Chapter

Modulating the Physicochemical Properties of Chitin and Chitosan as a Method of Obtaining New Biological Properties of Biodegradable Materials

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Abstract

Physical and chemical modifications of chitin and chitosan allow for obtaining new functional properties of the natural polymers. This is a particularly valuable feature for the design and manufacture of new materials for medical applications. Due to their wide and varied biological activity, chitin and chitosan materials are increasingly used as dressing materials with antibacterial and hemostatic properties and as materials accelerating the regeneration of damaged tissues because of stimulation of granulation tissue formation, re-epithelialization and reduction of the formation of scar tissue. In addition, chitosan derivatives have antifungal, antiviral, anticancer activity. The increasing use of chitin and chitosan also has a positive impact on the environment, as it is obtained as a result of chitin deacetylation, usually isolated from shellfish shells. The main source of chitin is waste coating of crustaceans. The annual natural reproducibility of chitin by biosynthesis is estimated at 2–3 billion tons. Our interest in the use of biodegradable biopolymers derived from chitin concerns the design, synthesis in laboratory scale, testing new material properties and the final implementation of new developments for industrial practice of new dressing materials useful in the treatment of bleeding wounds (haemostatic properties) as well as in the regeneration of wounds and ulcers of various etiologies. Examples of chitin-based dressing materials introduced by Tricomed SA are Medisorb R Ag, Medisorb R Membrane, Medisorb R Powder and Tromboguard®.

Keywords: chitin, chitosan, chemical modification, biological properties, dressing materials, manufacturing on an industrial scale

1. Introduction

Chitin and chitosan belong to the polymeric materials of natural origin from the polysaccharides group. The widely used polysaccharides of natural origin also include cellulose and derivatives of hyaluronic and alginic acid. Use for the production of medical devices (contact with the patient's body), makes them meet several requirements: they should maintain their physicochemical properties after

treatment at elevated temperature during sterilization, after exposure to X-ray, detergents and aseptic. Polysaccharide biopolymers, like most polymeric materials, degrade after some time of use, so it is also important that their decomposition products do not cause inflammation, allergic or immune reactions or any other interactions with patients' bodies.

Chitin is a polysaccharide composed of N-acetylglucosamine residues linked by β-1,4-glycosidic bonds. Chitin is the main component of the fungal walls and the shells of arthropods (crustaceans, insects, and arachnids), but is also present in sponges, corals, and mollusks. However, for laboratory and industrial purposes, it is obtained mainly from marine invertebrates such as: crabs, shrimps, lobsters and krill. The properties of chitin depend on its origin. Chitin used in the production of medical devices must come from certified, controlled fisheries and must be properly purified. The methods of isolating chitin from natural sources are strictly dependent on the choice of the organism from which it is isolated. This polysaccharide is similar in structure to cellulose. It differs in the presence of an acetyl amide group (-$NHCOCH_3$) in place of one of the hydroxyl groups (**Figure 1**). The presence of this group means that there are much stronger intermolecular hydrogen bonds in chitin, which results in its greater mechanical strength compared to cellulose [1, 2].

Depending on the origin source, chitin can occur in three amorphous forms: α, β and γ [2, 3]. The most widespread is α chitin found in fungi, shells of crustaceans and krill, and the skeletons of insects. The β form, which can mainly be isolated from squids, is much less common. The differences in the crystal structure of both amorphous forms of chitin affect their processing capabilities. The ordered crystal structure of chitin limits its solubility in commonly used solvents, and thus, reduces its use in industry. α-Chitin is moderately soluble in aqueous thiourea solution, aqueous alkaline urea solution, 5% LiCl/DMAC, some ionic liquids, hexafluoroacetone, hexafluoro-2-propanol, methanesulfonic acid [4, 5]. The form of β-chitin, on the other hand, swells in water (forms a suspension) and is soluble in formic acid. Chitin has no cytotoxic effect *in vitro*, is physiologically inert, biodegradable, has antibacterial properties and is characterized by a high affinity to proteins. During its biodegradation in the wound environment, its oligomers and units are released. Most often, it is used in the form of gel, membranes, fibers, polymer films or is a component of polymer blends. Chitin activates macrophages, stimulates the proliferation of fibroblasts and influences vascularization [6–11].

Despite the very good biological properties of chitin, its practical use is moderate, which is related to its low solubility causing difficulties in its processing. Therefore, chitin is used as a raw material to obtain new derivatives with better performance parameters. In terms of practical use, the most important chitin derivatives are its esters and chitosan, which is a product of chitin deacetylation, which can be classified into the group of amino-polysaccharides.

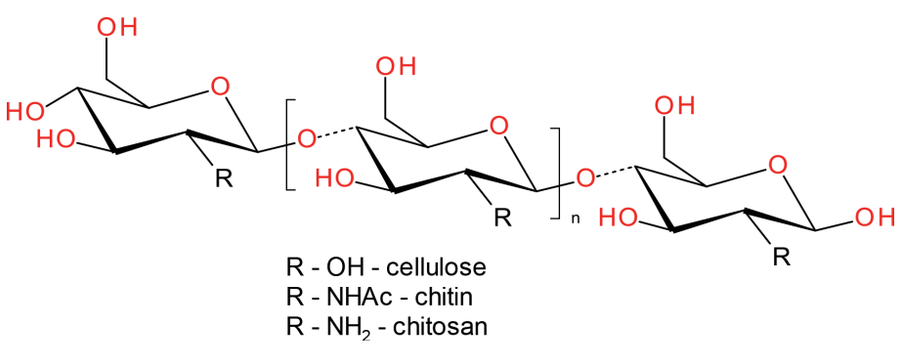

Figure 1.
Structural resemblance of cellulose, chitin and chitosan.

2. Chitin esters - materials with tailored functional properties

2.1 Chitin esters in dressing materials

The esterification of chitin hydroxyl groups allows to increase the utility potential of the polysaccharide by introducing various substituents, and thus, influencing the physical, chemical and biological properties of materials. The best known are chitin esters, in which the hydroxyl groups are esterified with one type of acylating reagent (presence of the same ester groups on both hydroxyl groups of chitin). Acetylated chitin derivatives (CH_3CO- substituent) are prepared with acetic anhydride in the presence of an acid catalyst. However, the physicochemical properties conditioning the processing of chitin acetate turned out to be unsatisfactory [12]. The use of a mixture of orthophosphoric acid and trifluoroacetic acid anhydride as a catalyst allowed to obtain a variety of chitin esters derived from: butyric acid, cyclopropanecarboxylic acid, cyclobutanecarboxylic acid, cyclopentanecarboxylic acid, cyclohexanecarboxylic acid and substituted benzoic acids. In the case of chitin butyrate, the process efficiency (DS (degree of substitution) included in the range 1.9–2.38) was dependent on the excess of butyric acid anhydride use [13–15]. Di-butyrylchitin (chitin di-butyrate, DBC) is an example of a chitin derivative soluble in typical organic solvents [16]. DBC is obtained by chitin esterification with butyric anhydride. Typically, it is a two-stage process. In the first step, chitin is purified from calcium salts with 2 M hydrochloric acid. The next stage is the process of proper esterification of purified chitin. The substrates of the reaction, apart from chitin, are butyric anhydride and the catalyst, which is most often chloric (VII) acid. The reaction is carried out in a heterogeneous system by adding powdered chitin in appropriate proportions to the reaction mixture consisting of butyric anhydride and chloric (VII) acid. The classical esterification process requires the use of substrates in a molar ratio of acid anhydride to N-acetylaminoglucose unit of 10: 1. It is also crucial to carry out the reaction at a temperature of 20°C. Increasing the reaction temperature to 40°C causes a rapid lowering of the molecular weight of the modified polymer. The catalyst concentration has a direct influence on the efficiency of the esterification reaction. The yield of the reaction is the higher when more concentrated chloric (VII) acid is used. However, it should be remembered that the use of too much concentrated chloric (VII) acid results in the macromolecule degradation. The esterification process is completed by adding diethyl ether to the reaction mixture. The isolated product is then heated with water to remove residual chloric (VII) acid. The product obtained in this way is treated for 24 hours with acetone, in which only di-butyrylchitin is dissolved. Then, the solution is concentrated to 5–6%. After the desired concentration is reached, the solution is poured into deionized water to precipitate the polymer, then the product is dried to obtain solid di-butyryl chitin. The above-described process of chitin esterification allows the conversion of free hydroxyl groups on the C3 and C6 carbon of the chitin into ester groups ($CH_3(CH_2)_2CO$- substituent). Di-butyrylchitin is composed of di-butyl-N-acetyl-amino-glucose units linked by 1,4-β-glycosidic bonds. The polymer is stabilized by hydrogen bonds between the polymer chains. Hydrogen bonds are formed with the participation of the hydrogen atom from the acetylamino group and the oxygen atom from the ester group. This kind of intermolecular interaction determines its good mechanical properties [12–15]. Di-butyrylchitin does not dissolve and does not swell in water, but it dissolves in many organic solvents such as: acetone, methanol, ethanol, tetrahydrofuran (THF), dimethylformamide (DMF), chloroform, methylene chloride and others. Di-butyryl chitin is not easily degraded, it is resistant to γ-radiation (possibility of radiation sterilization), while enzymatic degradation under the influence of lysozyme and CE econase occurs at a

slow rate, which causes a slight change in molecular weight. Di-butyrylchitin with a molecular weight above 100000 Da has film-forming and fiber-forming properties [1, 2, 12–15]. Thus, obtaining DBC with the desired molecular weights directly determines its further processing capabilities (in particular electrospinning and leaching). The most important biological parameters of di-butyrylchitin are: prolongation of blood clotting time and good wettability. The use of DBC dressings has a positive effect on the granulation process (increasing the level of glycosaminoglycans in the wound), collagen cross-linking (generating more durable tissue), accelerating the wound healing process to form a healthy epidermis without scarring and protecting the wound against excessive moisture loss (optimal moist environment) [1, 2, 6–11]. In the course of treatment, the dressing slowly bio-degrades and resorbs until it disappears completely, which eliminates the painful act of changing it. The spontaneous, anti-pain effect of the dressing was also noted. DBC does not show cytotoxicity or irritation, it is a biocompatible polymer [9]. Di-butyrylchitin fibers are obtained by two methods: wet and dry-wet. The choice of the method used determines the structure of the obtained fibers. The fibers obtained in the wet-spinning process are less regular in shape, with a greater surface development than in the case of dry-wet spinning. DBC fibers produced by wet spinning are used as a raw material for the production of nonwovens. The technique of producing nonwovens from DBC depends on cutting the fibers into 6 cm long sections, from which the fleece is produced using a mechanical method on carding machines, and then the fibers in the fleece are joined by needling and calendaring [16, 17].

The dry-wet method of forming fibers from DBC hinges on preparing a spinning solution with a concentration of 15 to 25% in ethanol, heating it to 60°C and squeezing it through a spinning die. Then, the incompletely solidified fiber is introduced into a water bath, where it is completely solidified. The fiber is then wound onto drums, stretched, and dried. A microporous DBC fiber with a linear mass of 1.7 to 5.6 g is obtained, depending on the concentration of the spinning solution used. The fibers obtained by the dry method have an elongated and curved cross-sectional shape, similar to a croissant. The degree of crystallinity of the fibers determined in X-ray examinations is similar in both methods and amounts to approx. 19%, and the transverse dimension of the crystallites approx. 23 Å. It is also easy to obtain chitin materials (the so-called regenerated chitin) from these materials without damaging their macro-structure after a mild alkaline treatment. Fibers made of regenerated chitin and di-butyrylchitin do not induce cytotoxic, haemolytic or irritating effects and cause minimal local tissue reaction after implantation [17–19]. Di-butyrylchitin and regenerated chitin fibers can be used to obtain dry dressing materials, as well as materials for other biomedical purposes. DBC-based woven dressings are biodegradable within the wound and do not need to be replaced during use [16, 17]. Chitin di-pentanoate (chitin divalerate, Di-O-Valeryl-Chitin, DVCH) is also used for the production of commercially available dressings, where chitin is esterified with two valeryl groups ($CH_3(CH_2)_3CO$- substituent) at the 3 and 6 positions of N-acetylglucosamine units. The high degree of DVCH esterification was achieved by using a large excess of valeric anhydride used both as acylating agent and reaction medium, and perchloric acid as catalyst. It turned out to be optimal to conduct the reaction where the molar ratio of valeric acid anhydride to chitin was 7:1, which also facilitated thorough mixing of the components during the reaction and temperature control. The performance of the reaction under conditions of high homogeneity of the solution has a great influence on the quality of the product. Insufficient mixing of the solution during the acylation step led to a local temperature rise, uneven chitin acylation and ultimately resulted in products with varying degrees of esterification and higher polydispersity. Additionally, at elevated temperature it was observed reduction of the molecular

weight of the biopolymer as a result of the acidic degradation of chitin that occurs in parallel with the acylation reaction in the presence of a strong acid. To obtain products with a high degree of esterification, 0.5 M perchloric acid was used (deacetylation of the N-acetyl group was not observed). The separation of the raw product from the reaction mixture takes place during the neutralization of the valeric acid excess with a 2.5% $NaHCO_3$ solution. The use of sodium bicarbonate as a weak base prevents deacetylation of the N-acetyl group. Depending on the reaction time and temperature, products with different parameters are obtained. The lower temperature leads to a product with a higher molecular weight. A longer reaction time increases the yield of the reaction, but is associated with a reduction in molecular weight due to acidic degradation of the polymer. The DVCH polydispersity index ranged from 1.47 to 2.06, suggesting a low molecular weight distribution. Due to the good solubility of DVCH in organic solvents such as acetone or ethanol, it is possible to prepare thin polymer layers by casting or porous structures by salt leaching. The DVCH shows a semi-ductile behavior and breaks when it exceeds the yield point. The stretching properties of DVCH films depend on the molecular weight. The modulus, yield stress, tensile stress as well as strain at break increase continuously with increasing DVCH molecular weight. The increase in the modulus with molecular weight results in higher mechanical strength of DVCH films. The elongation at break, although slightly increases with increasing molecular weight, remains low, not exceeding 4%. As a consequence, the higher DVCH molecular weight is, it behaves like a stiff plastic that can withstand relatively high stresses but does not withstand high elongation before breakage. Using the salt leach method, it is also possible to develop porous materials from DVCH. The structure of porous DVCH-based materials consists of a unified network of interconnected channels. This structure is characterized by a high content of open pores of various sizes. Two pore populations can be distinguished: large with a size in the range 150–780 μm (average pore size approx. 435 μm ± 168 μm) and small with a size in the range of 4–22 μm (average pore size 7.7 μm ± 3.3 μm [16–19]. Chitin divalerate is a technologically friendly biopolymer. The good solubility of DVCH in organic solvents (ethanol, DMAC, DMSO, acetone) due to the presence of hydrophobic valeryl groups in C-3 and C-6 positions enables its easy processing of its particles. The DVCH maintains the film-forming ability of chitin, so it can easily be used in the production of threads, films, foams and scaffolds, as well as non-woven fabrics. Biological data show that DVCH is not cytotoxic to fibroblasts and does not cause irritation or allergy to the skin of animals [20]. For the synthesis of chitin dihexanoate (DHCH) it is also possible to use appropriate acid anhydrides and methanesulfonic acid as a catalyst. In order to increase the homogeneity of the solution and better control the temperature in the process, by analogy to the synthesis of the valerate ester, an excess of acid anhydride and methanesulfonic acid are used, the mixture being the reagents and the reaction medium. Optimal methanesulfonic acid to chitin molar ratios are 16:1 and 10:1 for chitin di-hexanoate and chitin di-butyrate, respectively. This approach will result in a high degree of substitution of hydroxyl groups, equal to almost 2, and a low polydispersity. Moreover, under optimal conditions, no hydrolysis of the N-acetyl bond was observed. Good chitin solubility in methanesulfonic acid, even at low temperatures, allows the esterification process to be carried out under milder conditions. The key parameter is the intensity of agitation of the reaction suspension. Insufficient heat transfer due to poor mixing of the solution, similar to the synthesis of chitin di-pentanoate, leads to a lower degree of esterification, high polydispersity of the final product and a reduction in molecular weight. The neutralization process is carried out with a 4% sodium bicarbonate solution. The synthesis of DBC at a low temperature and short reaction time (temperature 0°C and 8°C) is ineffective due

to the low reaction yields and possibly incomplete esterification of the chitin hydroxyl groups, resulting in the formation of a significant amount of insoluble gel fractions when dissolved in acetone prior to precipitation with water. For DHCH, it is preferable to use low synthesis temperatures (0°C and 8°C). The yield of DHCH synthesis was relatively high (above 70%), with the highest efficiency observed at 21°C (84 to 95%). Unfortunately, carrying out the synthesis of DHCH at 21°C resulted in a low molecular weight product. A trend analogous to that of chitin di-pentanoate was observed, indicating that the longer the reaction time, the higher the reaction performance and the lower the molecular weight of the obtained biopolymers. Although in DHCH the hydroxyl groups of chitin are substituted with longer alkyl chains than in DVCH or DBC, it has been found that DHCH retains good solubility in solvents such as ethanol, acetone, dichloromethane, 1,2-dichloroethane, N,N-dimethylformamide, N,N-dimethylacetamide and ethyl acetate and no solubility in water. Good solubility, filmogenic and fiber-forming properties of DHCH give greater possibilities of its processing (film casting, washing method, electrospinning method) compared to chitin alone. The mechanical properties of DHCH and DBC in the form of thin solid layers poured from solution were investigated in relation to their molecular weights. DHCH and DBC showed semi-continuous properties and cracked rapidly upon exceeding the plasticity point, similar to that observed for DVCH. The elongation at break was small and did not exceed 4%. For both biopolymers, their tensile properties correlate with the molecular weight. Parameters such as modulus of elasticity, stress at yield, as well as stress and strain at break, were found to increase with increasing DHCH and DBC molecular weight. Comparing the mechanical properties of DHCH, DBC and DVCH, it can be concluded that Young's modulus decreases with increasing chain length of the acyl group of chitin diesters (a similar relationship is observed for chitin monoesters, where only one hydroxyl group is acylated). Due to the good solubility of hydrophobic chitin diesters in organic solvents and their insolubility in water, it is possible to obtain porous structures based on DHCH and DBC by using the salt leaching method. The prepared porous materials are characterized by a united network of interconnected channels and a high degree of open porosity with pores of various sizes (pore size in the range 78–421 μm, average pore size 253 μm ± 93 μm) [21–24]. Due to its physic-chemical properties, DHCH can successfully replace or support materials based on di-butyrylchitin (e.g. in the form of mixtures of both biopolymers) and thus it can be used as a material for medical and pharmaceutical applications, especially in tissue engineering scaffolds and in healing wounds. The described procedure of chitin esterification to obtain products of high purity. Moreover, this method is universal (the possibility of preparation various chitin diesters) and is easy to produce and is not time-consuming [21]. Another method of chemical modification of chitin is esterification leading to carboxymethylchitin (CMCht, CM-chitin) [22, 23] or dicarboxymethylchitin using monochloroacetic or mono-chloropropionic acid followed by substitution of halogen with a hydroxyl group. This modification leads to the loss of the supramolecular structure of chitin and the formation of water-soluble derivatives [24].

2.2 Mixed chitin esters (co-esters)

The presence of two hydroxyl groups at the C-3 and C-6 positions of chitin allows the introduction of two different acyl substituents, leading to the formation of mixed esters (co-esters) of chitin. This possibility is also due to the differential reactivity of the hydroxyl groups linked to the primary and secondary carbon atoms in chitin. Thus, under ideal conditions, it is possible to obtain mixed esters containing the same molar fraction of different acyl groups in the modified material.

Different ester groups (e.g., butyric and acetic acid residues) are present in mixed esters in a single polysaccharide chain. The replacement of some large bulky butyl groups with much smaller acetate groups in one polysaccharide chain causes that in a condensed state, it becomes possible to obtain favorable conditions for the formation of intermolecular bonds of the hydrogen bridge type. This effect cannot be expected when using a mixture of two different biopolymers (e.g. chitin diacetate and chitin di-butyrate). Thus, the term mixed polymers is not the same as mixed chitin esters. In order to obtain a polymer mixture, it is necessary to use at least two chemically different polymers (**Figure 2**). In contrast mixed ester/co-ester of chitin contains only one component. It was found that the differences between chitin mixed esters (co-esters) and a mixture of two species can be observed in NMR spectra (^{1}H and ^{13}C) (**Figures 3** and **4**). The studies used 50:50 butyryl-acetyl-chitin co-polyester (**2**) (mixed ester), 90:10 butyryl-acetyl-chitin co-polyester (**3**) and a 1: 1 mixture of chitin di-butyrate (**1**) and chitin diacetate (**4**).

A comparative analysis of the ^{13}C-NMR spectra of the 180–150 ppm range characteristic for the chemical shifts of carbon in carboxylic acid derivatives showed that the distribution in the mixed ester of chitin **2** and **3** is different from the carbon signals of the 1: 1 mixture of polymers **1** and **4** (**Figure 3**). A similar result is observed in the range of 10–40 ppm, characteristic for carbons of aliphatic ester residues introduced as a result of esterification of chitin with acetic anhydride and butyric acid (**Figure 3**).

Comparative studies of ^{1}H-NMR spectra in the range of chemical shifts 2.5 ppm to 0.5 ppm also allowed to state that in the case of butyryl-acetyl chitin co-polyesters (samples **2** and **3**) the recorded signals are different than in the case of the 1: 1 mixture of polymers **1** and **4** (**Figure 4**).

The possibility of forming the intermolecular hydrogen bonds that stabilize butyryl-acetyl chitin co-polyester structure translates into fiber-forming properties, and thus the possibility of modulating the functional properties of the final materials and their use in the manufacture of new materials for medical use. In addition to stabilization by hydrogen bonds, it is also possible to create weak interactions based on hydrophobic interactions. The participation of such various weak interactions in the stabilization of materials may translate into their ability to interact with both hydrophobic and hydrophilic structures, which affects biological activity.

2.2.1 Chitin co-esters in dressing materials

Acetate-formate mixed chitin ester was obtained using formic acid, acetic anhydride and trifluoroacetic acid as a catalyst [25]. It turned out that this ester is slightly soluble in typical organic solvents. This is one of the reasons why this derivative has not found practical use, even though its biological properties are comparable to those of chitin. A similar situation was observed in the case of trifluoroacetate-formate derivatives of chitin [26].

Attempts to obtain a mixed butyric acetic ester of chitin by reaction using acetic and butyric anhydrides and methanesulfonic acid or trifluoroacetic acid as catalysts have been unsuccessful. The final product was a mixture of chitin acetate, chitin butyrate and the expected acetate-butyrate of chitin [27, 28].

The approach to synthesize mixed chitin esters using a mixture of trifluoroacetic acid and the corresponding organic acid as catalysts also led to the formation of mixtures of chitin monoesters and mixed esters (co-polyesters) of chitin. It has been shown that carrying out the reaction at the temperature of 70°C for a short time (30 min) under homogeneous conditions allows for obtaining co-polyesters: acetate-butyrate, acetate-hexanoate, acetate-octanoate and acetate-palmitate of chitin. The final co-polyesters have molecular weights ranging from

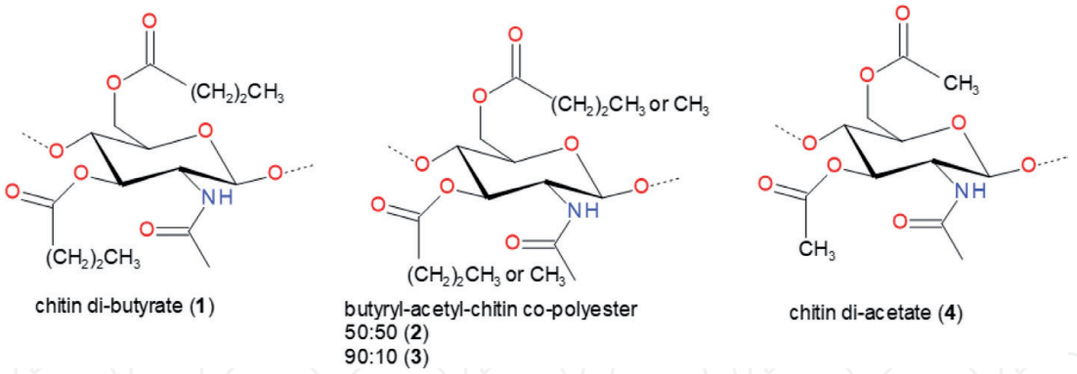

Figure 2.
Chemical structure of chitin di-acetate, chitin di-butyrate, butyryl-acetyl chitin co-polyester (mixture ester, co-ester).

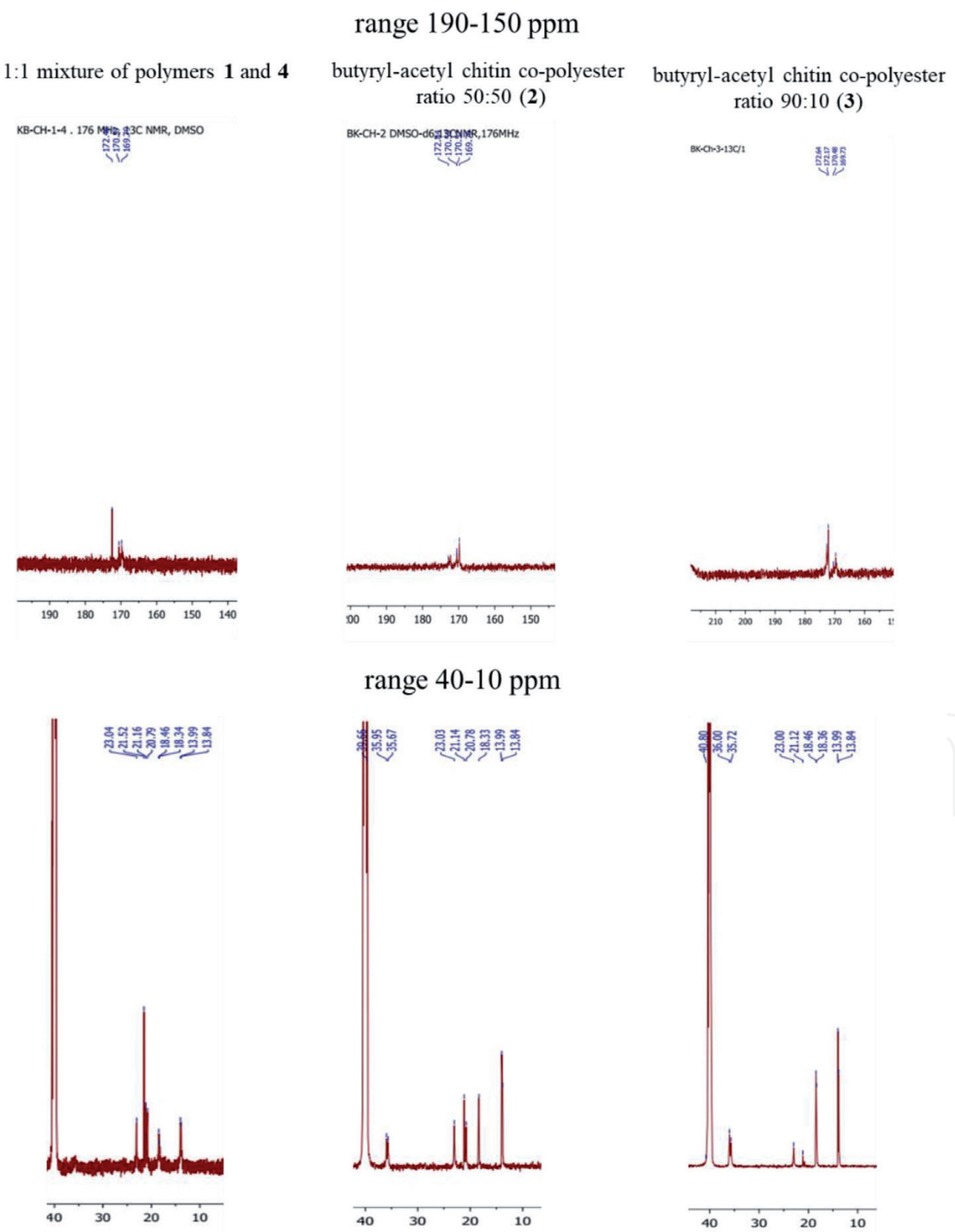

Figure 3.
Fragments of ^{13}C-NMR spectra of **1**, **2**, **3** *and* **4** *derivatives.*

30 to 150 kDa and the degree of esterification ranging from 1.0 to 2.0, depending on the raw materials used.

Another approach to obtain mixed butyryl-acetyl esters of chitin [29] is based on the use of butyric and acetic acid anhydrides and methanesulfonic acid as a catalyst. However, this method is not very friendly from the point of view of industrial stoppage. Thinking about the industrial synthesis of the butyryl-acetyl chitin derivative, it was necessary to establish reaction conditions that would eliminate the need to use methanesulfonic acid.

In the works on the development of a method for the production of the butyryl-acetyl chitin co-polyester on an industrial scale, it was necessary to develop, in the first stage, the conditions for the synthesis on a laboratory scale, which would later be transferred to an industrial scale. The esterification in laboratory conditions is carried out under heterogeneous conditions at 20-25°C, using chloric (VI) acid as a catalyst and a mixture of butyric and acetic acid anhydrides, used in a molar ratio of 90:10 [22, 23]. The product was obtained with a yield of 82 to 89% is soluble in DMF, DMSO and NMP, has a high molar mass (intrinsic viscosity of these products determined in DMF at the level of 2.0–2.05 dL/g) and a full degree of esterification. In the research on the development of a method of producing butyryl-acetyl chitin co-polyester in industrial conditions it was crucial to eliminate the possibility of formation an explosive mixture which may arise as a result of direct contact of acetic anhydride with perchloric acid. It turned out that the priority was to use an efficient cooling system so that the process temperature did not exceed 20°C. In laboratory conditions, it was sufficient to use an ice-water bath with NaCl (brine bath) and intensive mixing of the suspension. In laboratory conditions, diethyl ether is added to the slurry to remove excess unreacted anhydrides and formed carboxylic acids and the crude product is filtered off. The crude acetylation product is washed with water and dilute ammonia water, dried and finally dissolved in ethanol. The transfer of the conditions from the laboratory scale to the macro scale

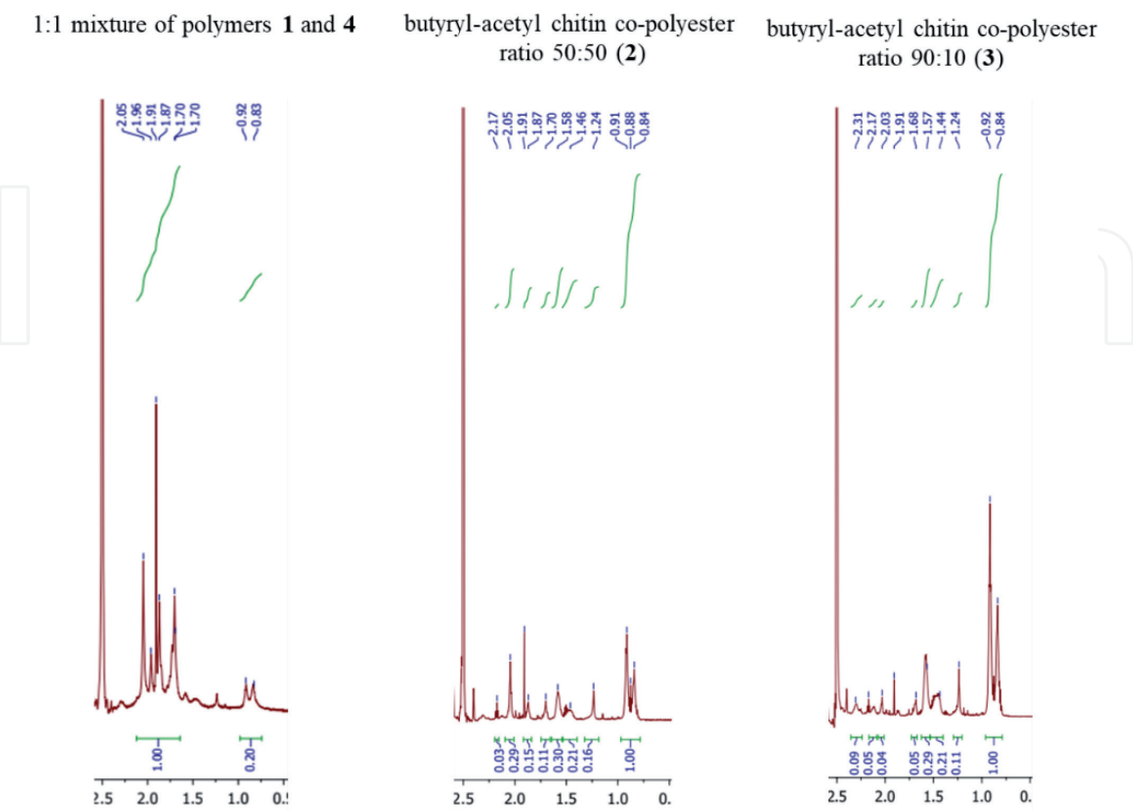

Figure 4.
Fragments of ^{1}H-NMR spectra of **1**, **2**, **3** *and* **4** *derivatives in the range 2.5–0.5 ppm.*

did not involve only increasing the amount of reagents and the size of the synthesizer. The key was to optimize the process conditions and the use of reagents that can be used in industrial conditions from the point of view of safety, environmental impact and economics. A reactor with a capacity of 60 dm^3 with an effective cooling system was used. A 3 kg chitin input was used for the synthesis. The remaining reagents (2 dm^3 of perchloric acid, 15 dm^3 of butyric anhydride and 1 dm^3 of acetic anhydride) were added in portions. The time required for all reagents to be introduced and for complete conversion was about 24 h. In place of diethyl ether, under industrial conditions, ethyl acetate was used to remove excess unreacted butyric and acetic anhydrides. In industrial conditions it was also necessary to replace the ammonia water to neutralize the acetic and butyric acid residues. It turned out that it is possible to use sodium carbonate for this purpose. Also, the step of separation of raw product required changes in the industrial process. In the synthesis under laboratory conditions, G4 Schott funnels were used for filtration. However, the use of this method on a large scale was ineffective. So suction filtration was used, the efficiency of which was 100 dm^3 per hour. The process efficiency on an industrial scale was comparable to that of a laboratory scale synthesis. The physical and chemical properties of the final products were also comparable. The conducted research guaranteed the reproducible and required parameters of the raw material for the production of new medical materials [22, 23]. **Figure 5** shows a set for the synthesis of butyryl-acetyl chitin co-polyester on an industrial scale.

The development of an efficient synthesis of the butyryl-acetyl chitin co-polyester (BAC 90/10) made it possible to demonstrate that the obtained chitin derivative has the ability to form fibers from a wet solution with a strength slightly above 20 cN/tex with high porosity. Fibers with a strength at this level can be the basis for the production of 3D polymer-fiber composites. BAC 90/10 fibers show a stronger predisposition to apatite crystallization; strong sorption tendencies of fibers allowing the possibility of local supersaturation favoring the nucleation of apatite. It has been also found that BAC 90/10 fibers degrade fast under *in vitro* conditions.

One application of the butyryl-acetyl chitin co-polyester (BAC 90/10) is its use to produce highly porous film materials [30].

The research work began with laboratory tests during which two methods of formation of porous materials were tested: (a) pouring a 5% ethanol BAC 90/10 solution on a layer of solid inorganic salt (porophor agent), which, after solidification, was exposed to water to wash out the porophor agent, and (b) the use of porophor

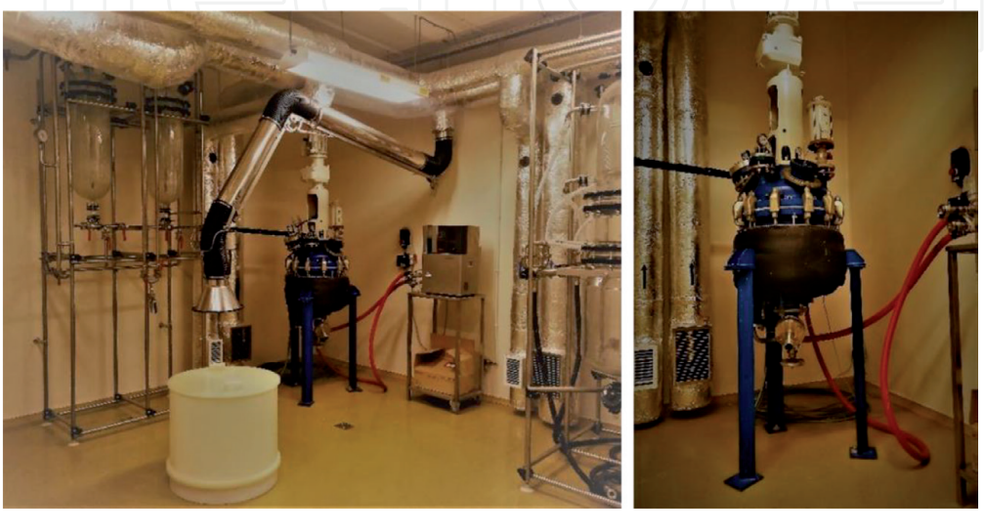

Figure 5.
Set for the synthesis of butyryl-acetyl chitin co-polyester on an industrial scale.

agent suspensions in BAC 90/10 solution, which was a mixture of solvents with a density close to the bulk density of the porophor agent. Various organic and inorganic salts (K_2CO_3, $KHCO_3$, $KHSO_4$, KNO_2, $(NH_4)_2CO_3$, $(NH_4)HCO_3$, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, Na_2CO_3, $NaHCO_3$, Na_2HPO_4, $Na_2S_2O_3$ x $5H_2O$, NaCl, di-ammonium citrate, di-ammonium oxalate were tested. It was found that all tested salts can be used as porophors. However, the most optimal porophor agent, in terms of porosity (95–99%) and tensile strength of 5 cN, was NaCl.

Based on laboratory work, it was possible to start work on optimizing the production of porous dressing materials (Medisorb R, Medisorb R Ag). In the industrial method, solid NaCl as porophor agents and a 3% solution of BAC 90/10 in ethanol were used. The membrane was formed by pouring a 3% solution of BAC 90/10 onto a layer of porophor agent to produce a spongy structure. After drying, the membrane is rinsed in distilled water at 40°C until the porophor agent is washed off. The product is then dried at 80°C. After packing, the obtained membrane dressings are subjected to a process of radiation sterilization (in the case of the variant without the addition of an antibacterial substance). To obtain a silver-coated membrane, the membrane is sprayed with a suspension of metallic silver dispersed in water by means of a spray nozzle. The silver particles are evenly distributed in the suspension using an ultrasonic device. After drying and then packing, the product is subjected to radiation sterilization. The powder dressing is obtained by grinding the byturyl-acetyl chitin co-polyester, which is then sterilized by radiation [23, 31]. **Figure 6** shows a scheme for the preparation of porous dressings based on butyryl-acetyl chitin co-polyester.

Dressing materials obtained by leaching salt from butyryl-acetyl chitin co-polyester (BAC 90/10) and sodium chloride with a diameter of 0.16–0.40 nm and/or microsilver were characterized by a high degree of porosity, pore size in the range of 275–305 nm and a degree of crystallinity in the range of 27.2–27.4%. SEM

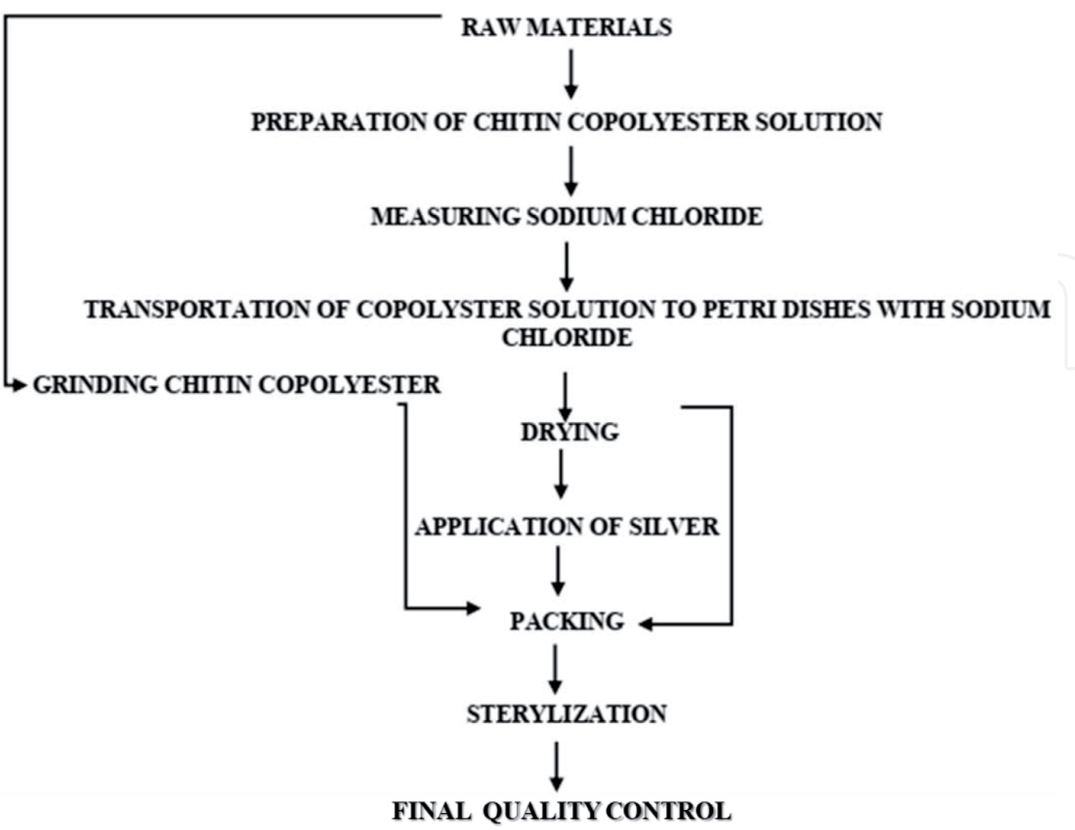

Figure 6.
A scheme for the preparation of porous dressings based on butyryl-acetyl chitin co-polyester.

tests confirmed the porous structure of pores, which are negative for the crystals of the inorganic porophor agent used. In addition, the pores are open pores, which increases the effectiveness of water adsorption. **Figure 7** shows microscopic picture of porous structures obtained by the porophor agent washout method.

Dressings made of butyryl-acetyl chitin co-polyester (**Figure 8**) are intended for wounds of various etiologies, including chronic wounds, where the healing process is disturbed by comorbidities. In order to accelerate the healing of deep wounds, a dressing in the form of a backfill has been designed. Wounds are often accompanied by a bacterial infection, therefore, apart from the dressing in the form of a membrane made of chitin co-polyester only, there is also a variant containing the addition of silver, showing a bactericidal effect in the wound environment. Silver may appear in various forms, however, it has been assumed that only the ionic form of silver has a bactericidal effect. Any other form of silver must be converted to its ionic form. Hence, metallic silver with a small particle size after oxidation and hydrolysis is characterized by the highest antibacterial activity. Silver in ionic form also has the ability to interact with proteins. It has been found that the ionic form of silver has a higher protein binding capacity compared to nanoparticles [32–36].

The presence of pores and microcapillaries in the structure of membrane dressings allows drainage of wound exudate. Dressings made on the basis of chitin co-polyesters are characterized by high biocompatibility. Biological tests confirmed the lack of cytotoxic, irritating and allergenic effects. These dressings are degraded

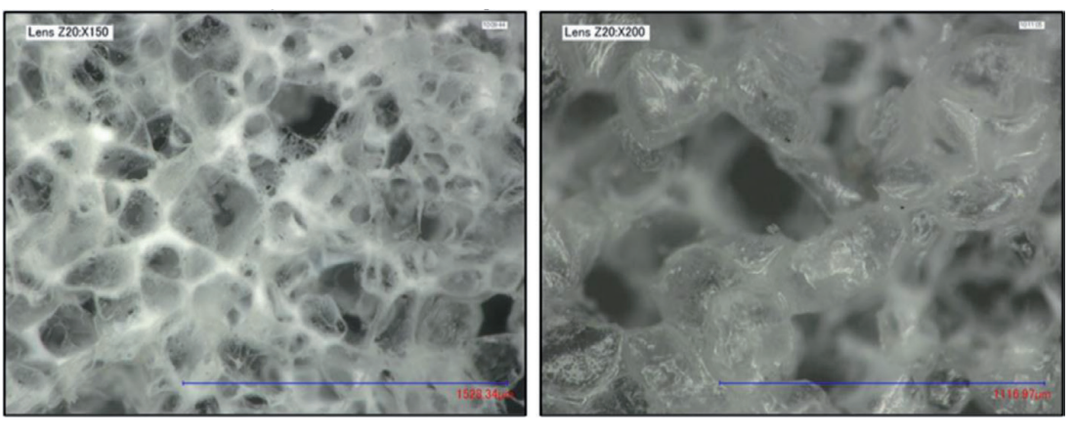

Figure 7.
Microscopic picture of porous structures obtained by the porophor agent washout method.

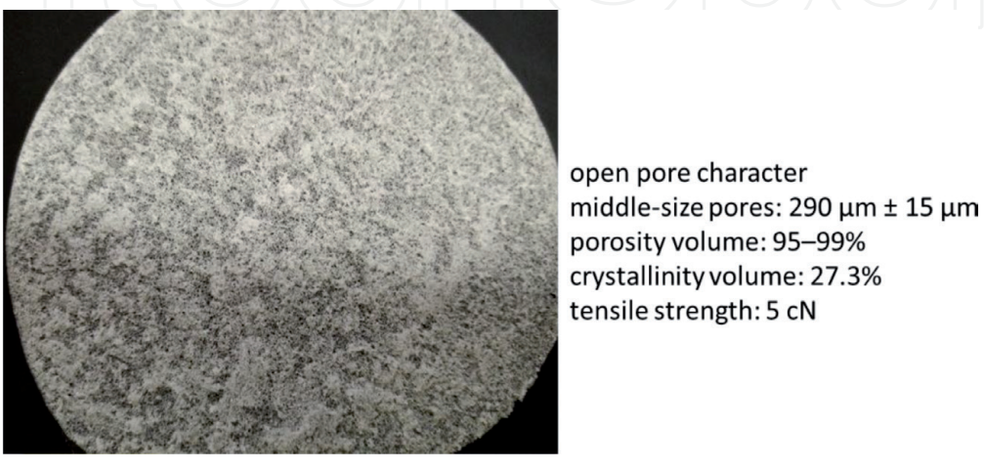

Figure 8.
Picture of porous structures obtained by the porophor agent washout method.

in the subcutaneous tissue and gradually become smaller. The dressing shortens and weakens the exudative phase, drains the wound and accelerates the productive phase. The epithelialization process under the butyryl-acetyl chitin co-polyester was completed faster compared to the control sample [37].

FTIR ATR analysis was made for samples of untreated Medisorb R dressings and material treated with fresh human plasma in order to test the dressing surface degradation and protein absorption on the dressing surface. Comparing spectra of samples treated with fresh human plasma and pure material, the decreasing of intensity of the vibration band of C=O at 1733 cm^{-1} in relation to the amide I signal at 1659 cm^{-1} was observed. It confirmed the sample surface degradation, which was connected to the hydrolysis of ester BAC 90/10 groups. In the *in vivo* tests, the dressings under macroscopic examination, in full thickness defects of subcutaneous tissue and skin caused wound healing with no inflammation, undergoing the most gradual biodegradation between weeks 4 and 8, and the observed differences were statistically significant [37].

The developed biodegradable dressings based on butyryl-acetyl chitin co-polyester were subjected to clinical evaluation using a wide range of patients. The use of dressings significantly accelerated the wound healing process caused by venous insufficiency and diabetes, also in patients whose healing process was disturbed by comorbidities. The improvement of the clinical condition of the wound depends on the individual patient and is most often observed after 30–60 days. The obtained results indicate that the tested dressings significantly reduce the time of wound healing. Medisorb R Ag is more effective than Medisorb R Membrane in the treatment of infected wounds. The powder form (Medisorb R Powder) allows the application of the dressing to deeper wounds. Thanks to their unique structure, dressings drain wound exudates beyond its environment, thus restoring the proper course of the cell reconstruction process. The ability to biodegrade in contact with the wound secretion eliminates the need to replace dressings, so the newly formed granulation tissue is not disturbed - cell reconstruction processes run smoothly [38].

3. Chitosan- raw materials obtained from chitin

Chitosan is obtained as a result of the hydrolysis of chitin N-acetylamide groups (partial deacetylation of chitin). The main advantage of chitosan is its much better solubility in aqueous acid solutions, especially organic acids. Chitin deacetylation by chemical or enzymatic methods allows for obtaining materials with various degrees of hydrolysis (**Figure 9**). However, it is assumed that at least 50% chitin deacetylation is necessary for the material to be classified as chitosan. The degree of deacetylation (DD) is defined as the ratio of the number of free NH_2 groups to the initial number of $NHCOCH_3$ groups present in chitin and is presented in the equation:

$$DD = \frac{N_{NH_2}}{N_{NH_2} + N_{NHCOCH_3}} \cdot 100\%$$

where N - the number of specific units (structural units) in the polymer.

The value of DD affects the biological and physicochemical properties of the polymer, such as solubility, swelling and stability, as well as reactivity.

Chitosan obtained by chemical (concentrated NaOH) or enzymatic (chitin deacetylase) deacetylation of chitin. The first step of preparation of chitosan

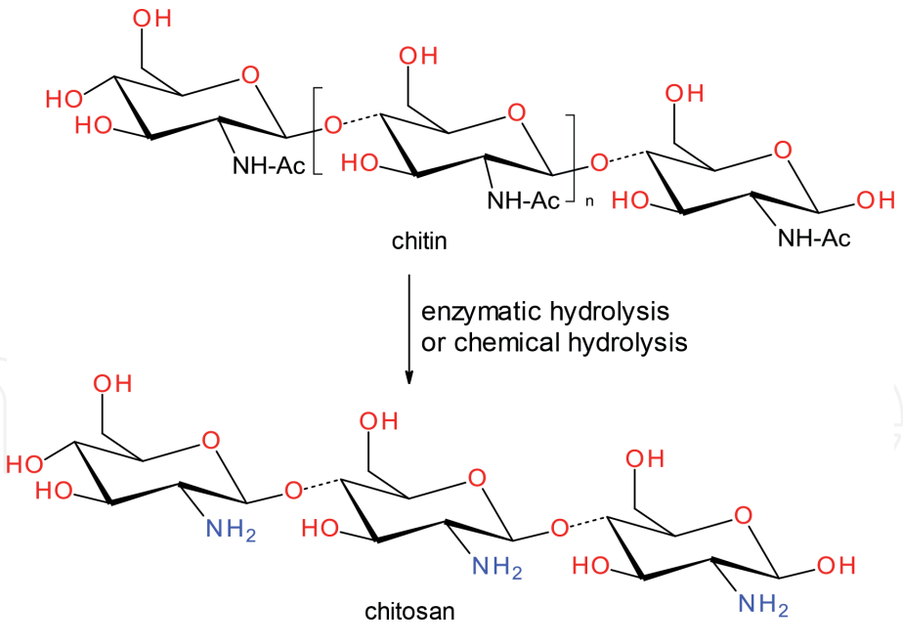

Figure 9.
Chitosan formation from chitin by chitin deacetylation.

is mechanical grinding of the raw chitin, and subsequent process of removing proteins, color compounds and inorganic salts takes place. The deproteinization process is usually performed with a dilute aqueous solution of sodium hydroxide at an elevated temperature [4, 5]. For protein removing also proteolytic enzymes were used [39, 40], but in the case of papain, trypsin and chymotrypsin, it was found that they did not completely remove the protein fraction. After deproteinization process, the residue is treated with dilute aqueous hydrochloric acid to dissolve the calcium carbonate. A similar effect can be obtained by using HCOOH, HNO_3, H_2SO_4 or EDTA [5]. The decolorization process is based on extraction with ethanol, acetone or treatment with oxidizing reagents ($KMnO_4$, NaOCl, H_2O_2). These activities allow to obtain chitin of required purity for its further transformation into chitosan. Chitosan from chitin obtained by chemical deacetylation is obtained at high temperature (above 100°C), under high pressure and in the presence of concentrated (40–50%) strong bases (usually NaOH). A typical industrial chitosan production process provides almost complete recovery of proteins, calcium oxide or calcium carbonate, carotenoid pigments and sodium acetate under the conditions of using sodium hydroxide as the deacetylating agent. However, this process has several disadvantages. It is not environmentally friendly as it consumes a large amount of energy and is also difficult to control leading to a heterogeneous product. The use of chitin deacetylase for the production of chitosan oligomers and polymers can potentially eliminate most of these drawbacks [41]. The advantages of enzymatic deacetylation are more pronounced in the processing of chitin oligomers, as these substrates are soluble in the aqueous medium and are therefore more susceptible to enzymes. The downside is the high cost of the enzyme and the requirement to use pre-processed chitin. The conditions of the chitin deacetylation process have a significant impact on the distribution of N-acetyl-D-glucosamine and D-glucosamine groups in the polysaccharide chain. Their location in the chain has a significant impact on the physicochemical properties of the material, such as crystallinity, solubility and susceptibility to degradation [3]. Depending on the final use, chitosan can be formed into a hydrogel, membranes, fibers, sponges and micro/nanoparticles [42].

4. Chitosan parameters

4.1 Physio-mechanical parameters of chitosan

Chitosan is a polysaccharide composed of randomly distributed acetylated and deacetylated units of D-glucosamine. Chitosan exists in five different crystal forms, four of which are hydrated and one is anhydrous. Microcrystalline chitosan is characterized by better biodegradability and bioactivity.

Most of the properties of chitosan depend on two parameters: degree of deacetylation and molecular weight distribution. Depending on the source and method of preparation, the deacetylation degree varies from 30 to 95%, and the molecular weight from 300 to over 1000 kDa [43]. The solubility of chitosan strongly depends on the deacetylation degree, which translates into the number of free amino groups. Chitosan is soluble in acidic solutions due to its susceptibility to protonation and formation of ammonium salts. It is soluble in acetic, formic, citric, lactic and hydrochloric acid and insoluble in most organic solvents. Chitosan, as a biodegradable polymer, is easily broken down by microorganisms into simple chemical compounds such as carbon dioxide (CO_2) and ammonia (NH_3). Like other biopolymers, it is susceptible to many chemical and physical factors leading to its degradation. The degradation process also depends on the degree of deacetylation and the molecular weight of the polymer [3, 5].

Chitosan has many valuable properties, such as: biocompatibility, biodegradability, non-toxicity, the ability to create polycations in an acidic environment, the possibility of modification, high affinity for metals, dyes and proteins, hydrophilicity, ability to create membranes and others [3, 5, 44]. These features make it applicable in medicine and pharmacy, in various industries, in environmental protection, including water treatment and separation processes. [5, 45, 46]. Chitosan also has a number of properties that limit its use in certain areas. It swells strongly in water (especially in an acidic environment), and in the swollen state it is characterized by low mechanical strength. The use of chitosan is also limited due to its high viscosity. The reduction of the viscosity of chitosan solutions can be achieved by increasing the deacetylation degree while reducing the molecular weight and increasing the temperature or ionic strength [5, 47]. The key problem with the use of chitosan is its susceptibility to external factors (humidity and temperature) and processing conditions (heating or sterilization), which can affect its structure and cause its degradation. Parameters such as molecular weight or the presence of impurities have a significant impact on the processing of chitosan [48]. This causes difficulties in maintaining the stability of chitosan (no changes in molecular weight) for a long time at room temperature [49]. The influence of many factors, such as increased temperature, the presence of strong acids, mechanical shear or radiation, on the molecular weight of chitosan was demonstrated. It is also believed that high molecular weight chitosan is more stable. The lack of reproducibility in the processing of chitosan is also due to significant differences in molecular weight, deacetylation degree and purity level depending on the source of the raw material. The level of chitosan purity may affect both biological properties, such as biodegradability or immunogenicity, as well as its solubility and stability [48, 50].

4.2 Biological parameters of chitosan

Chitosan is a non-toxic polysaccharide containing randomly distributed acetylated and deacetylated units of D-glucosamine. The results of many studies confirm

the antibacterial effect of chitosan. The mechanism explaining this feature is unknown [51]. The antimicrobial activity of chitosan is strongly dependent on many factors, such as molecular weight [52], degree of deacetylation (DD), pH of the dissolving medium and its ionic strength. Stronger antibacterial activity was observed with a high degree of deacetylation [53] and a low molecular weight of chitosan [54]. The antibacterial activity of chitosan is also associated with the form of the polymer (hydrogels, membranes, dissolved form) and the presence of other compounds [55]. One of the factors responsible for the antibacterial activity of chitosan is its cationic nature. The positively charged ammonium groups of chitosan may interact with negatively charged components of the bacterial cell wall, causing damage to the cell membrane and destruction of bacterial cells (a mechanism proposed for high molecular weight chitosan) [56]. Ultimately, this causes the formation of an impermeable layer around the bacterial cell, affecting permeability and transport to the cell [57, 58]. It has been suggested that low molecular weight chitosan can penetrate bacterial cell walls and eventually enter the cytoplasm and bind to DNA affecting DNA transcription, mRNA synthesis and finally protein biosynthesis [59].

The difference in the hydrophilicity and the negative charge of the cell surface of the bacteria makes gram-negative bacteria interact more strongly with chitosan, resulting in higher antibacterial activity against them.

The antibacterial activity of chitosan or its derivatives on gram-negative bacteria has been demonstrated for various strains: *Escherichia coli*, *E. coli* K88, *E. coli* ATCC 25922, *E. coli* O157, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella enteritidis*, *S. choleraesuis* ATCC 50020, *S. typhimurium*, *S. typhimurium* ATCC 50013, *Enterobacter aerogenes*, *Listeria monocytogenes* [60–64]. The antibacterial activity of chitosan or its derivatives on gram-positive bacteria has been demonstrated for: *Staphylococcus aureus*, *S. aureus* ATCC 25923, *Corynebacterium*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus cereus*, *Bacillus megaterium*. This indicates a broad spectrum of chitosan activity. It was also found that the effectiveness of chitosan binding to the bacterial cell wall depends on the pH value. At low pH, chitosan shows better adsorption to the bacterial cell wall due to the increased positive charge of protonated amino groups [65–67].

The antifungal activity of chitosan also depends on its molecular weight and degree of acetylation. It was found that chitosan shows antifungal activity against selected phytopathogenic fungi *Penicillium* sp. in citrus [68], *Botrytis cinerea* in cucumber [69], *Phytophthora infestans* [70], *Alternaria solani* and *Fusarium oxysporum* [71]. Chitosan is also active against fungal species pathogenic to humans, while being non-toxic to human cells. The antifungal activity of chitosan or its derivatives has been demonstrated against: *Candida albicans*, *Candida parapsilosis*, *Candida krusei*, *Candida glabrata*, *Penicillium digitatum*, *Penicillium italicum*, *Fusarium proliferatum*, *Hamigera avellanea*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Cryptus stolofattans*. The suggested mechanism of action is to create a permeable layer by chitosan which disrupts fungal growth [72–75]. It is believed to be related to the activation of defense processes, including chitinse accumulation, synthesis of proteinase inhibitors, callus synthesis and the lignification process [76].

The antiviral activity of chitosan derivatives is also suggested. The research focuses mainly on HIV. Peptide-chitosan conjugates (GlnMetTrp-chitosan and TrpMetGln-chitosan) show a protective effect on C8166 cells by counteracting the cytolytic effects of the HIV-1RF strain. These derivatives have the ability to suppress HIV-induced syncytium formation and reduce HIV load without inhibiting HIV-1 reverse transcriptase and protease *in vitro* [77]. Sulfated low molecular weight chitosan derivatives inhibit HIV-1 replication, HIV-1 induced syncytium formation, lytic activity and p24 antigen production. These derivatives are believed to influence the binding of HIV-1 gp120 to the CD4 cell surface receptor [78]. In comparative studies, chitosan conjugates with

thioglycolic acid, lactic acid, PEG and antiviral drugs, significantly higher efficacy was observed compared to the use of the drug alone [79–85].

Recent studies have shown that chitosan and its derivatives exhibit anti-tumor activity in both *in vitro* and *in vivo* models. It was found that chitosan derivatives increased the secretion of interleukin-1 and 2, influencing the maturation and infiltration of cytotoxic T lymphocytes [86]. The results have been confirmed in an *in vivo* study [87]. Moreover, a direct cytotoxic effect on neoplastic cells was found by inducing apoptosis. For A375, SKMEL28 and RPMI7951 cancer cell lines it was found to reduce cell adhesion, inhibit proliferation, inhibit specific caspases, regulate the activity of Bax as well as Bcl-2 and Bcl-XL, induce CD95 receptor expression through greater susceptibility to chitosan-induced apoptosis by FasL [88]. In the case of the PC3 A549, HepG2 and LCC cell lines, in the presence of chitosan, inhibition of the growth of neoplastic cells and inhibition of MMP-9 expression, hampering of cells in the S phase and reduction of the rate of DNA synthesis, p21 and PCNA regulation were found [89]. HepG2 and LCC xenografts in a mouse model showed inhibition of tumor growth and a reduction in the number of metastatic colonies at the dose of 500 mg/kg [90]. Carboxymethylated chitosan [91] inhibited hydrogen peroxide-induced apoptosis in Schwann cells by reducing caspase-3, –9 and Bax activity and increasing Bcl-2 activity.

Various biological properties of chitosan also include good adhesion to cells, macrophage activation, stimulation of fibroblast proliferation, stimulation of cytokinin production, stimulation of type IV collagen synthesis, promoting angiogenesis processes, haemostatic properties [92–95]. Moreover, it has a positive effect on granulation and epithelialization, and reduces scar formation. It is believed that many of the listed biological activities of chitosan are related to its unique feature, namely its cationic nature. Chitosan molecules with a positive charge interact with negatively charged erythrocytes and thrombocytes activating the extrinsic coagulation, effectively stopping bleeding.

Chemical modification of chitosan allows to modulate the biological activity of chitosan, for example, heparin inactivation or antiviral activity. Chitosan can be in the form of: gel, sponge, fiber or porous composition with ceramics, collagen or gelatin. Chitosan is used as a component of wound healing dressings, while in the case of scaffolds, it is usually used with other natural polymers (hyaluronic acid, alginic acid, poly-L-lactidic acid, elastin, collagen, gelatin) or additives (hydroxyapatite, calcium phosphate, ceramic components) [95–97].

Chitosan increases the inflow of phagocytic cells (segmented granulocytes and macrophages) to the site of infection, stimulates the migration and proliferation of endothelial cells and fibroblasts. The effect of chitosan on the proliferation of fibroblasts depends on the degree of deacetylation and molecular weight. Forms with a higher degree of deacetylation and lower molecular weight stimulate fibroblast proliferation to a greater extent [98–123]. Chitosan is widely researched for its use in bone and cartilage reconstruction. It has the ability to create porous structures, which makes it used in tissue engineering, orthopedics and bone regeneration. It has also been used in drug delivery systems or therapeutic substances (DNA plasmids, siRNA, nanosilver), for the production of surgical sutures, wound healing dressings and artificial internal organs [124–150].

An interesting chitosan feature is also its ability to bind with mucus and cross epithelial barriers, so that its use as an adjuvant or auxiliary adjuvant in vaccines is considered. It is also included among the auxiliary substances that enable the preparation of various forms of drugs with specific properties.

It is an excellent metal ion complexing agent. This parameter is useful due to the immobilization of metal ions with antibacterial activity and enabling their controlled release, depending on the needs [97].

Chitosan can also be an environmentally friendly agent used to obtain textiles with antibacterial properties. Attempts were made to introduce chitosan powder into cotton and polyester-cotton fabric. Chitosan was introduced after the fabric surface was activated by 20% NaOH. The performed studies confirmed that chitosan is well implemented in fabrics made of a cotton and polyester/cotton blend [151].

4.3 Chitosan in dressing materials

Due to its physicochemical and biological properties, chitosan and its derivatives are considered to be versatile biomaterials with various biological activities [152–159].

Chitosan and its derivatives as materials with antimicrobial activity and low immunogenicity are widely used in wound healing. They provide a three-dimensional matrix for tissue growth, activate macrophages and stimulate cell proliferation [160]. Chitosan improves the activity of polymorphonuclear leukocytes, macrophages and fibroblasts, which increase granulation and organization of repaired tissues [161]. Its degradation to N-acetyl-β-D-glucosamine stimulates the proliferation of fibroblasts, supports regular collagen deposition, and also stimulates the synthesis of hyaluronic acid at the wound site. These properties accelerate healing and prevent scarring [162]. The development of chitosan formation in the form of nanofibers with the assumed adhesive properties allowed to obtain a material useful for the creation of dressing materials [163]. Chitosan nanofibers obtained by electrospinning method are porous, have high tensile strength, large surface area combined with an ideal rate of water vapor and oxygen transfer. They are also compatible with stem cells derived from adipose tissue, which is beneficial for wound healing [164, 165].

A characteristic feature of chitosan dressings is their ability to effectively control bleeding [166]. The most important element of hemostasis is blood clotting, which leads to the formation of a clot consisting mainly of the fibrin network and platelets embedded in it. This process prevents further loss of fluid and electrolytes from the wound and reduces contamination of the wound. There is erythema around the wound, swelling, pain and locally increased temperature. Inflammation widens local blood vessels, which facilitates the penetration of macrophage cells and fibroblasts into the wound, which cleanse the wound of tissue residues, vascular clots and pathogenic bacteria. In the next phase of healing, fibroblasts synthesize collagen and other proteins needed to build and regenerate connective tissue and rebuild damaged blood vessels. In the course of scar formation, type III collagen fibers transform into type I collagen until they reach the balance characteristic of healthy skin and are necessary to restore skin continuity. The final remodeling process leads to a significant increase in the mechanical strength of the wound. The haemostatic effect of chitosan has been clearly documented. Chitosan in the form of a non-woven fabric has a positive effect on each stage of wound healing. The unique features of chitosan include: macrophage activation, stimulation of fibroblast proliferation, absorption of growth factors, stimulation of cytokinin production, stimulation of type IV collagen synthesis, support for angiogenesis processes, antibacterial and hemostatic properties. The positive effect of chitosan on granulation tissue, epidermis and reduction of scar formation has been proven. Like chitin, chitosan is susceptible to enzymatic biodegradation which produces biologically active oligosaccharides. The positively charged chitosan molecules react with negatively charged erythrocytes and thrombocytes to activate the external clotting pathway and effectively block bleeding. At the same time, chitosan can serve as a carrier of specific medicinal substances (DNA plasmids, siRNA, nanosilver particles), which enhance its positive effect on the healing process. Chitosan has also been found to significantly increase the adhesion and aggregation of platelets in the process of hemostasis [167, 168].

Currently, there are many chitosan materials available on the market that are used to heal wounds in patients undergoing plastic surgery [169], skin grafting [170, 171] and endoscopic sinus surgery [172]. Chitosan-containing materials in the form of nonwovens, nanofibers, composites, films and sponges are: HemCon®, GuardaCare®, ChitoFlex®, ChitoGauze®, Celox™ Granules, Celox™ Gauze, Chito-Seal™, Clo-SurPLUS PAD Tegasorb™, Tegaderm™ ChiGel, ChitopackC®, and TraumaStat™ [173–176].

Haemostatic dressings also include Tromboguard® - a multi-layer dressing made of three layers: semi-permeable polyurethane foil, hydrophilic polyurethane foam, and a layer containing chitosan. The film layer protects the dressing against seepage, allowing the wound environment to remain moist, ensuring optimal air permeability to its interior and creating a barrier against external factors. Polyurethane foam is a load-bearing layer and has strong absorbent properties thanks to the "pore-in-pore" structure. The polyurethane layer is responsible for storing exudate and keeping it outside the wound surface, ensuring adequate wound moisture. Additionally, it is a layer that protects the wound against mechanical damage.

The active layer, which is created by a unique composition of chitosan and alginates, activates the blood coagulation process, significantly reducing bleeding time. By reacting on the wound surface with erythro- and thrombocytes, chitosan significantly shortens the bleeding time. Calcium alginate accelerates the natural clotting process, and sodium alginate - by absorbing wound discharge - creates a layer of gel on the surface of the dressing that prevents it from sticking to the wound. Alginates are resorbable, non-toxic, non-carcinogenic, non-allergic and haemostatic [177]. When used as dressing materials, it is important that during contact with the wound, part of the alginate dressing passes in the form of a gel, which prevents the wound surface from drying out, and thus creates the possibility of creating a favorable, moist environment within the skin lesion [178]. At the same time, hemostatic properties result in a faster wound healing process and allow for more effective scarring. Patients also benefit from using these dressings to reduce pain when changing them. A significant advantage of using alginate-containing dressings is the elimination of the dressing sticking to the wound and high absorbency.

The Tromboguard® dressing (**Figure 10**) is used to stop bleeding in the case of: traumatic wounds, postoperative wounds, skin graft collection sites in surgery and reconstructive surgery - including combustiology, wounds requiring emergency care, gunshot and puncture wounds, wounds resulting from traffic accidents. It is characterized by a quick hemostatic effect (stops bleeding in 3 minutes), an antibacterial effect inside the product (protecting the dressing against the growth of microorganisms), and effective blood absorption even under pressure. It is not irritating, sensitizing and cytotoxic.

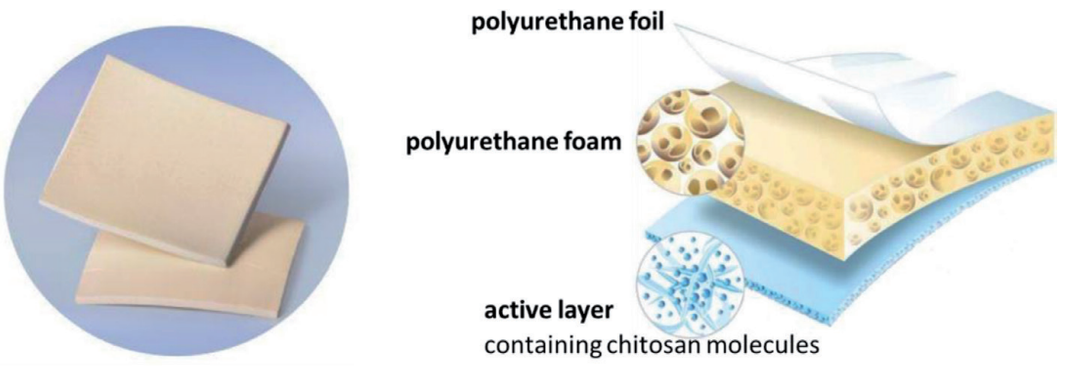

Figure 10.
Tromboguard® dressing structure.

Tests of operational parameters: tensile strength, the ability to adapt to the injury site or the transmission of moisture vapors have shown that this dressing has a tensile strength (for porous materials) of min. 75 kPa (according to PN-EN ISO 1798), which corresponds to the value recommended for dressing materials, and vapor permeability (transmission of moisture vapor) of min. 400 g/m^3/24h.

The results of clinical trials have demonstrated the high haemostatic efficacy of Tromboguard®. The high effectiveness and durability of the antihaemorrhagic effect was confirmed 24 hours after application, which allowed the introduction of an absorbent foam dressing [179] and a three-layer hemostatic dressing to the market [96, 97].

5. Conclusion

Chitin and its ester derivatives, as well as chitosan obtained as a result of chitin deacetylation, have many valuable chemical, physical and biological properties that determine their use in many areas, also in medicine.

The widest use of chitin and its derivatives is observed in biomedical sciences, in particular: in dressing materials (active dressings), active substance carriers (drugs and growth factors), in tissue engineering (cell scaffolds - scaffolds, mainly orthopedics) and in regenerative medicine (stem cell differentiation). Chitin accelerates the wound healing process by having a beneficial effect on processes such as angiogenesis, granulation, epithelialization and scar formation, which play a key role in the physiological wound healing process. It increases the inflow of phagocytic cells (segmented granulocytes and macrophages) to the site of infection, stimulates the migration and proliferation of endothelial cells and fibroblasts. Chitin derivative dressings are considered to be very effective medical devices in the healing of difficult-to-heal wounds [6–11].

The results of clinical trials with dressings based on butyryl-acetyl chitin co-polyesters have also shown their high effectiveness in healing wounds of various etiologies, mainly those caused by chronic venous insufficiency and diabetes. Their use leads to a reduction in the ulcer area and its depth. These dressings were assessed as having a high safety profile [38].

On the other hand, the results of clinical trials with chitosan dressings showed high effectiveness and durability of the anti-haemorrhagic effect. These studies also confirmed the safety of the dressing [96]. The antibacterial test confirmed that the dressing is bactericidal. Thus, there are currently many different hemostatic dressings based on chitosan on the market.

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Conflict of interest

The authors declare no conflict of interest.

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