THE USE OF CHITIN AND CHITOSAN IN MANUFACTURING DRESSING MATERIALS

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Abstract

Despite continuous progress in the development of advanced dressing materials, there is a constant need for dressings used in an environment of infected and hard-to-heal wounds. Dressings that meet the above described requirements are products based on chitin and its derivatives. Chitosan and chitin derivative dressings are now becoming a very effective medical device in healing hard-to-heal wounds, as well as in the control of severely bleeding wounds. Chitosan and chitin are particularly valuable raw materials that accelerate wound healing processes, and they are also biocompatible and antibacterial. Dressings made of butyric-acetic chitin copolyester are intended for treating wounds of various aetiologies, including chronic wounds in which the healing process is disturbed by concomitant diseases. Materials based on chitosan are also widely used in the area of heavily bleeding and chronic wounds.

Keywords: *chitin, chitosan, chitin copolyester, wound dressing, biological properties of wide spectrum of action*

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1. Introduction

Chitin and chitosan are polymeric materials, which are defined as natural or synthetic substances whose characteristic features are: low density, resistance to corrosion and – in a significant number of cases – the inability to conduct electricity. Due to their chemical structure, polymers are classified as macromolecular compounds, composed of organic or inorganic molecules that combine in order to form a polymer chain using covalent, coordinate covalent, hydrogen and ionic interactions. The mechanical, physicochemical, processing and functional properties of polymers depend on the nature of the bond that occurs between its structural units. A group of polymers of natural origin and a group of polymers of synthetic origin can be distinguished. Natural polymers are divided into three classes: polysaccharides, proteins and polyesters. Among the polysaccharides, the most well-known substances are: hyaluronic acid, chitin and chitosan and cellulose; whereas among proteins, it is collagen and elastin.

Owing to the fact that these polymers are raw materials for medical devices (that contact the patient's body), they must meet several requirements: they should retain their physicochemical properties after treatment at elevated temperature during sterilization, and they should also retain them after being in contact with X-rays, detergents or disinfectants. Polymers, like most materials, degrade after a certain period of time; hence, it is also important that their decomposition products do not cause inflammation, allergic and immunological reactions and do not enter in any other interaction with the human body.

2. Chitin as a Raw Material for Manufacturing Dressings

Chitin $(C_8H_{13}O_5N)_m$ is a polysaccharide made of glucosamine residues connected by β-(1→4)-glycosidic bonds. Chitin is the main component of the walls of fungi and armour of arthropods (crustaceans, insects and arachnids), but it is also present in sponges, corals and molluscs. However, for laboratory work and industrial purposes, it is obtained mainly from marine invertebrates such as crabs, shrimp, lobsters and krill. The methods of isolating chitin from natural sources are strictly dependent on the choice of the organism from which chitin is obtained. This polysaccharide has a similar structure to cellulose. It differs by the presence of the acetamide group ($-NHCOCH_3$) in place of one of the hydroxyl groups. Chitin has much stronger intermolecular hydrogen bonds, which results in its greater mechanical strength compared with cellulose [1, 2]. Fig. 1 shows structural similarity of cellulose, chitin and chitosan.

Depending on the origin source, chitin can occur in three amorphous forms: α, β and γ [2, 3]. The most common is α-chitin (found in fungi, shells of crustaceans and krill and insect skeletons). β-Chitin is much less common and can be mainly isolated from squids. 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 the medical industry. α-Chitin is moderately soluble in: aqueous thiourea solution, aqueous alkaline urea solution, 5% lithium chloride (LiCl)/dimethylacetamide solution, some ionic liquids, hexafluoroacetone, hexafluoro-2-propanol and methanesulfonic acid [4, 5]. On the other hand, β-chitin swells in water (forms a suspension) and it is soluble in formic acid. Chitin has no *in vitro* cytotoxic effect, is physiologically inert and biodegradable, has antibacterial properties and has a high affinity for proteins. During its biodegradation, oligomers and structural units are released in the wound environment. β-Chitin most often occurs in the following forms: gel, membranes, fibres, polymer films or is a component of polymer blends. Chitin activates macrophages, stimulates fibroblast

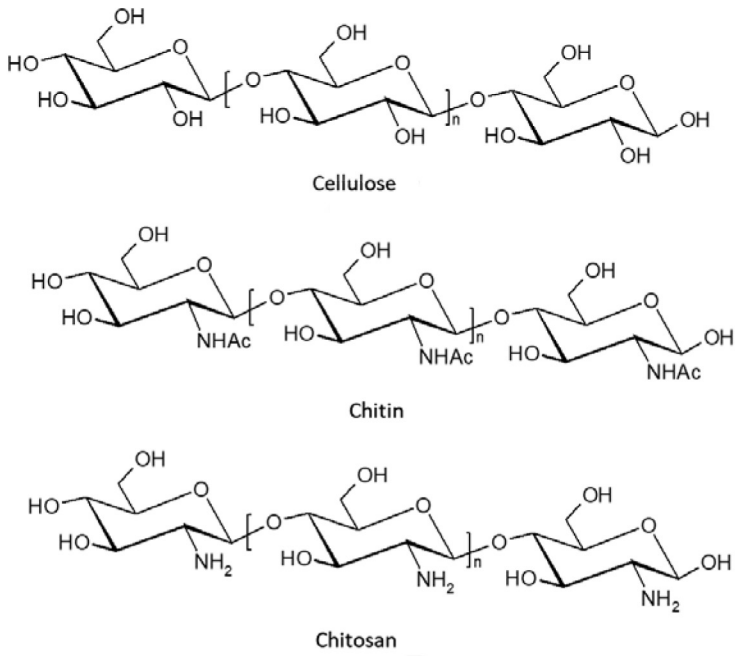

Figure 1. Structural similarity of cellulose, chitin and chitosan.

proliferation and affects vascularization [6–11]. The practical use of chitin relates to its ester and amine derivatives, which is due to chitin's low solubility in typical solvents.

2.1. Chitin Esters as a Raw Materials for Manufacturing Dressing Materials

Examples of chitin-based dressings are Dibucell (manufacturer: Celther Polska Ltc) and Beschitin (manufacturer: Unika), only available in Japan. The esterification of chitin hydroxyl groups increase the use potential of this polysaccharide by allowing the incorporation of various substituents. This modification affects the physical, chemical and biological properties of chitin esters.

The best known are chitin esters in which hydroxyl groups are esterified with one type of esterifying reagent. Acetylated chitin derivatives are obtained by reaction with acetic anhydride in the presence of an acid catalyst; however, the physicochemical properties of the final derivatives are not satisfactory [12]. The use of a mixture of orthophosphoric acid and trifluoroacetic anhydride as catalysts allows the preparation of various chitin esters with: butyric acid, cyclopropane carboxylic acid, cyclobutane carboxylic acid, cyclopentane carboxylic acid, cyclohexane carboxylic acid and substituted aromatic acids. In the case of chitin butyrate, the process efficiency (observed DS – degree of substitution of hydroxyl groups, from 1.9 to 2.38) depends on the excess of butyric acid anhydride used [13–15].

Dibutyrylchitin (DBC) is an example of a chitin derivative soluble in typical organic solvents [16]. DBC is a modern biodegradable biomaterial that is obtained as a result of the esterification of chitin with butyric acid anhydride. The process usually has two stages. In the first stage, chitin is purified from calcium salts at room temperature with 2 mol/dm^3 hydrochloric acid. The next stage is the process of proper esterification of purified chitin. The substrates of this reaction, besides chitin, are butyric anhydride

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and a catalyst, which most often is chloric (VII) acid. The reactions are carried out under heterogeneous conditions by adding chitin powder to the reaction mixture of butyric acid anhydride and chloric (VII) acid in the appropriate proportions. The classic esterification process requires the use of substrates in a 10:1 molar ratio of acid anhydride to N-acetyl amino glucose structural units, respectively, and it usually takes place at 20°C. Increasing the reaction temperature to 40°C causes a rapid reduction in the molecular weight of the obtained polymer. The catalyst concentration has a direct effect on the yield of the butyrylization reaction, which improves as the acid concentration increases. However, it should be remembered that the use of too much chloric (VII) acid results in a competitive reaction – degradation of the macromolecule. The esterification process is completed by adding ethyl ether to the reaction mixture. The isolated product is then heated with water in order to remove residual chloric (VII) acid. The crude product is treated for 24 h with acetone, in which only DBC dissolves. The solution is then concentrated to a 5%–6% solution. After reaching the assumed concentration, the solution is poured into deionized water to precipitate the polymer; subsequent filtration and drying leads to the solid DBC form. The process of butyrylization of chitin described above causes the conversion of free hydroxyl groups at the C3 and C6 carbon of the saccharide ring into ester groups (butyrate). Therefore, DBC is composed of dibutryl-N-acetyl amino glucose units connected by β-(1→4)-glycosidic bonds. The polymer is also stabilized by hydrogen bonds between polymer chains. The hydrogen bonds are formed with the use of a hydrogen atom of the acetylamino group and an oxygen atom of the ester group. This type of intermolecular interaction determines good mechanical properties of the prepared biopolymer. DBC does not dissolve or swell in water. However, it dissolves in many popular organic solvents such as: acetone, methanol, ethanol, tetrahydrofuran, dimethylformamide, chloroform, methylene chloride and others. DBC is not easily degraded; it is resistant to gamma radiation, while enzymatic degradation using enzymes such as lysozyme or econase CE occurs at a low rate and with a slight change in molecular weight. The bioactive properties of this biopolymer include: prolonged blood coagulation time, good wettability and the previously mentioned resistance to gamma radiation (this is important when materials made of this polymer are to be subjected to radiation sterilization).

DBC, with a molecular weight > 100,000 Da, has film-forming and fibre-forming properties. Preparation of DBC with the desired molecular weights directly determines its further processing capabilities (in particular, electrospinning and leaching). Fig. 2 presents a dressing composed of DBC. The use of DBC dressings has a positive effect on the granulation process (increasing the level of glycosaminoglycans in the wound) and collagen crosslinking (the formation of more durable tissue); it accelerates the healing process of wounds with the formation of a healthy epidermis without scars and protecting the wound from excessive moisture loss (optimal moist environment). During treatment, the dressing is slowly biodegraded and resorbed until its complete disappearance, which eliminates the painful dressing change. Spontaneous analgaesic effects of the dressing have also been observed. DBC does not show cytotoxicity or cause irritation, and it is a biocompatible polymer. A previous study described textile dressings containing DBC or regenerated chitin (RC) [17]. The obtained dressings were cut into 5 × 5 cm pieces, sterilised by ethylene oxide and then subjected to biological evaluation required for medical devices. The tests included cytotoxic effects, cytokine levels – tumour necrosis factor α (TNFα) and interferons (IFNs) – synthesis of nitrogen oxides (NO_2/NO_3), intracutaneous irritation and the influence of full thickness skin lesions on the healing process. DBC and RC did not cause cytotoxic effects or primary

irritation *in vitro* or *in vivo* and did not elevate TNFα, IFNs or nitrogen oxide levels; both had a positive influence on the wound healing process.

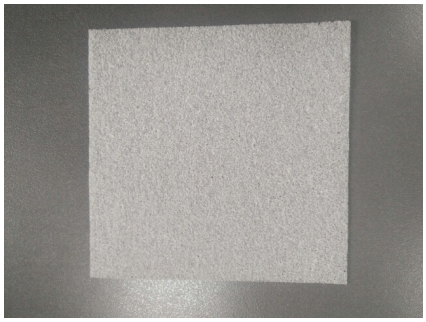

Figure 2. A dibutyrylchitin dressing.

DBC fibres can be obtained by two methods: wet and dry-wet. The choice of method determines the structure of the final fibres. The fibres obtained in the wet spinning process have a less regular shape with a larger surface development than in the case of dry-wet spinning. DBC fibres made by wet spinning are used as raw material for the production of nonwoven substrates. The technique of producing nonwoven substrates from DBC consists of cutting fibres in the form of a cable into 6 cm long sections, from which the fleece is made using a mechanical system on carding machines, and then the fibres in the fleece are combined by the needling and calendering techniques. Figs. 3 and 4 show microscopic photos of DBC and BAC fibres.

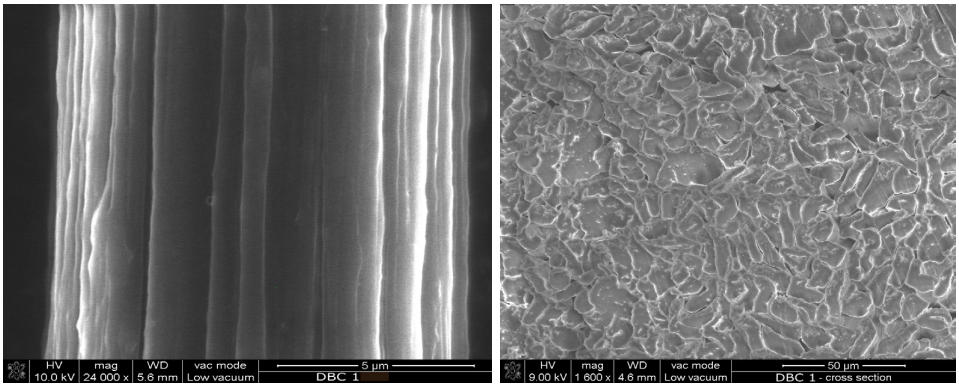

Figure 3. Micrographs of dibutyrylchitin fibres.

The dry-wet method of forming fibres from DBC comprises preparing a spinning solution (15%–25%) in ethanol, heating it to 60°C and pressing it through a spinning nozzle. The not fully solidified fibre is then introduced into a water bath, where it is completely solidified. The fibre is then wound on drums, stretched and dried. A microporous DBC fibre with a linear mass of 1.7 to 5.6 g is obtained depending on the concentration of the spinning solution used.

Fibres obtained by the dry method have an elongated and bent cross-sectional shape, similar to a croissant. The degree of fibre crystallinity determined in X-ray examinations

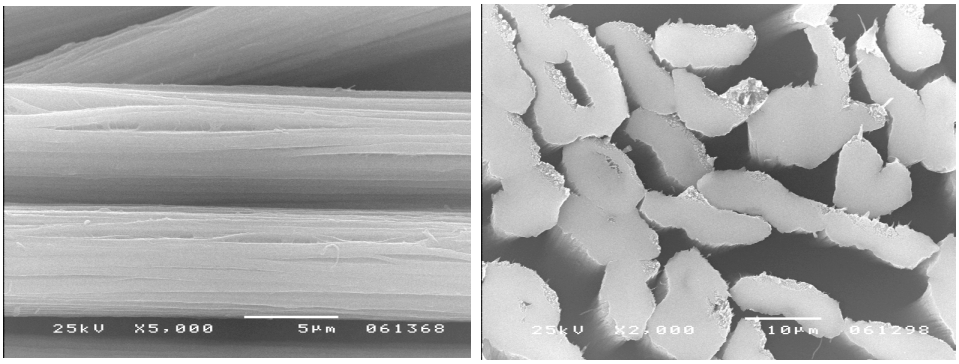

Figure 4. Micrographs butyric-acetic chitin copolyester (BAC) 90:10 (butyric and acetic anhydride, respectively) fibres.

is similar in both methods and amounts to approximately 19%, and the transverse dimension of crystallites is approximately 23 Å. It is equally easy to obtain chitin materials (regenerated chitin) after using mild alkaline treatment without damaging their macrostructure. Fibres from regenerated chitin and DBC do not cause cytotoxic, haemolytic or irritant effects and cause minimal tissue local reaction after implantation [18–20]. DBC and regenerated chitin fibres can be used to obtain dry dressing materials as well as materials for other biomedical purposes. Woven dressings based on DBC are biodegradable within the wound and do not require replacement during their use.

There have also been attempts to obtain difunctional derivatives of chitin (two types of groups attached to the biopolymer). Acetate-formate derivatives of chitin were obtained using formic acid, acetic anhydride and trifluoroacetic acid as a catalyst [21]. However, it turned out that the obtained chitin esters are poorly soluble in typical organic solvents. This is one of the reasons why this derivative has not found practical use, despite the fact that its biological properties are comparable to those of chitin. A similar situation was observed in the case of trifluoroacetate-formate derivatives of chitin obtained in the reaction of chitin, formic acid and trifluoroacetic acid [22]. Another method produces both mono- and diesters of chitin under the action of acetic and butyric acid anhydrides in the presence of an acid catalyst (methanesulfonic acid or trifluoroacetic acid). The final reaction product is a mixture of chitin acetate, chitin butyrate and chitin acetate-butyrate [23, 24]. A mixture of trifluoroacetic acid and the appropriate organic acid is used as a catalyst for the esterification of chitin hydroxyl groups, including a chitin monoesters and chitin copolyesters. The main component of the obtained derivatives is acetylchitin. It is also possible to obtain butyryl, hexanoate and octanoate derivatives by this method. The reaction is carried out at 70°C. After 30 min, while using this type of catalyst for the esterification, the reaction mixture becomes homogeneous. Using the same esterification method, acetyl-butyryl, acetyl-hexanoate, acetyl-octanoate and acetyl-palmitate chitins are obtained. The monoesters and copolyesters obtained in this way are 30–150 kDa with an esterification degree from 1.0 to 2.0, depending on the raw materials.

Another method of chemical modification of chitin is its esterification, which results in carboxymethyl chitin [25, 26] or N,N-dicarboxymethyl chitosan using monochloroacetic or monochloropropionic acid and subsequent reaction of halogen substitution with hydroxyl group. The modification leads to the loss of the supramolecular structure of chitin and the formation of water-soluble derivatives [27].

Butyric-acetic chitin copolyester (BAC, butyric and acetic acid ester with different mass fractions of each component) has similar properties to DBC. It is possible to modulate the BAC parameters, which, due to the fact that it is a raw material for the production of new materials, also affects the parameters of the final medical device. The esterification is carried out under heterogeneous conditions at 20–25°C, using chloric (VI) acid as a process catalyst and a mixture of butyric and acetic anhydrides, used in a 90:10 molar ratio [25, 26]. The products are obtained with a yield of 82% to 89% and are soluble in dimethylformamide, dimethyl sulfoxide and N-methyl-2-pyrrolidone. They are also characterized by high molar mass (intrinsic viscosity of these products determined in dimethylformamide at the level of 2.0–2.05 dL/g) and a full degree of esterification. The introduction of both butyryl groups and additional acetate groups with shorter aliphatic chain into chitin macromolecule causes an increase in the susceptibility of this raw material to deformation during fibre formation while maintaining their biological properties. The development of an efficient BAC synthesis has shown that the obtained chitin derivative has the ability to form fibres from a wet solution with a strength slightly above 20 cN/tex, while retaining high porosity. Fibres with strength at this level may form the basis for the production of multi-dimensional (MD) and three-dimensional polymer-fibre composites. BAC fibres have a stronger predisposition to crystallization of apatite; strong sorption tendencies of fibres create the possibility of local supersaturation conducive to apatite nucleation. The BAC fibres also degrade faster under *in vitro* conditions. As a result of the fact that the deacetylation reaction does not proceed to a 100% yield, copolymers including N-acetyl-D-glucosamine and 2-amino-2-deoxy-D-glucose are formed. The presence of strongly basic and primary amine groups formed in the hydrolysis reaction of chitin amide groups significantly facilitates the product's solubility in an acidic environment.

2.2. BAC as a Raw Material for Manufacturing Dressing Materials

BAC is one chitin derivatives that has been used in the production of dressings. Although the procedure for the preparation of mixed butyric-acetic esters of chitin [28] is known, the application of butyric and acetic anhydrides and methanesulfonic acid as a catalyst is problematic from the point of view of industrial production. Thinking about the industrial synthesis of butyric-acetic derivative of chitin, it was necessary to find reaction conditions that would eliminate the need for methanesulfonic acid. In the research on developing a method for the production of butyric-acetic chitin copolyester on an industrial scale, it was necessary to develop in the first stage synthetic conditions that could later be transferred to an industrial scale. It had been assumed that a heterogeneous method of synthesis would be developed. The optimal composition of the mixture of both anhydrides is 90:10 (molar ratio) of butyric and acetic anhydride. Perchloric acid was used as the catalyst. In order to eliminate the possibility of creating an explosive mixture formed in direct contact of acetic anhydride with perchloric acid, the key 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 intensively stir the suspension. To remove the excess of both anhydrides and the corresponding carboxylic acids, diethyl ether was added to the suspension and the crude product was filtered off. The crude acetylation product was washed with water and diluted with aqueous ammonia solution, dried and finally dissolved in ethanol. Its structure was confirmed by nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR).

The transfer of lab-scale synthesis conditions to the macro scale was not just about increasing the amount of reagents and the size of the synthesizer. A 60 dm^3 reactor with

an effective cooling system was used. Three kilograms of chitin 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 to introduce all the reactants and complete conversion was about 24 h. Instead of diethyl ether, in industrial conditions ethyl acetate was used to remove the excess of unreacted butyric and acetic anhydrides. In industrial conditions, it was also necessary to replace ammonia water to neutralize acetic and butyric acid residues. Sodium carbonate was employed for this purpose. In addition, the stage of draining the crude product required changes in the industrial process. G4 Schott funnels were used for filtration in the laboratory synthesis. However, using this method on a large scale was not very effective. Therefore, filtration was applied on the nutches, the capacity of which was 100 dm^3/h. The process efficiency on an industrial scale was comparable to that on a laboratory scale. The physicochemical properties of the final products were also comparable. The conducted tests guaranteed obtaining raw materials of preferred parameters for manufacturing medical materials, and the process was repeatable [25, 26]. Fig. 5 presents pictures of the equipment for industrial scale synthesis of BAC.

Figure 5. Butyric-acetic chitin copolyester industrial scale synthesis equipment.

2.3. Porous Dressing Materials Based on BAC

One application of BAC 90:10 is its use to manufacture highly porous film materials. Research work began with laboratory tests, where two methods of creating porous materials were tested: (a) pouring a 5% BAC 90:10 ethanol solution onto a layer of solid inorganic salt (pore former agent), which was solidified by washing with water; and (b) the use of pore former agent suspensions in BAC 90:10 solution, which was a mixture of solvents with a density close to the bulk density of the pore former agent. A variety of both organic and inorganic salts have been tested in laboratory tests (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, $Na_2S_2O_3 \cdot 5H_2O$, NaCl, diammonium hydrogen citrate, diammonium oxalate). All tested salts can be used as porophor agents. However, the optimal porophor agent – from the point of view 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 serves as the porophor agent and a 3% solution of BAC 90:10 in ethanol

is the substrate. BAC is dissolved in 96% ethanol. A 3% solution is prepared. The membrane is created by pouring a 3% copolymer solution on a porophor agent layer (sodium chloride) in order to form a spongy structure. After drying, the membrane is rinsed in distilled water at 40°C until the blowing agent is washed out. The product is then dried at 80°C. The obtained dressings in the form of a membrane after packaging are subjected to 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 using a spray nozzle. Silver particles are evenly distributed in suspension using an ultrasonic cleaner. After drying and then packaging the product, it undergoes radiation sterilization. The dressing in the form of a powder is obtained by grinding BAC, which is subsequently sterilized by irradiation [27, 29, 30]. The scheme for obtaining porous dressing materials based on BAC is shown in Fig. 6.

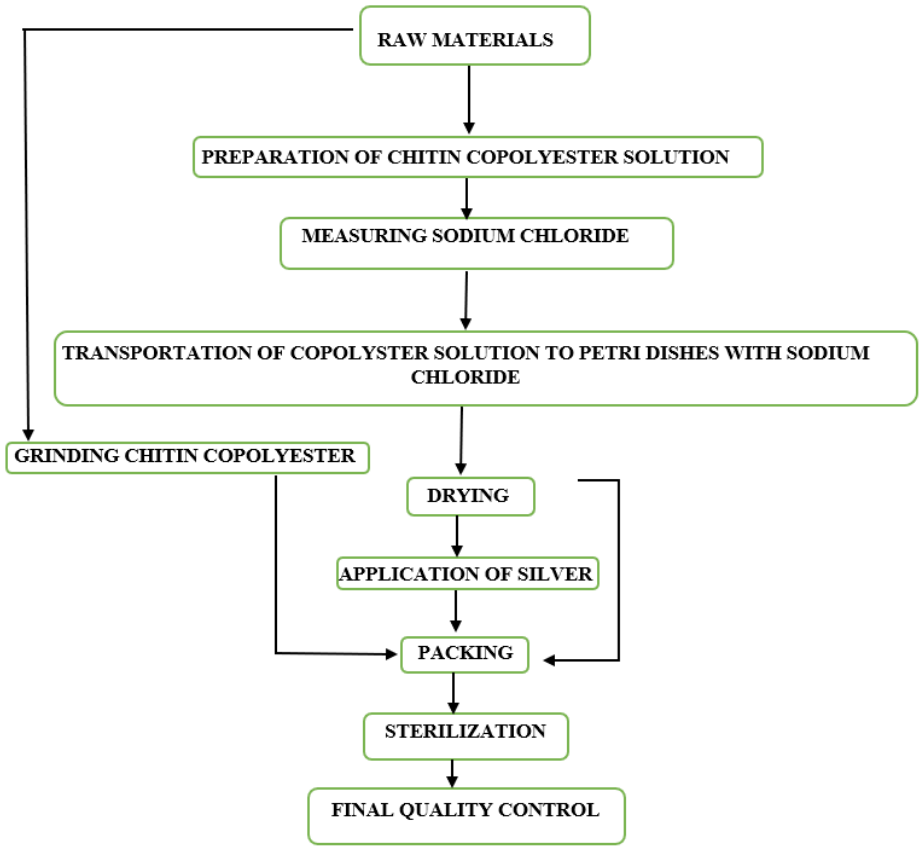

Figure 5. Butyric-acetic chitin copolyester industrial scale synthesis equipment.

Dressing materials obtained by the salt-leaching method from BAC 90:10 and sodium chloride – with a diameter of 0.16–0.40 nm and/or microsilver – are characterized by a high degree of porosity, with 275–305 nm diameter pores, and a 27.2%–27.4% degree of crystallinity. Fig. 7 presents scanning electron micrographs of porous structures obtained by the porophor agent salt-leaching method. Those studies

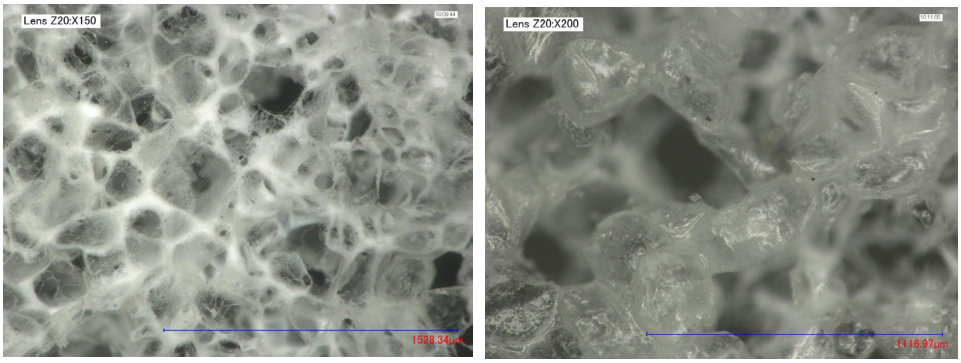

Figure 7. Scanning electron micrographs of the porous structures obtained by the porophor agent salt-leaching method.

confirmed the porous structure of the pores, which do not contain the crystals of the inorganic blowing agent. In addition, the pores are open, which increases the efficiency of water adsorption.

Dressings obtained from BAC (Fig. 8) are intended for treating wounds of various aetiologies, including chronic wounds in which the healing process is disturbed by comorbidities. A powder dressing has been designed in order to accelerate the healing process of deep wounds. Wounds are often accompanied by bacterial infection; hence, in addition to the dressing made in the form of a membrane only from chitin copolyester, there is also a variant that contains silver, which shows bactericidal effect in the wound environment. Microbes cannot become resistant to the effects of silver, unlike in the case of antibiotics. Silver can occur in various forms, but it has been assumed that only the ionic form is bactericidal. Any other form of silver must be 'processed' to an ionic form. The shorter this path, the greater the antibacterial effect of silver compounds. Hence, metallic silver with small particle sizes after oxidation and hydrolysis is characterized by the highest antibacterial activity. Ionic silver also has the ability to interact with proteins. Indeed, the ionic form of silver has a greater ability to bind to proteins compared with nanoparticles [31–35].

Figure 8. Butyric-acetic chitin copolyester dressings.

The presence of pores and microcapillaries in the structure of membrane dressings allows drainage of exudate from the wound. Dressings made from chitin copolyesters are characterized by high biocompatibility. Biological studies have confirmed the absence of cytotoxic, irritant and sensitizing effects. These dressings are degraded in the subcutaneous tissue and gradually reduced. The dressing affects the shortening and weakening of the exudative phase, dries the wound and accelerates the production phase. The epithelization process under a BAC dressing was completed faster compared with the control group [36].

The developed biodegradable dressings, the main component of which is BAC, have been clinically evaluated with a wide spectrum of patients. The use of dressings significantly accelerated the healing process of wounds resulting from venous insufficiency and diabetic conditions, as well as in patients in which the healing process was disturbed by comorbidities. The improvement of the clinical condition of the wound depends on each patient; it is usually observed after 30–60 days. The obtained results indicate that the examined dressings significantly reduce wound healing time. Medisorb R Ag is more effective than Medisorb R Membrane in treating infected wounds. The powdered form (Medisorb R Powder) enables application of the dressing in deeper wounds. Thanks to its unique structure, the dressing drains the exudate from the wound to the outer environment. Owing to this action, the dressing restores the proper course of the cell reconstruction process. The ability to biodegrade in contact with wound secretions eliminates the need to replace dressings, so the newly formed granulation tissue is not affected, and the cell reconstruction processes can proceed smoothly. The investigated dressings show high efficiency in healing wounds of various etiologies [37].

3. Chitosan as a Raw Material for Manufacturing Dressings

Chitosan is obtained due to hydrolysis of the acetylamino groups of chitin (partial deacetylation of chitin) (Fig. 9). Its main advantage is much better solubility in aqueous solutions of (especially organic) acids. Deacetylation of chitin by chemical or enzymatic methods allows obtaining materials with various degrees of hydrolysis. However, it is assumed that a 50% deacetylation level must be achieved.

The physicochemical properties of chitosan depend on the origin of the chitin substrate for the formation of chitosan and deacetylation conditions, which directly translates into the degree of deacetylation, molecular weight and biodegradability. Chemical modifications of chitosan (alkylation, hydroxyalkylation, acylation, phosphorylation, sulphation and many others) allow modulating the useful properties of polymers [38–40].

Chitosan occurs in five crystalline forms, four of which are hydrated and one of which is anhydrous. It is a polymer perfectly soluble in dilute aqueous solutions of inorganic and organic acids. Microcrystalline chitosan is in the form of a suspension or powder and, compared with unmodified chitosan, has many beneficial properties (e.g. better biodegradability or bioactivity). Chitosan is a nontoxic linear biopolymer, a polysaccharide composed of randomly distributed acetylated and deacetylated D-glucosamine units.

Although chitosan is a biodegradable, biocompatible and bioresorbable polymer, it has lower mechanical strength compared with chitin. It shows good adhesion to cells; has sorption and antibacterial properties; it activates macrophages; it stimulates fibroblast proliferation, cytokine production and type IV collagen synthesis; and it promotes angiogenesis and has haemostatic properties [41–44]. In addition, it has a positive effect on granulation and epidermalization and it reduces the formation of scars. A unique feature of chitosan is its cationic character. Positively charged

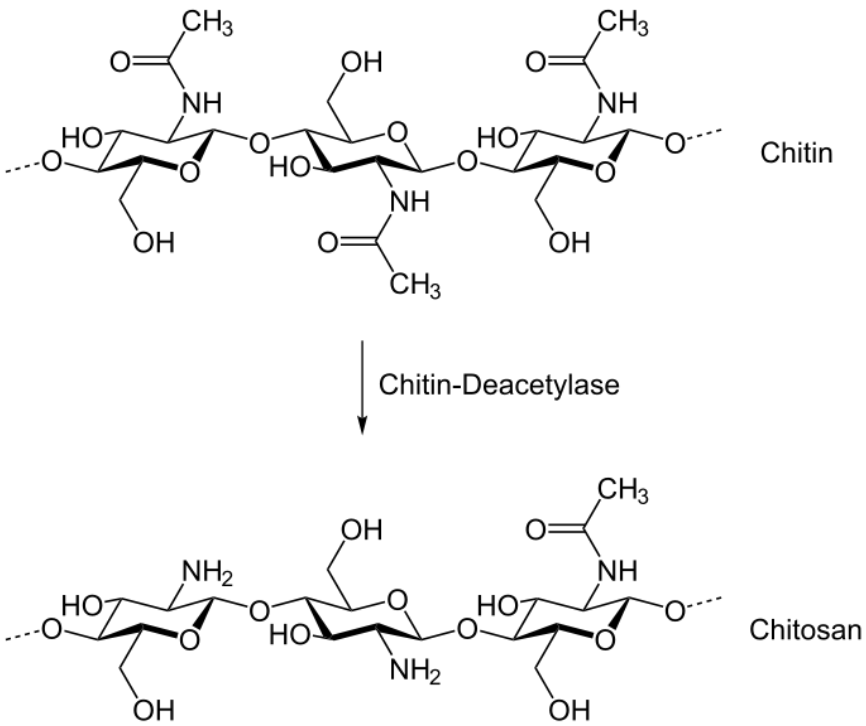

Figure 9. Formation of chitosan from chitin by deacetylation.

chitosan molecules interact with negatively charged erythrocytes and thrombocytes, activating the extrinsic coagulation pathway, which effectively stops bleeding. Chemical modification of chitosan can lead to completely new applications, such as heparin inactivation or antiviral activity. Chitosan can occur in the form of a gel, sponge, fibre or a porous composition with ceramics, collagen or gelatin. Chitosan is used as a component of dressings to accelerate wound healing; however, in the case of scaffolds, it is usually used together with other natural polymers (hyaluronic acid, alginic acid, poly(methyl methacrylate), poly-L-lactic acid, elastin, collagen, gelatin) or additives (hydroxyapatite, calcium phosphate, ceramic components) [44–46].

Chitosan increases the inflow of phagocytic cells (segmented granulocytes and macrophages) to the site of infection, it stimulates the migration and proliferation of endothelial cells and fibroblasts. The effect of chitosan on fibroblast proliferation depends on its degree of deacetylation and molecular weight. Forms with a higher degree of deacetylation and lower molecular weight stimulate fibroblast proliferation to a greater extent [47–72]. Chitosan has been widely studied for potential use in bone and cartilage tissue reconstruction. It has the ability to create porous structures, which enables its use in tissue engineering, orthopaedics and bone regeneration. Chitosan has also been investigated for its use in matrices for transport of medicines (drug delivery system) or therapeutic substances (DNA plasmids, small interfering RNA [siRNA], nanosilver), as well as for the production of surgical threads, wound dressing materials and artificial internal organs [73–99]. It has been shown to have bacteriostatic and even bactericidal effect on bacterial and yeast cells. An interesting property of chitosan is its

ability to bind to mucus and cross epithelial barriers, due to which its use as an adjuvant or auxiliary adjuvant in vaccines was considered. It is classified as an auxiliary substance, enabling the preparation of various forms of drugs with specific properties, e.g. lozenges that dissolve in appropriate sections of the gastrointestinal tract.

Chitosan is an excellent material that allows forming various shapes or continuously flat surfaces. It is an excellent complexing agent for metal ions. This property is useful due to the immobilization of metal ions with antibacterial activity and it enables their controlled release, depending on the needs. This process can be properly modelled in accordance with adopted algorithms [46]. Chitosan is also subject to alteration with various functional groups. There is ongoing research aimed at permanently binding peptides with chitosan [44].

Chitosan can also be an environmentally friendly agent used to obtain textiles with antibacterial properties. Attempts have been made to incorporate chitosan powder into cotton and polyester/cotton. The introduction of chitosan was carried out after activation of the fabric surface with 20% NaOH. The tests confirmed that chitosan is well implemented in cotton and polyester/cotton blends. The presence of chitosan was confirmed after five washing cycles [100].

3.1. Chitosan-Based Dressing Materials

Chitosan-based dressing materials are used in severely bleeding wounds and chronic wounds because this saccharide has antibacterial effects, maintains proper wound moisture and possesses good absorption of wound secretions [101]. Examples of chitosan-based dressings are HemCon®, Chito Gauze®, Celox Rapid Gauze® [102] and Kerlix® [103]. Chitosan promotes clot formation by affecting the ability to form crosslinks between erythrocytes [104]. In an acidic environment, it shows tissue adhesive properties. The positive charge resulting from protonation of the amino group is conducive and attracts negatively charged morphotic elements of blood [105]. In order to achieve an elevated water binding capacity (secretions) and simultaneous formation of a gel-like layer, it is also preferable to use alginates. Alginates are used in medical dressings in the form of nonwoven fabrics or so-called nonwoven absorbent materials in the form of compressed fibres (fibre plates). The loose fibre structure adapts to different types of wounds and effectively tampons exudates and even stops bleeding.

Haemostatic dressings based on the chitin derivative, chitosan, as well as alginates are currently very effective medical devices that stop bleeding. Their unique features used in medical dressings include: activation of macrophages, stimulation of cytokine production, and promoting angiogenesis. An example of such a dressing is Tromboguard, a tri-layer dressing with a semi-permeable polyurethane film, hydrophilic polyurethane foam and a layer containing the aforementioned polysaccharides. The film layer protects the dressing against percolation and thus allows the wound to maintain adequate humidity, ensures optimal air permeability into the interior and creates a barrier against external factors. The polyurethane foam is a supporting layer and has strong absorption properties thanks to the modern ‘pore-in-pore’ structure. It is responsible for storing exudate and keeping it out of the wound surface, ensuring adequate moisture in the wound. In addition, it provides a layer to protect the wound against mechanical injuries. The active layer, created with a unique composition of chitosan and alginates, activates the blood coagulation process and significantly reduces bleeding time. Chitosan reacts with erythrocytes and thrombocytes on the wound surface and significantly minimizes bleeding time. Calcium alginate accelerates the natural clot formation process, and sodium alginate – by absorbing wound secretions – forms a gel layer on the surface of the dressing that prevents it from sticking to the wound. Alginates are resorbable,

nontoxic, noncarcinogenic, do not cause allergic reaction and show haemostatic properties. When alginates are used as dressing materials, it is important to remember that during contact with the wound, part of the alginate dressing transforms into a gel, which prevents drying of the wound surface, and thus enables creating a favourable, moist environment within the damaged skin [106]. At the same time, haemostatic properties result in a faster wound healing process and allow for more effective scarring. For patients, the advantage of using these dressings is also the reduction of pain during dressing change. An important benefit of using sodium or calcium alginate-based dressings is that the dressing does not stick to the wound and is very absorbent.

Tromboguard dressing, presented in Fig. 10, is used to stop bleeding in the case of: traumatic wounds, postoperative wounds, skin graft donor sites in surgery and reconstructive surgery – including combustiology, wounds requiring emergency care, gunshot and bullet wounds and wounds resulting from traffic accidents. It is characterized by rapid haemostatic action (stops bleeding in 3 min), antibacterial effect inside the product (protecting the dressing against the development of microorganisms) and effective absorption of blood even under pressure. It does not cause irritation (according to PN-EN ISO 10993-10), sensitization (according to ISO 10993-10) or cytotoxic reactions (according to PN-EN ISO 10993-5).

Figure 10. Tromboguard dressing.

The tests of functional parameters, such as tensile strength (according to PN-EN ISO 1798), ability to adapt to the place of injury (according to PN-EN 13726-4) or moisture vapour transmission (according to PN-EN 13726-2), have shown that the obtained dressing has a minimum tensile strength (for porous materials) of 75 kPa, which corresponds to the recommended value for dressing materials; a minimum vapour permeability (moisture vapour transmission) of 400 g/m^3/24 h; and the ability to adapt to the place of injury in range of 2.0 to 5.0 N/cm, respectively. The results of PMCF (Post-Market Clinical Follow-up) clinical studies have documented the haemostatic properties of innovative haemostatic Tromboguard dressings. The high effectiveness and durability of the antihaemorrhagic effect was confirmed during 24 h after application. These studies have also confirmed the safety of using the dressing. An antibacterial study showed that the dressing is bactericidal against *Staphylococcus aureus* and *Escherichia Coli* [45]. A patent has been granted for the dressing [107]. Another variant currently being developed is a spray dressing, which has a similar composition to the active layer used in the dressing described above, but with the addition of cotton fibres [108]. The market launch of a foam absorbent dressing [109] and a three-layer haemostatic dressing [45] has been largely possible thanks to research on sorption-desorption of metal salts on a chitosan gel [46].

4. Conclusions

Chitin and its ester derivatives as well as chitosan have many valuable chemical, physical and biological properties that determine their use in many areas, including medicine. The most attention has been focused on the use of chitin in biomedical sciences, in particular: in dressing materials (active dressings), carriers of active substances (drugs and growth factors), in tissue engineering (cellular scaffolding, mainly in orthopaedics) and in regenerative medicine (differentiation of stem cells). Chitin accelerates the process of wound healing due to its beneficial effects on angiogenesis, granulation, epidermalization and scarring, all of which play a key role in the physiological healing process. It increases the inflow of phagocytic cells (segmented granulocytes and macrophages) to the site of infection, and stimulates migration and proliferation of endothelial cells and fibroblasts. Chitin-derivative dressings are now becoming a very effective medical device in the healing of hard-to-heal wounds. The results of clinical studies with dressings based on BAC have also demonstrated their high efficiency in wound healing of various aetiologies, primarily those caused by chronic venous insufficiency and diabetes. Patients had a reduction in ulcer surface and depth. The dressings were rated as having a high safety profile. The results of clinical studies with dressings including chitosan have shown high efficiency and durability of antihaemorrhagic activity. These studies have also confirmed the safety of use the dressings. An antibacterial study confirmed that the dressing is bactericidal.

5. References

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