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Chitin Nanocomposite Scaffolds for Advanced Medications

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Abstract: The rapid development of nanotechnology, especially in the biological material sciences, has stimulated the demand for new hybrid materials and biocomposites for wide range of applications. Chitin, the second most abundant polysaccharide after cellulose, is a naturally occurring biopolymer found in yeast, fungi and exoskeletal structures of numerous invertebrates. Chitin meets the several desirable properties of biomaterial, which includes mechanical strength, chemical and thermal stability, biocompatibility, etc. Both chitin and its deacetylated product chitosan are considered of great economic values because of their versatile biological activities which made them proper to various applications in personal care products, pharmaceutical, medicine, food, agriculture and environmental sectors. This chapter gives an overview of the extensive research and recent developments on chitin/chitosan based nanocomposite scaffolds for application as advanced medicines. Several kinds of nanocomposite scaffolds have been applicable or under investigations, such as chitin-chitosan-gelatin scaffolds, pectin-chitin/$CaCO_3$ nanocomposite scaffold, et al. Here we highlight the most recent research on different aspects of chitin based nanocomposite scaffolds, including their preparations, properties and applications, especially the applications of chitin nanocomposite scaffolds in tissue engineering, stem cell technologies, and vaccine preparations.

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1. Introduction

There has been rapid development of nanotechnology recently, especially in the biological material sciences, which stimulates the demand for new hybrid materials and biocomposites of specific and defined properties for a wide range of applications. Nanomaterials exhibit novel characteristics compared to bulk materials, such as high mechanical strength, high surface-area-to-volume ratio, and extremely miniaturized size [1].

The interest in chitin as a raw material began in the 1920s owing to market pressure for low cost fibers, which promoted research into artificial silks. In particularly, chitin's biocompatibility and wound-healing properties made it attractive for different biomedical applications including tissue engineering scaffolds, wound dressing and sutures, and biocompatible devices. Chitin is the second most abundant polysaccharide after cellulose. It is a natural biopolymer found in fungi, yeast and the exoskeletal

structures of numerous invertebrates including insects, sponges, worms and aquatic arthropods [2].

The industrial processing of shellfish such as crab, shrimp and krill for human food generates a huge amount of waste and it is said that 50–60% of the total weight is chitin. Annually, worldwide production is about 1.44 million metric tons dry weight [3]. These abundant and renewable marine wastes can be commercially exploited for the extraction of chitin [3]. However, traditional chitin extraction processes employ harsh chemicals and produce other waste-water or solvent. Advances in the biological extraction of this natural polymer, which reduces energy cost and waste water—producing valuable by-products—will certainly have high economic and environmental impacts [3]. Thus, the bio-extraction of chitin from crustacean shell waste has been increasingly studied in order to develop eco-friendly, cleaner, and economical processes.

Due to their molecular and supramolecular structures characterized for their intrinsic antimicrobial and wound healing properties, chitin and derived compounds (chitosan and chito-oligosaccharides) have been identified as suitable bio platforms to make specialized polymers functionalized for the advanced medicine.

What is chitin? As a derivative of glucose, it is a long-chain natural occurring polymer of *N*-acetyl-D-glucosamine with a molecular length varying from 5000 to 8000 *N*-acetyl-D-glucosamine units found in crabs, to up to only 100 units contained in yeast.

Chitosan, a chitin-derived compound, is usually produced by chitin deacetylation with concentrated alkali solutions at elevated temperatures. This process of deacetylation, which does not proceed to completion, implies that chitosan, obtained from commercial sources, is in reality chitin with a low degree of acetylation. When the number of the units is higher than 50%, the polymer is termed chitin, when less than 50% it is termed chitosan.

In conclusion, chitin and chitosan may be considered as two points of a continuum material that share the same basic structure differing in their acetylation degree. As to the function of its cell structural support and defense against environmental aggressions, chitin may be compared to human keratin, which supports skin, hair and nails [4].

It has therefore proven useful for several medical and industrial purposes from tissue engineering to making advanced medications for wounded and burned skin [5], to producing smart and innovative colored dressings, imitating the iridescent colors used by birds and butterflies in nature. Birds' plumage and butterfly wing scales, in fact, are often organized into stacks of nano-sticks or nano-layers made of chitin nano-crystals, which produce various iridescent colors thin-film interference (Figure 1) [6,7].

Figure 1. The different colors and iridescences are due to chitin nanocrystals. Source: Morganti 2012 [6].

Chitin is insoluble in aqueous solution and relatively difficult to process. To improve its processability, many different approaches have been studied for the successful employment of this polymer in the areas of advanced medicine including stem cells, tissue engineering etc.

Chitosan displays interesting physicochemical properties, differing in its properties and organized structure. In a solid state, relatively rigid crystals are formed due to its regularly arranged hydroxyl and amino groups, while in solution the hydrogen bondings drive the formation of microfibrils, depending on the chitosan concentration. Furthermore, chitosan is easy to develop into various designs, i.e., films, sponges, scaffolds and hydrogels, which result in the ability to make various kinds of tissue-engineering materials and wound dressings [7]. Due to their unique structural, physico-chemical and functional properties, both chitin and chitosan are good candidates for the preparation of scaffolds and dressing materials for tissue regeneration [8].

In recent years, considerable attention has been attracted to ameliorating the functionality of these biopolymers for improving their properties by increasing their solubility or introducing selective active functions or making blends with other intrinsically bioactive polymers [9].

This chapter tries to give an overview of the recently reported chitin and chitosan nanocomposite scaffolds, emphasizing their characteristics and applications in tissue engineering and other applications.

2. Characteristics of Potential Chitin/Chitosan Nanocomposite Scaffolds

2.1. Nanohydroxyapatite/Gelatin/Carboxymethyl Chitin Composite Scaffold

Sagar et al. developed a novel 3D scaffold from the unique combination of nanohydroxyapatite/gelatin/carboxymethyl chitin (*n*-HA/gel/CMC) for bone tissue engineering by using the solvent-casting method combined with vapor-phase cross-linking and freeze-drying [10]. An optimized (composition and processing parameters) ratio of *n*-HA:gel:CMC (1:2:1) exhibited an ideal porous structure with regular interconnected pores (75–250 μm) and a higher mechanical strength.

Their results suggested that the divalent (Ca^{2+}), carboxyl (COO^{-}), amino (NH_4^{+}), and phosphate (PO_4^{3-}) groups created favorable ionic interactions which facilitated structural stability and integrity of the composite scaffold. Further, hemocompatibility and biocompatibility with MG-63 osteoblast cells indicated that the structural and dimensional stability of a composite scaffold provided an optimal mechanosensory environment for the enhancement of cell adhesion, proliferation, and network formation [10].

2.2. Pectin-Chitin/$CaCO_3$ Nanocomposite Scaffold

Kumar et al. developed a nanocomposite scaffold using a mixture of chitin, pectin and nano $CaCO_3$ through the technique of lyophilisation, with specific biomedical applications for tissue engineering and drug delivery [2].

The developed composite scaffold showed controlled swelling and degradation, compared to the control scaffold. Moreover, cells attached onto the scaffolds started to proliferate after 48 h of incubation and demonstrated negligible toxicity towards cells. Drug delivery through the scaffold was confirmed using a bisphosphonate called Fosamax. The results suggested that the developed composite scaffold possessed the essential requisites for their applications in the fields of drug delivery and tissue engineering.

2.3. α-Chitin Hydrogel/Nano Hydroxyapatite Composite Scaffold

In another paper, Kumar et al. synthesized α-chitin hydrogel/nano hydroxyapatite (*n*HAp) composite scaffold using a freeze-drying approach [11] with nHAp and α-chitin hydrogel. Hydroxyapatite [(HAp), $Ca_{10}(PO_4)_6(OH)_2$] is a major inorganic ceramic material and an essential component of bone. nHAp is used for various applications like dental filling material, bone tissue engineering, etc. Because it offers a high surface area to volume ratio, a small concentration is sufficient to enhance

its bioactivity and osseointegration. Hap nanoparticles were mixed with α-chitin hydrogel at concentrations of 0.5% and 1% (*w*/*w*), stirred for 30 min, frozen at −20 °C and lyophilized to get a microporous nanocomposite scaffold [11]. The prepared composite scaffolds were characterized using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Thermogravimetric analysis and differential thermal analysis (TG-DTA) and scanning electron microscopy (SEM). Porosity, swelling ability, protein adsorption, in vitro biodegradation and biomineralization of the scaffolds were evaluated. These supports showed interconnected micro-pores, sufficient swelling ratio of 15–20, good protein adsorption, controlled degradation and wonder biomineralization ability.

2.4. Chitin-Chitosan/Nano ZrO_2 Composite Scaffolds

According to de Moraes et al. [11], innovative biomaterials can provide a promising new direction for the treatment of bone defects, stimulating a proper repair process with no damage to the adjacent tissues [12]. Collagen, in fact, is one of the most used biomaterials due to its biocompatibility and bioactivity [13]. Thus, Soares et al. [14], evaluating the odontogenic potential of human dental pulp cells in contact with a porous system of chitosan-collagen mineralized with calcium-aluminate, concluded that this scaffold seems to be an interesting candidate for in vivo applications to exposed pulp tissue.

Owing to its good mechanical strength and biocompatibility, zirconia is also considered to be one of the most used materials after titanium over a period of about 20 years, especially in dentistry. Cultured osteoblasts proliferate and differentiate in zirconia with no adverse reaction. Jayakumar R et al. tried incorporation of nano ZrO_2 onto the chitin-chitosan scaffold to enhance osteogenesis [15]. They fabricated a nanocomposite scaffold using a lyophilisation technique with chitin-chitosan and nano ZrO_2. The prepared nanocomposite scaffolds were characterized by FTIR, SEM, XRD and TGA. The swelling, degradation, cell attachment, cell viability and biomineralization of the composite scaffolds were also detected. The results showed better swelling and controlled degradation compared to the control scaffold [15]. Cytocompatibility studies proved the non-toxic nature of chitin-chitosan/nano ZrO_2 scaffolds against MG-63, L-929 and hMSCs [15]. Additionally, cell attachment studies showed the nanocomposite scaffold significantly increased the cell attachment when compared to control scaffolds. All these results suggested that the developed nancomposite scaffolds possess the prerequisites for tissue engineering scaffolds and could be used for bone tissue engineering and other bio-engineering purposes of human interest.

3. Chitosan/Gelatin/nSiO$_2$ Composite Scaffold

Kavya et al. [16] fabricated a 3D nanocomposite scaffold of chitosan, gelatin and nano-silica by lyophilisation to produce a better candidate for bone tissue engineering compared to pure chitosan and chitosan/gelatin scaffolds.

To prepare composite scaffolds with better biological compatibility and hydrophilicity, they added gelatin to enhance the performance of chitosan. Unique biocomposites of amorphous silica, chitin, and crystalline aragonite have been discovered in marine sponges. Silica is believed to be essential in skeletal development so that critical amounts of silicon ions are found to up-regulate genes like collagen type-, BSP, osteocalcin and osteopontin in osteoblasts.

To this purpose, these authors developed a 3D composite scaffold of chitosan/chondroitin sulfate/nSiO$_2$ to bring out the combined properties of chitosan, gelatine and nSiO$_2$ to facilitate bone regeneration. Porosity, swelling, density, mechanical integrity, degradation, biomineralization and protein adsorption studies, favored it in comparison to the conventional chitosan and chitosan/gelatin scaffolds. In vitro cyto-compatability, cell attachment-proliferation and ALP activity studies by using MG-63 cells, advocated its remarkable performance. These cumulative results indicate the chitosan/gelatin/nSiO$_2$ nanocomposite scaffold as a suitable candidate for bone tissue engineering.

Chitosan-Graphene Oxide Network Structure Scaffold

Graphene—a single layer of sp2 bonded carbon atoms in a two-dimensional hexagonal lattice—has attracted considerable attention as a potential biomaterial because of its physic-chemical properties such as a large surface area, high hydrophilicity and dispersibility. Chitosan-graphene network structure scaffolds were synthesized by covalent linkage of the carboxyl groups of grapheme oxide with the amine groups of chitosan [10].

The covalent incorporation of graphene oxidant into a CS network favorably modulated the biological response of osteoblasts, such that cell attachment and growth were significantly enhanced. Thus, related to a combination of a number of physic-chemical factors, including a large surface area, nanoscale roughness, the presence of pendant groups, a hydrophilic nature and high water retention ability, this network is believed to be a promising material for tissue engineering applications in regenerative medicine (Figure 2) [17,18].

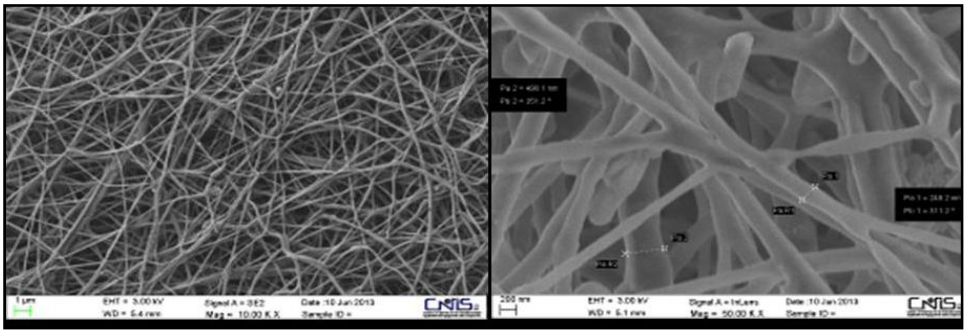

Figure 2. Engineered non-woven tissue made by chitin nanofibrils with scanning electron microscopy (SEM). Source: Morganti et al. [19].

4. Applications of Nanocomposite Scaffolds for Advanced Medications

Tissue Engineering

Extensive research is in progress to develop biosmart materials in the field of tissue engineering, being considered of prime importance for biomedical and microbiological applications.

Chitin, chitosan and its derivatives have received much attention because of their biocompatibility and other advantages. Chitin composite scaffolds have sufficient mechanical strength. And due to their viscoelasticity in wet conditions, they can be cut, deformed, and fitted according to bone defects. This allows us to use them in cases of bone cysts and large defects with smaller openings.

These scaffolds can spontaneously grow by consuming the calcium and phosphate ions from the surrounding fluid which render them osteoinductive structures, analogous to living bone.

Besides being a matrix of a composite material, chitin has also been confirmed to promote osteogenesis in mesenchymal stem cells, accelerate wound healing, enhance cell migration, and form granulation tissue with angiogenesis [19–22]. These effects might be mediated by the production of cytokines and growth factors by fibroblasts that come into contact with the chitin material. For tissue engineering applications, the ideal situation would be gradual but complete scaffold degradation concomitant to tissue remodeling, while foreign material might elicit an adverse host tissue response in the long term, so the degradability of polymeric scaffolds should be of primary concern.

Chitin is susceptible to lysozyme and chitotriosidases, which are ubiquitously present in humans. In this regard, it is interesting to underline that this natural polymer has to be considered as a pathogen-associated molecular pattern (PAMP) regulating the macrophage function and inflammation, depending on its size [23].

Moreover, it has been shown that chitin nanofibrils (240 × 7 × 5 nm) stimulate the defensive production of defensine-2, contextually reducing the production of metallo-proteinases and inflammation [24,25]. This is another advantage for chitin in the application of tissue engineering. Combinations of chitin/chitosan with other polymers have been fabricated to nanocomposite scaffolds for tissue engineering as we mentioned above. Recent work established that a chitin-hydroxyapatite composite, loaded with mesenchymal stem cell-induced osteoblasts, was able to support bone regeneration when implanted into bone defects in a rabbit femur [26].

Composite scaffolds of chitin with nanohydroxyapatite and nanotitania, obtained by dispersing the particles in a chitin hydrogel cast from a $CaCl_2$/methanol solvent system and freeze-drying of the mixture, supported apatite deposition and adhesion of a variety of cell lines [11,15]. Composite scaffolds, prepared with *n*-HA/gel/CMC, have sufficient mechanical strength and, along with their viscoelasticity in wet conditions, they can be cut, deformed, and fitted according to bone defects which allows them to be used in cases of bone cysts and large defects with smaller openings [10].

Another composite scaffold, based on α-chitin hydrogel/nano hydroxyapatite composite scaffold, showed that its viability in the presence of scaffold leachables and *n*HAp did not affect its cyto-compatibility. It was also found that the scaffolds were cytocompatible and cells were well attached and distributed throughout these structures [11]. Moreover, scaffolds made by chitin nanofibrils developed antibacterial and anti-inflammatory activity and increased the production of defensine-2, speeding up the skin repairing process in wounded and burned skin [17,24–27].

All these results suggested that these scaffolds can be used for bone and wound tissue engineering also because of their effectiveness and safeness. However, biopolymers offer a highly effective flexibility to design porous matrices by chitosan, chitin, collagen, alginate and other natural compounds. Moreover, when at the nanodimension, they have to be considered the best candidates for drug delivery applications due to their controllable pore sizes, high surface area with favorable properties.

5. Stem Cell Technologies

Because of the fast development in technologies, these years, stem cell had bright prospects in regenerative medicine and organ transplantations. Chitin and other biomaterials can help to realize the vast potential of stem cells in regenerative medicine by: (1) playing a role in providing substrates that support stem cell self-renewal, while maintaining stem cell pluri- or multipotency; (2) favoring the provision of a matrix permissive to stem cell differentiation; (3) representing a matrix of composite material as physical support of cells to regenerate organs; and (4) guiding their differentiation

when appropriate signals are provided [28]. To this purpose, the advantages of a chitin matrix in supporting stem cell proliferation and differentiation has extended its applications in vivo, where chitin has been shown to be effective as a carrier material for mesenchymal stem cells in the treatment of large physical defects.

Mesenchymal stem cells seeded or encapsulated in water-soluble chitin-alginate fibrous scaffolds have been differentiated into chondrogenic and osteogenic lineages by immersion in the respective differentiation media [29]. Nanocomposite scaffolds of chitin or chitosan would definitely have more broad application prospects.

6. Vaccine Preparation

Vaccination is one of the major keys to maintaining a good public health and wellbeing status of society. It induces specific adaptive immune responses and memory responses against infections, tumors, etc. [30]. Unfortunately, not all vaccines are as effective, often showing low efficiency of antibody production with a weak host T cell response and T cell memory, which require repeated booster injections to obtain longer host memory immune responses.

To solve this problem, adjuvants [31] are often used to augment the effects of a vaccine by stimulating the immune system to respond to the vaccine more vigorously, thus providing increased immunity to a particular disease. There are many adjuvants, such as aluminium salts, virosomes, etc. Aluminium—the only adjuvant approved by the Food and Drug Administration (FDA) for clinical use—stimulates B cell response for antibody production but is not very effective at inducing host T cell responses and does not work well with all antigens.

Chitin nanocomposite scaffolds are a biodegradable polymer, which not only has immune adjuvant effects on its own [23,24] but is also able to release incorporated cytokines and antigens in a controlled manner, therebby synergistically boosting adjuvant effects. Chitin also accelerates macrophage migration and fibroblast proliferation with a particular role in vascularisation [32].

For clinical applications, chitin/chitosan is frequently cited to possess low immunogenicity as an advantage, and at the same time, the ability to act as an immunoadjuvant. Glycolic acid-g-chitosan-gold nanoflower nanocomposite scaffolds, for example, were confirmed to be used for the sustained delivery of drug [33]. This property would also help chitin/chitosan nanocomposite scaffolds to be used in vaccine preparation as an adjuvant.

7. Conclusions

Recent progress in chitin and chitosan nano-composite scaffolds highlights great potential usage in wound-healing and tissue engineering due to their unique structural, functional, physical and chemical properties. Another area of particular interest is also gene-therapy, referring to methods aimed at influencing gene

expression in living organisms through the delivery of integrating exogenous DNA or RNA to treat or prevent disease [34]. For these reasons, many innovative approaches are emerging in recent years with the use of novel materials and technologies. However, biomedical modifications of these biopolymers and their preparation in different designs have been reported extensively, but until now with limited commercial impact. Further studies on the clinical applications of these chitin and chitosan nanocomposite scaffolds would be the most important issue in this field.

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