Efficiency and Biocompatibility of Antimicrobial Textile Material of Broad Spectrum Activity

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ABSTRACT: The subject of this article is to study the behavior of the antimicrobial textile material. The material is in the form of plaster wound dressing which is consisting of a nonwoven textile base containing polypropylene (PP)/viscose, a polymer carrier, and an antimicrobial active substance. The polymer carrier is the polysaccharide D-glucosamine (chitosan), and the active antimicrobial substance is antibiotic gentamicin sulfate. The amount of gentamicin sulfate immobilized into the polymer matrix of the polymer carrier was 0.15–0.20 mg/cm^2 or 2.0–2.5% of the mass of the nonwoven textile material. The antimicrobial textile material has been studied *in vitro* and *in vivo* conditions through the efficiency of the antimicrobial effects on different kinds of pathogenic microorganisms, as well as the biocompatibility in preclinical research. The results of these experiments indicate that all bioactive textile materials were biocidal *in vitro* for all pathogenic test organisms. Good biocompatibility, the existence of the correlation between the *in vitro* and *in vivo* results, concerning efficiency, qualifies these antimicrobial biomaterials for clinical use. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 120: 1459–1467, 2011

Key words: drug delivery system; biocompatibility; biological applications of polymers; polysaccharides

INTRODUCTION

Biomedical textiles are fibrous structures designed for use in specific biological environments, where their performance depends on biocompatibility with cells and biological tissue or fluids. Apart from this, different shaping and combining possibilities of these materials make them multifunctional.[1–4]

Over the last few years, the polymer-active substance systems with the controlled release have been able to reach a long term, efficient release of the active substance so easily so that the drug level in the blood does not exceed the maximum concentration, which can present the toxicity level, or the minimum desired concentration when the drug loses its efficiency.[5–11]

The antimicrobial textile material, which has been the subject of this article, is obtained by the adhesion of chitosan as a polymer carrier of the gentamicin sulfate active substance on a nonwoven textile material as base. The elaboration of the bioactive layer and its form (film, cream, or gel) demands the choice of the polymer which possesses a suitable molecular and macromolecular structure and by means of its optimal features stimulates the process of the epidermal regeneration and healing of wounds. Chitosan, as a natural polymer carrier of the antimicrobial active substance, has excellent biostimulating features which enables the regeneration and vascularization of the damaged tissue. Besides, the unique combination of features such as biodegradability, nontoxicity, nonallergic effect, cathion nature, and antimicrobial activity determine the use of this polymer in medicine and veterinary medicine.[12,13] One of the important aspects of the application of chitosan and its derivates in medicine and veterinary medicine is related to the treatment of different kinds of wounds and dermatoses where the treatment effect and the tissue regeneration is much better when combined with anti inflammatory, antibacterial, and analgesic effect of, for example, neomycin, tetracycline, gentamicin, lidocaine, C vitamin, etc.[4] During the last two decades, chitosan has often been used as a safe drug polymer carrier. Because of its hydrophilic nature as the ability to bind water molecules chitosan, which is a polycation in acid environment, forms the gel suitable for making the system for controlled release of the active substance.[12–15]

The aim of this article is the study of the behavior of the developed antimicrobial textile material in the form of a plaster wound dressing, consisting of a nonwoven textile base which contains polypropylene (PP)/viscose, a polymer carrier, and the gentamicin

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sulfate antimicrobial active substance *in vitro* and *in vivo* conditions. The efficiency of the formed antimicrobial textile materials has been tested by classic qualitative and quantitative microbiological methods. The growth inhibition has been tested for different gram-positive and gram-negative microorganisms: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Candida albicans*.[16–19]

Within the research, the efficiency of the formed antimicrobial textile materials has been tested by means of determining the size of the inhibition zone of pathogenic microorganisms and the number of existing microorganisms. Apart from this, the biocompatibility of the antimicrobial composition has been examined by testing the following: cytotoxicity, sensitivization, and irritation.[20–22] The tests have been performed according to the matrix for testing the biocompatibility and to recommendations and guides published in Guidelines ISO 10993. Moreover, the standing (aging) effect of this antimicrobial textile material has been examined.

MATERIALS AND METHODS

Bioactive antimicrobial textile material is a composite material and consists of

a. nonwoven textile base
b. a polymer carrier
c. an active antimicrobial substance

Nonwoven textile material

The nonwoven textile material used in designing this transdermal system has all necessary characteristics of the certified material for the medical use, hygroscopic, air and moisture permeability; appeal, flexibility, and comfort in contact with wound surface or skin; little shrinkage; nontraumatic nature; sterilization ability; and possibility to apply polymeric composition.

Nonwoven textile base presents an inert medical textile material made of mixture polypropylene 50%/viscose 50% fibers which are thermally bonded. Linear density of fibers is 1.7/1.7 dtex, surface weight is 84 g^{-2}, and thickness is 0.9 mm. The increase in length under maximal stress is 40*N*/50 mm 20%. Sterilization: Dry procedure, with gas ethylene oxide. Manufacturer: Vilmed® Nonwovens, Freudenberg.

Polymer carrier

Polysaccharide (poly-D-glucosamine), chitosan presents the product of deacetylation of chitin with the degree of deacetylation 78–82% of purity for bio-

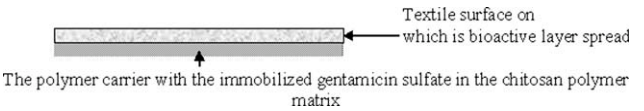

Figure 1 The scheme of bioactive textile material.

medical use. Manufacturer: ς-Aldrich, St. Louis USA. Chitosan with molecular weight 200,000. Chitosan gels were prepared at 2 wt/wt % concentration in dilute acetic acid solution (20 wt/wt %), pH 5.

Active antimicrobial substance

Gentamicin sulfate, 2.0% distilled water solution, Manufacturer: Galenika—Belgrade, Serbia, Pharma Generi CSBV. The concentration of the active antimicrobial substance on nonwoven textile material: 0.15 mg/cm^2, 0.20 mg/cm^2, or 2.0% and 2.50% of the mass of the nonwoven textile material.

Indicator pathogenic microorganisms: *Staphylococcus aureus* ATCC 25923; *Pseudomonas aeruginosa* ATCC 9023; *Escherichia coli* ATCC 2592; *Candida albicans* ATCC 2443; and *Klebsiella pneumoniae* ATCC 4352.

Procedure of obtaining antimicrobial textile material

The bioactive antimicrobial textile material was obtained by fixing the antimicrobial probe into the porous structure of the textile material by inclusion, i.e., physical–chemical modification. Gentamicin is immobilized in a polymer matrix of chitosan (hydrogel), as a polymer carrier, in desirable concentration, which is through adhesion process spread on the surface of the nonwoven textile foundation, Figure 1.[23] Release of drug from polymer matrix has multistage characteristics. Such complex physiological–chemical process has been divided in following several stages:

- Diffusion of water molecules or physiological solution in polymer carrier of active substance and swelling,
- Breaking up primary and secondary bonds of drug and polymer matrix, and
- Diffusion of drug from polymer hydrogel through membrane to physiological solution (water, buffer).

Several mechanisms have been elucidated to describe gentamicin sulfate release from polymer hydrogel systems including diffusion, swelling, and chemically controlled release. Diffusion controlled is the most widely applicable mechanism for describing drug release from hydrogels.[23]

Gentamicin sulfate was incorporated into the formulations at 2 wt/wt % concentrations. Procedure of formation of this antimicrobial textile material has

been developed at the Fiber Laboratory Faculty of Technology and Metallurgy, University in Belgrade.[12]

Bioactive layer of bandage: polymer carrier with immobilized gentamicin sulfate in chitosan (hydrogel) polymer matrix. Concentration of active substance in polymer matrix, on nonwoven textile material: 0.15 or 0.20 mg/cm^2.

Determination of inhibition activity by diffusion method on an agar plate

The inhibition activity was determined by the diffusion method (qualitative method) on an agar plate. The preparation of the antimicrobial textile material samples preceded the determination of inhibition activity. The samples of 1 cm radius were used. The antimicrobial efficacy of this bioactive antimicrobial textile materials was examined on the agar plate (0.6% agar) seeded with indicator microorganisms. The samples were tested after 24 h incubation at 37°C, and the presence or absence of the visible colonies on the agar surface directly above the fibrous textile material was recorded. After visual inspection, the agar surface inhibition zone (bactericidal and bacteriostatic) was measured.[24] The monitoring of antimicrobial activity by this method was carried out after the samples were kept in physiological solution (0.9% wt/vol of NaCl) for a different period of time. The samples were put in the test tubes with 9 mL physiological solution and after different time intervals (5, 10, 15, 30, 60, 120 min, 24 h, or a month), they were taken out and dried at 37°C, 24 h.

The monitoring of antimicrobial activity by this method was carried out after 6 months, 1 year, and 2 years to determine the stability of the bond between bioactive substance and nonwoven fabric. The bioactive antimicrobial textiles were kept at room temperature. These pieces of research were conducted to determine and predict the durability and permanence of the formed bioactive antimicrobial textile material.

The stability of the bond between a nonwoven textile material and an active substance was tested by putting the samples of the antimicrobial textile material of the mentioned dimensions onto the moistened agar plate in sterile conditions and by keeping them for a particular period of time (5, 10, 15, 30, 60, 120 min, 24 h or 7, 14, 21 and 30 days). After the end of the particular time periods the samples were moved to Petri dishes. The presence of the inhibition zone of the microorganism growth in the place, where there used to be samples, is indicative of the release of the active substance from the samples during a particular period of time.

Determination of bacterial count

The *in vitro* antimicrobial effect of each textile fibrous material was examined in physiological solution (0.9% wt/vol of NaCl).

The bacterial count was measured by standard method of decimal dilution (quantitative method) in sterile physiological solution and the plastic Petri plates were seeded by test microorganisms on agar. There are several recognized protocols in the literature for evaluating the efficiency of biocidal surfaces (ASTM E 2149-01—Dynamic Shake Flask Test, JIS Z 2801 : 2000, and AATCC-100-2004).[24,25] For the purpose of this experiment, a method for determination of bacterial count on the basis of ASTM E 2149-01 and AATCC-100 standard methods was used. One milliliter of inoculate of the test microorganism was added into 9 mL sterile physiological solution (phosphate buffer, pH 7.0). Then, an antimicrobial textile material sample of 1 cm diameter was added in solution and shaken at 120 rpm ("Shake-flask" method) for 24 h at 37°C. Viable cells (log TBC/mL) were enumerated on agar by pour plating 1 mL of dilution of physiological solution followed by incubation at 37°C for 24 h. After 24 h has passed, colonies were counted assuming that each cell provides one colony. The result was an average value of the two dilutions with recorded colony growth. The total bacterial count (TBC) is calculated according to the eq. (1)[25]:

$$\mathrm{TBC} = n \cdot a \cdot r \tag{1}$$

where TBC (count cell/mL) is the total bacterial count, n is the number of Petri dish parts, a is the cell count in one part, and r is the dilution.

The samples of the antimicrobial textile material of the radius 1 cm were put in the test tubes with 9 mL physiological solution and after different time intervals (5, 10, 15, 30, 60, 120 min, 24 h or 7, 14, 21 and 28 days), they were taken out and dried at 37°C for 24 h, after which the colonies were counted.

Methods for biocompatibility testing

The methods of cytotoxicity *in vitro*, sensitivization and primary cutaneous irritation *in vivo*.

Assessment of biocompatibility *in vitro* and *in vivo*

The sensitivization testing and primary cutaneous irritation have been performed on experimental animals according to recommendations of ISO 10993 standards (ISO 10993-10 : 2002/Amd 1: 2006 Tests for irritation and delayed-type hypersensitivity) and Ethics Committee Faculty of Medicine, University of Belgrade (License no. 1943/2).

Testing cytotoxicity in vitro. Test has been performed according to the procedure for laboratory testing according to the requests of ISO 10993-5 : 1992(E) standard, as follows: sterilized antimicrobial textile material in the form of the plaster wound dressing (2.5 × 2.5 cm) has been placed on adherent layer of

L929 cells; a material non causing cytotoxic reaction has been used as the control sample (stomatologic sucker SALIGAL^R); positive control-sterile water solution of phenol, 4%, special control—fibroblast culture cells without test material; medium for culture of cells—the complete medium for cultures: RPMI 16-40 (Sigma) with additives, 10% of fetal veal serum (ICN), 2 m*M* of glutamine (Sigma), 100 IU/mol penicillin, and 0.5% of streptomycin; cells used—NCTC, clone L929, adizotropic tissue of mouse. Conditions: incubation on 37°C with 5% CO_2, thermostat Heraeus. Procedure has been performed under sterile vertical laminar.

Cytotoxicity index (0–5) has been determined on the basis of lysed cells %: 0—nondetectable cytotoxicity; 1—very small cytotoxicity, less than 20% of lysed cells; 2 and 3—moderate cytotoxicity, more than 40% but less than 60% of lysed cells; 4—serious cytotoxicity 60 to 80% of lysed cells; 5—very serious cytotoxicity more than 80% of lysed cells.

Testing sensitivization. Data about animals: species—guinea pig. Number of animals: 10 + 5 (control).

Application: Antimicrobial textile materials in the form of a plaster wound dressing (2.5 × 2.5 cm) was applied dorsolaterally (cranially—nearer to scapula) on the surface of shaved skin of each of 10 guinea pigs. In the control sample, sterile nonwoven textile material has been applied on five control animals. Test and control samples were in contact with the skin for a period of 6 h and then removed. The second application has been performed 7 days after the first one, the third application—7 days after the second, the fourth application—7 days after the third, and the fifth application—14 days after the last one.

The sensitivization index has been determined on the basis of the numerical scale for edema and erythema assessment, each on a 0–4 grading scale. For erythema: 0—no erythema; 1—very slight erythema, barely perceptible; 2—well-defined erythema; 3—moderate to severe erythema; 4—severe erythema (beet redness) to slight eschar formation (injuries in depth). For edema: 0—no edema; 1—very slight edema, barely perceptible; 2—slight edema (edges of area well defined by raising); 3—moderate edema (raised ~ 1 mm); 4—severe edema (raised more than 1 mm and extending beyond the area of exposure).

The calculation of the index of sensitivization index has been performed on the basis of the results which were obtained by using the previous numerical scale for assessment of erythema and edema.

The sensitivization degree = (edema + erythema)/number of measurements.

The index of sensitivization (IS) = the sensitivization degree/number of animals.

Testing primary cutaneous irritation. Data about animals: species—rabbits (New Zealand White).

Number of animals: 3.

TABLE I
The Inhibition Activity of the Test Samples of the Antimicrobial Nonwoven Textile Material Treated by Gentamicin Sulfate After Being Kept in Physiological Solution for a Different Period of Time

Time	Inhibition zone (mm)				
	E. coli	*S. aureus*	*Klebsiella*	*P. aeruginosa*	*C. albicans*
0 min control sample	8	11.5	9	15	11.5
5 min	7	10	10	11	10.5
10 min	6.5	10	10	12	10
15 min	7.0	7. 5	10	12	10
30 min	6	9.5	7.5	12	9.5
60 min	5.5	8	4	11	8.5
120 min	3.5	8	4.5	9	7
24 h	3.5	6	4.5	8	5.5
30 days	–	1	–	–	3

Application: The antimicrobial textile material in the form of a plaster wound dressing (2.5 × 2.5 cm) was placed directly on the surface of the shaved skin on the left and right side cranially on each of rabbits, such a procedure enabling six experimental places (application). To control, the same dimension samples of sterile nonwoven textile material have been placed on the bodies of animals, on six surfaces caudally from the experimental places. Test and control samples remained in contact with skin during 4 h. The calculation of primary cutaneous irritation index has been performed on the basis of the results which were obtained by using the previous numerical scale for assessment of erythema and edema.

The irritation degree = (edem + eritem)/number of measurements.

Primary cutaneous irritation index (PII) = the irritation degree/Number of application.

The calculation of primary cutaneous irritation index has been performed on the basis of the results obtained, using the previously numerical scale for assessment of erythema and edema. The primary cutaneous irritation index: from 0.0 to 0.4—nonirritating; from 0.5 to 1.9—very small irritation; from 2.0 to 4.9—irritation; and from 5.0 to 8.0—very hard irritation.

RESULTS

Antimicrobial activity

The results of measuring the test-indicator microorganism growth inhibition zone (clear zone, bactericidal effect) around the samples of antimicrobial textile material after being kept in physiological solution and without exposure to physiological solution (control sample) are shown in Table I. The quantity of the added gentamicin sulfate was 0.15 mg per cm^2 of nonwoven textile material. Each value, given in Table I, represents the average value of five experimental results for each indicator microorganisms. All results

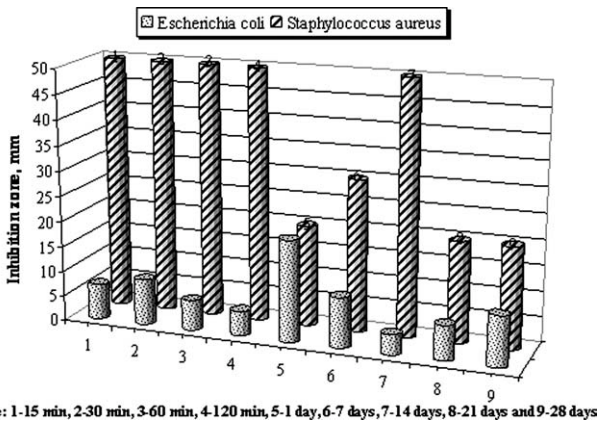

Figure 2 The width of the inhibition zone by using gentamicin sulfate on *E. coli* and *S. aureus* depending on the time spent in physiological solution.

are within the range of experimental error allowed for the measuring method used with variation coefficient lower then 5% and SD less than 0.07.

Figure 2 shows the results of the inhibition zone caused on *E. coli* and *S. aureus* depending upon the time, which the samples spent in the physiological solution. The samples were exposed to the effects of the physiological solution to monitor the dynamics of the release of gentamicin sulfate. The quantity of added gentamicin sulfate in this case was 0.20 mg cm^{-2} of the nonwoven textile material.

Figure 3 show the growth inhibition zone of the indicator sort *S. aureus* and *E. coli* on contact place (3a) and after 15, 30, 60, and 120 min or after 24 h (3b).

The quantitative analysis of the antimicrobial activity of a nonwoven textile material treated by gentamicin sulfate and concerning the number of survived microorganisms in the physiological solution where the test samples of a nonwoven antimicrobial textile material had previously been kept for 5, 10, 15, 30, 60, 120 min, and 24 h) is shown in Table II.

The starting count of microorganisms 10^6 per mL, inoculum, has also been determined. The microorganisms count has been used to test the stability of the bond between an antimicrobial substance and a fiber, i.e., to determine whether the release of an active substance in the contact with a moistened environment can be controlled. The results of the count of microorganisms (N) in 1 cm^3 of physiological solution are shown in Table II.

The antimicrobial activity by the method of diffusion on an agar plate has been tested after 6 month, 1 year, and 2 years to determine the stability of the bond between an antimicrobial nonwoven textile material and a bioactive substance. These pieces of research have been done to determine and predict the permanence of the formed antimicrobial biomedical textile materials, and the efficiency evaluated by determining the zone of growth inhibition on *S. aureus* is presented in Figure 4.

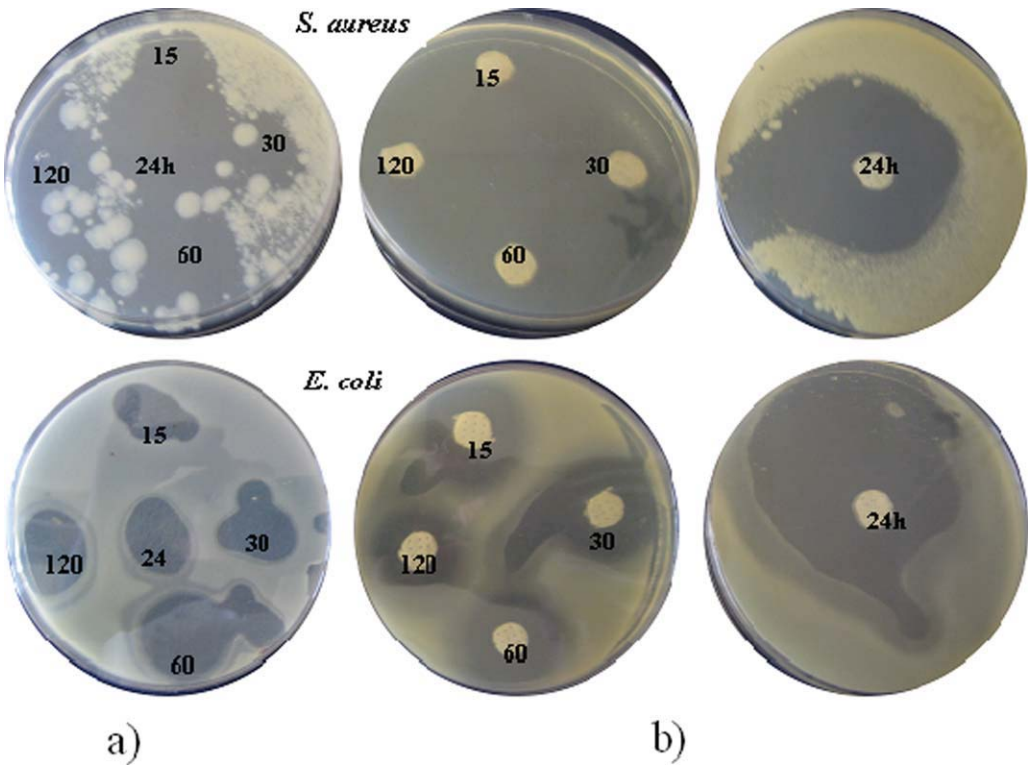

Figure 3 The growth inhibition of the indicator sort *S. aureus* ATCC 25923 and *E. coli* ATCC 25923 (a) on contact place and (b) after 15, 30, 60, and 120 min and after 24 h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE II
The Count of Microorganisms in Physiological Solution as a Function of Time

	Count of microorganisms (N/cm^3)				
Time (min)	*E. coli*	*S. aureus*	*Klebsiella*	*P. aeruginosa*	*C. albicans*
Inoculum (0)	1.0×10^6	6.98×10^6	2.0×10^6	1.0×10^6	1.0×10^6
5				1.0×10^2	2.6×10^3
10					2.6×10^3
15				1.0×10^2	2.6×10^3
30	0	0	0	0	2.0×10^2
60					0
120					
1440					

Results of biocompatibility testing

Preclinical studies of cytotoxicity *in vitro* and primary cutaneous sensitivization and irritation have been done *in vivo*. The samples containing gentamicin sulfate 0.20 mg/cm^2 have been selected considering the fact that they have shown greater efficiency regarding the width of the zone of the growth inhibition, especially for the pathogenic microorganisms *S. aureus*.

Results of cytotoxicity testing

Cytotoxic effect of antimicrobial textile material has been determined on the basis of lysed cells percent or cytotoxicity according to ISO 7405 : 1997 (E). Results of qualitative change of cells morphology index are presented in Table III and the quantitative analysis of cytotoxicity in Table IV.

Results of sensitivization testing

In all test animals, erythema or edema was not present immediately, as well as 24 h and 48 h after the removal of test material in treated and control sites. There was no difference between treated and control sides ($P > 0.05$). The sensitivization degree after application of the antimicrobial textile material was calculated to be 0. The index of sensitivization obtained from the results of antimicrobial textile material has also been valued at IS = 0. Identical results have been obtained for the control group of guinea pigs.

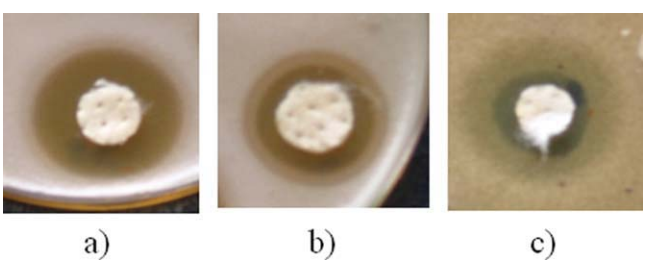

Figure 4 The efficiency of antimicrobial nonwoven textile material after: (a) 6 month, (b) 1 year, and (c) 2 years. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Results of primary cutaneous irritation tests

In all test animals, erythema or edema was not present after 24, 48, and 72 h in treated and control sites. There was no difference between treated and control sides ($P > 0.05$). Score of erythema and edema, e.g., the score of primary irritation (SPI) after application of the antimicrobial textile material was calculated to be 0 on a scale of 0.00 to 8.00.

The primary irritation index (PII) was also calculated to be 0 and antimicrobial textile material was evaluated as no irritation. Identical result has been obtained for control group of rabbits.

The results of examining the biocompatibility of an antimicrobial textile material contain cytotoxicity, sensibilization, and irritation tests.

Figure 5(a,b) show the cell cultures with no effect of antimicrobial textile materials [Fig. 5(a)] and with the effect of suction which also gives negative control [Fig. 5(b)], i.e., it does not show cytotoxicity, and with the effect of the antimicrobial textile material [Fig. 5(d)]. Negative control, Figure 5(a), shows the cells of normal appearance, adherent, with a confluent layer, whereas in the presence of the antimicrobial textile material, Figure 5(d), we can notice fibroblastoid appearance of cells and inhibited proliferation, which implies slight degenerative changes. The provoked cytotoxic effect is very mild, unlike the phenol solution which has a 100% cytotoxic effect, provoking explicit degenerative changes [Fig. 5(c)].

TABLE III
Results of Qualitative Change of Cells Morphology

	Index of cells morphology changes			
Kind of sample	Sample 1	Sample 2	Sample 3	Average value
Positive control (phenol solution)	3	3	3	3
Negative control (SALIGAL^R)	0	0	0	0
Negative control (cells theirselves)	0	0	0	0
Antimicrobial textile material	0	1	0	0.33

TABLE IV
Results of Cytotoxicity Quantitative Analysis

	Lysis percent (cytotoxicity)				
Kind of sample	Sample 1 (%)	Sample 2 (%)	Sample 3 (%)	Average value (%)	Index
Positive control (phenol solution)	100.0	100.0	100.0	100.0	5
Negative control (SALIGAL^{R})	4.0	5.0	5.0	4.66	0
Negative control (cells themselves)	4.0	3.0	5.0	4.00	0
Antimicrobial textile material	11.0	13.0	14.0	12.33	1

DISCUSSION

The results in Table I reveal that the antimicrobial textile material shows inhibition activity toward all indicator test microorganisms, which is indicative of a wide range of effects. The strongest effect of gentamicin sulfate is obvious with *P. aeruginosa* and *C. albicans*. The width of the inhibition zone drops negligibly with the increase in time which the sample spent in physiological solution, which can be explained by the release of the active substance. This is exactly why the width of the inhibition zone is at its maximum for control samples (the samples which were kept in physiological solution), except for *Klebsiella pneumoniae*. In this case, the width of the inhibition zone of the samples, which were kept for 5, 10, and 15 min, is bigger than the inhibition zone of the control sample. The reason for this can be manifold. The first reason is certainly the question of the active substance diffusion through a base, and the second reason can be the sensitivity of *Klebsiella pneumoniae* to gentamicin sulfate concentrations which were achieved on the edges of the inhibition zone.

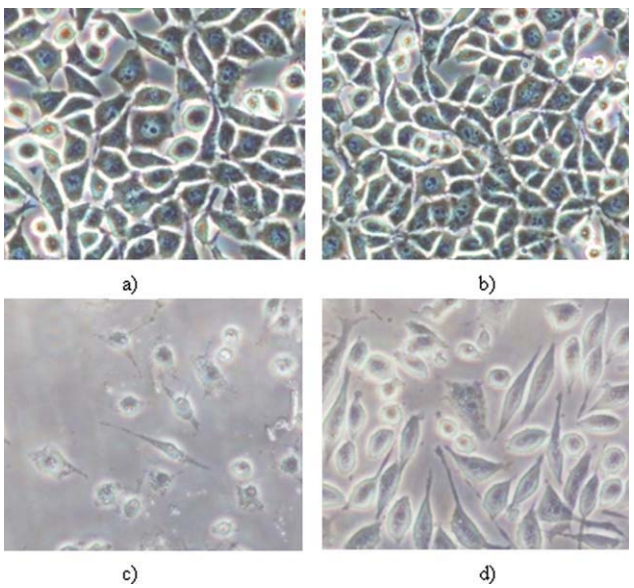

Figure 5 (a) Negative control (layer L929 cells without test sample, magnification ×250), (b) Negative control (L929 cells and (SALIGALR)), (c) Positive control (layer L929 cells and sterile water solution of phenol, 4%), and (d) Test sample of antimicrobial textile material in the form of plaster on layer L929 cells. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The bacterial inactivation efficiencies of gentamicin sulfate and their combinations on textile fabrics were investigated to evaluate the disinfectant action on *S. aureus, Klebsiella, E. coli, C. albicans*, and *P. aeruginosa*. The inactivation performance was seen to depend on the bacterial count in physiological solution as a function of time (5, 10, 15, 30, 60, 120, and 1440 min) Table II.

The results of these experiments indicate that all materials were biocidal *in vitro* for all five test organisms. The phenomenon of continuous and sustained release of gentamicin sulfate is clinically very important. Since agents that function by constant, prolonged release of gentamicin sulfate are more clinically effective in both preventing and treating infections than are agents that release rapidly.[26]

The 25% increase in the quantification of the active substance completely inhibited the growth of *S. aureus* in the Petri dish whose radius was 5 cm (Figs. 2 and 3). Figures 2 and 3 show that the inhibition zone of the growth of *S. aureus* can be noticed only after 24 h [Fig. 3(b)] spent in the physiological solution. When it comes to *E. coli*, the zones of the growth inhibition are generally smaller in comparison to *S. aureus* and there are no significant differences during the monitored period of time. The mentioned behavior can be the result of a sensitivity degree of the indicator sort to gentamicin sulfate. It can also be the result of a higher concentration of gentamicin sulfate as well as the increase in the sensitivity degree of the applied test method. Figure 3(a) also clearly shows the growth inhibition of pathogenic microorganisms at the places where the samples were added and stayed for a particular period of time to be removed afterward. It is obvious that the released quantity of gentamicin sulfate was enough to inhibit the growth of microorganisms at the contact place as well as further. Apart from this, the photos confirm the sufficient efficiency of the antimicrobial textile material for both sorts of bacteria. Similar results in the respect of the prolonged release and the efficiency of formulations with gentamicin sulfate have been demonstrated in some other researchers' studies.[26]

Tables III and IV show the results of cytotoxic changes on the culture of cells examined *in vitro*. As the results shown through the change index and the lyse percentage, the cells have undergone minimal

changes ranging between 0 and 1, 11.0–14.0%, Table IV, of the change index, which implies that the examined antimicrobial textile materials are slightly cytotoxic. Figure 5(a,b) show the cell cultures with no effect of antimicrobial textile materials [Fig. 5(a)] and with the effect of sucker which also gives negative control [Fig. 5(b)], i.e., it does not show cytotoxicity, and with the effect of the antimicrobial textile material [Fig. 5(d)]. Negative control, Figure 5(a), shows the cells of normal appearance, adherent, with a confluent layer, whereas in the presence of the antimicrobial textile material, Figure 5(d), we can notice fibroblastoid appearance of cells and inhibited proliferation, which implies slight degenerative changes. The provoked cytotoxic effect is very mild, unlike the phenol solution which has a 100% cytotoxic effect, provoking explicit degenerative changes [Fig. 5(c)].

The *in vivo* research results on guinea pigs and rabbits have shown that the examined antimicrobial textile material neither shows irritating effects nor provokes sensibilization, and as such is acceptable for using in a direct contact with a tissue of a living organism. This means that these materials are suitable for application on human skin, that they do not cause any harmful effects and that they can be used for clinical purposes. The literature data, related to the examination of the irritation and cytotoxicity of nonwoven textile materials made of PP and viscose modified by chitosan are also indicative of the nonexistence of the irritation and cytotoxicity effect.[27] Moreover, certain different formulations, such as a polymer in the form of an implant, containing higher concentrations of gentamicin sulfate with controlled release have not shown any inflammatory reactions or tissue irritation.[27] The skin of the examined animals at the contact place with the tested samples seemed satisfactory and acceptable.

CONCLUSIONS

The results of these pieces of research have shown that the obtained antimicrobial nonwoven textile materials are of broad-spectrum activity and that they are capable of inhibiting the growth of pathogenic microorganisms. These materials are suitable for making plasters of different shape and dimensions which can be used for the prevention of wound infections as well as for the treatment of injected skin changes and wounds. They can be very efficient within the corresponding antimicrobial therapy in the long run regarding both their application and their long standing effects.

According to the obtained research results and the calculated sensitivization index, it can be concluded that the antimicrobial textile materials in the form of plaster do not cause sensitivization. The results of the examination of cutaneous irritation have shown that a fibrous antimicrobial textile material behaves as a nonirritating bioactive material. Our results of the index of the quantitative morphology cell changes within the cytotoxicity examinations have shown that the provoked cytotoxic effect is very mild, so the obtained antimicrobial textile material can be characterized as efficient and nontoxic biomaterials. Good biocompatibility, the existence of the correlation between the *in vitro* and *in vivo* results, concerning efficiency, qualifies these antimicrobial biomaterials for clinical use.

References

1. Ramachandran, T.; Rajendrakumar, K.; Rajendran, R. Jornal-TX 2004, 84, 42.
2. Petrulyte, S. Dan Med Bull 2008, 55, 72.
3. Oltarzhevskaya, N. D.; Krichevskii, G. E. Pharm Chem J 2005, 39, 42.
4. Lin, T. L.; Lu, F. M.; Conroy, S.; Sheu, M. S.; Su, S. H.; Tang, L. Med Device Technol 2001, I, 489.
5. Mehdizadeh, A.; Toliate, T.; Rouini, M. R.; Abashzadeh, S.; Dorkoosh, F. Acta Pharm 2004, 54, 301.
6. White, W. C.; Monticelio, A. R. AATCC New England Regional Technical Conference, Mystic, CT, May 1–3, 2003.
7. Skundric, P.; Simovic, Lj.; Kostic, M.; Medovic, A.; Milosevic, K.; Dimitrijevic, S. Antimicrobial Bioactive Band-Aids with Prolonged and Controlled Action, VII International Conference MEDTEX 2007, Bolton, July 16–18.
8. Skundric, P.; Medovic, A.; Simovic, Lj.; Dimitrijevic, S.; Kostic, M.; Janicijevic, M.; Milakovic, B. V International Conference MEDTEX 2005, Portoroz, June 27–29, Book 1, p 232.
9. Skundric, P.; Medović, A.; Kostic, M. AUTEX Res J 2002, 2, 78.
10. Skundric, P.; Medovic, A.; Simovic, Lj.; Dimitrijevic, S.; Kostic, M. 'Development and Characterization of Antibacterial Bioactive Fibers as Transdermal Therapeutic Systems,' 5th World Texrile Conference AUTEX 2005, Portorož, Slovenia, June 27–29, Book 1, p 232.
11. Mihailovic, T.; Asanovic, K.; Simovic, Lj.; Skundric P. J Appl Polym Sci 2006, 103, 4012.
12. Skundric, P.; Kostic, M.; Lukic, S.; Mihailidi, T.; Milosavljevic, S.; Stankovic, S.; Mihailovic, T.; Asanovic, K.; Dimitrijevic, S.; Siler-Marinkovic, M.; Lj, S.; Racin, M.; Popovic, K.; Medovic, A.; Simovic, L. J.; Janicijevic, M.; Simeunovic, S.; Tasic, G.; Milakovic, B.; Uscumlic, G.; Hadzi-Djokic, J.; Stupar, M. Project: Development of the Biomedical Textile Materials and Products with Programmed Properties; TR 6713-B; Ministry of Science of the Republic Serbia: Belgrade, Serbia, 2005.
13. Lim, S. H.; Hudson, S. M. Carbohydr Polym 2004, 56, 227.
14. Wisnewska-Wrona, M.; Neikraszewicz, A.; Struszczyk, H.; Guzinska, K. Fiber Text East Eur 2002, 10, 82.
15. Senel, S.; McClure, S. Adv Drug Deliv 2004, 56, 1467.
16. Giannola, L. I.; De Caro, V.; Adragna, E.; Giandelia, G.; Giannola, G.; D'Arpa, N.; Napoli, B. Ann Burns Fire Disasters 1999, XII, Available at: http://www.medbc.com/annals/review/vol_12/num_1/text/vol12n1p14.htm.
17. White, C. W.; Olderman, J. M. Antimicrobial Techniques for Medical Nonwovens: A Case Study, ÆGIS Environments of Midland, Michigan, 1–14, Available at: http://www.aegiscanada.ca/Literature/Antimicrobial_Performance_of_Medical_ Textiles-IFAI_4A9.pdf.
18. Hayes, S. F.; White, C. How Antimicrobial Treatment Can Improve Nonwovens, ÆGIS Environments of Midland, Michigan, 1–12, Available at: http://www.aegisasia.com/Antimicrobial-Treatment-Improve.html.
19. Wollina, U.; Heide, M.; Müller-Litz, W.; Obenauf, D.; Ash, J. Textiles and the Skin, Karger 2003, 31, 82.

20. Rajendran, S.; Anand, S. C. Medical Textiles: Proceedings of the 2nd International Conference, 24th and 25th August 1999; Bolton Institute: UK, Edited by Subhash Anand, Woodhead Publishing, 2000, p 107.
21. Müller, U. Available at: http://www.devicelink.com/mdt/archive/08/03/006.html.
22. Sevastianov, I. V. Biocompatible Biomaterials: Current Status and Future Perspectives, Trends Biomater Artif Organs 2002, 15, 20.
23. Simovic, Lj.; Skundric, P.; Pajic-Lijakovic, I.; Ristic, K.; Medovic, A.; Tasic, G. J Appl Polym Sci 2010, 117, 1424.
24. Beth, J. G. Am Chem Soc 2001, 12, 201.
25. Harrigan, W. F. Laboratory Methods in Food Microbiology, 3rd ed.; Academic Press: London, 1998.
26. Krasko, M. Y.; Golenser, J.; Nyska, A.; Nyska, M.; Brin, Y. S.; Domb, A. J. J Control Release 2007, 117, 90.
27. Strusycyzk, M. H.; Brzoza-Malczewskaya, K.; Szalcyźnska, M. Fiber Text East Eur 2007, 15, 163.