Polysaccharide films modified by compounds of natural origin and silver having potential medical applications

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Abstract In the first stage, seven polysaccharide films were prepared with varying compositions of starch, carboxymethyl starch (CMS), and carboxymethyl cellulose (CMC), together with glycerol and citric acid. From these samples, the most suitable film for possible future applications in medicine in the form of patches was selected based on tensile strength tests, solubility in water, moisture sorption, swelling, and wetting angle. This film (labelled as “V”) contained (in wt%) CMS (2.5), CMC (2.5), citric acid (3), and glycerol (3). In the second stage, the selected film was used for further applications by individually introducing into it appropriate additives with known antimicrobial activity.

These additives consisted of terpene compounds of natural origin (α -pinene, menthol, and eugenol) as well as colloidal silver. These four films were then studied to determine the influence of the appropriate additive on moisture absorption, solubility in water, swelling, and antimicrobial activity. The results of the microbiological tests confirmed the antimicrobial activities, and also the anti-infective and regenerative effect of these films. In general, the films containing terpenes showed comparable or better medicinal properties than the film obtained with colloidal silver. A patch prototype (with a medical net) based on the optimal film (“V”) was developed, and its flexibility is apparent in that it can take the shape and size of the area on the human body that it should cover.

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Graphical Abstract

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Introduction

The use of natural substances in the treatment of skin diseases and wounds has been known for centuries. Examples of such natural compounds include honey, butter, milk, myrrh, and resin. Gauze with carbolic acid (phenol), invented by surgeon Joseph Lister (1860s), and wound dressings sterilized with dry heat, steam and pressure, implemented by Robert Wood Johnson (1890s), co-founder of the Johnson & Johnson company, made the first steps in the development of patches. In the 1960s, the first generation of dressings appeared, which were polymer films made of polyethylene, polypropylene, or polyester. They were not permeable to moisture and caused an accumulation of body fluids on the surface of the wound, which delayed the healing process (Queen et al. 2004). Subsequently, the second generation of dressings made of pure polyurethane or carboxymethyl cellulose (CMC) and polyisobutylene (hydrocolloid dressings) appeared. However, they did not have a curative effect as their main disadvantage was that they adhered to the newly formed tissue, which could lead to its damage. The third, and latest, generation of dressing materials includes polyurethane

films and hydrogels. The most advantageous feature of hydrogels is keeping the wound moist, which speeds up the healing process, allows for a painless change of the dressing, and prevents excessive loss of body fluids. The three-dimensional structure of the hydrogel supports the creation of a protective barrier against infection and traps the bacteria from the wound within the macromolecule. Such patches make it possible to absorb the exudate with the contamination, and, thus, the wound is constantly cleaned. In addition, the patch is an oxygen-permeable layer that facilitates cell regeneration and protects against the development of dangerous microbial growth (Petkow and Górkiewicz-Petkow 2002; Bialik-Wąs and Pielichowski 2011; Wachal et al. 2018).

Polysaccharides are biopolymers and can be good alternatives to the conventional polymers obtained from the petrochemical industry. These materials meet the “principles of 5E”: efficiency, energy saving, enabling, economy, and environmental friendliness. Films obtained on the basis of biopolymers are very often used in the production of containers, bags, and packages, but at the same time new applications are being sought for these materials (Liu et al. 2004; Tey et al. 2007). One of the ways of modifying this type of material is the incorporation of colloidal silver or compounds of natural origin (terpenes) into them, which extends their applications in medicine and agriculture due to the antibacterial and antifungal

properties of this type of material (Spychaj et al. 2013a; Drewnowska et al. 2017; Hassan et al. 2018).

Starch is a polysaccharide compound that has been used to protect skin against moisture, to prevent abrasions, and to accelerate the regeneration of the epidermis. Potato flour has also found widespread use in the form of aqueous solutions (Hovgaard and Brondsted 1996; Lee et al. 2009). Baths in starch accelerate the reconstruction of damaged skin and help fight pricks and skin changes occurring, e.g., in acne or seborrheic dermatitis. In the case of hands and feet, the use of starch soothes the skin, speeds up the renewal of the epidermis, and inhibits excessive sweating (Gebelein et al. 1991; Alberta Araújo et al. 2004; Lautenschläger 2009). In addition, carboxymethyl cellulose has found applications in medicine, and, e.g., is used as an emulsion stabilizer for injections, an adhesion and film-forming agent in tablets, for the relief of burning and itching of eyes, and as a safe and reliable carrier of anticancer drugs (Guo et al. 1998; Kamel et al. 2008). The films prepared on the basis of carboxymethyl cellulose can also be effective in the control of wound infections (Wong and Ramli 2014).

Starch, carboxymethyl starch (CMS), and carboxymethyl cellulose (CMC) are green polymers with great importance in pharmaceuticals, medicine, cosmetics, environmental protection, and the food industry (Spychaj et al. 2013b; Basta et al. 2013). Additional examples of their usage include as thickening and sizing agents in the textile industry, personal care products, and in surfactant systems, as flocculants, drilling fluids, and coating materials (El-Saied et al. 1994; Liu et al. 2008; Zdanowicz and Spychaj 2014).

The use of biopolymers from different sources has been investigated for many years for pharmaceutical and biomedical applications. Polymers such as alginate, chitin, chitosan, pectin, and gelatin are used as wound healing materials and their properties make them beneficial for use in wound dressings (South and Lyon 2010; Smith et al. 2016). The multifunctional behavior and tenability of biopolymers—from woven fibers that swell on contact with exudate, to water-rich hydrogels, which can encourage and maintain a moist wound environment in dry wounds—facilitate their application to a wide variety of wound types (South and Lyon 2010; Smith et al. 2016). Film properties are increasingly being designed to obtain physiological compatibility and the ability to control the release of

drugs when exposed to various biological environments. This would lead to the development of smart, physiologically responsive biopolymers (Singh et al. 2017; Koneru et al. 2020).

Films obtained from polysaccharides, glycerol, and citric acid may also have medicinal properties similar to those of starch and carboxymethyl cellulose (Antosik et al. 2017; Antosik and Wilpiszewska 2018). Moreover, the incorporation of metals (e.g. gold, silver, and copper) or compounds of natural origin (such as, α -pinene, menthol, and eugenol) to these films, may additionally increase the application potential of these types of materials in dermatology (Kaur et al. 2010; Bachiega et al. 2012; Kamatou et al. 2013). Both metal compounds and also α -pinene, menthol, and eugenol have anti-inflammatory and bactericidal properties. The latter can also have a regenerating and moisturizing effect on the epidermis (Rufino et al. 2014; Ali et al. 2015; Midha et al. 2017; Lin et al. 2017). The use of the material obtained on the basis of carboxymethyl cellulose and with the addition of silver as a material with high microbiological activity that can be used to prepare wound dressings has been previously described (Hassabo et al. 2015; deBoer et al. 2015).

In consideration of the previously published literature data, the aim of our work was to first develop antimicrobial polysaccharide films consisting of various compositions of starch, CMS, and CMC, together with glycerol and citric acid. Following that, the most suitable of these films was chosen for further development based on tensile strength, solubility in water, moisture sorption, swelling, and wetting. This optimal film was then individually imbued with additives consisting of terpene compounds of natural origin (α -pinene, menthol, and eugenol) or colloidal silver, and these four new films were studied and compared. The best film was also mechanically stabilized with a medical net in order to form a medicinal patch that can be used for the treatment of, e.g., EB (*Epidermolysis bullosa*), burns, eczema, acne, and abrasions. It should be emphasized that such patches containing terpenic compounds of natural origin have not yet been described in the literature. The purpose of these films will be not only to protect against the development of infection but also to provide adequate hydration and accelerate the regeneration and healing of the skin.

Experimental

Raw materials

In the preparation of the polysaccharide films, the following raw materials were used: starch (technical grade, Nowamyl S.A. Nowogard, Poland, CAS number 9005–25-8); monohydrate citric acid (analytical grade, Chempur, Poland, CAS number 5949–29-1); glycerol (analytical grade, Chempur, Poland, CAS number 56–81-5); carboxymethyl cellulose–CMC (degree of substitution 0.7, average molecular weight, $\bar{M}_n$, $250 \cdot 10^3$ g/mol, technical grade, Pronicel Sp. Z o. o., Poland, CAS number 9000–11-7); and carboxymethyl starch–CMS (CAS number 9057–06-1). The latter compound was prepared according to the method described in the literature (Spychaj et al. 2013b, a), with degree of substitution 0.8, and average molecular weight $680 \cdot 10^3$ g/mol, both determined according to the methods described below. The following additives to the film compositions were used: colloidal silver (analytical grade, Alter Medika, Poland, CAS number 7440–22-4); α -pinene (98%, Sigma-Aldrich, Poland, CAS number 80–56-8); menthol (99%, Sigma-Aldrich, Poland, CAS number 2216–51-5); and eugenol ($\geq 98\%$, Sigma-Aldrich, Poland, CAS number 97–53-0). All chemicals required for this work were commercially available and used as received. The medical net–Premilene® Mesh—used for converting the optimal film to a patch, was produced by B. Braun Melsungen AG, Germany.

Determination of the degree of substitution for CMS

The degree of substitution, DS, was measured according to the method described in the literature (Zdanowicz and Sychaj 2014). The CMS sample was moisturized in 1 mL of ethanol and then dissolved in 50 mL of distilled water. Next, 20 mL of 0.187 M aqueous solution of NH_4Cl was added to neutralize the pH of the mixture, and then the whole was poured into the flask with 50 mL of CuSO_4 (0.039 M solution). After 15 min, water was added to the slurry, the contents were filtered, and the filtrate was titrated with EDTA solution.

Determination of the number average molecular weight for CMS

The number average molecular weight of the CMS was determined using Gel Permeation Chromatography (GPC) analysis. For this purpose, use was made of the Shodex OHpak SB-806 M MQ column with Shodex OHpak SB-G pre-column in the Merck Hitachi Liquid Chromatograph (pump L-7100, interface D-7000) with RI detector (Merck LaChrom RI L-7490). Moreover, dextran standards for the chromatographic column calibration with sodium nitrate solution (0.1 M) as eluent (flow rate 0.5 mL/min) were applied.

The preparation of polysaccharide-based films

The polysaccharide-based films were prepared by the method of outpour of a solution followed by solvent evaporation. The following raw materials were introduced into the glass reactor with the capacity of 250 cm³: 200 g of deionized water, glycerol, citric acid, and 5 g of polysaccharides added according to the combinations presented in Table 1. These included starch, mixtures of starch with CMS and/or CMC, and mixtures of CMS with CMC. Next, the mixture was

Table 1 Components of the produced polysaccharide-based films

Film names	Polysaccharide content [wt%]					Additives [wt%]				
	CMS	CMC	S	CA	G	Ag	P	M	E	
I	–	–	5	–	3	–	–	–	–	
II	2.5	–	2.5	1.5	3	–	–	–	–	
III	–	2.5	2.5	1.5	3	–	–	–	–	
IV	1.25	1.25	2.5	1.5	3	–	–	–	–	
V	2.5	2.5	–	3	3	–	–	–	–	
VI	2.5	2.5	–	3	10	–	–	–	–	
VII	2.5	2.5	–	3	15	–	–	–	–	
VIII	2.5	2.5	–	3	3	3	–	–	–	
IX	2.5	2.5	–	3	3	–	3	–	–	
X	2.5	2.5	–	3	3	–	–	3	–	
XI	2.5	2.5	–	3	3	–	–	–	3	

where: wt%—weight percent, CMS—carboxymethyl starch, CMC—carboxymethyl cellulose, S—starch, CA—citric acid, G—glycerol, Ag—colloidal silver, P— α -pinene, M—menthol, E—eugenol

stirred until a homogeneous solution was obtained, and for the composition with starch, stirring was made in a water bath at a temperature of 90 °C. Following that, the additive compounds (colloidal silver, α -pinene, menthol, and eugenol) were introduced individually. The mixture was stirred for 30 min until a homogeneous mixture was obtained, and next this mixture was outpoured into the Teflon mold. The mold was put into a drying oven for 48 h at 70 °C, and the prepared film was then taken from the mold.

Methods of testing the properties of the polysaccharide-based films

Tensile strength

The tensile strengths of the fabricated films were measured using the INSTRON testing machine. The initial length of the sample was 50 mm, 10 mm wide, and about 0.2 mm thick. The speed of the mobile clamp was 10 mm/min. Seven samples of each material type were tested (Roy et al. 2012; Antosik and Wilpiszewska 2018).

Moisture absorption

Moisture absorption tests were performed for each film as follows. Three samples (1.5 × 1.5 cm) were prepared and placed for two weeks in a desiccator to dry. Dry samples were weighed and subsequently transferred to a climatic chamber (55 ± 2% humidity, 25 ± 2 °C). The samples were then weighed after being in the climate chamber for 3, 5, 7, 24, 48, and 72 h. Moisture absorption was calculated using the following equation (Ghanbarzadeh and Almasi 2011; Wilpiszewska et al. 2015):

$$A_t = \frac{M_t - M_0}{M_0} \cdot 100\%$$

where: A_t —moisture absorption after time t [%]; M_0 —mass of dry sample [g]; M_t —mass of sample after time t [g].

Solubility in water

Solubility in water was evaluated for each film. Three samples (1.5 × 1.5 cm) were prepared and placed for two weeks in the desiccator to dry. Dry samples were weighed and transferred to test tubes filled with 50 mL

of distilled water. After 24 h, the samples were dried and weighed. Solubility was calculated using the following equation (Ghanbarzadeh and Almasi 2011; Wilpiszewska et al. 2015):

$$TSM = \frac{M_1 - M_2}{M_1} \cdot 100\%$$

where: TSM—Total Soluble Matter (in water) [%]; M_1 —mass of dry sample [g]; M_2 —mass of sample after drying [g].

Swelling tests

Swelling tests were aimed at determining the increase in mass and size of the materials. For this purpose, 4 × 4 cm material samples were weighed and then placed in 50 mL of saline solution at room temperature for 24 h. After this time, the test material was removed from the solution, dried on a paper towel, weighed and measured. Unfortunately, some samples disintegrated after 24 h, which made it impossible to determine their weight and size.

Wetting angle tests

Wetting angle tests were performed using the Goniometer OCA 15EC with the sitting drop method. Measurements were made in the time range of 5–300 s. These tests were performed only for the film selected as the optimal one for further studies with the additives (film V).

Microbial tests

The antimicrobial tests were performed with the gram-negative bacteria *Escherichia coli* strain K12 (ACCT 25,922), gram-positive bacteria *Staphylococcus epidermidis* (ACCT 49,461), and candida yeast—*Candida albicans* (isolated from patients with immunodeficiency disorders from the Department of Chemical and Process Engineering, DChPE, collection). The antimicrobial activities of colloidal silver, α -pinene, menthol, and eugenol were preliminarily investigated using the “disk agar plate diffusion method”. Plate Count Agar (PCA, BioMaxima S.A, PL) for *E. coli*, Brain Heart Infusion Agar (BHI, BioMaxima S.A, PL) for *S. epidermidis* and Sabouraud Dextrose Agar (SDA, BTL Sp. z.o.o., PL) for *C. albicans* were used. The 24-h microbial cultures in the recommended

liquid media were centrifuged at about 1000 rpm for 10 min. Then, the entire sediments were transferred to sterile bottles containing 0.85% sodium chloride solution. Microbial cultures were appropriately diluted to obtain an inoculation level of 0.5 in McFarland Standards (BioMerieux, France) corresponding to a cell density of approximately 1.5×10^8 CFU/mL. The plates containing the microorganism media in sterile plastic Petri dishes ($\varnothing = 9.0$ cm) were inoculated with 0.25 mL of microorganism suspensions. The sterile paper discs (Whatman No.1, diameter 5.0 mm), impregnated with each substance (10 μ L/disc), were placed at different locations on the surface of culture plates and incubated at 37 °C for 24 h. The disc soaked with sterile sodium chloride solution was used as a control experiment.

The zones of inhibition (ZOI) of growth around the paper discs were measured. The sensitivity to the tested agents was classified by the diameter of the inhibition halos as: not sensitive (–) for diameters less than 3 mm; sensitive (+) for diameters 3–6 mm; very sensitive (++) for diameters larger than 6 mm and less than 9 mm and super sensitive for diameters larger than 9 mm (+++). Two discs per plate were used, and each plate was set up in triplicate. Additionally, the polysaccharides-based films (numbered V, VIII–XI) were cut to the size of 25 × 25 mm, and about 1–2 mm in thickness, and put directly on the agar plates with the inoculum of tested bacteria or yeast. The Petri dishes were incubated at 37 °C for 48 h, and, afterwards, the microbial growth—or absence of growth—around these films was determined.

Results and discussion

Mechanical and physical properties of fabricated films

In order to select the optimal polysaccharide film composition, 7 film variants (without additives) were produced that differed in the content of polysaccharides (CMS, CMC, S) and the amount of cross-linker compound (citric acid, CA) and plasticizer (glycerol, G). These films were examined in terms of tensile strength, Young's modulus and elongation at break. In addition, solubility in water, moisture sorption (after 120 h), and swelling was determined. The results of these tests are collected in Table 2. For the selection of the optimal polysaccharide film, the correlation of all tested properties was taken into account. (We decided that for films containing additives the mechanical properties will not be measured.)

It was established that due to specific requirements (use for medical purposes) more emphasis should be placed on moisture sorption (after 120 h) and swelling. Despite relatively good mechanical results, films containing starch (I–IV) were characterized by low moisture absorption. During the swelling tests, the presence of starch in these materials caused the films to significantly increase in size in one direction only, and simultaneously, no volume swelling was observed. This phenomenon could damage the medical patch composed of such a film during use. This linear increase in size is related to the layered structure of starch and the structure of the polysaccharides. Water molecules coming into contact with the polysaccharide chains increase the volume of the film

Table 2 The mean measured properties of the produced polysaccharide-based films

Film number	Mechanical properties			Moisture		
	Elongation at break [%]	Young's modulus [MPa]	Tensile strength [MPa]	Solubility in water [%]	sorption after 120 h [%]	Swelling [wt%]
I	43.0 ± 0.1	49.5 ± 0.1	15.9 ± 0.1	36 ± 1	13 ± 1	156 ± 1
II	33.8 ± 0.1	N/A	0.7 ± 0.1	62 ± 1	16 ± 1	266 ± 1
III	37.2 ± 0.1	10.2 ± 0.1	5.9 ± 0.1	60 ± 1	17 ± 1	170 ± 1
IV	59.0 ± 0.1	25.7 ± 0.1	7.2 ± 0.1	51 ± 1	15 ± 1	165 ± 1
V	22.1 ± 0.1	4.8 ± 0.1	1.9 ± 0.1	73 ± 1	19 ± 1	182 ± 1
VI	N/A	N/A	0.3 ± 0.1	80 ± 1	23 ± 1	185 ± 1
VII	N/A	N/A	< 0.1 ± 0.01	84 ± 1	25 ± 1	190 ± 1

Fig. 1 Wetting angle test results for film V after various times of contact

matrix. Then, the absorption of water molecules increases and the layers undergo separation in one direction (Mahomed et al. 2017). In contrast, films V–VII, which did not contain starch, were characterized by low and very low values of the mechanical properties, which may prevent proper use of these films.

Moreover, it was observed for films with high concentration of glycerol (films VI and VII), that they were too delicate and degenerated by simply rubbing the film with gentle finger pressure. Hence, we decided that the optimal film for the medical applications in the form of patches and for the introduction of additives (colloidal silver, α -pinene, menthol, and eugenol) will be film V, which contains 3 wt% of glycerol of the dry polysaccharide mass. Therefore, we decided to prepare this film with a medical net inside, as discussed below, to improve its stability and, thus, this modification allows this film to be used in medical patches.

Fig. 2 Influence of different additives on the moisture absorption of films VIII–XI, based on film V (the “pure” material), at a temperature of 25 ± 2 °C

Wetting properties

The literature describes that the contact angle is a quantitative measure of the wetting of the solid (the film) by a liquid (water), and so the wetting angle tests were performed for film V, selected as the most suitable for further studies with the additives (Fig. 1). The value of the contact angles corresponds to the value gained at zero time, i.e., it is the initial contact angle at first droplet contact. Films characterized by good wetting of their surfaces by water should have a contact angle of less than 90° in order to be able to be used as wound dressings (Basiak et al. 2018). The literature also indicates that this contact angle is dependent on the amount of glycerol added to the film composition. Generally, the more glycerol, the lower the wetting angle and the better the water distribution on the film surface. These studies were performed for the contents of 33 and 50% glycerol (Basiak et al. 2018).

The wetting angle tests showed that the contact angle was 51.25° (average of two measurements, 50.8 and 51.7) after 5 s of applying the drop, which suggests very good wetting of the obtained materials. It can also be seen that by extending the contact time of the drop with the surface, the drop material is completely absorbed. This phenomenon is very desirable considering the medical use of the film as a patch.

Fig. 3 Influence of different additives on the solubility of the films based on “pure” film V

Incorporating additives in the films

In the second stage of our studies the selected film V (the “pure” material) was modified by the individual additions of the appropriate amounts of the additives—colloidal silver, α -pinene, menthol, and eugenol—to form films VIII–XI (Table 1) in order to study the effect of the appropriate additive on moisture absorption, solubility in water, swelling, and antimicrobial activity.

Moisture absorption of modified films

Figure 2 presents the influence of the different additives on the moisture absorption of modified films based on film V. It is noticeable that in all cases, the additives generally caused an increase in the moisture absorption in comparison to the pure film V. The film with menthol (X) exhibited a lower moisture absorption at the beginning of the measurements (up to 7 h), and then it showed the highest values (about 24–25%) among the compositions tested. This may be due to the specific properties of this compound (at room temperature it is a solid and forms white crystals), so that the film is in the form of agglomerates (Daryadel et al. 2018; Nesil et al. 2019). The presence of menthol agglomerates in the film can lead to an uneven absorption of moisture, and, consequently, can lead to fine cracks in the film and increased water absorption. The explanation for this behavior of the menthol film, however, requires further research.

For the film with colloidal silver as the additive (VIII) and for the film with α -pinene (IX), the increase in the moisture absorption to 23–25% (from 19% for pure film V) was observed. The addition of eugenol (film XI) did not have a significant influence on the moisture absorption after 120 h, and has a similar ability to absorb moisture as the base film V. Taking this into account, it can be assumed that both films will have similar durability when used to absorb wound exudates. However, in the case of other films, it can be assumed that they will be characterized by greater durability due to the increased ability to absorb water. Therefore, the application time per wound may be longer for them. These observations, however, require further study.

Table 3 Results of swelling tests for the films with additives (VIII–XI) and without (V)

Film number	Weight before [g]	Weight after [g]	Weight gain [%]	Size before [cm]	Size after [cm]	Size gain [%]	Disintegration
V	0.667 ± 0.001	1.215 ± 0.001	182.16 ± 0.01	4 ± 0.1	4.4 ± 0.1	110 ± 0.1	–
VIII	1.181 ± 0.001	3.231 ± 0.001	177.69 ± 0.01	4 ± 0.1	4.8 ± 0.1	120 ± 0.1	–
IX	2.619 ± 0.001	6.7528 ± 0.001	257.82 ± 0.01	4 ± 0.1	5.6 ± 0.1	140 ± 0.1	–
X	1.275 ± 0.001	2.548 ± 0.001	199.84 ± 0.01	4 ± 0.1	4.7 ± 0.1	118 ± 0.1	–
XI	1.628 ± 0.001	3.189 ± 0.001	195.88 ± 0.01	4 ± 0.1	4.8 ± 0.1	120 ± 0.1	–

Fig. 4 Antimicrobial activity as the growth of inhibition zone [mm] of the pure components—colloidal silver, terpenes (α -pinene, menthol and eugenol), and control (sodium chloride) – determined by the agar plate diffusion method

Water solubility of the films

The solubility in water of the prepared polysaccharide films with different additives is presented in Fig. 3. Firstly, it should be emphasized that the studied samples maintained their integrity—did not break or dissolve during the solubility test. Films IX (α -pinene) and X (menthol) exhibited an increased solubility in comparison to the film without additives (film V). Only the addition of colloidal silver caused a significant decrease in the solubility in water of the obtained film. This may be caused by the increase in the network density of this cross-linked film as the introduction of the colloidal silver to the film produced a more compact film structure.

Swelling tests

Table 3 summarizes the results obtained during the swelling tests for films V and VIII–XI. The table shows that the mass increase and size gain (volume increase) was the highest for film IX, which was obtained with α -pinene as the additive. Films with other additives showed these two parameters to be about similar in value or slightly higher than for the pure film V. This comparison shows that α -pinene film is best suited for the role of the patch. The increase in volume with simultaneous absorption of moisture (wound exudates) effectively produces dual beneficial properties—a “protective cushion” and “a soft compress”. The slightly inferior values of volume and mass increases for other films with additives do not preclude their use as patches, because unlike film IX, they may have better properties of inhibiting the growth of various microorganisms. At the same time, it should be emphasized that none of the examined films disintegrated during these tests.

Microbial tests

The results obtained in the microbial tests presented in this work were consistent with the literature data (Leite et al. 2007; Karapandzova et al. 2012; Zeng et al. 2012; Silva et al. 2012), and these results are the proof of antibacterial and anti-yeast activities of α -pinene and eugenol. Moreover, these tests also showed that the colloidal silver exhibited a low antimicrobial activity, only against such microorganisms as gram-positive *S. epidermidis* and yeast *C. albicans*, whereas menthol was active against gram-positive and gram-negative bacteria. Additionally, no inhibitory effect on the tested microorganisms was observed for the sodium chloride solution used as a control during the

tests (Fig. 4). We did not show the results of the zone of inhibition (ZOI) for α -pinene and menthol against *S. epidermidis* and *C. albicans*, because these two terpenes were not active against these microorganisms. According to the literature data (Semeniuc et al. 2017) the size of the ZOI varies depending on bacterial strain used for the tests. The mechanism of the action of essential oils remains unclear, and some studies have shown that, e.g., phenols, such as carvacrol and eugenol, disrupt the cellular membrane and react with the active sites of enzymes. All enzymes play a key role in the emergence of resistance, which could be an explanation of the observed differences.

The lowest concentration of an antibacterial agent necessary to inhibit visible growth of microorganism is defined as MIC (Minimum Inhibitory Concentration). These minimum concentrations were found for eugenol and α - and β -pinene, and they depend on the source of terpene compound, the strains of microorganism, and the methods used (Leite et al. 2007). Additionally, the MIC values of pinene depend on the type of enantiomers—only positive exhibited antimicrobial effect (Silva et al. 2012). Considering the above arguments, and the many variables encountered, a comparison of the antimicrobial activity results obtained by varied research teams is very difficult. However, literature data show (Leite et al. 2007; Pavese et al. 2018) that eugenol provided the most intense antibacterial effect (MIC values $> 5 \mu\text{L/mL}$ and with growth inhibition zones higher than 10 mm). Similar results are presented for menthol, which has been regarded as a potential source of a natural antimicrobial compound against staphylococcal infections, including MRSA strain (Horváth and Koščová 2017). It has been proven that menthol is a safe product that can be used topically or orally to treat minor aches, pains, inflammation, and congestion.

By contrast colloidal silver has caused much controversy. Due to its low stability in solvents, it

found only limited applications. The antibacterial action of colloidal silver against gram-negative and gram-positive pathogens as well as drug resistance is not entirely clear (Dakal et al. 2016). The results from this study indicated that colloidal silver presented a lack of action against gram-negative bacteria while Afonina et al. (2010) reported that these bacteria are more susceptible to the action of colloidal silver. Recently published studies showed that prolonged exposure of microorganisms to colloidal silver may result in the development of their resistance (Graves et al. 2015). Additionally, it was reported exposure of human cells to silver nanoparticles induces cytotoxicity, genotoxicity, and inflammatory response in human cells (Maneewattanapinyo et al. 2011). These results indicate that caution should be taken into account regarding the use of silver as the antimicrobial agent.

To the best of our knowledge, the mechanism of terpenes action has not been explained. It is hypothesized that terpene compounds inhibit internal enzymatic reactions or disrupt the outer cell membranes of microorganisms (Romeo et al. 2008). It is well known that the biocidal character of silver is better investigated and understood. Silver ions interact with the bacterial cell envelope and with molecules inside the cell (e.g., nucleic acids and enzymes) or produced reactive oxygen species (Kędziora et al. 2018).

The results of the microbial tests performed on the control film (pure film V) and on films with additives (VIII–XI) were collected in Table 4.

As expected, no inhibition zones against microorganisms were observed for the control film V. The results obtained for the other films indicated that the antimicrobial activities of the tested films depended on the type of microorganism as well as on the terpene compound used as the additive. It was assumed that the main reason for the varied susceptibilities of the tested microorganisms to the action of the films were

Table 4 Average growth inhibition zones around the studied films

Studied films	Microorganisms growth inhibition zones [mm]		
	<i>E. coli</i>	<i>S. epidermidis</i>	<i>C. albicans</i>
V	0.0 $\pm$ 0.0* (–)	0.0 $\pm$ 0.0 (–)	0.0 $\pm$ 0.0 (–)
VIII (silver)	7.1 $\pm$ 1.0 (+ +)	4.9 $\pm$ 1.0 (+)	0.0 $\pm$ 0.0 (–)
IX (α -pinene)	7.6 $\pm$ 1.0 (+ +)	0.0 $\pm$ 0.0 (–)	0.0 $\pm$ 0.0 (–)
X (menthol)	9.8 $\pm$ 0.5 (+ + +)	0.0 $\pm$ 0.0 (–)	0.0 $\pm$ 0.0 (–)
XI (eugenol)	11.5 $\pm$ 1.0 (+ + +)	8.25 $\pm$ 1.5 (+ +)	20.5 $\pm$ 0.5 (+ + +)

*sd–standard deviation (the sensitivity of the organisms)

Fig. 5 Samples of film XI (with 3.0 g of eugenol) evaluated by inhibition of growth against *E. coli*, *S. epidermidis* bacteria, and yeast, *C. albicans*

caused by differences in the composition and structure of the cell envelopes (e.g. lipid and protein composition of membranes) and species-specific physiology. Comparing the microorganisms, it can be concluded that yeast, *C. albicans*, and gram-negative *E. coli* bacteria, were more sensitive to all tested substances since inhibition zones were observed and these zones were significantly larger than those obtained for the others microorganisms. During our studies, the best overall antimicrobial activity was exhibited by film XI (the film containing 3.0 g of eugenol). For this film, the inhibition of microorganism growth was also

observed (Fig. 5). The broad-spectrum of the activity of eugenol against a varied group of microorganisms is confirmed by the results described in the literature (Marchese et al. 2017). However, the mechanism of action may require further investigations.

Unexpectedly, the films enriched with silver, α -pinene, and menthol did not present anti-yeast activity. It must be emphasized that films containing eugenol, α -pinene, menthol, and colloidal silver showed almost the same antibacterial activities, respectively, in comparison with those of the pure substances (Fig. 4). It can be assumed that various tested agents diffuse very easily through polymer composite films.

Due to the increase of bacterial resistance to antibiotics, there is considerable interest in developing novel natural antimicrobial agents for medical applications. It is noteworthy that components of essential oils derived from plants (mainly terpene compounds) do not have an influence on somatic human cells. Moreover, these compounds are used very often as antioxidant agents (Romeo et al. 2008). It was shown that eugenol from clove had high activity against some representative skin microorganisms, especially *S. epidermidis* and *C. albicans*. The latter microorganism is a commensal fungus that colonizes healthy human skin and the reproductive tract. However, for some immunocompromised people *C. albicans* is a predominantly opportunistic fungal pathogen, causing disseminated candidiasis and chronic mucocutaneous candidiasis (CMC).

Over the past few years, various polymers enriched with natural substances (such as, isolates from marjoram, cinnamon, and clove) have been extensively investigated. In order to form antibacterial materials,

Fig. 6 The appearance of film V with the medical net inside: (a) and (b)

Fig. 7 The patch design

Fig. 8 The patch prototype

methyl-cellulose, chitosan, gelatin, and starch films were used (Fisher and Phillips 2008; Sánchez-González et al. 2011; Hernández-Ochoa et al. 2012; Shaaban et al. 2014; Wu et al. 2017). Sánchez-González et al. defined various factors that can affect the antimicrobial action of films (Sánchez-González et al. 2011). These factors are: the nature of substances (non-volatile or volatile), characteristics of the film matrix, and the method of film preparation. Our results confirmed these claims, so further studies are needed, before producing patches with the incorporation of terpene compounds or silver. The exact mechanism of their action must be fully understood and effective dosage should be determined.

The design and preparation of patch prototype

The optimal film chosen (V) was formed on the medical net to strengthen its mechanical properties, and is shown in Fig. 6.

The film obtained with the medical mesh inside was glued onto the fabric with an acrylate pressure-sensitive adhesive (acrylic PSA) so that the glued fabric attached to the hydrogel could remain attached to the body. The whole was protected with siliconized paper. The design of this patch is depicted in Fig. 7, and the self-adhesive protective patch prototype was prepared and is shown in Fig. 8. It is flexible and able to take the shape and size of the area on the human body on which it is placed.

Conclusions

This work has shown that polysaccharide films based on starch, CMS and CMC together with glycerol, citric acid, and imbued with terpene compounds of natural origin can be good materials for the preparation of medicinal patches. The properties of these films are comparable to—or even better than—those same polysaccharide materials containing colloidal silver.

Moisture absorption tests showed that colloidal silver and α -pinene films had comparable results, while much better results were obtained for menthol. On the other hand, the addition of eugenol did not significantly change the moisture absorption value compared to the pure film without additives. The parameter of moisture absorption by the films is very important, because the designed patches should absorb moisture from infected or damaged places on the skin. As a result, the development of various infections can be inhibited, and as a consequence, the treatment will be accelerated.

Tests on the water solubility of the obtained films showed that none of the films fell apart but the films

with terpenes showed increased solubility in comparison to the film with colloidal silver, which is not favorable. On the other hand, the dissolution of materials containing terpene compounds may release these molecules from the film structure, which will be able to directly affect the infected skin, and, consequently—considering their therapeutic effect—accelerate the healing process.

The mass and volume increases were the highest for film IX (α -pinene as the additive). Films with other additives, including colloidal silver, showed lower values for these two parameters but slightly higher than for pure film V (without any additives). These results confirmed that with the help of appropriate terpene compounds it is possible to obtain comparable or better materials for preparing patches than with colloidal silver. The increase in volume with simultaneous absorption of moisture (wound exudates) makes this film behave simultaneously like a “protective cushion” as well as a “soft compress”.

During the microbiological tests, it was found that the film with eugenol showed the best antimicrobial activity. Films with other terpenes, however, showed similar antimicrobial activity as the film with colloidal silver. Moreover, test results should allow them to be used as patches for the treatment of EB (*Epidermolysis bullosa*), burns, eczema, acne, and abrasions. These films should not only protect against the development of infections but also, with the regenerative properties of the terpenes, provide adequate hydration and accelerate the regeneration and healing of the skin.

Considering the mechanical properties of this kind of terpene-included film, the medical patch based on it should be prepared with the help of a medical net. In the future, taking into account the medical uses of the developed patches, the overall analysis of biocompatibilities including cytocompatibility, tissue compatibility or hemocompatibility should also be performed. The great benefit of using terpene compounds as additives for materials intended to serve as patches should also be emphasized, since these compounds are substances of natural origin obtained from plants, are relatively cheap and renewable raw materials.

Declarations

Conflict of interest The authors declared that they have no conflict of interest.

Ethical approval This study following Compliance with Ethical Standards; this study does not involve human participants, animals, and potential conflicts of interest.

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