" where $CaCO_3$ is added to chitosan solutions to generate chitosan–$CaCO_3$ gels in specific shapes by using suitable molds (Chow and Khor, 2000). The interconnected-porous structure is very important, so that numerous cells can be seeded, migrate into the inside, increase the cell number and should be supplied by sufficient amounts of nutrient. The porous structure of chitosan is a promising characteristic for the development and optimization of a variety of tissue scaffolds and regeneration aids. Regulation of porosity and pore morphology of chitosan-based scaffolds is critical for controlling cellular colonization rates and organization within an engineered tissue. In addition, angiogenesis required for some scaffold application scenarios can be affected by scaffold porosity and pore morphology (Madihally and Matthew, 1999). Second, the cationic nature of chitosan also allows for pH-dependent electrostatic interactions with anionic glycosaminoglycans (GAG) and proteoglycans distributed widely throughout the body and other negatively charged species. This property is one of the important elements for tissue engineering applications because numbers of cytokines/growth factors are known to be bound and modulated by GAG including heparin and heparan sulfate. A scaffold incorporating a chitosan–GAG complex may serve means of retaining and concentrating desirable factors secreted by colonizing cells. Moreover, Nishikawa et al. (2000) reported that chitosan, structurally resembling with GAG consisting of long-chain, unbranched, repeating disaccharide units, regarded to play a key role in modulating cell morphology, differentiation, and function.

The mechanical properties of chitosan-based scaffolds are dependent on the pore sizes and pore orientations. Tensile testing of hydrated samples shows that porous membranes have greatly reduced elastic moduli (0.1–0.5 MPa) compared to non-porous chitosan membranes (5–7 MPa). The extensibility (maximum strain) of porous membranes varied from values similar to nonporous chitosan (approximately 30%) to greater than 100% as a function of both pore size and orientation. Porous membranes exhibited a stress–strain curve typical of composite materials with two distinct regions: a low-modulus region at low strains and a transition to a 2–3-fold higher modulus at high strains. The tensile strengths of these porous structures were in the range of 30–60 kPa (Madihally and Matthew, 1999). Chen and Hwa (1996) reported effect of the molecular weight of used chitosans and their crystallinity on the mechanical property of chitosan membrane. That is, the lower molecular weight of chitosan used, the lower the tensile strength of the chitosan membrane prepared due to the chance of entanglement differences. The use of lower molecular weight chitosan produced less entanglement. Crystallinity difference of chitosan may be attributed to another factor. The lower the molecular weight of chitosan used, the lower the enthalpy of the resulting membrane. These implied that the lower tensile strength of the membrane was a result of less crystallinity in the chitosan membrane prepared from low molecular weight of chitosan.

Chitosan has been shown to degrade in vivo, which is mainly by enzymatic hydrolysis. The degradability of a scaffold plays a crucial role on the long-term performance of tissue-engineered cell/material construct because it affect many cellular process, including cell growth, tissue regeneration, and host response. If a scaffold is used for tissue engineering of skeletal system, degradation of the scaffold biomaterial should be relatively slow, as it has to maintain the mechanical strength until tissue regeneration is almost completed. Lysozyme is the primary enzyme responsible for in vivo degradation of chitosan, which appears to target acetylated residues (Hirano et al., 1989). The final degradation products are biocompatible chitosan oligosaccharides of variable length. The degradation rate is inversely related to the degree of crystallinity which is controlled mainly by the DD. Highly deacetylated forms (DD > 85%) exhibit relatively a low degradation rate and may last several months in vivo, whereas the forms with lower DD degrade more rapidly (Paradossi et al. 1992; Lee et al., 1995; Kamiyama et al., 1999). the degradation rate also inherently affect both the mechanical and solubility properties.

One of the properties of chitosan is that it confers considerable antibacterial activity against a broad spectrum of bacteria. Aimin et al. (1999) has shown that chitosan can reduce the infection rate of experimentally induced osteomyelitis by *Staphylococcus aureus* in rabbits. The cationic nature of chitosan by amino group is related to anions on the bacterial cell wall. The interaction between positively charged chitosan and negatively charged microbial cell wall leads to the leakage of intracellular constituents. The binding of chitosan with DNA and inhibition of mRNA synthesis occurs via the penetration of chitosan into the nuclei of the microorganisms

and interfering with the synthesis of mRNA and proteins. Due to this antibacterial property chitosan has been blended with other polymers (Hu et al., 2003).

The field of wound healing has been another major emphasis in chitosan-based medical applications research. A number of researchers have examined the host tissue response to various chitosan-based implants. In general, these materials have been found to evoke a minimal foreign body reaction, with little or no fibrous encapsulation. It observed the typical course of healing with formation of normal granulation tissue, often with accelerated angiogenesis. Suh and Mattew (2000) reported that chitosan and its fragments on immune cells may stimulate the induction local cell proliferation and ultimately integration of the implanted material with the host tissue. Actually, chitosan possesses the properties favorable for promoting rapid dermal regeneration and accelerate wound healing suitable for applications extending from simple wound coverings to sophisticated artificial skin matrices. Okamoto et al. (1995) reported that chitosan influenced all stages of wound repair in experimental animal models. In the inflammatory phase, chitosan has unique hemostatic properties that are independent of the normal clotting cascades. In vivo these polymers can also stimulate the proliferation of fibroblasts and modulate the migration behavior of neutrophils and macrophages modifying subsequent repair processes such as fibroplasias and reepithelialization (Okamoto et al., 1995; Kosaka et al., 1996). Kosaka et al. (1996) reported that the cell binding and cell-activating properties of chitosan play a crucial role in its potential actions. These studies have added further to the body of evidence that chitosan are suitable as wound healing materials. These results suggest that cell-seeding onto chitosan-based scaffolds would provide tissue engineered implant being biocompatible and viable.

3. Chitosan derivatives for tissue engineering applications

The practical use of chitosan has been mainly restricted to the unmodified forms in tissue engineering applications. Recently, there has been a growing interest in modification of chitosan to improve its solubility, introduce desired properties and widen the field of its potential applications by choosing various types of side chains. Although some of the properties have been altered by these modifications, it is possible to maintain biological properties such as biocompatibility, biodegradability, antibacterial activity, mucoadhesivity and wound healing.

Modification of chitosan for tissue engineering applications has been performed to introduce the specific recognition of cells by sugars. The synthesis of sugar-bound chitosan had been investigated mainly in drug delivery system, gene therapy and tissue engineering since the specific recognition of cells, viruses, and bacteria by sugars was studied. Li et al. (2000) reported the synthesis of sugar bound chitosans, such as those with

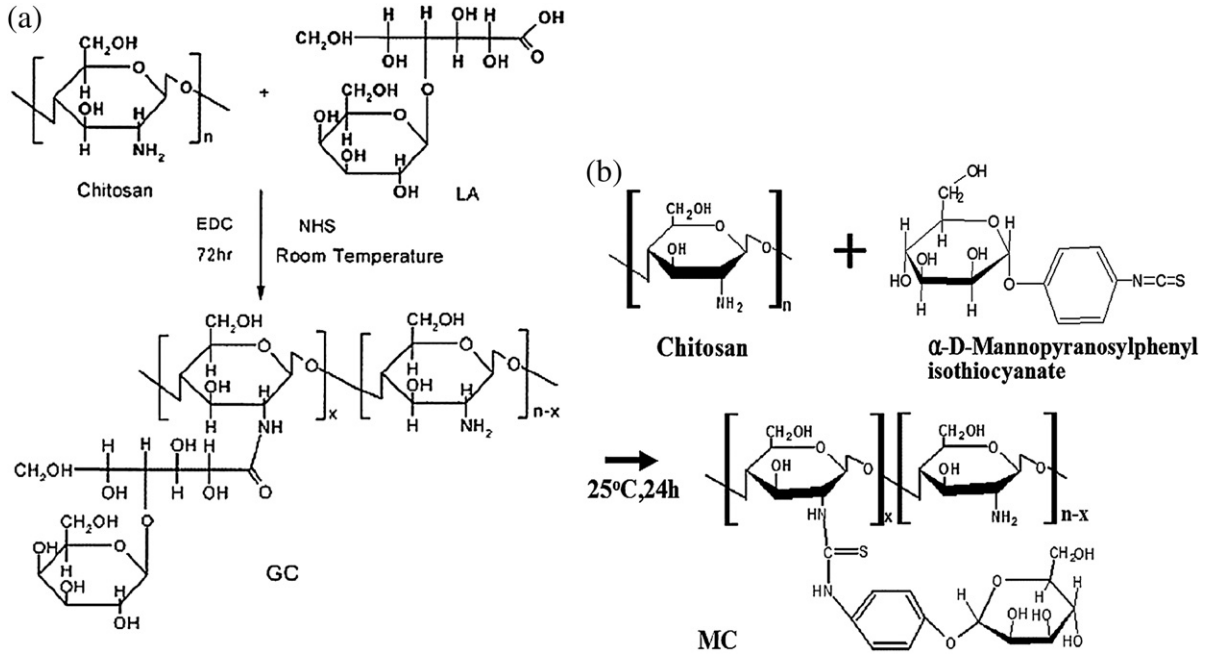

Fig. 1. Synthesis scheme of galactosylated chitosan (Park et al., 2003) (a) and mannosylated chitosan (Kim et al., 2006b) (b).

D- and L-fucose, and their specific interactions with lectin and cells. Also, galactosylated chitosan (GC) prepared from lactobionic acid and chitosan with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) as activating agents showed possibility of a synthetic extracellular matrix for hepatocyte attachment (Fig. 1(a)) (Park et al., 2003). In an approach similar with this, Kim et al. (2006b) prepared mannosylated chitosan (MC) having the specific recognition to antigen presenting cells such as B-cell, dendritic cell and macrophage (Fig. 1(b)). Also, as potent inhibitors of influenza viruses or blocking agents for acute rejection, Gamian et al. (1991) prepared sialic acid bound chitosan as a new family of sialic acid containing polymers using *p*-formylphenyl-a-sialoside by reductive *N*-alkylation (Fig. 2).

Graft copolymerization, chemical grafting of chitosan, is important for its functionalization and development of practically useful derivatives. A variety of routes for grafting have been investigated, such as ceric ion, Fenton's reagent, gamma-irradiation, various radicals, and ring-opening (Jenkins and Hudson, 2001). Ding et al. (2004) reported that chitosan graft-polymerized onto poly (L-lactide) (PLA) surface by plasma coupling reaction can be used to control the morphology and function of cells, and has potential applications in tissue engineering. Generally, poly (α-hydroxyacid)s, homopolymers and copolymers based on glycolide and lactide, have been widely used as a biomaterial in sutures, drug release systems and tissue engineering owing to their biocompatibility and biodegradability (Morita and Ikada, 2002). Poly(glycolide) (PGA) and its copolymers such as

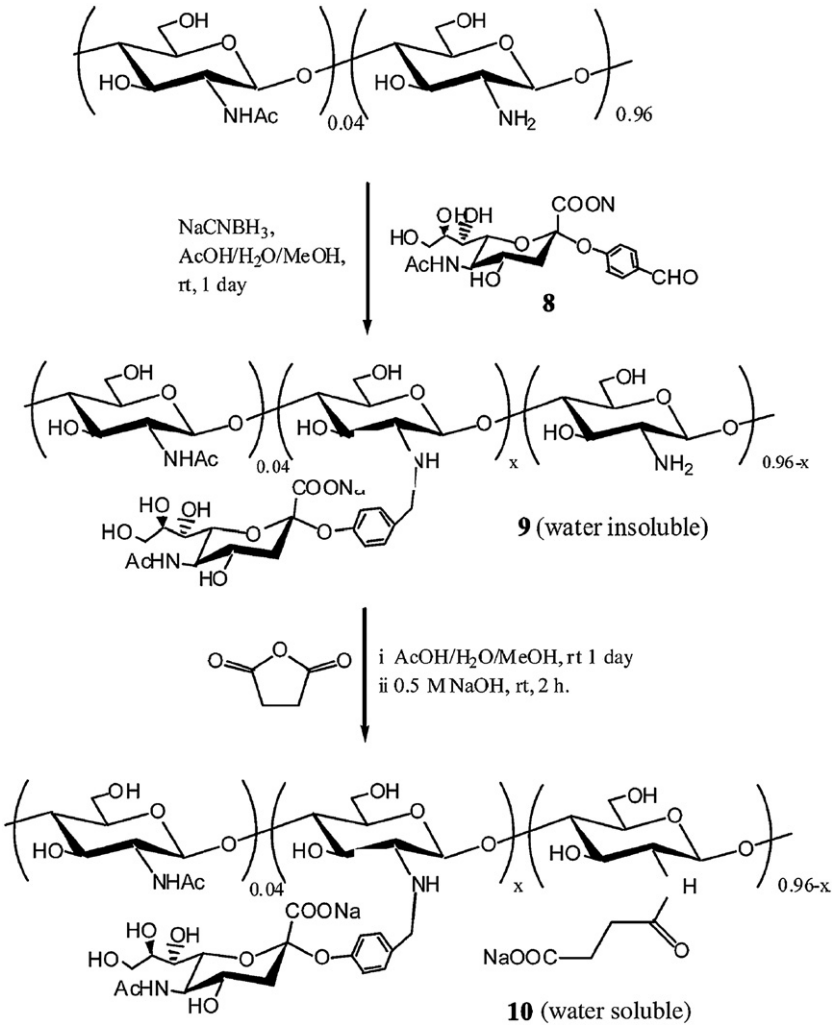

Fig. 2. Synthesis of sialic acid–chitosan and its *N*-succinylation (Gamian et al., 1991).

lactide–glycolide copolymer (PLGA) degrade too quickly when used as a scaffold, because their tensile strength reduces to the half within two weeks. In contrast, PLA degrades too slowly, requiring 3–6 years for complete resorption. Owing to this inadequate resorption property of PGA and PLA, naturally derived-polymers such as alginate, collagen, hyaluronic acid and chitosan have preferably employed in recent studies on tissue engineering. Moreover, poly (α-hydroxyacid)s degrade through non-enzymatic hydrolysis, whereas naturally derived polymers undergo enzymatic hydrolysis. Most of naturally derived-polymers are hydrophilic and yield products with low mechanical strength in comparison with poly (α-hydroxyacid)s, which leads to limited applications of these biopolymers (Morita and Ikada, 2002; Ikada, 2006). They can be cooperatively complemented through graft copolymerization or blending with poly (α-hydroxyacid)s. Zhu et al. (2002a) used a photosensitive heterobifunctional crosslinking agent attached to chitosan for coating onto PLA film surfaces (Fig. 3). Improved cell attachment was obtained with this approach whereas chitosan modified with heparin inhibited platelet adhesion and activation. In another approach, the solubility of chitosan in water was increased and the biocompatibility of trimethyl chitosan (TMC) was improved by PEGylation of TMC (Fig. 4). The PEG–g-TMC copolymer was water-soluble over the entire pH range and led to increased biocompatibility by PEGylation (Mao et al., 2005). Adekogbe et al. (2005) also reported chitosan crosslinked with dimethyl 3-3, dithio bis propionimidate (DTBP) for overcoming the rapid degradation of chitosan and its low mechanical strength in skin tissue engineering applications.

The combination of chitosan with other materials appears to be a common theme in various reports. Blending with other polymers is widely investigated. Blends with synthetic and natural polymers can imbibe the wide range of physicochemical properties and processing techniques of synthetic polymers as well as the biocompatibility and biological interactions of natural polymers. Huang et al. (2005) blended chitosan with gelatin to improve the biological activity since (i) gelatin contains Arg-Gly-Asp (RGD)-like sequence that promotes cell adhesion and migration, and (ii) forms a polyelectrolyte complex. Addition of gelatin affected the stiffness of 2D and 3D scaffolds, facilitated the degradation rate and maintained the dimension in the presence of lysozyme. Sarasam and Madihally (2005) reported the effect of blending chitosan with poly(ε-caprolactone) (PCL). As previously mentioned, these blending membranes improved mechanical properties as well as

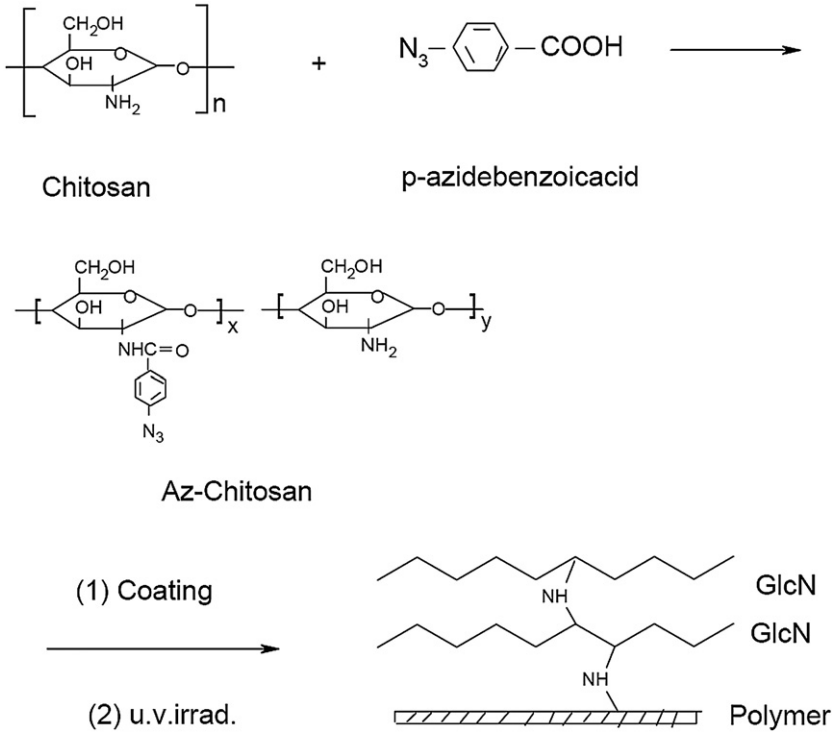

Fig. 3. Reaction scheme of immobilization of chitosan on PLA film surface (Zhu et al., 2002a).

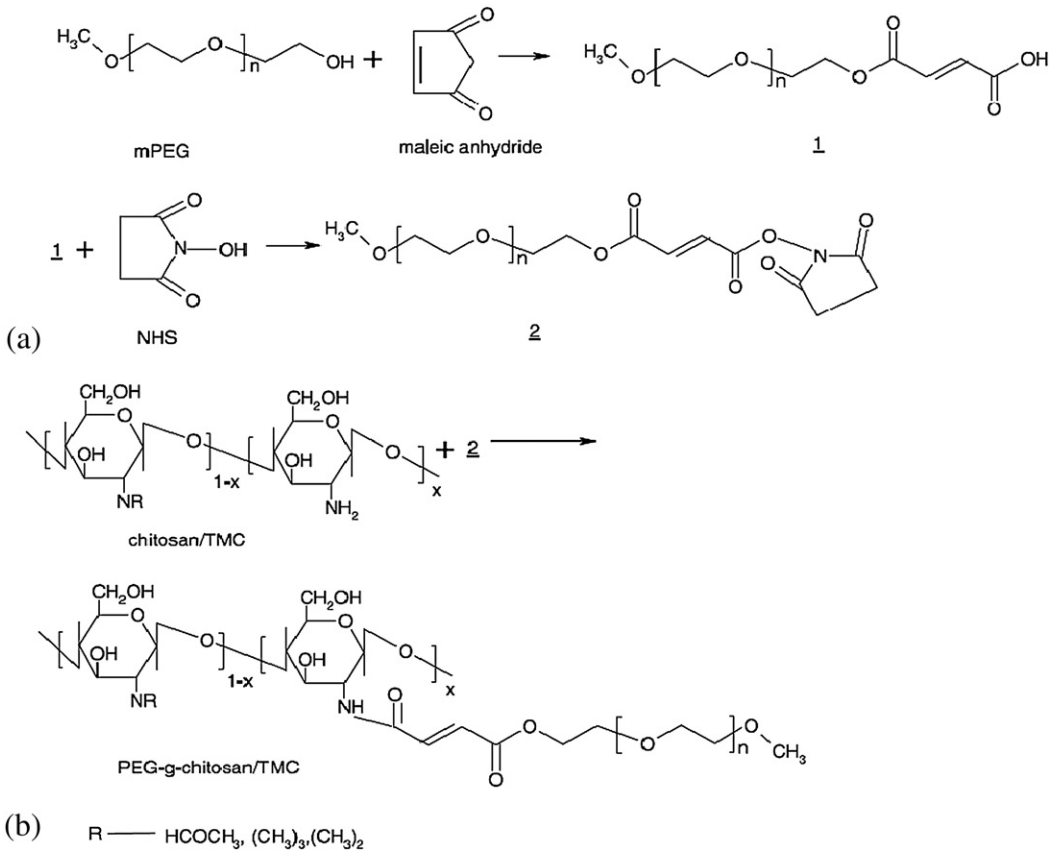

Fig. 4. Synthetic route of PEGylated chitosan derivatives (Mao et al., 2005).

cellular support. The γ-poly (glutamic acid) (γ-PGA), a hydrophilic and biodegradable polymer, was also used to modify chitosan matrices and the γ-PGA/chitosan composite matrix was found to enhance hydrophilicity and serum proteins adsorption, and to increase the maximum strength through addition of γ-PGA in tissue engineering applications (Hsieh et al., 2005). Chung et al. (2002a,b) prepared galactosylated chitosan-based scaffolds by combining with alginate to improve mechanical properties and biocompatibility. The scaffolds exhibited the usual pore configurations, and the pore sizes were dependent on the freezing pre-treatments, the molecular weight of chitosan and amount of galactosylated chitosan.

Many studies have attempted to immobilize specific sequences that can promote cell adhesion. RGD isolated from adhesive proteins is the most widely used one (Albelda and Buck, 1990; Mooney et al., 1994; Schugens et al., 1995). Chung et al. (2002) recently reported effect of cell adhesive peptides photochemically grafted onto chitosan surfaces on growing human endothelial cells on chitosan. Chitosan surfaces containing the grafted peptides were found to support the proliferation of human endothelial cells compared to chitosan itself without adherence. In another approach, Zhu et al. (2002b) utilized the reaction between the amino group on chitosan and the carboxylic acid group on amino acids with glutaraldehyde to attach various amino acids (lysine, arginine, aspartic acid, phenylalanine) onto chitosan. These amino acid functionalized chitosan moieties were entrapped onto PLA surfaces. Fig. 5 depicts this process where a solvent swells the surface of the PLA to permit penetration of the amino acid–chitosan derivatives solution that becomes trapped upon putting a non-solvent for the chitosan (Eugene and Lee, 2003).

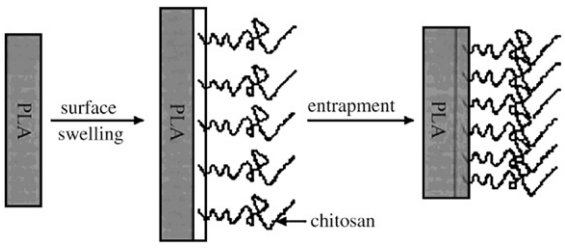

Fig. 5. Schematic representation of the entrapment of functionalized chitosan onto a PLA (Chung et al., 2002).

Recently, much attention has been focused on making polymeric nanofibers by electrospinning process as a unique technique because it can produce chitosan nanofibers with diameter in the range from several micrometers down to tens of nanometers, depending on polymer and processing conditions. Electrospinning applies high voltages to a capillary droplet of polymer solution or a melt to overcome liquid surface tension and thus enables the formation of much finer fibers than conventional fiber spinning methods. These nanofibers that mimic the structure and function of the natural extracellular matrix (ECM) are of great interest in tissue engineering as scaffolding materials to restore, maintain or improve the function of human tissue, because they have several useful properties such as high specific surface area and high porosity. The recent attempts have been made to prepare chitosan-based nanofibrous structures by electrospinning, with varying degrees of success (Li and Hsieh, 2006). Min et al. (2004) produced chitin and chitosan nanofibers with an average diameter of 110 nm and their diameters ranged from 40 to 640 nm by the SEM image analysis (Fig. 6). Bhattarai et al. (2005) further concluded that these chitosan-based nanofibers promoted the adhesion of chondrocyte and osteoblast cells and maintained characteristic cell morphology.

Up to the present, the most effective delivery route for the administration of macromolecules is the parenteral one. Most polymeric systems used for the extravascular parenteral delivery of drugs or vaccines are microspheres (Bittner et al., 1998) or implants (Davis, 1974; Bodmer et al., 1992). In those systems, the active compound is generally encapsulated by using organic solvents or by submitting it to relatively high temperatures which can cause a loss of activity. Moreover, the insert of an implant requires surgery which adds to the costs and the risks of this system. Those problems oriented research towards injectable thermosensitive in situ gelling formulations. Some synthetic polymer aqueous solutions, such as Poloxamer (Malmsten and Lindman, 1992), Poly(ethylene glycol) (PEG)/poly(DL-Lactic acid-co-glycolic acid) (PLGA) graft/triblock copolymers (Jeong et al., 1999; Jeong et al., 2000), Poly(ethylene glycol) (PEG)/poly(capro-lactone) (PCL) triblock copolymers (Bae et al., 2005), poly(phosphazene)s (Lee et al., 2002a,b) and PEG/poly(propylene fumarate) triblock copolymers (Chenite et al., 2000), are known to exhibit temperature-dependent

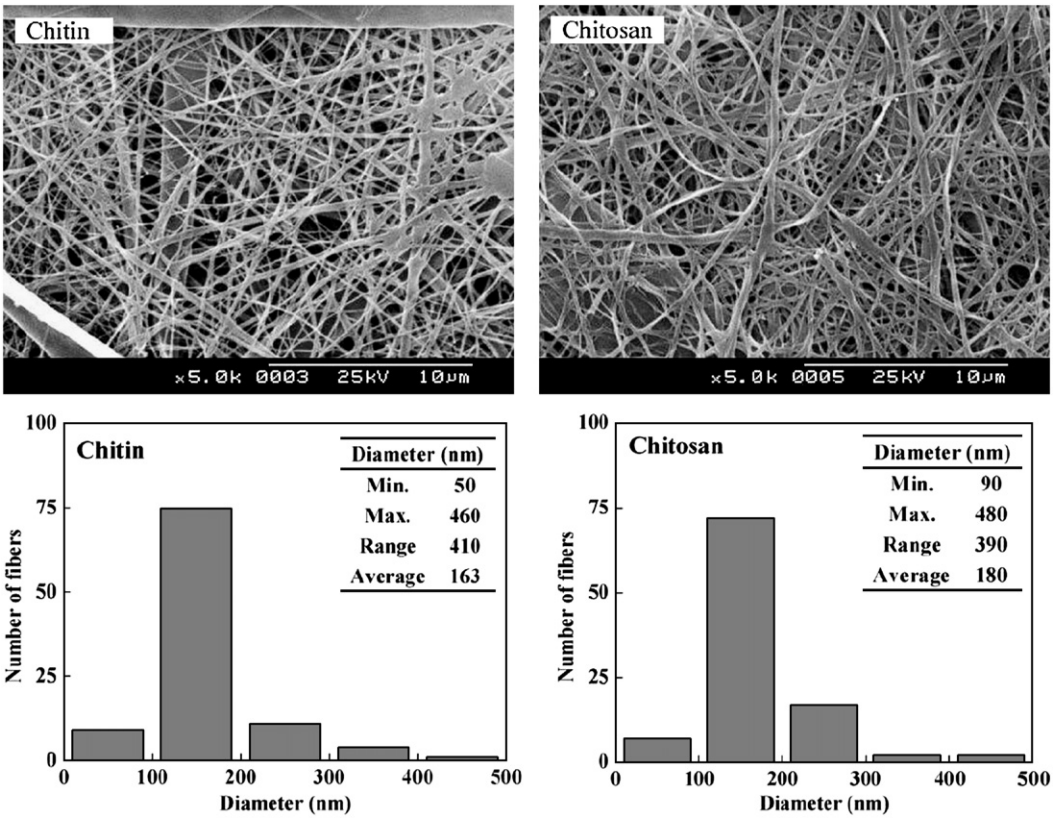

Fig. 6. SEM micrographs of chitin and deacetylated chitin (chitosan) nanofibrous matrix, before and after deacetylation reaction for 150 min at 100 °C (Min et al., 2004).

reversible sol–gel transitions. The polymeric aqueous solutions can be injected while kept above or below their transition temperature and form a gel as they reach body temperature. Although those copolymers are biocompatible and biodegradable, the need to heat the solution to incorporate the drug and inject the system makes this approach less practical. In this context, Chenite et al. (2000) developed the thermally gelling chitosan system through neutralizing highly deacetylated chitosan solutions with glycerol phosphate (GP) to retain chitosan in solution at physiological pH. The biodegradable thermogelling chitosan/GP solutions that can form a gel in body temperature are especially attractive as injectable implant systems in tissue engineering. Injectable implant systems, a more recent concept of tissue engineering, offer the following advantages over the use of preformed scaffolds: liquid gels are able to fill any space or shape of a defect site, living cells and therapeutic agents are incorporated prior to the injection within the solution, and more importantly, the systems can be implanted in the site without surgery. Clearly, the success of these systems strongly depends on the polymer gelation kinetics in the microenvironment involved. Furthermore, this chitosan/GP hydrogel system showed the site-directed, injectable, and controlled-release formulation of paclitaxel, one of the best antineoplastic drugs found from nature in the past decades, as an effective treatment for localized solid tumors (Ruel-Gariepy et al., 2004). After 17 days, this chitosan/GP hydrogel containing 64 mg/ml of paclitaxel had released 32% of its drug load in vitro and the animals that received this formulation intratumorally showed a marked tumor growth inhibition.

These chitosan derivatives have been used to create various tissue analogs including skin, bone, cartilage, liver, and so on in the past decades. The chitosan derivatives modified by various methods will be introduced in several organs.

4. Application of chitosan and its derivatives for artificial organs

4.1. Skin

The healing of a skin wound is complicated courses, including a wide range of cellular, molecular, physiological, and biological processes. Immediate coverage using wound dressing is a cornerstone of wound management. The wound repairs in cases of acute, chronic, more extensive wounds, or skin loss of the oldest would be inevitable unless some skin substitutes are used. The ultimate goal of skin tissue engineering is to rapidly produce a construct that offers the complete regeneration of functional skin, which should allow the skin to fulfill its many normal functions: barrier formation; pigmentory defence against UV irradiation; thermoregulation; and mechanical and aesthetic functions (Metcalfe and Ferguson, 2007). Some of all the functions can be restored with existing skin substitutes. In past decades, many skin substitutes such as xenograft, allografts, and autografts have been employed for wound healing. However, due to the antigenicity or the limitation of donor sites, the skin substitutes cannot accomplish the purpose of the skin recovery and hence not used widely (Yanas and Burke, 1980; Bell et al., 1981; Schul et al., 2000; Ma et al., 2003). The main role of skin substitutes is to promote wound healing by simulating the host to produce various cytokines. These cytokines play an important role not only in preventing dehydration and increasing inflammation, but also promoting the formation of granulation tissue in wound healing processes. Fibroblast in a dermal equivalent enhances epidermal differentiation and dermal regeneration by secreting cytokines. Therefore, a skin substitutes containing cultured fibroblasts can accelerate the healing process owing to the injection of fibroblasts into the wound tissue, and promote the synthesis of new tissue in the initial stage (Lee et al., 2003).

Many studies have been reported on the use of chitosan as a skin substitute material in skin tissue engineering due to its many advantages for wound healing such as hemostasis, accelerating the tissue regeneration and stimulating the fibroblast synthesis of collagen (Taravel and Domard, 1995; Taravel and Domard, 1996; Cho et al., 1999; Ma et al., 2001). Ueno et al. (1999) demonstrated that chitosan in the form of chitosan-cotton, accelerate wound healing by promoting infiltration of polymorphonuclear (PMN) cells at the wound site which is an essential event in rapid wound healing. Recently, Mizuno et al. (2003) also reported that chitosan was a good wound healing material and incorporation of that to basic fibroblast growth factor (bFGF) accelerated the rate of healing. Howling et al. (2001) demonstrated that highly deacetylated chitosan are more biologically active than chitin and less deacetylated chitosans. As mentioned earlier, these results are closely related to the electrostatic interaction of chitosan with anionic GAG, depending on the DD of chitosan and pH environment. The GAG distributed widely throughout the body is known to bind and modulate a number of cytokines/growth factors. Further studies emphasized on the combination of chitosan with other materials which have a potential way of achieving rapid wound healing. Yan et al. (2000) prepared chitosan in combination with alginate as polyelectrolyte complex (PEC) membranes. These biodegradable chitosan–alginate PEC membranes showed greater stability to pH changes and hence more

effective as controlled-release membranes than either chitosan or alginate itself (Wang et al., 2002). The PEC membranes were found to promote accelerated healing of incisional wounds in a rat model. Ma et al. (2003) fabricated porous chitosan/collagen scaffold by their cross-linking with glutaraldehyde and freeze-drying to improve biostability and good biocompatibility. They also reported that the potential cytotoxicity of glutaraldehyde might be decreased through the presence of chitosan. That is, chitosan can function as a bridge to increase the cross-linking efficiency of glutaraldehyde in the collagen-based scaffolds owing to the large number of amino groups in its molecular chain (Fig. 7). The glutaraldehyde-treated chitosan/collagen scaffold retained the original good biocompatibility and could successfully induce the fibroblasts infiltration from the surrounding tissue.

In next generation skin substitutes, biomaterial scaffolds would be carefully engineered to release in a time-dependent fashion and various signal molecules including growth factors and protein domains for cell migration, adhesion, proliferation and differentiation. Chitosan is the most potent candidate as a scaffold for skin substitutes due to its physico-chemical and biological properties. Due to free from the antigenicity and the limitation of donor sites, recent studies in embryonic stem cell biology will be fertilized to next generation skin substitutes with the exploitation of biomaterial engineering.

4.2. Bone

In bone tissue engineering, the biodegradable substitutes act as a temporary skeleton inserted into the defective sites of skeleton or lost bone sites, in order to support and stimulate bone tissue regeneration while they gradually degrade and are replaced by new bone tissue. Also, for being suitable for use in treating vertebral fracture or related conditions, bone cements must possess: proper injectability, a rapid setting time, appropriate stiffness, bioactivity, low setting temperature, and radiopacity (Service, 2000). Both bioactive ceramics and polymers have been developed and analyzed for use as tissue engineering scaffolds. Bioactive ceramics are chemically similar to natural bone which allows osteogenesis to occur, and can provide a bony contact or bonds with host bone (Hench and Wilson, 1984). However, brittleness and low biodegradability restrict clinical applications of these bioceramics. A number of natural and synthetic polymers have been studied for overcoming the weak points as bone substitutes. Especially, chitosan has been also extensively used in bone tissue engineering since after exploring its capacity to promote growth and mineral rich matrix deposition by osteoblasts in culture. Also, chitosan is biocompatible (additional minimizes local inflammation), biodegradable, and can be molded into porous structures (allows osteoconduction) (Martino et al., 2005). Several studies have been focused on chitosan–calcium phosphates (CP) composites for this purpose in bone tissue engineering. Beta-tricalcium phosphate (β-TCP) and hydroxyapatite (HA) of CP bioceramics are excellent candidates for bone repair and regeneration because of their similarity in chemical composition with inorganic components of bone. Zhang and Zhang (2002, 2003) prepared CP bioceramic embedded with chitosan sponge which enhanced mechanical property of the ceramic phase via matrix reinforcement and preserving the osteoblast phenotype.

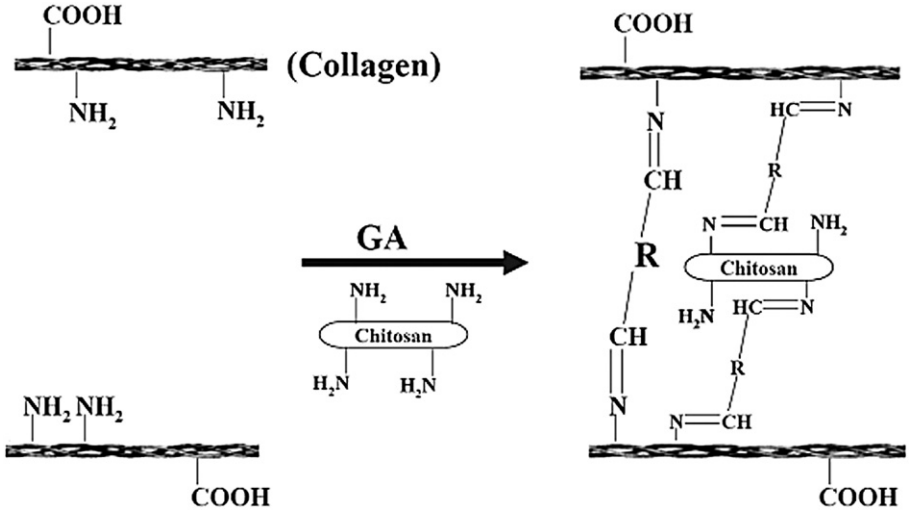

Fig. 7. Schematic presentation of collagen cross-linked with glutaraldehyde in the presence of chitosan (Schul et al., 2000).

Similarly, gentamycin-conjugated macroporous chitosan scaffolds reinforced with β-TCP showed that MG63 osteoblast cells were attached and proliferated on the surface of these composite scaffolds and migrated onto the pore walls. Calcium-HA is the main component of teeth and bones in vertebrates. Good mechanical properties with superior biocompatibility of sintered HA make it well preferred bone and tooth implant material. Kawakami et al. (1992) studied the in vivo effect of HA/chitosan materials through its application on the surface of the tibia after periosteum removal. Formation of new bone was observed after 1 week and continued during a 20-week follow-up, indicating suitability of this material for further clinical studies as a bone filling material. Zhao et al. (2002) used phase separation technique to fabricate biomimetic HA/chitosan–gelatin network composites in the form of 3D-porous scaffolds and they showed improved adhesion, proliferation and expression of rat calvaria osteoblasts on these highly porous scaffolds. Ge et al. (2004) reported that, HA–chitin material which is osteoinductive and exhibited rapid degradation and neovascularization in vivo during a 3-month period.

Chitosan was studied as an adjuvant to improve injectability of the cement while keeping the physico-chemical properties suitable for surgical application: setting time convenient for surgery, minimum disintegration of the cement in biological fluids, and mechanical properties suited to the kind of operation. Octo-CP obtained from calcium phosphate cements (CPC) with chitosan was shown to improve injectability and the strength of the cement (Leroux et al., 1999). The use of chitosan as adjuvant can be explained in response of chitosan solution gel by pH change from slightly acid to physiological pH. Actually, the chitosan–CP composites address the need to develop bone fillers that set in response to physiological conditions but do not set in vitro, upon mixing of the components. The composites may be useful for the regeneration of larger, nonload-bearing bone defects although in vivo evaluation of the composites is still under progress (Gutowska et al., 2001).

Xu et al. (2004) studied the feasibility of creating macropores in CPC for vascular ingrowth without significantly compromising the strength and toughness of the CPC scaffold, to further maximize these mechanical properties. The reinforcing effects were investigated by using chitosan and/or absorbable mesh in CPC. This injectable, bioabsorbable composite material possessed interconnected macropores (osteoconductive) and provided strength and elasticity for the implantation during tissue regeneration. The intra-molecular hydrogen bonds of chitosan provide interacting macromolecules with a good resistance to heat. Kim et al. (2004) showed the application of this property through composites of chitosan with poly methyl-methacrylate (PMMA). This specially developed composite material exhibited lower exothermic curing temperatures and possessed higher inter-connected porosity with a pore size suitable for osteoconduction with better anchorage to the surrounding bone. It was observed that the pore size of this composite material increased with time due to biodegradation of the chitosan. Also, chitosan has been used to modify the surface properties of prosthetic materials for the attachment of osteoblasts (Lee et al., 2002a,b). Bumgardner et al. (2003) showed that titanium (Ti) surface coated with chitosan via silane-glutaraldehyde chemistry exhibited increased osteoblast attachment and proliferation. In conclusion, although the chitosan-based composite biomaterials need to improve their mechanical properties for bone tissue engineering, no doubt that chitosan is a promising candidate scaffold material in clinical practice due to the worthiest ability to bind anionic molecules such as growth factors, GAG, DNA. Especially, the ability to link chitosan to DNA may render this material a good potential as a substrate for gene activated matrices in gene therapy application in orthopedics.

4.3. Cartilage

Tissue engineering of articular cartilage involves the isolation of articular chondrocytes or their precursor cells that may be expanded in vitro and then seeded into a biocompatible matrix, or scaffold, for cultivation and subsequent implantation into the joint (Suh and Matthew, 2000). In cartilage repair, the choice of biomaterial is very critical for the success of tissue engineering approaches (Grande et al., 1997). The ideal cell-carrier substance should mimic the natural environment in the articular cartilage matrix. It has been shown that cartilage-specific extracellular matrix (ECM) components such as type II collagen and GAGs play a critical role in regulating expression of the chondrocytic phenotype and in supporting chondrogenesis in vitro as well as in vivo (Kosher et al., 1973; Kosher and Church, 1975). Structural similarity of chitosan with various GAGs found in articular cartilage makes it an elite scaffolding material in articular cartilage engineering. Due to structural resemblance, chitosan thus shares some characteristics with various GAGs and hyaluronic acid present in articular cartilage (Suh and Matthew, 2000).

Hypothesizing that chitosan and some of its degraded products could be involved in the synthesis of the articular matrix component such as chondroitin, chondroitin-sulfate, dermatane-sulfate, keratan-sulfate and hyaluronic

acid, their synergy was examined in vivo. Lu et al. (1999) has demonstrated that the chitosan solution injected into the knee articular cavity of rats led to a significant increase in the density of chondrocytes in the knee articular cartilage, indicating that chitosan could be potentially beneficial to the wound healing of articular cartilage. Mattioli-Belmonte et al. (1999) showed that the bone morphogenetic protein (BMP)-7, associated with *N*,*N*-dicarboxymethyl chitosan induces or facilitates the repair of artificial cartilage lesions in rabbit, hypothesizing a synergism of their respective biological effect.

The cationic nature of chitosan allows formation of insoluble ionic complexes or complex coacervates with a wide variety of water-soluble anionic polymers. Especially, the formation of ionic complexes of chitosan with the negatively charged GAGs has regarded further important property in cartilage tissue engineering. This ion complexing mechanism can be used to immobilize chondroitin sulfates with hydrogel materials which mimic the GAG-rich ECM of their articulation because chitosan has a protective effect against GAGs hydrolysis by their specific enzymes. Sechriest et al. (2000) demonstrated biocompatibility and the chondrogenic characteristics of such GAG-augmented chitosan hydrogel surfaces. After one week of seeding, chondrocytes attached to the chondroitin 4-sulfate (CSA)-augmented chitosan maintained a spherical or polygonal morphology. The primary chondrocytes also cultured on CSA–chitosan maintain the synthesis of cartilage-specific collagens (Suh et al., 1998; Sechriest et al., 2000).

Chitosan was also conjugated with hyaluronan to obtain a biomimetic matrix for chondrocytes. Chondrocyte adhesion, proliferation, and also the synthesis of aggrecan and type II collagen were significantly higher on the hybrid fiber than on chitosan (Yamane et al., 2005). Similarly, in order to increase cellular adhesiveness of chitosan, Hsu et al. (2004) studied chitosan–alginate–hyaluronan scaffolds with or without covalent attachment with RGD containing protein. Cell-seeded scaffolds showed neocartilage formation in vitro. When chondrocyte seeded scaffolds were implanted at the site cartilage defects in rabbit knee, partial repair was observed after 1 month both in presence or absence of RGD, indication of potential of this composite material for cartilage regeneration.

In addition to the properties of chitosan mentioned above, chitosan-based scaffolds can provide the release of specific growth factors in a controlled fashion to promote the ingrowth and biosynthetic ability of chondrocytes. Lee et al. (2004) reported porous collagen/chitosan/GAG scaffolds loaded with transforming growth factor-β1 (TGF-β1). This scaffold exhibited controlled release of TGF-β1 and promoted cartilage regeneration. Moreover, addition of chitosan to the collagen scaffold was seen to improve mechanical properties and stability of the collagen network due to inhibition of the action of collagenases (Taravel et al., 1996; Lee et al., 2004). TGF-β1, a 25 kDa protein composed of two polypeptide chains held together by disulfide linkages, has been shown to promote protein synthesis (van Beuningen et al., 1994; Denuziere et al., 1998) and cell proliferation (Morales, 1997) in articular cartilage. It also inhibits the actions of matrix metalloproteinases (Xu et al., 1996) that play an important role in the digestion of the ECM in both normal and degenerative articular cartilage. Although TGF-β1 appears to be a powerful molecule to repair damaged cartilage, high dose of intra-articular injection is known to induce chemotaxis and activation of inflammatory cells, resulting in fibrosis and osteophyte formation in cartilage defects (Border and Noble, 1994; Denuziere et al., 1998; Hulth et al., 1996). Thus, it is evident that TGF-β1 should be administered in a controlled manner to minimize adverse effects. As a strategy to develop the controlled release system capable of safely delivering TGF-β1, Kim et al. (2003) prepared TGF-β1-containing chitosan microspheres with bovine serum albumin (BSA), slowly releasing TGF-β1 from them, and stably entrapped it into the porous freeze-dried chitosan scaffold for the treatment of cartilage defects. The sustained release of TGF-β1 resulted in the promotion of chondrocyte proliferation and matrix synthesis.

Recently, as a noteworthy accomplishment, Buschmann et al. showed that microfractured ovine defects are repaired with more hyaline cartilage when the defect is treated with in situ-solidified implants of chitosan–GP mixed with autologous whole blood, compared to microfracture alone in an ovine model at 6 months (Hoemann et al., 2005). Since bleeding has been identified as an initiating event in post-surgical repair, they hypothesized that microfracture-based repair could be improved by stabilizing the clot formed in the lesion with chitosan that is thrombogenic and actively stimulates the wound repair process. Furthermore, these chitosan–GP/blood clots are adhesive and contract much less than whole blood clots, thereby maintaining a voluminous scaffold (Hoemann et al., 2005). Chitosan–GP/blood implants were applied to marrow-stimulated chondral defects in rabbit cartilage repair models (Hoemann et al., 2007), where they induced greater fill of chondral defects with repair tissue compared to marrow-stimulation alone (Hoemann et al., 2005) and, in addition, produced a more cellular and hyaline repair cartilage well integrated with a porous subchondral bone structure (Hoemann et al., 2005; Hoemann et al., 2007; Chevrier et al., 2007).

Tissue engineering approach with more expanded understanding of articular cartilage and associated pathologies may provide the chitosan-based material that supports chondrogenesis, which can improve the quality of neocartilage produced and the integration with the host tissue as well as the long-term outcomes of cartilage repair in clinical settings.

4.4. Liver

Insufficient donor organs for orthotopic liver transplantation worldwide have urgently increased the requirement for new therapies for acute and chronic liver disease (Kim et al., 2006a). Bioartificial liver (BAL) is a promising application of tissue engineering for the treatment of fulminant hepatic failure (FHF). The principal goal is to develop a BAL device in which patient plasma is circulated extracorporeally through a bioreactor that houses metabolically active liver cells. One of the important issues for BAL devices is the proper choice of cell sources, such as primary hepatocytes, hepatic cell lines, and liver stem cells. The primary hepatocyte of these cells represents the most direct approach to BAL devices.[15] Many researchers are attempting to develop BAL devices in which hepatocytes are optimally maintained so that they carry out many activities as possible (Hoekstra and Chamuleau, 2002). BAL devices require a suitable ECM for hepatocyte culture because hepatocytes are anchorage-dependent cells and are highly sensitive to the ECM milieu for the maintenance of their viability and differentiated functions (Ben-Ze'ev et al., 1988; LeCluyse et al., 1996; Kang et al., 2005). Hepatocytes in vivo survive in a three-dimensional system that is formed by various kinds of ECMs such as collagen, proteoglycan, fibronectin, and laminin. In vitro cells must adhere to certain culture substrate in order to migrate, proliferate, and differentiate (Tingwu et al., 1993). Porous scaffolds with large surface-to-volume ratio are relevant to cell attachment.

Chitosan as a promising biomaterial can be applied in liver tissue engineering due to its various properties. One of the reasons for selecting chitosan as a scaffold for hepatocytes culture is that its structure is similar to GAGs, which are components of the liver ECM (Lindahl and Hook, 1978; Li et al., 2003a,b). Chupa et al. demonstrated that chitosan and chitosan complexes with GAGs had significant potential for the design of new biologically active biomaterials which can modulate the activities of vascular endothelial and smooth muscle cells in vitro and in vivo [136].

Li et al. (2003a,b) showed that the micro-structure of porous scaffolds provided large surface area for cells to adhere and facilitate nutrient and oxygen transportation. Wang et al. (2003) prepared chitosan/collagen matrix (CCM) by cross-linking agent EDC in NHS buffer system. The EDC cross-linked CCM showed moderate mechanical strength, good hepatocyte compatibility as well as excellent blood compatibility. On the other hand, implantable bioartificial liver (IBL) can restore, maintain or improve liver functions or offer the possibility of permanent liver replacement. Unlike the general approach for bioartificial skin, bone and cartilage, development of IBL has extreme difficulties. Appropriate design of the complex architecture, as well as the anti-thrombogenic extracellular component, are necessary for developing this blood-contacting device, because thrombus formation can lead to occlusion and decrease membrane efficiency. Wang et al. (2005) showed a superior blood compatibility through chitosan/collagen / heparin matrix in implantable bioartificial liver (IBL) applications.

Another strategy in liver tissue engineering focuses on the ability of highly concentrated, multivalent galactose residues to bind to the asialoglycoprotein receptor (ASGPR) expressed on the surface of hepatocytes. Typical cell–matrix interaction is mediated by adhesion receptor such as integrin which specifically binds RGD sequence (Adams, 2002). The ASGPR was the first reported mammalian lectin, or carbohydrate-binding protein. It was discovered in the mid-1960s by Ashwell et al. in their studies of the metabolism of plasma glycoproteins in mammals (Ashwell and Morell, 1974; Ashwell and Harford, 1982). Since then, hepatic ASGPR has been a classical system for studying receptor-mediated endocytosis. The ASGPR mediates the endocytosis and degradation of a wide variety of desialylated glycoproteins and neoglycoproteins that contain terminal galactose or *N*-acetylgalactosamine residues on their N-linked carbohydrate chains. Although ASGPR does not physiologically function as an adhesion receptor, galactose-containing polymers have been used to induce the selective adhesion of primary hepatocytes (Weigel, 1980; Kobayashi et al., 1986; Gutsche et al., 1994). Chitosan modified with galactose residues can improve hepatocyte attachment and maintain viability. Park et al. (2003) demonstrated galactosylated chitosan (GC) as a new synthetic ECM for hepatocyte attachment through the specific interaction between ASGPR on hepatocytes and galactose ligands of GC. Furthermore, Chung et al. (2002) suggested a potential ability to improve hepatocyte attachment to alginate (AL)/GC scaffolds for short-term culture. In our previous study (2006), we further showed enhanced hepatocyte functions in AL/GC scaffolds for long periods. That is, hepatocyte culture in AL/GC

scaffolds could enhance the functions through its spheroid formation (Fig. 8) in coculture condition with fibroblast. Li et al. (2003a,b) conjugated fructose onto the porous chitosan scaffold by the reaction between amino and aldehyde group. Fructose is also known as a specific ligand of ASGPR in hepatocyte. They showed that the chitosan surface modified with fructose induced the formation of cellular aggregates with enhancing liver-specific metabolic activities and cell density to a satisfactory level.

4.5. Nerve

More than any other form of trauma, nerve injuries complicate successful rehabilitation because mature neurons (like many other cells in the body) have little capacity for replication, that is, they do not undergo cell division. Once the nervous system is impaired, its recovery is difficult and malfunction of other parts of the body occurs (Heath and Rutkowski, 1998). The repair of nerve lesions has been attempted in many different ways, which have in common the goal of directing the regenerating nerve fibers into the proper endoneurial tubes. The strategies developed for nerve repair can be roughly classified into two categories: (1) bridging, which includes grafting and tubulization techniques, (2) end-to-end suturing of the nerve stumps. The former technique has been shown to be more effective, as it avoids tension across the repair site (Ciardelli and Chiono, 2006). A wide variety of materials have been suggested for the production of artificial tubes for nerve repair, including biocompatible, non-degradable and degradable materials. In recent years, bio-engineered nerve, especially biodegradable, has been the focus of most researches. A variety of artificial tubes have been used to repair nerve injuries, but the artificial tubes do not have enough internal surface area for nerve fibers and Schwann cells (SCs) to cohere. Moreover, biodegradable tubes may collapse when implanted in vivo due to the thin walls of the tubes, lack of internal support, the surrounding scar tissue constriction, body weight, and muscle contraction. Thus, artificial tubes to bridge large defects in nerve repair should contain a biodegradable matrix, which can provide an optimal structural, cellular, and molecular framework for SCs and neurite migration across a nerve gap.

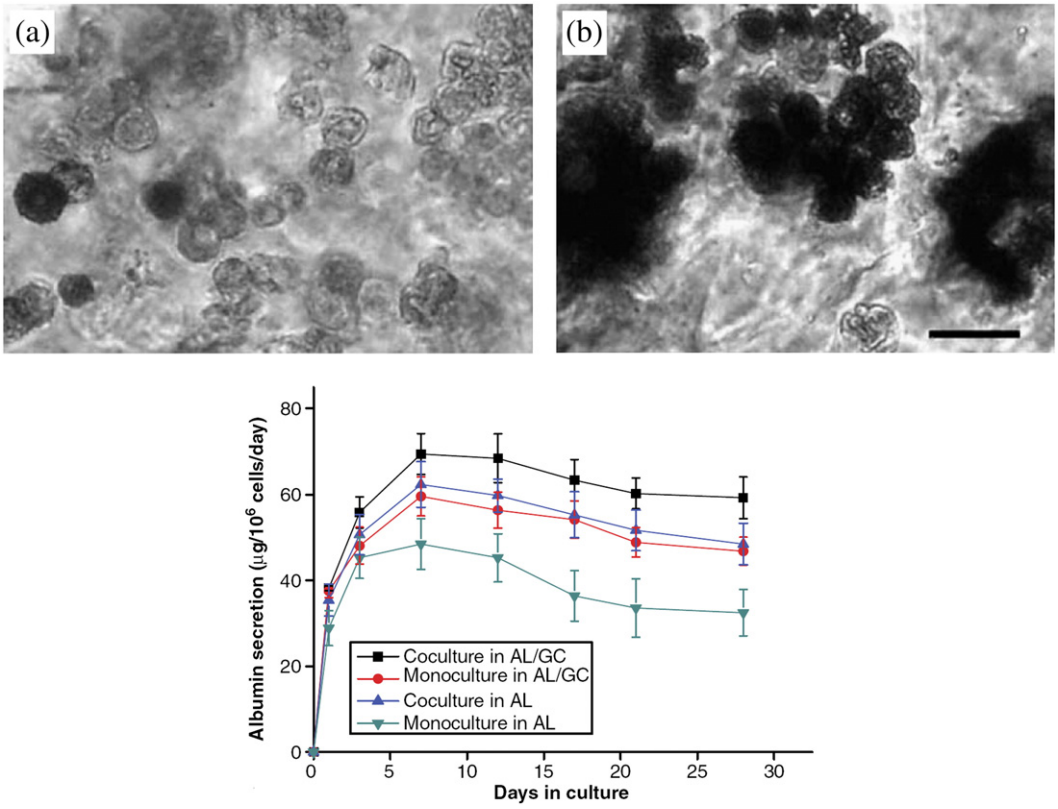

Fig. 8. Phase-contrast micrographs of hepatocytes within the AL (A) and AL/GC (B) scaffold stained with MTT and comparisons of liver-specific albumin secretion function (Seo et al., 2006).

Chitosan has been studied as a candidate material for nerve regeneration due to its properties such as antitumor, antibacterial activity, biodegradability and biocompatibility. Jianchun et al. reported that neurons cultured on the chitosan membrane can grow well and that chitosan tube can greatly promote the repair of the peripheral nervous system (Haipeng et al., 2000). Yuan et al. (2004) also showed that chitosan fibers supported the adhesion, migration and proliferation of SCs, which provide a similar guide for regenerating axons to Büngner bands in the nervous system (Bunge, 1994; Yuan et al., 2004).

In another strategy, Itoh et al. prepared hydroxyapatite-coated chitosan tubes including laminin-1 or laminin peptides as scaffolds for peripheral nerve reconstruction (Itoh et al., 2003). In their study, chitosan tubes were obtained from crab tendons, after removing calcium phosphate and proteins. They were heat pressed into a triangular shape and surface modified with hydroxyapatite, to improve their mechanical properties and to avoid excessive swelling during in vivo implantation. Laminin-1 and IKVAV (Ile-Lys-Val-Ala-Val) and YIGSR (Tyr-Ile-Gly-Ser-Arg) containing peptides were adsorbed on the tubes. When the bridging of a 15 mm defect in the sciatic nerve was evaluated, laminin peptides and laminin-1 improved the growth of regenerating axons. Furthermore, Matsuda et al. (2005) developed a new biomaterial for nerve regeneration through immobilization of laminin peptide in molecularly aligned chitosan by covalent bonding. Kato et al. (2002) identified neurite outgrowth promoting sites on the human laminin α3 chain LG4 module (A3G75 and A3G83) and then prepared peptide-conjugate chitosan membranes for tissue engineering applications. The peptides on the chitosan were flexible and interacted more effectively with cellular receptors than peptides alone. Also, it was reported that neurosteroids such as progesterone and pregnenolone, which are synthesized by Schwann cells, accelerate axonal regeneration in nerve repair. Chávez-Delgado et al. (2003) showed that progesterone delivered from chitosan prostheses provides better facial nerve regenerative response of the rabbits than chitosan prostheses without progesterone. Cao et al. (2005) further studied the physical, mechanical and degradation properties of chitosan films and the affinity between SCs and the films. Three kinds of cross-linked chitosan films were prepared with hexamethylene diisocyanate (HDI), epichlorohydrin (ECH) and glutaraldehyde (GA) as cross-linking agents, respectively. Crosslinking decreased the swelling degree and the degradation rate of the chitosan films, whereas it increased their hydrophilicity and elastic modulus. Especially, HDI cross-linked chitosan films of those enhanced the spread and proliferation of SCs while the other cross-linked films delayed the cell proliferation.

Also, in some studies, chitosan blended with a peptide to make the mechanical properties of scaffolds more similar to those of nerve tissues and to enhance nerve cell attachment. Mingyu et al. (2004) showed an improved attachment, differentiation and growth on the chitosan/poly(L-lysine) composite materials when compared to cells cultured on chitosan membranes. The improved nerve cell affinity on the chitosan/poly(L-lysine) composite materials had been attributed to the increased hydrophilicity by the abundant hydroxyl group and the positive surface charge of chitosan. Moreover, as shown in Fig. 9, composite film with 3 wt.% poly(L-lysine) is an even better material in nerve cell affinity than collagen, a substrate that is already widely used in tissue engineering. Cheng et al. (2003) added gelatin to chitosan for preparation of soft and elastic complex that has good nerve cell affinity. The chitosan/gelatin composite film showed a lower modulus and a higher percentage of elongation at break compared with chitosan film. Also, PC12 cells cultured on the composite films differentiated more rapidly and extended longer neutites than on chitosan films. Frier et al. (2005) also developed chitin hydrogel tubes which were fabricated from chitosan solutions using acylation chemistry and mold casting techniques for the preservation of the natural chemical composition of chitin, and no toxic crosslinking agent was necessary for the hydrogel preparation (Fig. 10). Chitin and chitosan support nerve cell adhesion and neurite outgrowth, making these materials potential candidates for scaffolds in neural tissue engineering.

4.6. Blood vessel

Vascular disease is the major cause of death in Western society (American Heart Association, 2004). Coronary artery and peripheral vascular disease are the largest causes of mortality, necessitating surgical interventions including small-diameter bypass grafting with autologous veins or arteries. Commonly, vascular transplantation has been used for the treatment of vascular disease. The search of substitute materials for vascular grafting has been a half-century endeavor. Of these endeavors, Poly(ethylene terephthalate) (PET, Dacron) and expanded polytetrafluoroethelene (ePTFE) have been regarded as the standard biomaterials for prosthetic vascular grafts. Examined over decades of use, both PET and ePTFE grafts have been shown to perform well at diameters >6 mm, but neither material has been suitable for small-diameter (<4 mm) applications. Furthermore, such vascular conduits with proper size and

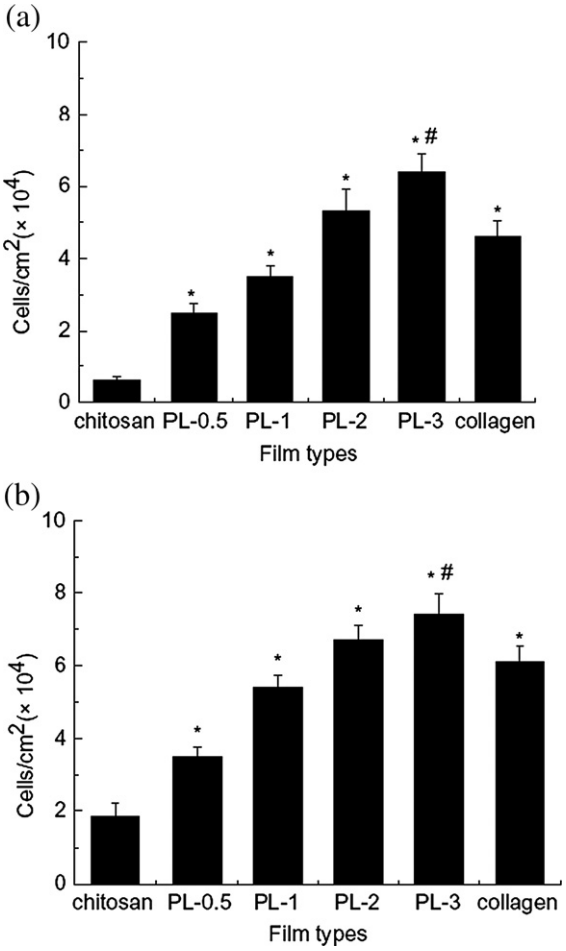

Fig. 9. Attachment of PC12 cells to the six types of material (initial seeding density was 1×10^5 cells/cm^2): (a) in serum free medium, (b) in medium containing 5% serum. *denotes a statistically significant, greater number of attached PC12 cells ($p < 0.05$) compared to chitosan. #denotes a statistically significant, greater number of attached PC12 cells ($p < 0.05$) compared to collagen (Mingyu et al., 2004).

length are often inadequate in many patients, and the operation also causes morbidity at the donor site (Xue and Greisler, 2003). Also, PET and ePTFE are foreign materials that lack the ability to grow, repair, or remodel. This disadvantage greatly limits their application in long-term treatment. Thus, finding a solution for small-diameter bypass grafting has become a major focus of attention.

Tissue engineering provided new possibilities in reconstructive vascular grafting. The scaffolds made of different materials, such as synthetic polymers, natural materials, and decellularized xenogenous tissues, have been utilized in blood vessel tissue engineering. Especially, chitosan of natural materials has been widely investigated in this field due to its structure similar to GAGs, which are the components of an ECM. Chupa et al. (2000) has made the effort to overcome both incomplete endothelialization and smooth muscle cell hyperplasia, which are two of the problems contributing to the poor performance of existing small-diameter (<4 mm) vascular grafts, through complexation of GAGs with porous chitosan scaffolds. GAG-based materials hold promise because of their growth inhibitory effects on vascular smooth muscle cells and their anti-coagulant activity. However, few data regarding chitosan as a scaffold of tissue engineered blood vessels have been reported. Chitosan itself was documented to promote migration of endothelial cells and fibroblasts so as to accelerate wound healing (Mori et al., 1998; Okamoto

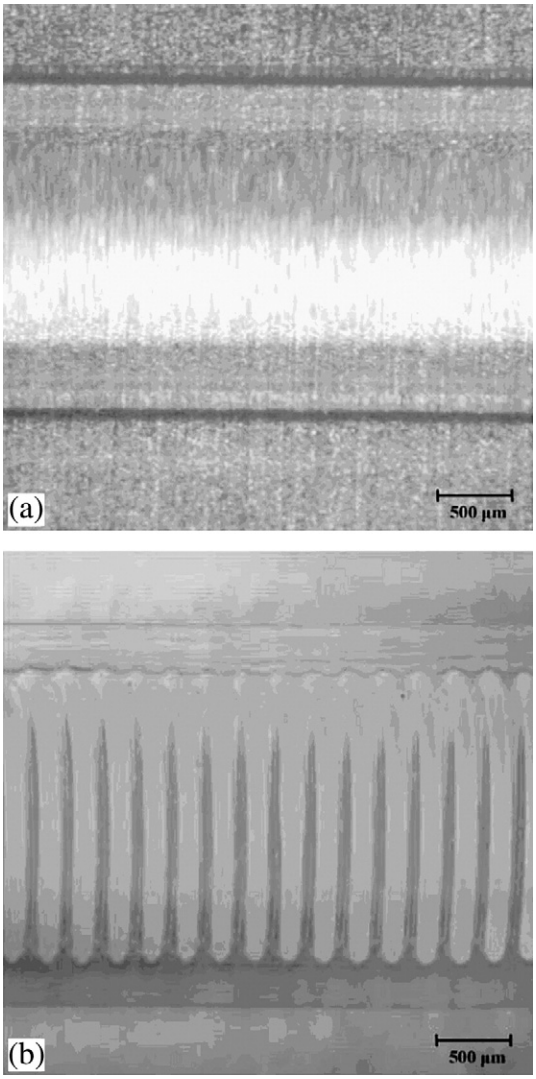

Fig. 10. Optical microscope longitudinal view of (a) a chitin hydrogel tube and (b) a chitin gel tube reinforced with a PLGA coils embedded in the wall (Freier et al., 2005).

et al., 2002). Because of the nature of the molecule, chitosan may be modified by covalent and ionic reactions, which, in turn, allow extensive adjustment of mechanical and biological properties. Madihally et al. (1999) fabricated a family of chitosan scaffolds, including heparin-modified porous tubes, which had the potential for application in blood vessel tissue engineering. Kratz et al. (1997) also prepared insoluble ionic complexes with heparin. The heparin–chitosan scaffolds showed excellent biocompatibility as shown by the reduced activation of coagulation, complement and blood cells (Fukutomi et al., 1996; Bannan et al., 1997; Kagisaki et al., 1997; Svenmarker et al., 1997; Belboul and Al-Khaja, 1997). Heparin plays an important role in blood vessel tissue engineering because heparin have anti-thrombogenic property, inhibit the proliferation of vascular smooth muscle cells, attract and protect many heparin binding growth factor, such as bFGF, VEGF, and PDGF, and help to control the release of these growth factors (Park et al., 2000). Heparin remains the gold-standard inhibitor of the process involved in the vascular response to injury. Furthermore, the complex of chitosan/heparin was supposed to have good blood compatibility according to the in vivo results that heparin–chitosan scaffolds were observed to stimulate cell proliferation and the formation of a thick, dense and highly vascularized granulation layer (Chupa et al., 2000).

5. Conclusions

Tissue engineering as termed 'Regenerative Medicine' is regarded as an ultimately ideal medical treatment for diseases that have been too difficult to be cured by existing methods. This biomedical engineering is designed to repair injured body parts and restore their functions by using laboratory-grown tissues, materials and artificial implants. For regeneration of failed tissues, this biomedical engineering utilizes three fundamental tools: living cell, signal molecules, and scaffold. Chitosan is one of the most promising biomaterials in tissue engineering because it offers a distinct set of advantageous physico-chemical and biological properties that qualify them for a variety of tissue regeneration. In this review, we presented the examples of the various types of chitosan derivatives modified for tissue engineering application and also introduced the strategies for using them as a scaffold in various kinds of organ such as skin, bone cartilage, liver, nerve and blood vessel. This survey has demonstrated the utility of chitosan as potential materials for various artificial tissue and organs. However, there are still many challenges such as improving its poor mechanical property as an artificial substitute, effective delivery strategy of growth factors to chitosan-based scaffold, demonstrating biocompatibility as well as sterility that must be addressed in various implant applications.

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