Medical Applications of Chitin

Subjects: Medicine, Research & Experimental | Biochemistry & Molecular Biology

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Chitin is a universal biopolymer that is found in microbes, plants, fungi, the exoskeleton of insects, various species of algae, and bottom-feeding crustaceans. This (1–4)-linked N-acetyl-ß-D-glucosamine polysaccharide can be readily processed with simple chemical procedures without putting a species at risk. Chitin has garnered interest as an alternative substance that can be used in the medical, environmental, and agricultural sectors.

Marine chitin | Medical | Chitosan | polysaccharide | Biopolymer

1. Tissue Engineering

To understand tissue regeneration using active bio-materials, there must be an understanding of how chitin works with damaged tissues and how it enables regrowth. The foundation of tissue engineering is built upon the idea of a biomaterial scaffolding that gives a cell the desired shape and structure [1][2]. Biomaterial scaffolding goes through changes where, for example, chitin is combined with smaller molecules such as sugars and peptides [1][2]. Previous hybrid studies on binding fibers of chitin with silk-fibroins and polyglycolic acid have demonstrated the connection and spreading of human keratinocytes and fibroblast. Both keratinocytes and fibroblasts are important cell types that are responsible for the innate inflammatory response in cutaneous repair and regeneration processes [3][4].

Using the information above, researchers are now looking at whether or not chitin can be useful in supporting the regeneration of cartilage [1]. This would be attributed to the repeating subunits of N-acetyl-ß-D-glucosamine that are the foundation of other glycosaminoglycans, like keratin sulfate and hyaluronate, which are key components of articular cartilage [1]. Cartilage is a very promising nominee for tissue engineering research programs due to chondrocytes being easily isolated through enzymatic digestion [5][6]. It has been well documented that cartilage grafts grow on biodegradable scaffolds, like polylactic and polyglycolic acids [5][6]. However, chitin has an advantage over these synthetic polymers as a result of the biopolymer's ability to bind with the glycosaminoglycans of cartilage [5][6]. Furthermore, previous studies have also shown that chondrocytes have a unique spherical morphology and exhibit type II collagen and aggrecan when developed on chitosan films [5][6].

Another promising avenue is the development of chitin-based tubes for tissue engineering found in the nervous system [7][8]. One study focused on using straightforward acylation chemistry in combination with molding techniques to produce chitin and chitosan gel tubes which can have positive mechanical properties, acting as nerve guides on in vitro dorsal root ganglion (DRG) neurons [7][8][9]. The study found that chitosan tubes were much stronger when measuring transverse compression than chitin tubes in terms of the various degrees of acetylation, enabling to support displacing loads [7][8][9]. In vitro analysis confirmed that both biopolymers showed adhesion and

differentiation found in the DRG neurons, but chitosan caused better neurite outgrowth than chitin [7][8][9]. Simply changing the amine content can promote better adhesion and neurite extension, both of which are key for tissue engineering in the nervous system [7][8][9].

2. Wound Dressing

Wounding healing is another avenue where researchers are looking at potential applications of chitin to be used as an alternative to conventional methods. The healing process of a wound is continuous and complex, as there are five important stages: homeostasis, inflammation, migration, proliferation, and maturation [10][11]. One of the many problems plaguing the wound healing process would be a bacterial infection that, if not managed correctly, could lead to life-threatening complications [12]. When picking the ideal candidate for wound dressing material, many different factors go into finding the best options, like allowing for gaseous exchange, providing protection from microorganisms, and ensuring no harm is done to the patient [12]. The current market has many different products that not only maintain the microenvironment on the surface of the wound, as newer technologies have started using bioactive products that also control and initiate the healing process in a safe manner [10][11].

Chitin and its derivatives can act as a wound healer accelerator by controlling the inflammatory response through interleukin 8, prostaglandin E, and interleukin 1 ß. This characteristic, alongside chitin's ability to be utilized in different sectors, allows chitin to be a promising source for wound dressing. [13][14]. As mentioned before, the use of chitin and chitosan scaffolds in patients with severe burns and wounds has shown promising results [13][14]. In conjunction with chitin having excellent antibacterial properties, metallic silver and silver sulphadiazine ointments have also been illustrated to have good antibacterial resistance [6][8]. Alone, each material can have a profound effect on wound treatment, but researchers are now looking at the possibility of combining the two [13][14]. A previous study found that the combination of α-chitin and nano-silver into scaffold composites has above-average antibacterial activity and great blood clotting ability against different microbes, such as *Staphylococcus aureus* and *Escherichia coli* [13][14]. A similar composite scaffolding was also made by using ß-chitin and nano-silver, demonstrating anti-bactericidal efficacy on microbes, whole blood clotting, and the composites showed good cell attachment [13][14]. The slight difference between both types of chitin can be attributed to differences in the intermolecular interactions [13][14]. The intermolecular interactions in ß-chitin are drastically weaker than those commonly found in α-chitin, causing ß-chitin to allow for better dissolution in many different solvents and making them more reactive and adaptable [13][14].

The above information has allowed researchers to test the idea of wound healing using biopolymers like chitin on live animal subjects under controlled conditions. A study using a rat model saw animals being anesthetized and shaved to reveal the skin surface [1]. After this was performed, a circular wound was surgically created on the left and right dorsal sides of the rodent [1]. Chitin films were created by taking raw powered chitin and dissolving it in 5% LiCl/N, N-dimethylacetamide, producing a 0.5% solution [1]. This solution was later poured into molds which created chitin gels, producing the films that were placed on the wounds [1]. After four days of healing, the traditional gauze dressing had noticeable hardening which caused tissue damage to occur, affecting the healing process that was not noted in chitin films [1]. After seven days, the regeneration of the wound's epidermal surface was 70%

healed, but there was a stark difference between wounds that were dressed with chitin films, as they healed much faster than traditional dressing [1]. After 14 days, the inflammatory phase and regeneration of the epidermal surface had not fully healed in both dressings, but it was recorded that chitin films had a stronger inflammatory response [1]. Overall, the use of chitin films can allow wounds to heal dramatically faster and stronger than conventional wound dressing materials [1].

Hu et al. [15] created a composite sponge for the purpose of wound dressing. In this study, chitosan-based sponges are considered an effective hemostatic dressing due to their interconnected porous structures, good swelling capacity, antibacterial activity, and hemostatic ability. However, the sponges themselves cannot preserve the moistness of the wound and could cause secondary damage when used as a wound dressing due to their poor hydrophilicity [15]. Hydroxybutyl chitosan is created when hydroxybutyl groups are conjugated to the C-6 hydroxyl and C-2 amino groups of chitosan. These modifications provide chitosan with water solubility, temperature-sensitive properties, and ensure that the transition from an aqueous to hydrogel phase is reversible. Furthermore, the hydroxybutyl sponge still retains properties such as high porosity, good hydrophilicity, non-toxicity, and tissue adhesion [15]. While all of these characteristics provide a solid basis for wound healing, due to the hydroxybutyl modification, this sponge lacks effective antibacterial properties and mechanical strength in hydrated states [15]. Therefore, the authors decided to combine chitosan and hydroxybutyl chitosan, at different ratios, to determine whether their individual characteristics could cover each other's failings [15]. It was determined that the composite sponge had better water retention, erythrocyte aggregation, and antibacterial activity when compared to the pure chitosan sponge and the hydroxybutyl sponge. Furthermore, the composite sponge was also able to induce higher cell proliferation and could support epithelial cell growth. Overall, the authors proved that the composite sponge had great potential to act as a wound dressing [15].

Ehterami et al. [16] attempted to create a hydrogel that was comprised of alginate, chitosan, and vitamin E due to their high performance in skin tissue engineering. The complexation between alginate, an anionic polymer typically derived from brown seaweed, and chitosan is an effective method to create microspheres and improve chitosan's properties regarding medical applications [16][17]. Furthermore, vitamin E, typically recognized for its role as an antioxidant, can also regulate gene expression, transcription, and the expression of connective tissue growth factor [16]. These properties enable the protection of wounds from infections. The authors found, based on their testing of thirty-six male Wistar rats that had been administered wounds, that the prepared hydrogel dressings had a higher wound closure than the wounds treated with gauze (the control) [16]. Furthermore, one of the hydrogels, the Chit/Alg/400 IU Vit E, demonstrated the most promise regarding cell proliferation rate and accelerated wound healing [16]. The authors were able to demonstrate, through this study, the effectiveness of their prepared hydrogels in wound healing, with chitosan being one of the primary components [16].

While these are only a few examples of chitin and chitosan in the wound healing process, it is clear that both these polymers have immense potential, especially when combined with other materials.

3. Drug Delivery

With all the advancements in the biomedical field using chitin, new developments have allowed the biopolymer to be used for drug delivery. Biopolymers, as mentioned before, have multiple applications due to their multifunctionality, applied as beads, microparticles, nanoparticles, micelles, hydrogels, tablets, and capsules [18][19]. Biopolymers, such as chitin have the ability to be formed into nanogels, which are hydrogels restricted to the nanoscopic field [18][19]. These nanogels have intensive properties, including size tunability, a large surface area for bioconjugation, drug loading abilities, controlled release, and an excellent ability to respond to environmental stimuli [18][19]. Through these properties, novel uses for chitin in drug delivery have been developed, such as water-soluble carboxymethyl chitin (CMC) and polylactic acid(PLA)/chitosan(CS) nanoparticles.

CMC nanoparticles can be used as an innovative way to deliver drugs to different cell types and areas within the cell [3]. Studies have shown that CMC can lower the adsorption of blood components without causing an antibody response that could be induced by the body [3]. The raw chitin, before the initial reaction, is pretreated with a solution of 60% sodium hydroxide at −20 °C for roughly 12 h [20][21]. Once this is completed, the main preparation of CMC is prepared by mixing raw chitin powder and monochloroacetic acid in an isopropyl alcohol solution that produces a condensation reaction [20][21]. The water solubility that makes CMC unique is determined by the concentration of sodium hydroxide that is available during the preparation of alkali chitin in certain freezing processes [20][21]. CMC and drug delivery operate on a two-step hydrolysis process that allows for controlled drug delivery [20][21]. The drug is bound to a peptide spacer which is then bound to CMC and is hydrolyzed by lysozymes, allowing the drug to be released [20][21]. Another study that combined CMC gels and doxorubicin, an anticancer drug, found the release of the drug from the gels was sustained over a period, which resulted in researchers wondering whether CMC can be applied to other drugs and vaccines [20][21].

Another novel technique that has garnered interest among researchers as an alternative method for drug delivery is the use of polylactic acid (PLA)/chitosan (CS) nanoparticles. PLA is another biodegradable polymeric material that is known to have low toxicity, good biocompatibility, and bio-absorbability in many in vivo studies [22][23]. Due to the low hydrophilicity and increased crystallinity of the biopolymer, the rate of degradation is lower due to bad soft tissue compatibility [22][23]. A previous study sought to find out if PLA/CS nanoparticles were an adequately controlled system to deliver the drug lamivudine, an antiretroviral drug used in the prevention and treatment of HIV/AIDS [22][23].

4. Cancer Diagnosis and Treatment

The diagnosis, treatment, and prevention of cancer using the emerging field of nanotechnology has changed significantly over time [24]. The use of nanoparticles, which are both biocompatible and capable of labeling certain molecules, has received much attention in the discussion of cancer diagnosis [24]. Semiconductor nanocrystals (or quantum dots (QD)) are engineered nanoparticles that can be used as fluorophores for in vivo imaging, including the bioimaging of cancerous tissues [24][25]. Although QD is a novel method for biomedical applications, its use faces challenges, including its toxicity due to the heavy metals (e.g., cadmium) that are released into the body. Other factors related to QD, including size, surface charge, concentration, and photodegradation also contribute to their cytotoxicity [24].

For these reasons, there is a need to develop other methods for diagnosing cancer. The expression of mannose receptors has been found to increase on cancer cells, and as a result, these receptors can serve as targets for nanoparticles [13]. Zinc sulfide (ZnS) nanoparticles surrounded by chitosan and functionalized with D-mannose can emit strong fluorescent radiation when targeting mannose bearing KB tumor cells [13]. These nanoparticles do not attach to the normal cells because normal cells do not increase mannose receptor expression [13].

In addition to the efficacy of mannosylated ZnS nanoparticles encapsulated by chitosan as in vivo fluorescent imaging for cancer diagnosis, chitosan derivatives may also have antitumor effects. Colonization of lung cancer is prevented by 6-O-sulfated chitin, and interestingly, the degree of sulfation is proportional to the inhibition of colonization [25]. In addition, the antitumor effects against sarcoma 180 solid tumors in BALB/C mice and MM-46 solid tumor implanted in C3H/HC mice are recognized by hexameric and heptameric N-acetyl chitosan oligomers, which have been shown to increase the cytotoxic proliferation of T-lymphocytes due to the increased production of lymphokines by hosts, such as Interleukin 1 and Interleukin 2 [25].

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