Development of antimicrobial hydrogels for burn wound treatment

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LAURA CHIRILĂ
ALINA POPESCU
MĂDĂLINA IGNAT
MANUELA AVADANEI
CRISTINA MIHAELA LITE

ABSTRACT – REZUMAT

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To obtain biomaterials with the potential for use in the treatment of first-burn injuries, this study focused on the development of several polymeric systems based on collagen-polyvinyl alcohol-active principles. The hydrogels were prepared using polymeric matrices formers (collagen and polyvinyl alcohol), water, and glycerol in the presence of the nonionic surfactant polysorbate 80 (Tween 80®) under proper homogenization. For the development of multifunctional textile materials designed for topical application, ciprofloxacin, chlorhexidine, tea tree essential oil, and curcumin were used as active principles. The obtained hydrogels were then immobilized by the padding method on 100% plain weave cotton. The functionalized textile materials were characterized in terms of their physico-mechanical and comfort characteristics, hydrophilicity, and antibacterial activity. The mass of all the functionalized textile materials increased compared to that of the untreated fabric, due to the amount of polymeric systems remaining after the functionalization process. The water vapour permeability and air permeability of the functionalized materials were lower than those of the untreated samples. Antibacterial activity was observed for all analysed samples, with inhibition zones between 14 mm (CUC-11 code in the presence of S. aureus) and 27 mm (CUC-1 code in the presence of E. coli), obtained for the textile materials treated with the hydrogels containing ciprofloxacin, exhibiting the most pronounced antibacterial effect compared to analogous samples containing chlorhexidine. The obtained experimental data suggest that these hydrogels are appropriate candidates for application in burn wound management.

Keywords: *cotton, antibacterial activity, drugs, curcumin, tea tree essential oil*

Dezvoltarea de hidrogeluri antimicrobiene pentru tratamentul arsurilor

Pentru a obține biomateriale cu potențial de utilizare în tratamentul arsurilor de gradul I, acest studiu s-a concentrat pe dezvoltarea mai multor sisteme polimerice pe bază de colagen-alcool polivinilic-principii active. Hidrogelurile au fost preparate folosind formatori de matrici polimerice (colagen și alcool polivinilic), apă și glicerol în prezența surfactantului neionic polisorbat 80 (Tween 80®) sub omogenizare corespunzătoare. Pentru dezvoltarea materialelor textile multifuncționale destinate aplicării topice au fost folosite ca principii active ciprofloxacina, clorhexidina, uleiul esențial de arbore de ceai și curcumina. Hidrogelurile obținute au fost ulterior imobilizate prin metoda fulardării pe o țesătură din 100% bumbac. Materialele textile funcţionalizate au fost caracterizate din punct de vedere al caracteristicilor fizico-mecanice şi de confort, al hidrofiliei şi al activității antibacteriene. Masa tuturor materialelor textile funcţionalizate a crescut faţă de cea a ţesăturii netratate, datorită cantităţii de sisteme polimerice rămase după procesul de funcţionalizare. Permeabilitatea la vapori de apă și permeabilitatea la aer a materialelor textile funcționalizate au fost mai mici decât cele ale probelor netratate. Pentru toate probele analizate a fost observată activitate antibacteriană, cu zone de inhibiție între 14 mm (CUC-11 în prezența S. aureus) și 27 mm (CUC-1 în prezența E. coli), obținute pentru materialele textile tratate cu hidrogelurile care conțin ciprofloxacină și care prezintă cel mai pronunțat efect antibacterian în comparație cu eșantioanele analoge care conțin clorhexidină. Datele experimentale obținute sugerează că aceste hidrogeluri sunt candidați adecvați pentru aplicații în managementul rănilor cauzate de arsuri.

Cuvinte-cheie: *bumbac, activitate antibacteriană, medicamente, curcumină, ulei esențial de arbore de ceai*

INTRODUCTION

The antibacterial property is one of the most frequently desired properties from a wound dressing, as the growth of microorganisms is controlled or eliminated by the presence of antimicrobial agents that are embedded into the textile material`s structure. Incorporating antimicrobial agents into wound dressings has been the most effective way to control the spreading of bacteria from wound sites. Different antimicrobial materials, which include chlorhexidine, hydrogen peroxide, chitosan, essential oils, honey, proflavine, iodine and silver have been in use in different wound dressings [1, 2].

Hydrogels are polymers designed to retain up to 96% water [3]. They possess a 3D network structure formed through chemical or physical cross-linking. The mechanism of water retention is based on the hydrophilic nature of the functional groups present in the polymer, such as -OH, -COOH, NH_2, -CONH, and SO_3H. Hydrogel fabrication is achieved by either physical cross-linking (through ionic interactions, crystallization, hydrogen bonding between chains, amphiphilic block copolymer combination, and protein interactions) or chemical cross-linking (through chain-growth polymerization, reactions of complementary groups, or thiol-alkene reaction or by using high energy radiation) [4]. The most common hydrogels used are polyacrylamide, polyvinylpyrrolidone, polyvinyl alcohol, polyethylene glycol, polyethylene oxide, and their derivatives [5].

Natural hydrogels consist of polypeptides (such as collagen, fibrin, gelatine, etc.) or polysaccharides (e.g., cellulose, chitosan, alginate, hyaluronic acid, etc.) and present advantages in biocompatibility and biodegradability. However, synthetic hydrogels are more versatile since they possess tunable properties by design, such as elasticity, porosity, viscosity, and swelling [6]. Hydrogel dressings are used in burn injury treatment because they are biocompatible materials, exhibit good oxygen and water permeability, are able to adhere to tissues and protect them from harmful environmental factors, absorb wound exudates, and even contribute to pain relief [7].

Research on improving hydrogel performance has led to the promotion of multifunctional hydrogels that minimize infections and maximize healing by delivering antimicrobial and antiseptic agents [8]. Moreover, engineered hydrogels with different scaffolds can be used for tissue regeneration in skin repair strategies [9, 10].

The enrichment of hydrogels with antimicrobial compounds has led to many studies advancing the field of burn wound management. Furthermore, the progress in manufacturing multilayer hydrogel dressings has enabled improvements in healing properties. Additionally, drug-loaded hydrogels provide controlled drug release for longer durations, reducing the need for frequent changing of wound dressings [11]. Tamahkar et al. reported the antibacterial efficiency of a multilayer hydrogel dressing loaded with ampicillin that exhibited antibiotic release for seven days [12]. Another wound-healing dressing was produced by encapsulating cefazoline into coaxial polylactic acid (PLA)-based electrospun nanofibers. Using this material, Hajikhani studied the rate of wound closure for samples containing 10% and 20% collagen [13]. Similarly, Shi studied the healing properties of a wound dressing based on a dual-release system for gentamycin sulphate and platelet-rich plasma. The release of the active components was achieved by loading gelatine microspheres with covalent bonding to carboxymethyl chitosan in the matrix of carboxymethyl chitosan [14]. The most recent studies aimed to use different *green* antimicrobial agents, such as herbal extracts [15] or essential oils [5,16], for loaded hydrogel construction.

Despite their reported activity against both gram-positive and gram-negative bacteria, the main disadvantage of essential oils is their high volatility and susceptibility to degradation under different mild conditions (such as temperature, light, or oxidation) [17]. Hence, the integration of essential oils into hydrogel matrices constitutes an attractive approach for maintaining their properties and controlling their release [18]. Wang and collaborators reported using eucalyptus essential oil, ginger essential oil, and cumin essential oil to prepare effective antibacterial hydrogels physically cross-linked by carboxymethyl chitosan and carbomer 940. They demonstrated that burn wound repair in a mouse model was significantly accelerated when the developed hydrogel dressing was applied [19]. Huma Mahmood performed the co-encapsulation of tea tree or lavender with oil ofloxacin in gellan gum-based hydrogel films as wound dressings [20]. Lu also used thyme oil to manufacture an antibacterial-loaded zwitterionic hydrogel [21]. Other essential oils incorporated into hydrogel dressing systems for their antimicrobial properties include *Hypericum perforatum* oil [22], *Eupatorium adenophorum* essential oil [23], clove essential oil [24], *Galium verum* essential oil [25], and chamomile oil [26].

Another active component used in hydrogel enrichment is curcumin due to its antimicrobial, antioxidant, and anti-inflammatory properties [27, 28]. Wafa Shamsan Al-Arjan developed a pH-responsive dressing material capable of curcumin release for burn and chronic wound healing [29]. Babaluei also formulated a hydrogel dressing based on sodium carboxymethylcellulose, polyacrylamide, and mussel-inspired polydopamine containing vitamin C and curcumin to promote full-thickness burn regeneration [30].

In the field of smart textile research, there are only a few publications regarding the use of hydrogels for burn wound management. Michael Rodrigues tested a textile-based hydrogel dressing containing usnic acid for its antimicrobial and antibiofilm properties. The material has up to 99.9% microbial reduction percentages against *Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans*, and *Aspergillus niger* [31]. Türkoğlu et al. also reported the development of textile-based sodium alginate and chitosan dressings and investigated the physico-mechanical properties of the resulting products [32].

Given the progress in research on the use of hydrogels for burn wound management, the further application of these materials in textiles is worth exploring. This study aimed to develop textile materials with functional properties by applying bioactive polymeric systems based on collagen-polyvinyl alcohol-active principles (ciprofloxacin, chlorhexidine, curcumin, and tea tree essential oil) that have been tested on textile fabrics for their antimicrobial and drug-releasing properties as potential candidates for first-degree burn treatment. Ciprofloxacin is an antibiotic from the fluoroquinolone class, patented by Bayer [33], while chlorhexidine is an antiseptic widely used as a disinfectant in oral hygiene [34]. The polyvinyl alcohol was selected as a polymeric matrix due to its biocompatibility and ability to undergo self-crosslinking due to the high density of hydroxyl groups on its side chains

Table 1

BIOACTIVE PRINCIPLE CONSTITUENTS FOR EACH EXPERIMENTAL HYDROGEL						
Code	**PVA**	**Collagen**	**Ciprofloxacin**	**Chlorhexidine**	**Essential oil**	**Curcumin**
CUC-0	*	*				
CUC-1	*	*	*		*	*
CUC-2	*	*	*		*	
CUC-3	*	*	*			*
CUC-4	*	*			*	*
CUC-5	*	*	*			
CUC-6	*	*			*	
CUC-7	*	*				*
CUC-8	*	*		*	*	*
CUC-9	*	*		*	*	
CUC-10	*	*		*		*
CUC-11	*	*		*		

[35]. Moreover, collagen was incorporated to provide regenerative properties, along with improving biocompatibility. For this purpose, formulations of different hydrogels have been carried out. The aim was to identify the main factors influencing the formulation process and the most appropriate composition for developing textile materials designed for the treatment of first-degree burns.

MATERIALS AND METHODS

Materials

Collagen (ZENYH, Romania) and polyvinyl alcohol (87-90% hydrolyzed, average mol wt 30,000–70,000, Sigma Aldrich) were used as embedding agents for the bioactive principles. Ciprofloxacin (Sigma Aldrich), chlorhexidine digluconate (Sigma Aldrich), tea tree essential oil (Mayam, Romania), and curcumin (PuraSana, Romania) were used as bioactive principles. Polysorbate 80 - Tween 80® (Sigma Aldrich) was used as a nonionic surfactant, and glycerol (Riedel-de haën|Honeywell, USA) was used as a solubilizing agent. Glutaraldehyde (Sigma Aldrich) was used as a cross-linking agent for the polymeric systems. Bleached 100% cotton woven fabric with a weight of 168 g/m^2 was used for the functionalization processes.

Synthesis of the hydrogels

First, stock solutions of 5% collagen and 10% PVA were prepared and mixed through continuous stirring at 600 rpm for 30 minutes at room temperature. The collagen-polyvinyl alcohol mixture ratio (by volume) was fixed at 1:1 (20 ml of collagen and 20 ml of PVA) (*Solution 1*). To obtain the essential oil/curcumin system, 10 mg of curcumin was mixed under continuous stirring with 2.4 ml of tea tree essential oil until complete homogenization (*Solution 2*). Afterwards, over the previously prepared polymeric matrix (*Solution 1*), 0.2 g ciprofloxacin or 4 ml chlorhexidine (20 µl/ml) was added, and magnetic stirring was continued for another 30 minutes. To improve the flexibility of the hydrogels, 10 ml of glycerol was added to the resulting mixture, and after homogenization, 4 ml of Tween 80 was added. Furthermore, the oil/curcumin system (*Solution 2*) was added dropwise to the above polymeric mixed solution under continuous stirring for 30 minutes. Then, 0.2 ml of glutaraldehyde was added as a cross-linking agent as the final step of hydrogel synthesis. The succession of PVA-collagen hydrogel preparation stages is presented in figure 1, and the selected experimental variants are presented in table 1.

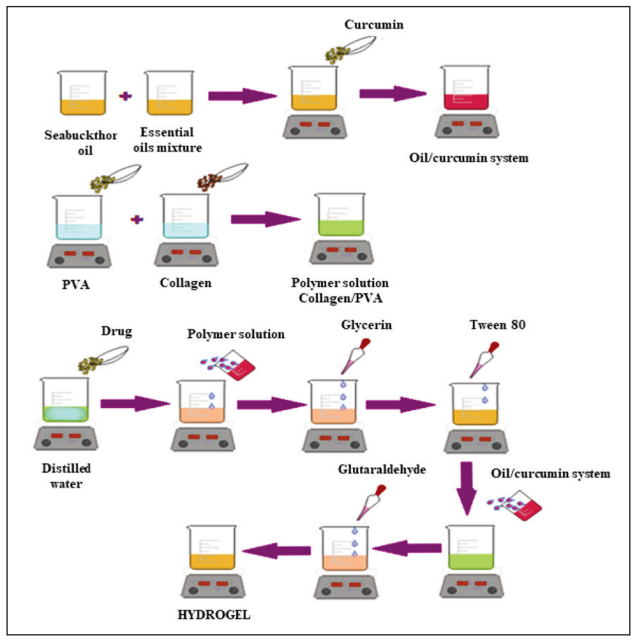

Fig. 1. The succession of PVA-collagen hydrogel preparation stages

Methods

Dynamic light scattering – DLS

The hydrogel samples (CUC-0 – CUC-10) were analysed using Zetasizer Nano ZS equipment (Malvern) by dynamic light scattering (DLS) technique. For each sample, three measurements were made to determine the particle size and the zeta potential. All eleven samples were measured without any further preparation at 25°C using specific standard operating procedures (SOPs) and disposable folded capillary cells (DTS 1070).

Physico-chemical and physico-mechanical characteristics

The treated woven fabrics were characterized in terms of the main physico-chemical and physico-mechanical characteristics, respectively: mass per unit area (SR EN 12127-2003), water vapour permeability (STAS 9005:1979), permeability to air (SR EN ISO 9237:1999) and hydrophilicity based on wettability (measured by the drop test method according to the Romanian Standard SR 12751/1989 standard).

Assessment of antibacterial activity

The antibacterial activity of the functionalized 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 liquid cultures of the ATCC 6538 *Staphylococcus aureus* and ATCC 11229 *Escherichia coli* test strains replicated at 24 h. Inhibition zones were calculated using the following formula:

$$H = (D - d)/2 \quad (1)$$

where H is the inhibition zone diameter (mm), D – the total diameter of the specimen and inhibition zone (mm) and d – the diameter of the specimen (mm).

After incubation, the obtained results were assessed based on the absence or presence of bacterial growth in the contact zone between the agar and the sample and based on the eventual appearance of an inhibition zone. Following the standard method, the inhibition zone was measured in mm, and the degree of bacterial growth was estimated in the nutrient medium under the specimen. The criteria for inhibition zones according to the standard SR EN ISO 20645:2005 are presented in table 2.

Table 2

CRITERIA FOR INHIBITION ZONES ACCORDING TO THE SR EN ISO 20645:2005 STANDARD

Inhibition zone (mm)	Growth	Evaluation
>1	absence	satisfactory effect
1–0		
0		
0	little	efficiency limit
0	moderately	unsatisfactory effect

RESULTS AND DISCUSSION

Dynamic light scattering (DLS)

DLS is a suitable technique for the determination of the size distribution profile of particles in a dispersion. Although it is recommended for measuring molecules and particles typically in the submicron range, it has also been shown to be a useful tool for the characterization of hydrogels. The results obtained for the hydrogel samples are presented in table 3.

Hydrogel samples have different sizes, depending on their composition and component interactions.

Ciprofloxacin is a fluoroquinolone antibiotic used to treat many bacterial infections and contains a large molecule, which was also reflected in the DLS results, where samples that contained ciprofloxacin, CUC-1, CUC-2, CUC-3 and CUC-5, had large sizes. PdI represents the polydispersity of the sample, and values <0.2 are preferred. The obtained PdI values suggest that the samples had high polydispersity. The zeta potential is an indicator of sample stability. The values obtained for the hydrogel samples suggest a tendency for deposition and particle agglomeration and the sign represents the type of charge on the surface of the particles. From a stability perspective, the most stable dispersion seems to be CUC-5, with positively charged particles. The obtained hydrogels have good conductivity, which makes them feasible for applications in various other fields, such as soft electronics, sensor and actuator fabrics, and biomedicine.

Table 3

SIZE DISTRIBUTION AND ZETA POTENTIAL FOR HYDROGEL SAMPLES

No.	Sample	Size (nm)	PdI	Zeta potential (mV)	Conductivity (mS/cm)
1	CUC-0	43.7	0.3	–1.63	0.282
2	CUC-1	$3.4*10^4$	0.47	–0.4	0.344
3	CUC-2	$2.9*10^4$	0.71	–0.2	0.293
4	CUC-3	$5.1*10^4$	0.34	–0.5	0.304
5	CUC-4	163	0.71	–0.5	0.331
6	CUC-5	$5.5*10^4$	0.14	25.1	0.297
7	CUC-6	3.2	0.67	–0.3	0.364
8	CUC-7	38.63	0.43	–1.44	0.317
9	CUC-8	91.31	1	0.218	0.313
10	CUC-9	620.9	0.91	–0.522	0.268
11	CUC-10	39.83	0.34	–1.11	0.328

Physico-chemical and physico-mechanical characteristics

The obtained values for the main physico-chemical and physico-mechanical characteristics are presented in table 4.

According to the results presented in table 4, the mass of all functionalized textile materials increased compared with that of the untreated fabric, and the highest increase of 23.5% was observed for the textile material treated with the polyvinyl alcohol-collagen-chlorhexidine-tea tree essential oil-based hydrogel (code CUC-9). There was no distinct correlation between the number and type of hydrogel components and the value of the mass obtained for each functionalized textile material. Lower values were recorded for the air and water vapour permeability of the functionalized materials than for those of the untreated fabric due to the polymeric systems deposited at the surface of the fabrics, indicating a decrease in comfort for all experimental variants. The most pronounced decrease in air permeability (by 46%) was obtained for the textile material treated with the hydrogel based on polyvinyl alcohol-collagen-chlorhexidine-tea tree essential oil (code CUC-9), which was closely related to the increase in the mass of this sample. This indicates that the hydrogel deposited on the surface of the fabric in the form of a semipermeable film leads to an increase in the mass of the functionalized sample and implicitly decreases its air permeability. The performed functionalization treatments led to a decrease in the water vapour permeability for all functionalized samples, with small nonsignificant variations among the analysed variants. Analysis of the hydrophilicity values obtained for the textile biomaterials showed that they possessed excellent moisture absorption capacity, absorbing excess exudate while maintaining a moist environment that stimulated the wound-healing process.

Table 4

PHYSICO-CHEMICAL AND PHYSICO-MECHANICAL CHARACTERISTICS

Code	Mass (g/m^2)	Permeability to air ($l/m^2/s$)	Water vapour permeability (%)	Hydrophilicity (s)
M	204	223.4	35.3	Immediate
CUC-0	247	126.8	31.2	Immediate
CUC-1	243	121.2	33.1	Immediate
CUC-2	249	119.4	31.7	Immediate
CUC-3	249	109.1	29.5	Immediate
CUC-4	239	109.0	25.8	Immediate
CUC-5	248	120.0	30.1	Immediate
CUC-6	229	123.9	29.0	Immediate
CUC-7	241	120.1	31.4	Immediate
CUC-8	249	115.3	30.4	Immediate
CUC-9	252	102.8	32.1	Immediate
CUC-10	244	104.9	33.3	Immediate
CUC-11	250	125.2	31.9	Immediate

Antibacterial activity

Images of Petri plates after 24 h of incubation are shown in figures 2 and 3, and an assessment of antibacterial activity is shown in table 5.

By analysing the obtained results, it can be concluded that the textile materials treated with synthesized emulsions based on polyvinyl alcohol-collagen-active principles had an antibacterial effect against both test strains (*E. coli* and *S. aureus*), with inhibition zones between 14 mm (CUC-11 in the presence of *S. aureus*) and 27 mm (CUC-1 in the presence of *E. coli*). All textile biomaterials obtained by treating textile materials with hydrogels containing ciprofloxacin as an active principle (codes CUC-1, CUC-2, and CUC-3) had the most pronounced antibacterial effect compared to analogous samples containing chlorhexidine (CUC-8, CUC-9, and CUC-10).

Table 5

EVALUATION OF THE ANTIBACTERIAL ACTIVITY

Code	*E. coli*		*S. aureus*	
	Inhibition zone (mm)	Evaluation	Inhibition zone (mm)	Evaluation
M	0	Unsatisfactory effect	0	Unsatisfactory effect
CUC-0	16.0	Satisfactory effect	17.0	Satisfactory effect
CUC-1	27.0	Satisfactory effect	20.0	Satisfactory effect
CUC-2	23.5	Satisfactory effect	21.0	Satisfactory effect
CUC-3	24.5	Satisfactory effect	24.0	Satisfactory effect
CUC-4	20.0	Satisfactory effect	26.0	Satisfactory effect
CUC-5	21.0	Satisfactory effect	17.0	Satisfactory effect
CUC-6	22.0	Satisfactory effect	20.0	Satisfactory effect
CUC-7	23.5	Satisfactory effect	22.0	Satisfactory effect
CUC-8	15.5	Satisfactory effect	18.5	Satisfactory effect
CUC-9	18.0	Satisfactory effect	20.5	Satisfactory effect
CUC-10	16.0	Satisfactory effect	15.0	Satisfactory effect
CUC-11	15.0	Satisfactory effect	14.0	Satisfactory effect

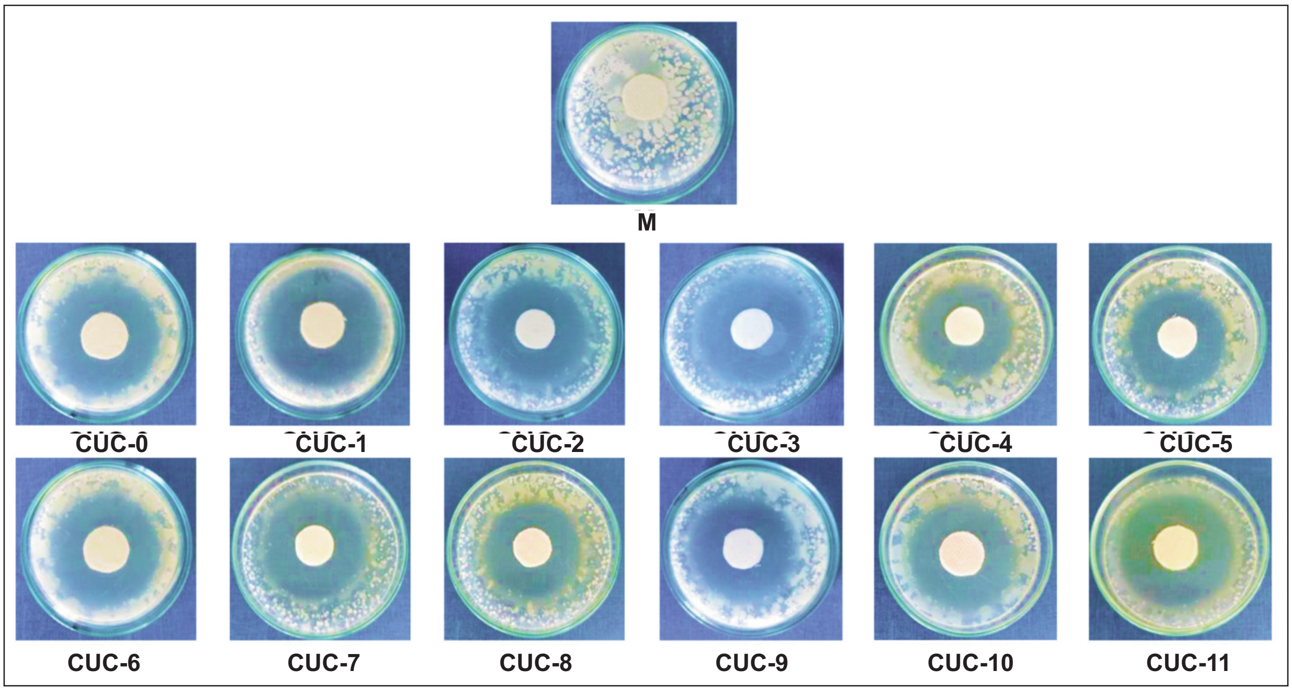

Fig. 2. Images of Petri plates showing an antibacterial effect after 24 h against the *E. coli* test strain

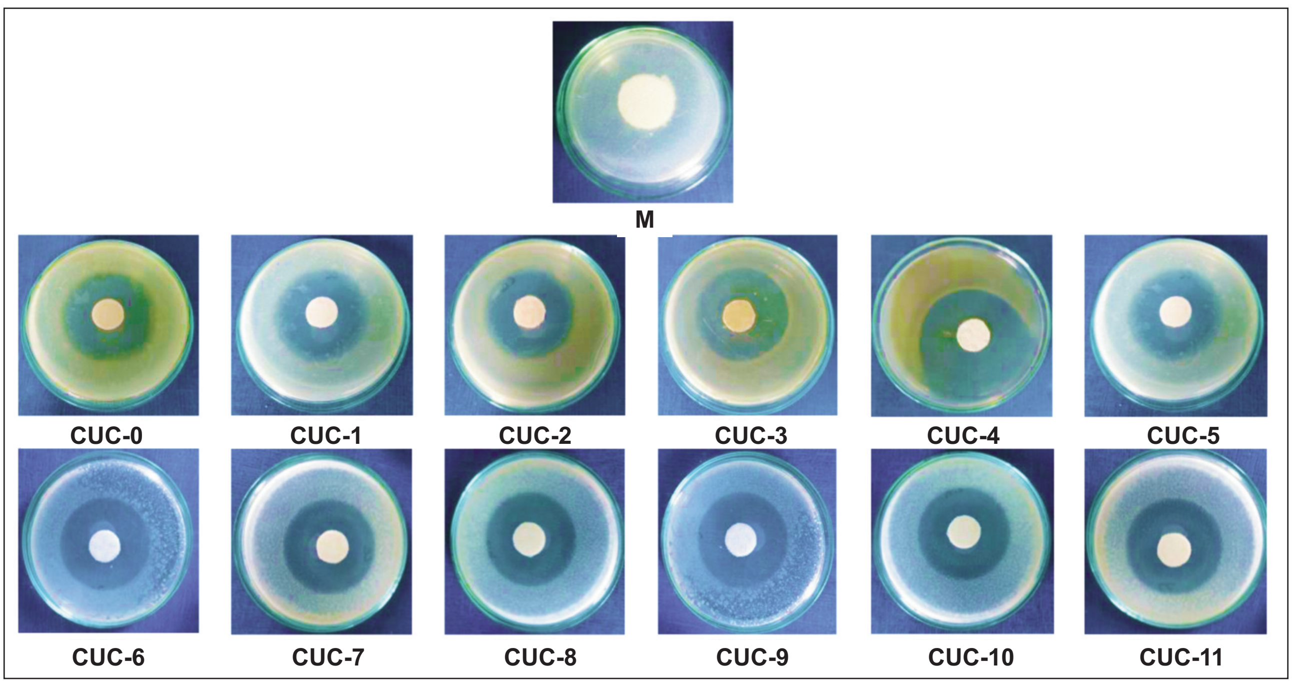

Fig. 3. Images of Petri plates showing an antibacterial effect after 24 h against the *S. aureus* test strain

Among samples treated with hydrogels based on ciprofloxacin and tested against *the E. coli* test strain, a slightly higher level of antibacterial activity was observed for the sample treated with a hydrogel containing all active principles (code CUC-1, inhibition zone = 27 mm) in comparison with samples treated with the hydrogels based only on ciprofloxacin (CUC-5, inhibition zone = 21 mm), tea tree essential oil (CUC-6, inhibition zone = 22 mm) or curcumin (CUC-7, inhibition zone = 23.5), highlighting the synergistic effect of the three active principles. However, the opposite behaviour was observed for these samples when tested against the *S. aureus* test strain, for which higher antibacterial activity was obtained for the sample treated with the hydrogel without ciprofloxacin, based on tea tree essential oil and curcumin (code CUC-4), with a 26 mm inhibition zone.

For both tested strains, the textile materials group which was treated with the hydrogels containing chlorhexidine (CUC-8 – CUC-11), showed a slightly lower antibacterial efficiency in comparison to the samples treated with hydrogels based on ciprofloxacin, the most evident antibacterial effect being obtained for the sample treated with the hydrogel based on polyvinyl alcohol-collagen-chlorhexidine-tea tree essential oil (code CUC-9, inhibition zone = 20 mm), tested against the *S aureus* test strain. Additionally, for all samples from this group, the inhibition zone diameter was higher than that for samples treated with hydrogel-based only on chlorhexidine.

CONCLUSIONS

Textile materials with healing and antimicrobial properties were obtained by applying hydrogels containing ciprofloxacin, chlorhexidine, tea tree essential oil and curcumin on cotton fabric. In this context, several hydrogel formulations based on PVA and collagen, which were used as polymeric matrices, were prepared and evaluated. The developed hydrogels applied on cotton fabrics influence the comfort indices, decreasing the air and water vapour permeability due to the polymeric systems deposited at the surface of the fabrics. Textile materials treated with hydrogels based on PVA and collagen containing different bioactive principles showed antibacterial activity against both test strains (*S. aureus* and *E. coli*). Samples of textile material treated with hydrogels containing ciprofloxacin exhibited higher antibacterial activity than analogous samples treated with hydrogels containing chlorhexidine. From the results obtained, and taking into account that the developed polymeric systems contain the active principle designed for the treatment of first-degree burns, it can be concluded that through the application of hydrogels containing ciprofloxacin, chlorhexidine, tea tree essential oil and curcumin on cotton fabrics, it is possible to obtain a varied range of biomaterials with antibacterial properties that can be used for burn wound management. Research on potential bioactive systems based on PVA and collagen for obtaining multifunctional textile materials for topical application is in progress for studying the potential of biocompatibility in terms of skin irritation.

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Authors:

LAURA CHIRILĂ[1], ALINA POPESCU[1], MĂDĂLINA IGNAT[2], MANUELA AVADANEI[3], CRISTINA MIHAELA LITE[1]

[1]National Research and Development Institute for Textiles and Leather – INCDTP,
Lucretiu Patrascanu 16, 030508 Bucharest, Romania

[2]Leather and Footwear Research Institute (ICPI) Division, National Research & Development Institute for Textiles and Leather, 93 Ion Minulescu Street, 031215 Bucharest, Romania

[3]“Gheorghe Asachi” Technical University of Iasi, Faculty of Industrial Design and Business Management,
29 Blvd D. Mangeron, 700050, Iasi, Romania
e-mail: mavad@tex.tuiasi.ro

Corresponding author:

LAURA CHIRILĂ
e-mail: laura.chirila@incdtp.ro