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BIOACTIVE HYDROGEL TYPE CARRIER SYSTEMS AIMED FOR TEXTILE WOUND DRESSING

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Abstract: *Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration. Polysaccharide gels are usually biocompatible and show several peculiar physical-chemical properties that make them suitable for a variety of biomedical applications. The hydrogels based on these types of polymers can cool the wound and reduce pain, which is helpful for burns or painful wounds. This paper aims to study the antibacterial activity of a layer-by-layer hydrogel "carrier" system based on sodium alginate and chitosan, which is further functionalized with active principles, like hyaluronic acid, or ZnO nanoparticles, or bacitracin. The synthesis of the hydrogels system was followed by the immobilization on textile woven fabrics made of fibrous blends of different chemical compositions, in order to achieve bioactive wound dressing for application in the treatment of inflammatory skin conditions. The textile materials used to produce the wound dressings have the same weave and warp thread (100% cotton, Nm 50/2), but different weft thread (100% acetate 130 dtex or 100% Lenpur Nm 34/1). For this purpose, several experimental variants of hydrogels were prepared and the treated textile materials were characterized from a physical-chemical and comfort point of view. The antibacterial activity and the biocompatibility of the textile materials functionalized with the hydrogels and active ingredients were also investigated. The textile materials treated with the synthesized hydrogels and subsequent bacitracin addition show an antibacterial effect on both S. aureus and E. coli test strains, while the hydrogels followed by ZnO nanoparticles addition show an antibacterial effect against S. aureus.*

Keywords: *bioactive textiles, ZnO nanoparticles, bacitracin, hyaluronic acid, antibacterial activity*

1. INTRODUCTION

Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration [1]. Various biopolymers are used in the production of wound dressings; an ideal skin wound dressing must accomplish the following requirements: provide a physical barrier to prevent further contaminations and injuries, absorb generated metabolites and enhances wound healing capacity by maintaining a moist environment [2]. In hydrogel-based scaffolds, biopolymers like alginate and chitosan are used due to their biocompatibility, lack of toxicity and relatively low price [3]. The hydrogels can also cool the wound and reduce pain, which is helpful for burns or painful injuries [4]. Polysaccharide gels are biocompatible and show several physical-chemical properties that make them suitable for a variety of biomedical applications [5].

Among wound dressings, the bi-layer composite which consists of a dense outer layer and porous sub-layer has a good ability to promote the healing process and can be synthesised using a variety of biopolymers. Usually, the outer layer is designed for the prevention of bacterial invasion, and possibly to act as a rate-controlling layer for water vapor permeation. Meanwhile, the inner layer is designed for attachment to wound tissue and the drainage of wound exudates [1].

Chitosan is a natural polymer that is widely applied in tissue engineering [3] and present several advantages, such as: low cost, high biocompatibility, biodegradability and ease of chemical modification. Alginate is a linear anionic polysaccharide derived from brown algae or bacteria. The hydrophilicity, excellent biocompatibility, and huge liquid-absorbing capacity make alginate an attractive material for wound dressings [4].

Among various biomaterials for wound management, hyaluronic acid plays a major role in the wound healing and tissue regeneration process [6]. It is a primary component of the extracellular matrix and its molecules strongly bind to water molecules and get heavily hydrated to form a viscous gel. This is the mechanism through which it regulates the viscoelasticity of biofluids and controls tissue hydration [5]. Wound dressings impregnated with antibacterial nanoparticles made of silver, gold, and zinc oxide (ZnO) have been extensively used in the literature. The unique properties of ZnO are to improve epithelialization, to enhance the local defence system and to reduce bacterial infection and inflammation [6].

2. MATERIALS AND METHODS

2.1 Materials

The following chemicals and auxiliaries were required for the development of layer-by-layer hydrogel ”carrier” systems: medium molecular weight chitosan (Sigma Aldrich, Germany), sodium alginate (Sigma Aldrich, Germany), vegetable glycerine (99.5% purity) used to give plasticity to the bilayer polymeric structure (SC Herbavit SRL Roumania), hyaluronic acid obtained from Streptococcus equi (Sigma Aldrich, Germany), bacitracin obtained from Bacillus licheniformis, ≥65 IU/mg, (Sigma Aldrich, Germany), zinc oxide nanoparticles with a size distribution less than 50nm (Sigma Aldrich, Germany), deionized water served as a solvent for the preparation of the polymeric solution.

As for the textile materials used to produce the wound dressings, two types of fabrics were used, both of them presenting the same weave and warp thread (100% cotton, Nm 50/2), but different weft thread (100% acetate 130 dtex-sample code S_1 or 100% Lenpur Nm 34/1-sample code S_2).

2.2 Hydrogels Synthesis

Sodium alginate is the polymer selected for the first layer of the hydrogel structure. The polymeric solution was synthesized by dissolving a 5% concentration of sodium alginate in distilled water. The obtained solution was maintained under continuous magnetic stirring of 250-300 rpm for one hour until homogenization, followed by the addition of 10% vegetable glycerine, with the role of increasing the plasticity (elasticity) of the polymer matrix and increasing the stability of the structure. The second polymeric layer of the layer-by-layer structure is represented by chitosan. This polymeric solution was achieved by solubilizing 1.5% chitosan in deionized water and subsequently by adding 1% acetic acid. The solution obtained was maintained under magnetic stirring at 300–400 rpm over a two-hour time interval. The obtained hydrogels were used to perform the functionalization treatments of textile materials (**Table 1**).

2.3 Functionalization Treatments

The first step of the textile materials functionalization consists in the application of the first polymeric layer (hydrogel based on sodium alginate) by the padding method (4 passes through the squeezing rollers, pressure 2,7 bar), followed by drying operation at 50°C for 5 minutes, using a laboratory oven for drying-heat setting operations (ROACHES, UK). The textile material treated in the first step with sodium alginate hydrogel was then treated in the second step with the polymeric solution of chitosan, using the same equipment's and process parameters.

In order to add active principles to the textile surfaces, solutions containing certain amounts of hyaluronic acid, or ZnO nanoparticles, or bacitracin were obtained and subsequently applied on the textile materials by the padding, under the conditions described above. The technological flow can be seen in **Fig. 1**.

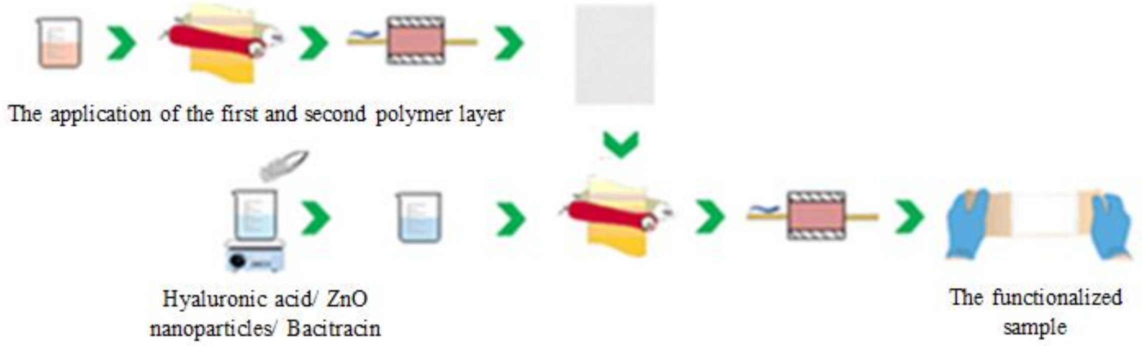


Fig. 1. *The technological flow used for the functionalization of the textile woven fabrics by adding active ingredients in the multilayer polymer system immobilized on the textile surface*

Table 1. Experimental variants of textile materials treated with hydrogel type carrier systems

Woven sample code	Fibrous composition		"Carrier" system	Functionalized sample code
	Warp	Weft		
S_1	100% cotton, Nm 50/2	100% acetate, 130 dtex	Chitosan–sodium alginate–hyaluronic acid	S_1-HA
S_2	100% cotton, Nm 50/2	100% Lenpur, Nm 34/1	Chitosan–sodium alginate–hyaluronic acid	S_2-HA
S_1	100% cotton, Nm 50/2	100% acetate, 130 dtex	Chitosan–sodium alginate–ZnO nanoparticles	S_1-ZnO
S_2	100% cotton, Nm 50/2	100% Lenpur, Nm 34/1	Chitosan–sodium alginate–ZnO nanoparticles	S_2-ZnO
S_1	100% cotton, Nm 50/2	100% acetate, 130 dtex	Chitosan–sodium alginate–bacitracin	S_1-B
S_2	100% cotton, Nm 50/2	100% Lenpur, Nm 34/1	Chitosan–sodium alginate–bacitracin	S_2-B

3. CHARACTERIZATION METHODS

3.1. Physical-mechanical and comfort characteristics evaluation

In order to evaluate the influence of the functionalization treatments on the main physical-mechanical and comfort characteristics of textile woven fabrics, they were analysed before and after functionalization from the point of view of: mass (SR EN 12127:2003), hydrophilicity (by drop test) according to STAS 12751/89, water vapor permeability (STAS 9005:1979), air permeability (SR EN ISO 9237:1999).

3.2. Antibacterial activity evaluation

For the testing of antibacterial activity, the diffusion method in agar was used according to the standard SR EN ISO 20645:2005. For the experimental part, cultures of ATCC 6538 strains of Staphylococcus aureus (gram positive) and ATCC 8739 Escherichia coli (gram-negative) in a liquid medium were used. The evaluation method allowed the qualitative assessment of antibacterial activity based on the measurement of the inhibition zone obtained on the tested strains.

3.3. Biocompatibility evaluation

For the biocompatibility tests, the level of nitric oxide (NO) released in the culture environment was determined and the MTT test (based on the reduction of a yellow tetrazolium salt-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT, to purple formazan crystals by metabolically active cells) was used to determine cellular viability. To perform these tests, the following preliminary steps were implemented: 1) Cultivation of HaCaT keratinocytes; 2) Exposure of HaCaT keratinocytes to textile material: in 24 well-plated and in inserts for cell culture. All results were calculated with average values of at least three independent experiments (n=3).

4. RESULTS AND DISCUSSIONS

4.1. Physical-mechanical and comfort characteristics evaluation

From the point of view of the influence of the functionalization treatments on the mass characteristic, it was found that regardless of the type of active principle used as final treatment layer (hyaluronic acid, or ZnO nanoparticles, or bacitracin), the multilayer polymer functionalization system as a hole generates an increase of the values of mass characteristics per surface unit, with about 35-40% in the case of woven fabric containing 100% acetate yarns and with about 26% in the case of woven fabric containing 100% Lenpur yarns in the weft direction.

From the comparative analysis of the air permeability values obtained for the treated and untreated textile materials it can be shown that the functionalization treatments cause a significative decreasing in the air permeability characteristic due to the constituent polymeric substances deposited on the surface of the textile support in the form of a semi-permeable film. The same conclusion can be drawn in the case of comparative analysis of the values of water vapor permeability, for which, a reduction between 28-37% of the value of this characteristic occurred in the case of woven fabric containing acetate yarns and 38-42% reduction in the case of fabric containing Lenpur yarns.

In terms of hydrophilicity determined by the drop test, it was found that the textile materials of both series treated with the polymeric system chitosan-alginate sodium-ZnO provide the highest values of hydrophilicity in the series studied. The determined characteristics are presented in **Table 2**.

Table 2. Mass, air permeability and water vapor permeability of the textile materials functionalized with the layer-by-layer hydrogel type carrier systems

Sample code	Fibrous composition of yarns		Mass (g/m^2)	Air permeability ($l/m^2/sec$)	Water vapor permeability (%)	Hydrophilicity, drop test (seconds)
	Warp	Weft				
S_1*	100% cotton, Nm 50/2	100% acetate, 130 dtex	136	260.4	42.4	instantaneous
S_1-HA			191	23.42	30.4	>300
S_1-ZnO			188	22.42	26.3	26.95
S_1-B			184	25.08	27.2	270.58
S_2*	100% cotton, Nm 50/2	100% Lenpur, Nm 34/1	172	386	45.5	instantaneous
S_2-HA			217	89.66	27.8	>300
S_2-ZnO			217	95.88	26.5	12.59
S_2-B			218	82.56	27.8	45.4

** Control samples: Textile woven fabrics before the application of functionalization treatment*

4.2. Antibacterial activity evaluation

Analysing the data obtained for the antibacterial testing (**Table 3**), it can be concluded that in the case of the untreated textile structures, considered control samples, the test strains had a significant growth, the inhibition zones of bacterial growth being absent.

Among the functionalized treatments studied, the variants treated with the chitosan-sodium alginate-bacitracin system, shows antibacterial activity on both tested strains, respectively *E. coli* and *S. aureus*, for which the biggest inhibition zone were highlighted (16 mm). The textile materials treated with the polymeric chitosan-sodium alginate-ZnO nanoparticles polymeric system show low antibacterial activity against on *S. aureus* strain only, with a inhibition zone of 2.5 mm (**Table 4**).

The samples treated with chitosan-sodium alginate-hyaluronic acid system have an insufficient antibacterial effect, exhibited by a significant bacterial growth and the absence of inhibition zones of bacterial growth.

4.3. Biocompatibility evaluation

In the case of keratinocytes grown in the presence of textile materials impregnated with bacitracin, the cell viability was better compared to the results obtained in the case of keratinocytes grown in the presence of textile materials impregnated with hyaluronic acid or zinc oxide nanoparticles (**Fig. 2**).

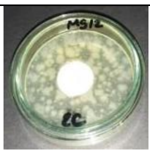
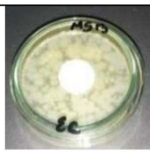
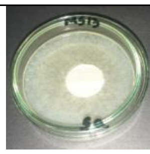
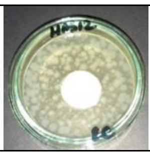
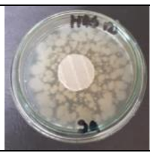
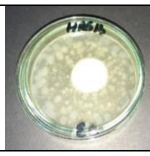
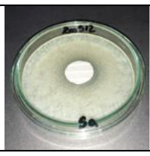
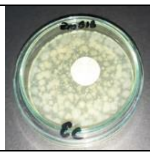
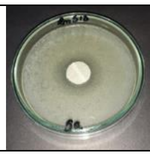
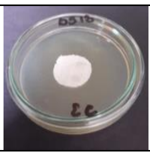
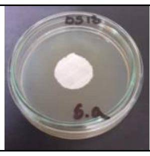
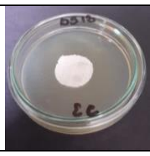
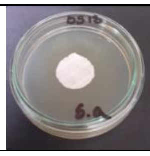
Table 3. Evaluation of the antibacterial activity for the obtained biomaterials

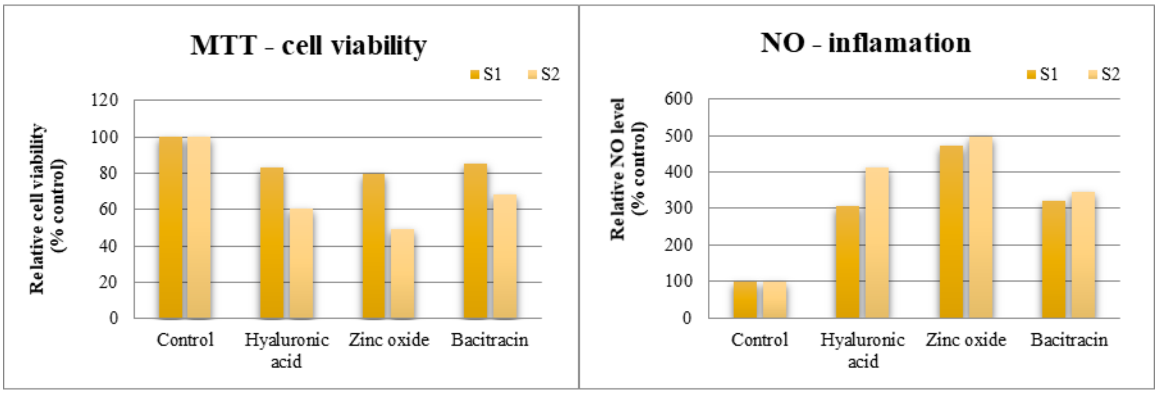
Sample code	Inhibition zone (mm)		Evaluation	
	E. coli	***S. aureus***	***E. coli***	***S. aureus***
S_1*	0	0	Insufficient effect	Insufficient effect
S_1-HA	0	0	Insufficient effect	Insufficient effect
S_1-ZnO	0	H=2.5 mm	Satisfactory effect	Satisfactory effect
S_1-B	H=16 mm	H=16 mm	Satisfactory effect	Satisfactory effect

S_2*	0	0	Insufficient effect	Insufficient effect
S_2-HA	0	0	Insufficient effect	Insufficient effect
S_2-ZnO	0	H=2.5 mm	Satisfactory effect	Satisfactory effect
S_2-B	H=16 mm	H=16 mm	Satisfactory effect	Satisfactory effect

** Control samples: Textile woven fabrics before the application of functionalization treatment*

Table 4. Images of Petri dishes after the exposure to the *E.coli* and *S. aureus* strains of the S_1, S_2 woven fabrics before and after the functionalization

E. coli	***S. aureus***	***E. coli***	***S. aureus***
S_1		**S_2**	
			
S_1-HA		**S_2-HA**	
			
S_1-ZnO		**S_2-ZnO**	
			
S_1-B		**S_2B**	
			



a) b)

Fig. 2. *Graphical representations of: a - Analysis of the cellular viability using the MTT test; b - Level of NO released in the culture environment to observe the pro-inflammatory effect after 5 hours of incubation in a two-dimensional system*

Cellular viability values were close to those of the control, with some exceptions, such as bacitracin–treated textiles. Increased levels of nitric oxide were observed in textiles impregnated with bacitracin, in comparison with the other treated samples, suggesting a possible pro-inflammatory potential in the skin (**Fig. 2**).

5. CONCLUSIONS

Layer-by-layer hydrogel carrier systems based on sodium alginate, chitosan and certain bioactive substances were applied on the textile woven fabrics by the padding method to accomplish textile wound dressing for the application in the treatment of inflammatory skin conditions. The functionalized textile materials were characterized from several physical-chemical and comfort point of view. Also, antibacterial activity and biocompatibility were evaluated in order to prove the safety application of the developed wound dressing on the skin. It was found that regardless of the type of active principle used as a final treatment layer (hyaluronic acid, or ZnO nanoparticles, or bacitracin), the multilayer polymer functionalization system as a whole generates an increase of the values of mass characteristics per surface unit up to 40%, and a significative decreasing of air and vapor permeability and of the hydrophilicity. Biocompatibility evaluation demonstrates that the textile material functionalized with the studied hydrogels do not present skin irritation. Taking into consideration the antibacterial activity data, it was proved that the textile samples containing bacitracin show antibacterial effect for both *S. aureus* and *E. coli* test strains, while those that contain ZnO nanoparticles show antibacterial effect against *S. Aureus* only.

To sum up, wound dressings based on hydrogels are innovative materials that exhibit antibacterial activity and biocompatibility and are a suitable solution for the treatment of inflammatory skin conditions.

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