HYDROGEL DRESSINGS WITH ANTIMICROBIAL AND HEALING PROPERTIES

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The current study aimed to develop hydrogels as delivery systems based on a mixture of biodegradable and biocompatible polymers (i.e., gelatin and collagen) for encapsulating different active principles (i.e., chamomile and plantain tincture, aloe vera and propolis) to obtain dressings for treating first-degree burns injuries. The synthesized hydrogels were then immobilized on a textile material made from 100% cotton fibers. The functionalized textile materials were analyzed in terms of physical-mechanical characteristics, water absorbency and antibacterial activity. SEM analysis was used to investigate the morphology of the cotton fibers after the functionalized treatment. The antibacterial activity of the treated samples was qualitatively assessed through the Agar diffusion method by using cultures in a liquid medium of *S. aureus* and *E. coli* test strains. The obtained overall results indicated that incorporating of these active principles into the polymeric hydrogels can significantly enhance the potential efficiency of the fabrics as dressings with antimicrobial and healing accelerating properties and can be an appropriate option for treating first-degree burns injuries.

Keywords: dressings, first-degree burns injuries, hydrogels

INTRODUCTION

Wound dressings with antimicrobial and wound healing accelerating properties are emerging as valuable options to prevent wound infection and improve the wound healing process being an appropriate option for treating first-degree burns injuries (Laurano *et al*., 2022; Fan *et al.*, 2022). Compared to other types of polymeric systems, hydrogels are 3D networks consisting of physically or chemically cross-linked hydrophilic polymers, which are the best choice for a dressing material (Tavakoli and Klar, 2020; Jaya and Vivek, 2014). Dressings based on hydrogels demonstrated advanced functions in the wound process such as antimicrobial properties, adhesion and hemostasis, anti-inflammatory effects, drug delivery, self-healing, and stimulus-response (Luneva *et al.*, 2022). In order to obtain biomaterials with potential use in the treatment of first-degree burns injuries, this experimental study approached the immobilization on 100% cotton fabric of hydrogels based on collagen-gelatin as polymeric matrix which embedding different active principles, in certain formulations.

EXPERIMENTAL PART

Materials

Collagen (*ZENYH, Romania*) and gelatin (bovine skin, type B, *Sigma Aldrich, Germany*) were used as embedding agents for the bioactive agents. Tween 80 (*Sigma Aldrich, Germany*) was used as surfactant and glycerol (*Riedel-de haën|Honeywell, USA*) has been used as a solubilizing agent. Aloe vera (organic powder *Mayam, Romania*), propolis (alcoholic solution 25%, propolis extract – hydroglyceric solution 60%, propolis extract 48% – with colloidal silver 50 ppm, *Dacia Plant, Romania*),

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plantain tincture (70% ethanolic solution, *Hofigal, Romania*) and chamomile tincture (70% ethanolic solution, *Hofigal, Romania*) were used as bioactive agents. Calcium chloride (anhydrous, *Fluka|Honeywell, USA*) was used as a cross-linking agent. For the preparation of bioactive systems, distilled water has been used. Bleached 100% cotton fabric was used for the functionalization processes.

Synthesis of the Hydrogels

For the achievement of the bioactive systems, initially, stock solutions of 5% collagen and 10% gelatin were prepared and then mixed under magnetic stirring, the mixture ratio of collagen:gelatin (by volume) was fixed at 1:1 (20 mL collagen and 20 mL gelatin). Further, over the previously prepared polymeric matrix, 13.3 mL of 5% Aloe vera solution was added, followed by the incorporation of 10 mL glycerol, which was added dropwise. The system was maintained under magnetic stirring for 10 minutes. After that, 4 mL Tween 80 and 28.5 mL distilled water separately were added under vigorous magnetic stirring. Further, after the complete homogenization of the solution, 2 mL propolis (alcoholic or hydroglyceric solution or with colloidal silver), 2 mL tincture (plantain or chamomile) were separately added, maintaining the magnetic stirring for 10 minutes at each stage. As a final step in the synthesis of the hydrogels, a solution of 1% of calcium chloride was added as a cross-linking agent. The selected experimental variants are presented in Table 1.

Table 1. The hydrogel's bioactive principle constituents for each experimental variant

Code	Active Principles					
	Propolis				Tincture	
	Alcoholic solution	Hydroglyceric solution	Colloidal silver	Aloe Vera	Plantain	Chamomile
TAP1	×			×	×	×
TAP2		×		×	×	×
TAP3			×	×	×	×
TAP4		×		×	×	
TAP5		×		×		×

Immobilization of Hydrogels on the Textile Materials

The synthesized hydrogels were immobilized on the textile materials by padding method on the laboratory padder (ROACHES, UK). The treated textile materials were then subjected to the drying operation at 50°C, for 3 minutes.

Methods

pH Analysis

The pH of the synthesized hydrogels was measured using a Multiparameter benchtop meter inoLab® Multi 9310 IDS (*Germany*). The measurements were made in triplicate.

Optical Microscopy

The synthesized hydrogels were analyzed microscopically using an OLYMPUS BX51 optical microscope (*Philippines*) equipped with the OLYMPUS UC30 photo digital camera.

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Air and Vapor Water Permeability

The water vapor permeability was performed according to STAS 9005:1979 standard and the air permeability was performed according to SR EN ISO 9237:1999 standard.

Hydrophilicity

Hydrophilicity of functionalized textile materials was evaluated according to Romanian Standard SR 12751-89 determining fabric wettability by drop method.

Assessment of Antibacterial Activity

The antibacterial activity of the treated samples was qualitatively assessed by the Agar diffusion method according to the SR EN ISO 20645:2005 standard - Determination of antibacterial activity-agar diffusion plate test, by using cultures in liquid medium replicated at 24 hours of ATCC 6538 *Staphylococcus aureus* and ATCC 11229 *Escherichia coli* test strains. Inhibition zones were calculated using the following formula:

$$H = (D - d)/2 \tag{1}$$

where: H – the inhibition zone [mm]; D – the total diameter of specimen and inhibition zone [mm]; d – the diameter of specimen [mm]. The criteria for inhibition zones according to the standard SR EN ISO 20645:2005 are presented in the Table 2.

Table 2. Criteria for inhibition zones according to the standard SR EN ISO 20645:2005

Inhibition zone [mm]	Growth	Evaluation
>1 1-0 0	absence	satisfactory effect
0	little	efficiency limit
0 0	moderate important	unsatisfactory effect

Electron Microscopy

Visualization of the morphology of the cotton fiber surfaces treated with the bioactive systems was performed by scanning electron microscopy using the Quanta 200 electron microscope (*FEI, The Netherlands*).

RESULTS AND DISCUSSION

Optical Microscopy

The images obtained by optical microscopy are shown in Figure 1. According to microscopic images, the dispersed molecule phase is presented as a compact, dense small globule mass. Normally, the destabilization of the solutions begins with the drop's flocculation of different tinctures followed by the phenomenon of coalescence and formation of two distinct phases (separation).

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TAP1 TAP2 TAP3 TAP4 TAP5

Figure 1. Optical microscopy of the resulted bioactive systems

pH Analysis

The pH values for synthesized polymeric systems are presented in Table 3.

Table 3. The pH values of the synthesized hydrogels

Code	pH value
TAP1	4.91
TAP2	4.92
TAP3	4.92
TAP4	4.89
TAP5	4.99

The pH of the polymeric solutions is between 4.89-4.99. It is known from the literature that the skin without any injuries has a pH between 4-6 and the wound can have a pH of up to 8.9. Also, a study on the pH of commercial dressings on the market showed that the pH value is around 4-5. In conclusion, the polymeric solutions which will be used to obtain dressings for first-degree burns injuries, fall within the accepted limits for the field of application from the point of view of the pH value.

Air and Vapor Water Permeability

The values obtained for water vapor permeability and air permeability are presented in Table 4.

Table 4. Air permeability, vapor water permeability and the hydrophilicity of the obtained biomaterials

Code	Vapor water permeability	Air Permeability [l/m^2/s]		Hydrophilicity
		100 Pa	200 Pa	
TAP1	29,93	155	208.6	instantly
TAP2	26.13	201	350.2	instantly
TAP3	27.56	217.8	401	instantly
TAP4	27.74	235.2	445.8	instantly
TAP5	25.77	204.6	375.8	instantly
M	30.52	362.2	450.3	instantly

Air and water vapor permeability recorded lower values compared to the untreated fabric due to the polymeric substances deposited at the surface of the fabric in the form of a semi-permeable film, indicating a comfort decrease for all experimental variants. The most pronounced decrease in air permeability was obtained for the textile material treated with the hydrogel based on collagen-gelatin-propolis (alcoholic solution)-aloe vera-plantain tincture-chamomile tincture (code TAP1), highlighting thus the fact that the use of propolis in the form of an alcoholic solution causes a more pronounced decrease in the comfort indices compared to the hydroglyceric solution. This behavior can be attributed to the increase in alcohol concentration in the polymeric system determined by the using several alcoholic based tinctures which leads to a sealing of the

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free zones in the fabrics geometry thus limiting the free zones in the textile substrate and subsequently the volume of air penetrating the samples.

Hydrophilicity

According to the results presented in Table 4, all the analysed samples present the good hydrophilicity, the functionalization treatments not having a negative influence on this feature. The glycerin content in a dressing improves the ability to absorb excess moisture and exudate. At the same time, glycerin has the ability to be absorbed in the fluids of the body, being diluted quickly without causing toxicity to the body. The surface of the hydrogel is not completely dry due to its high affinity to water, and the polymer is sufficiently viscous to remain on the surface of the lesion, finally allowing easy removal without causing skin damage.

Antimicrobial Activity

Images of Petri plates after 24h incubation are shown in Figure 2 and the assessment of antibacterial activity is shown in Table 5.

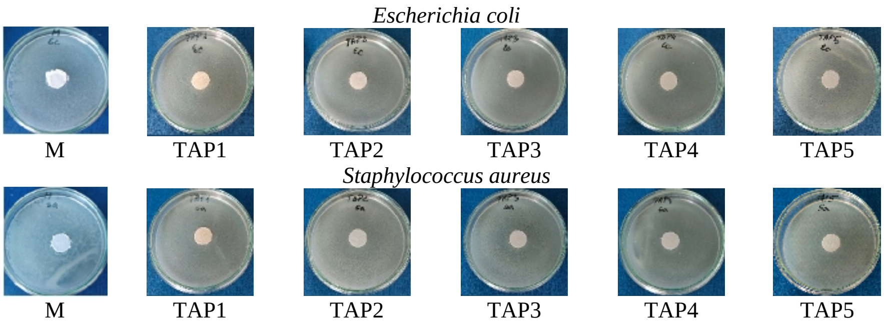

Figure 2. Images of Petri plates showing antibacterial effect after 24 h of incubation

Table 5. Evaluation of the antibacterial activity

Code	Inhibition zone (mm)		Evaluation	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
TAP1	0	0.5	Satisfactory effect	Satisfactory effect
TAP2	0	0	Satisfactory effect	Satisfactory effect
TAP3	0	0	Satisfactory effect	Satisfactory effect
TAP4	0	0	Satisfactory effect	Satisfactory effect
TAP5	0	0	Satisfactory effect	Satisfactory effect
M	0	0	Unsatisfactory effect	Unsatisfactory effect

According to ISO 20645 standard method, in the case of no inhibition zone (0 mm), without bacterial growth in the nutrient medium under the specimen, the antibacterial effect is evaluated as “satisfactory”, which indicates antimicrobial activity. By analyzing the obtained results, it can be concluded that the textile materials treated with synthesized hydrogels have an antibacterial effect against both test strains.

Scanning Electron Microscopy

The selection of the images obtained at a magnification of ×50, ×1000, and ×2000 for the textile material treated with a bioactive system are shown in Figure 3. The

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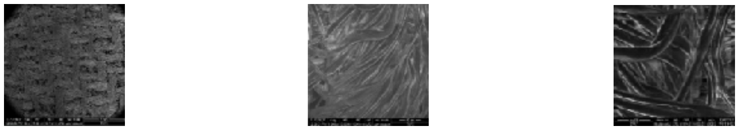
resulting micrographs reveal that the surface of the cotton fibers is covered with a thin polymeric layer deposited both on the surface of the fibers and inside the space between the fibers. However, the micrographs cannot provide information about the thickness of the deposited layer and its uniformity.



×50 ×1000 ×2000

Figure 3. Electronic images recorded for the TAP 3 variant

CONCLUSIONS

The current study was set to prepare a delivery system based on a mixture of biodegradable and biocompatible polymeric hydrogels for encapsulating different active principles. The enriched hydrogels were used to functionalize textile materials. The obtained wound dressings were subjected to different analyzes using scanning electron microscopy, physical-mechanical investigations and antibacterial tests. Results suggest that the samples enriched with the alcoholic propolis solution exhibited a more pronounced decrease in the comfort indices compared to the hydroglyceric solution. The pH of the synthesized hydrogels varies between 4.89 and 4.99, values which according to the specialized literature are suitable for wound dressings. The textile materials treated with synthesized hydrogels based on collagen-gelatin-bioactive principles have antibacterial effect against both test strains.

These overall results indicate that incorporating these active principles into the polymeric hydrogels can significantly enhance the potential efficiency of the fabrics as wound dressing material, the researches being in progress.

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