

Research Article

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Development of wash-resistant antimicrobial compression covers for amputated limbs

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Abstract: Medical textiles for amputee care during post-operative, rehabilitation, and prosthetic period play an important role in the effective correction of stump formation. Treatment of amputations involves not only initial stabilization, but also create conditions for the formation of a stump optimal for effective prosthetics. At the stage of amputation, the use of functional compression covers that provide uniform pressure from the distal to the proximal area of the stump is particularly effective. Additional antimicrobial treatment of the covers can prevent the growth of pathogenic bacteria on the wound surface of the amputated limb. The article deals with the issue of antimicrobial activity of functional knitted covers for rehabilitation purposes with a combination of compression and antimicrobial activity resistant to repeated washings. To impart antimicrobial properties, two methods of surface modification of knitted fabrics were used: treatment with copper sulphate and hydrazine sulphate as reducing agents, and use of phenyl phenolic intensifier in a dye bath. Furthermore, an effect of multiple washing on the compression alteration and antimicrobial activity of knitted compression covers was investigated. It was found that the most

effective method to ensure the wash-resistant antimicrobial activity of knitted compression covers is surface modification using a phenyl phenolic intensifier in the dye bath. After multiple washes, compression class of the most of the developed compression knitted covers did not change, what ensures proper compression treatment of amputated limbs.

Keywords: knitted covers; compression; surface modification; antimicrobial activity; shrinkage

1 Introduction

In recent years, military conflicts and terrorist attacks in various parts of the world have influenced an increase of injuries caused by explosions. A significant number of injuries are accompanied by severe damage leading to amputation of limb segments. Treatment of traumatic amputations involves not only initial stabilization of the victim's condition and stopping bleeding to save patient's life, but also creating optimal conditions for the formation of a stump optimal for effective prosthetics. At the stage of primary amputation in military mobile hospitals and in hospitals located near the military conflict zone, the use of functional compression covers that provide uniform pressure from the distal to the proximal area of the stump is particularly effective. Pre-prosthetic rehabilitation includes compression therapy, scar massage, stump hygiene, and phantom pain relief. Textile materials that are in direct contact with affected skin can serve as a medium for the accumulation, preservation, and spread of pathogenic microorganisms, including fungal and bacterial [1]–[4]. This property of the textile material contributes to the colonization and further reproduction of the microflora. Therefore, medical textiles for the care of an amputated limb in the postoperative period should combine compression with antimicrobial properties to prevent the spread of infectious agents during prolonged contact with the skin.

To provide textile materials or products with antimicrobial effect, special raw materials are used or antimicrobial treatment is applied. It is important to take into account the

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need to maintain antimicrobial properties after repeated washings. The development of functional knitted compression covers with additional antimicrobial property for stumps provides a solution to the extremely urgent issue of effective postoperative care of an amputated limb and during further rehabilitation at the stage of prosthetics.

Cotton fibers are characterized by their comfort, breathability, and permeability to vapor. However, due to their hydrophilic properties, cotton-based textile materials in contact with a wound surface can serve as a favorable environment for the growth of bacteria and fungi [1], [2], [5]–[7]. Textile materials used for the treatment of postoperative wound surfaces should ensure inhibition and destruction of the growth of microorganisms that can cause serious health problems for the patient, ranging from simple discomfort to infections and diseases [8]–[10]. This has contributed to the development of new functional textile materials with antimicrobial properties [11]–[16]. There are several studies on the use of antibacterial substances in textiles. It has been experimentally proven that textiles impregnated with environmentally friendly tea tree essential oil, nutmeg, and birch extracts have the highest antimicrobial activity against Gram-positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus cereus*. However, such impregnation is not resistant to repeated washing [17]. Scientific papers present a number of studies on the use of antibacterial agents for textile modification. In particular, the use of metals [18], such as silver [19]–[23], copper and zinc oxides [24], or stainless-steel wires integrated into the fabric structure by wrapping around natural fibers [25]. Special attention has been paid to the use of biopolymers, like chitosan [26]–[28] and triclosan [29] for finishing of textile materials. Triclosan is widely used in antimicrobial compounds because it demonstrates a wide range of antibacterial activity and meets European Union and United States requirements for toxicity hazards [30]. The mechanism of antimicrobial action of triclosan is based on the inhibition of enzymes involved in fatty acid synthesis of fatty acids through mimicry of a natural substrate [31]. Triclosan is more active against Gram-positive bacteria than against Gram-negative bacteria [32]. Studies have shown the antibacterial activity of cellulose knitted fabrics treated with metal salts such as $\text{Zn}(\text{NO}_3)_2$, CuSO_4 , and AgNO_3 [18].

Antimicrobial agents can be applied to textile materials using such technological methods as impregnation, coating, spraying, foaming, immersion in an extraction bath, dry tamping, etc. As a rule, antimicrobial treatment is carried out in the final stages of finishing textile materials. However, in some cases biocidal substances are integrated directly into the structure of synthetic fibers at the stage of extrusion of the polymer mass [33]–[38].

To date, a significant number of studies have been conducted to investigate the effectiveness of fixation and resistance of antimicrobial substances to external factors in textiles. In particular, the antimicrobial activity of textile fabrics made of cotton, wool, polyamide, and polyester fibers, as well as their combined variants after appropriate treatment, has been analyzed [39]–[42]. Studies have shown that knitted fabrics with the same fiber composition and similar overall linear yarn density, but with different number of folded yarns in the knitting structure, demonstrate different level of antimicrobial activity. This is due to the fact that an increase in the number of folded yarns in the loop leads to an increase in the surface area, which, therefore, increases the effectiveness of the antimicrobial effect [43].

The effectiveness of textile fabrics in the destruction of microorganisms is assessed by determining the level of antibacterial or antifungal activity. This involves comparing the number of colonies of microorganisms before and after fabric treatment. One of the key quality criteria is the preservation of antimicrobial activity after repeated washing, which allows to assess the strength of the active substance's fixation on the fibers. However, achieving both high antimicrobial effectiveness and resistance to mechanical stress (including repeated washing) remains a technologically challenging task. This is due to the need to take into account the environmental, health, and safety aspects of the use of antimicrobial agents [31], [44], [45].

The aim of this work was to develop functional wash-resistant antimicrobial compression knitted covers for postoperative compression care of amputated limbs and compare effectiveness of two methods of antimicrobial treatment: direct antimicrobial treatment and treatment combined with the dyeing process. Furthermore, changes of compression level generated by the covers after repeated washings were investigated to ensure the optimal compression treatment of amputated limbs in the postoperative and pre-prosthetic period [46].

2 Materials and methods

2.1 Materials

For antimicrobial surface modification, six variants of tubular cotton-based knitted structures with integrated elastomeric yarn laid in the form of tuck-miss with the layout 1×1 and 1×3 were developed. Elastomeric yarn was used to generate compression of the cover on a limb. The prototypes were knitted on a 13E gauge circular weft-knitting machine with a cylinder diameter of 3.75 inches. A single-plated pattern was chosen for the formation of the main knitted structure: cotton

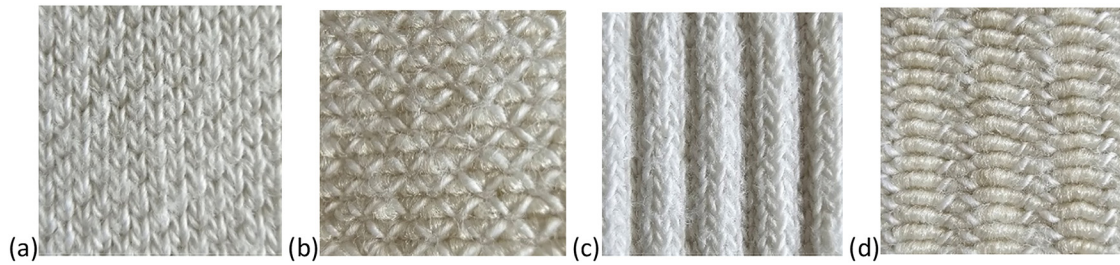


Figure 1: Surface view of knitted specimens: Technical face side (a) and technical back side (b) of the structure with 1 × 1 tuck-miss repeat of elastomeric inlay yarn; technical face side (c) and technical back side (d) of the structure with 1 × 3 tuck-miss repeat of elastomeric inlay yarn.

Table 1: Main structural parameters and technical indicators.

Sample code	Wale density, cm ⁻¹	Course density, cm ⁻¹	Area density, g/m ²	Loop length of plating cotton yarn, mm	Loop length of textured-elastomeric PA-PU ground yarn, mm	Average length of textured-elastomeric PA-PU inlay-yarn per one wale, mm
Tuck-miss repeat of the elastomeric yarn 1 × 1						
1 × 1/11	8.5	11	402.4	5.6	5.2	1.1
1 × 1/10		10	397.6	6.2	5.8	1.2
1 × 1/9		9	400.8	6.8	6.4	1.1
Tuck-miss repeat of the elastomeric yarn 1 × 3						
1 × 3/11	9.0	11	450.0	6.1	5.5	1.2
1 × 3/10		10	456.8	6.7	6.1	1.3
1 × 3/9		9	417.6	7.3	6.7	1.2

yarn with a linear density of 20 tex was used as the plating yarn and textured polyamide (PA) yarn of 4.4 tex with a polyurethane (PU) core of 2.2 tex was used as the ground yarn. Three variants of course densities (9, 10, and 11 cm⁻¹) were used during the knitting. These prototypes of knitted compression covers were produced at the rotation speed 110 min⁻¹ of a wheel that feeds elastomeric yarn into the knitting zone. The developed knitted compression covers provide the second compression class according to the standard RAL-GZ 387/1:2008 [47] at the 30 % elongation. A principal surface view of the developed knitted structures is presented in Figure 1.

The main structural parameters of the developed knitted structures are presented in Table 1 (relative error of measurement was up to ±3 %).

2.2 Methods

2.2.1 Wash and shrinkage calculation method

The washing test was carried out according to the standard test method EN ISO 6330:2021 [48], SIEMENS varioPerfect iQ700 washing machine. Washing characteristics: samples were washed in a detergent solution with a detergent concentration of 3 g/L for 15 ± 0.5 min at a temperature of 40 ± 2 °C; after the main wash cycle, three times of rinsing with 20 ± 2 °C water and 1 ± 0.1 min duration of each rinse

were used. After washing, the samples were centrifuged for 1 ± 0.1 min at 1,000 rpm and then dried on a flat horizontal surface for 24 h under standard atmospheric conditions [49].

The shrinkage after washing the knitted samples was measured in longitudinal and transverse directions after each washing and drying cycle. Shrinkage values were calculated according to the formula:

$$\lambda = \frac{L - L_0}{L_0} \cdot 100 \% \quad (1)$$

where λ is the shrinkage after washing in %; L_0 is the initial dimension of the sample in mm (before washing and drying); L is the dimension in mm after washing and drying.

Five samples were prepared for each knitted structure and two measurements were performed on each sample and each direction; the average value obtained from 10 measurements for each knitted variant was recorded.

2.2.2 Compression evaluation method

Compression was calculated according to the theory of Laplace's law [46]:

$$P = \frac{2\pi F}{S} \quad (2)$$

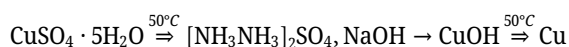
where: P is the compression in Pa; F is the tensile force in N; S is the area of the sample in mm².

Compression, calculated in SI units of measurement Pa, is then converted to mmHg, as these units of measurement are most commonly presented in medical literature and in the specifications of medical devices.

The tensile test was performed in the direction of courses (transverse) corresponding to the deformation of the compression cover of its use. Tensile force was measured using the universal test machine ZWICK/Z005 (Germany) according to the standard LST EN ISO 13934-1:2013 [50]: with the 200 N force sensor; the constant 100 mm/min displacement rate of the moving clamps; the (100 × 80) mm of the tested sample are; the fixed elongation of 30 %. The data were analysed by using the testxpert® software. Five elementary tests were performed for each knitted structure. Relative standard deviation of measurements did not exceed 4 %.

2.2.3 Copper treatment method

Surface modification of the developed compression knitted covers with copper nanoparticles was carried out by chemical reduction of metal ions to metals directly in the structure of the textile fabric and on its surface [51]. The sequence of stages in the process of copper reduction from a copper sulphate solution can be represented in the form of the diagram:



A solution of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) with the concentration of 10 % was used to reduce copper ions. Hydrazine sulphate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$) was used as a reducing agent - 10 % solution. The reduction of copper ions in the structure of knitted fabrics was carried out in the following sequence:

- preparation of a 10 % solution of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$);
- preparation of a 10 % solution of hydrazine sulphate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$);
- preparation of a 15 % alkali solution (NaOH).

The knitted samples were soaked in the 10 % solution of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) for 60 min. The copper sulphate solution with the knitted samples was heated to 50 °C temperature. A 10 % solution of hydrazine sulphate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$) and a 15 % alkali solution (NaOH) were added to the heated solution. The reaction mixture continued to be heated at a temperature of 50–60 °C until copper was completely reduced. The modified samples were washed with water and dried in air.

2.2.4 Combined biocidal treatment and dyeing method

To impart antibacterial properties to the developed compression knitted fabrics in the process of their dyeing, an intensifier of the phenyl phenolic series was introduced into the composition of the dye bath. The knitted compression covers were dyed using 5-chloro-2 (2,4-dichlorophenoxy) phenol as an intensifier, which has proven antibacterial and antimicrobial properties in a broad spectrum. The use of this component as an intensifier of the textile dyeing process allows not only to reduce the temperature, but also to eliminate bactericidal treatment at the final finishing stage. The sequence of the whole process is as follows:

- Preparation of the intensifier emulsion: OS-20 surfactant and the intensifier were added to a beaker, and then warm distilled water was gradually added, thoroughly to a volume of 40 cm³. The mixture was heated to the temperature at which the intensifier completely melted and stirred until a homogeneous emulsion was formed.
- Pre-treatment for dyeing: The finished intensifier emulsion was poured into a container with knitted samples. This ensures penetration of the intensifier into the fibres, which improves subsequent fixation of the dye. After the working bath cooled to 40 °C, the knitted samples were removed.
- Dyeing: After pre-treatment, the knitted samples were dyed according to the standard cotton dyeing method without any changes in the process.

2.2.5 Antimicrobial activity evaluation method

The antimicrobial efficacy of the treated samples was evaluated against representatives of Gram-negative and Gram-positive microflora: *Escherichia coli* (strain KMY1T) and *S. aureus* (strain ATCC25923). The qualitative determination of antimicrobial activity was carried out according to the requirements of EN ISO 20645:2004 (Agar diffusion plate method) [52]. Antimicrobial tests for each variant of specimens were performed in triplicate ($n = 3$).

After plating the samples on the nutrient medium, the Petri dishes were incubated for 18 h at 37 °C. After incubation, antimicrobial activity was determined by measuring the width of the zone of inhibition of microbial growth around the samples according to the established method:

$$H = \frac{D - d}{2} \quad (3)$$

where H is the inhibition zone in mm, D is the total diameter of the sample and the inhibition zone in mm, and d is the diameter of the sample in mm.

After antimicrobial treatment, knitted samples were subjected to five washing cycles (according to washing procedure described above) after which the antimicrobial activity was evaluated.

3 Results and discussion

3.1 Antimicrobial activity

The results of antimicrobial activity against Gram-positive and Gram-negative bacteria of the treated compression knitted covers, expressed as the inhibition zone, are presented in Table 2.

The visualization of the experimental studies of antimicrobial activity is shown in Figures 2 and 3.

All knitted specimens treated with antimicrobial agents exhibited pronounced antimicrobial activity. Analysis of the experimental data demonstrates an inhibition zone around the samples and indicates a higher level of antimicrobial activity against Gram-positive bacteria *S. aureus* compared to Gram-negative *E. coli*. There are clear differences in antimicrobial activity depending on the treatment method (see Table 2). However, after antimicrobial treatment, all tested knitted fabrics, regardless of the antimicrobial treatment, demonstrated a stable effect, which is confirmed by the presence of an inhibition zone with a diameter of 11–31 mm for *S. aureus* and 4–16 mm for *E. coli*. The results obtained indicate the formation of stable bonds between the antimicrobial agent and the knitted material, regardless of the knitted structure.

However, as noted above, the main challenge in the field of antimicrobial modification of textile materials is to ensure an antimicrobial effect after repeated washing. Based on the data presented (Table 2), it can be argued that both applied antimicrobial treatments are effective and suitable for use on cotton-based knitted fabrics. In particular, it was found that the antimicrobial activity of knitted fabrics modified with copper nanoparticles as well as dyed by introducing a phenyl-phenol series intensifier against Gram-positive bacteria *S. aureus* remains at a high level (with a highly expressed inhibition zone) for at least five washing cycles. After five washing cycles, antimicrobial activity is still recorded at a sufficiently high level (more than 16 mm for fabrics dyed with phenyl phenolic intensifier and more than 5 mm for copper treated fabrics). Fabrics dyed with phenyl phenolic intensifier also demonstrate very good antimicrobial activity against Gram-negative bacteria *E. coli* as well as good resistance of activity after multiple washings (with inhibition zone greater than 12 mm after five washings). Copper-treated fabrics also demonstrate antimicrobial activity against *E. coli* bacteria after five washing cycles; but it is rather low (with 0.1–0.3 mm inhibition zone). However, despite this, the growth of bacterial colonies on the sample is still inhibited.

It was found that knitting structure, i.e., the pattern repeat of the elastomeric inlay yarn and the loop density do not have statistically significant effect on the antimicrobial effectiveness of the treated knitted fabrics (Figures 4 and 5). This is an important finding because it means that the structure of the knitted compression cover can be designed taking into account only the compression requirements.

Table 2: Antimicrobial activity of tested knitted fabrics.

Sample code	Inhibition zone, mm							
	Before washing		After 1st washing cycle		After 2nd washing cycle		After 5th washing cycle	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
Copper treatment								
1 × 1/9	11.8 ± 0.7	4.0 ± 0.2	11.5 ± 0.6	1.5 ± 0.1	11.3 ± 0.5	1.0 ± 0.1	8.2 ± 0.4	0.2 ± 0.03
1 × 1/10	11.8 ± 0.6	4.1 ± 0.2	11.0 ± 0.5	1.7 ± 0.2	10.5 ± 0.5	0.8 ± 0.1	8.7 ± 0.5	0.1 ± 0.02
1 × 1/11	12.1 ± 0.7	4.1 ± 0.2	11.5 ± 0.6	1.8 ± 0.2	10.8 ± 0.6	1.0 ± 0.2	8.5 ± 0.4	0.3 ± 0.04
3 × 1/9	11.1 ± 0.4	4.2 ± 0.3	10.0 ± 0.5	2.0 ± 0.3	8.5 ± 0.4	0.7 ± 0.1	7.0 ± 0.4	0.1 ± 0.03
3 × 1/10	11.6 ± 0.5	4.0 ± 0.2	10.7 ± 0.5	2.6 ± 0.3	8.3 ± 0.4	1.0 ± 0.1	5.3 ± 0.3	0.3 ± 0.04
3 × 1/11	11.8 ± 0.6	4.1 ± 0.3	10.3 ± 0.5	1.7 ± 0.2	7.0 ± 0.5	1.5 ± 0.2	5.7 ± 0.4	0.3 ± 0.03
Biocidal treatment with phenyl phenolic intensifier								
1 × 1/9	30.2 ± 1.3	16.9 ± 0.8	29.7 ± 1.1	16.2 ± 0.7	20.7 ± 1.5	15.5 ± 0.9	19.2 ± 1.1	12.2 ± 0.6
1 × 1/10	31.2 ± 1.5	15.8 ± 0.9	31.0 ± 1.6	15.2 ± 0.9	23.8 ± 1.2	14.8 ± 0.7	19.7 ± 1.2	13.3 ± 0.8
1 × 1/11	31.8 ± 1.9	16.7 ± 1.1	28.2 ± 1.3	16.5 ± 0.9	20.7 ± 1.4	14.8 ± 0.8	20.0 ± 1.5	13.7 ± 0.8
3 × 1/9	28.7 ± 1.3	16.0 ± 0.8	26.2 ± 1.4	15.2 ± 0.8	16.3 ± 0.9	13.8 ± 0.6	16.2 ± 0.9	13.0 ± 0.7
3 × 1/10	29.2 ± 1.4	15.3 ± 0.6	27.0 ± 1.5	15.2 ± 1.0	17.2 ± 1.5	15.2 ± 0.9	16.5 ± 1.0	14.2 ± 0.9
3 × 1/11	28.8 ± 1.1	16.2 ± 0.8	27.0 ± 1.3	15.3 ± 1.4	17.5 ± 1.3	15.0 ± 0.7	17.2 ± 1.4	12.3 ± 0.6

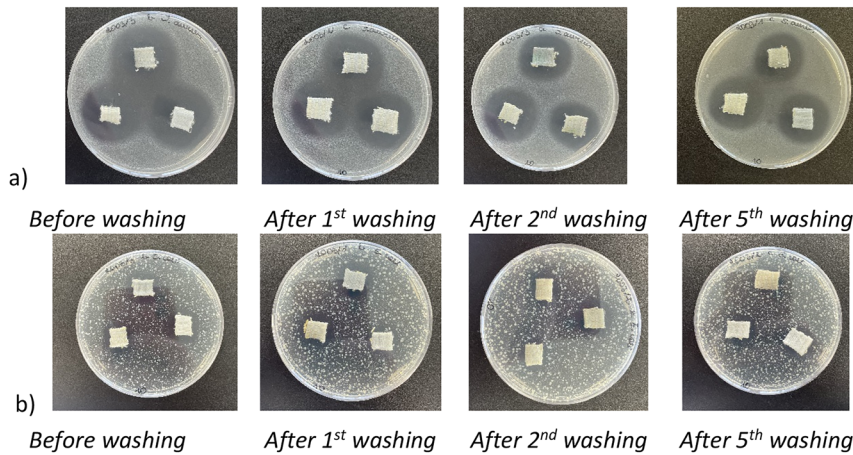


Figure 2: Antimicrobial activity of knitted fabrics with elastomeric yarn repeat 1×3 against *S. aureus* (a) and *E. coli* (b) bacteria after surface modification with copper nanoparticles.

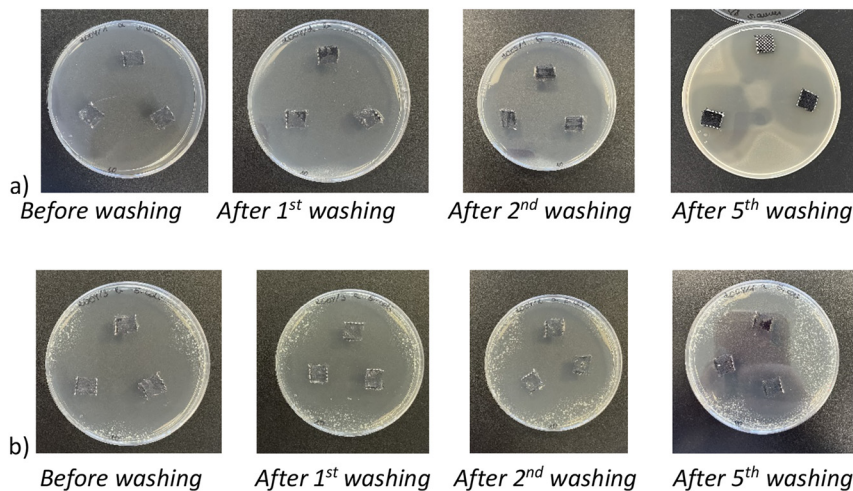


Figure 3: Antimicrobial activity of knitted fabrics with elastomeric yarn repeat 1×3 against *S. aureus* (a) and *E. coli* (b) bacteria after dyeing with phenyl phenolic intensifier.

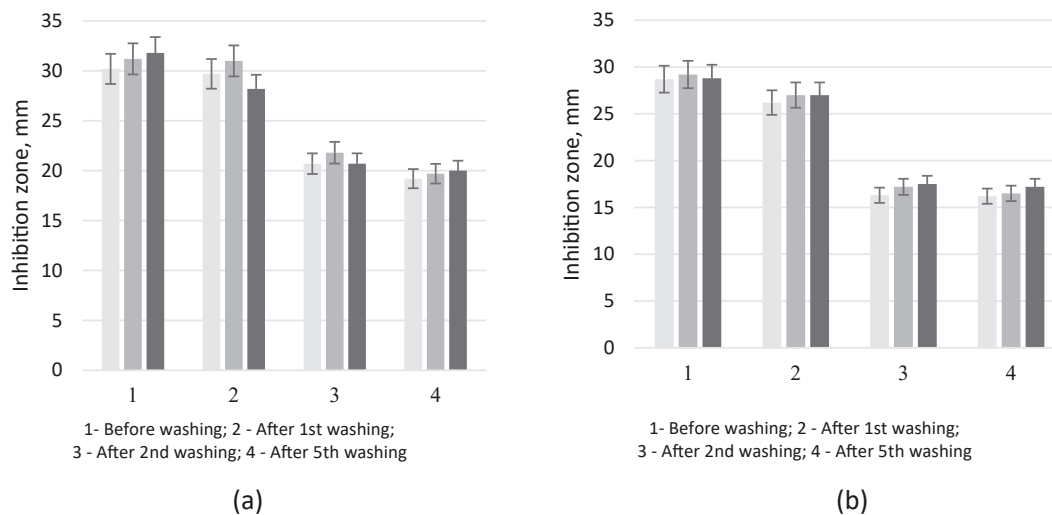


Figure 4: Antimicrobial activity of knitted fabrics dyed with phenyl phenolic intensifier against *S. aureus* bacteria: (a) pattern repeat 1×1 ; (b) pattern repeat 1×3 : Course density 9 cm^{-1} , 10 cm^{-1} , 11 cm^{-1} .

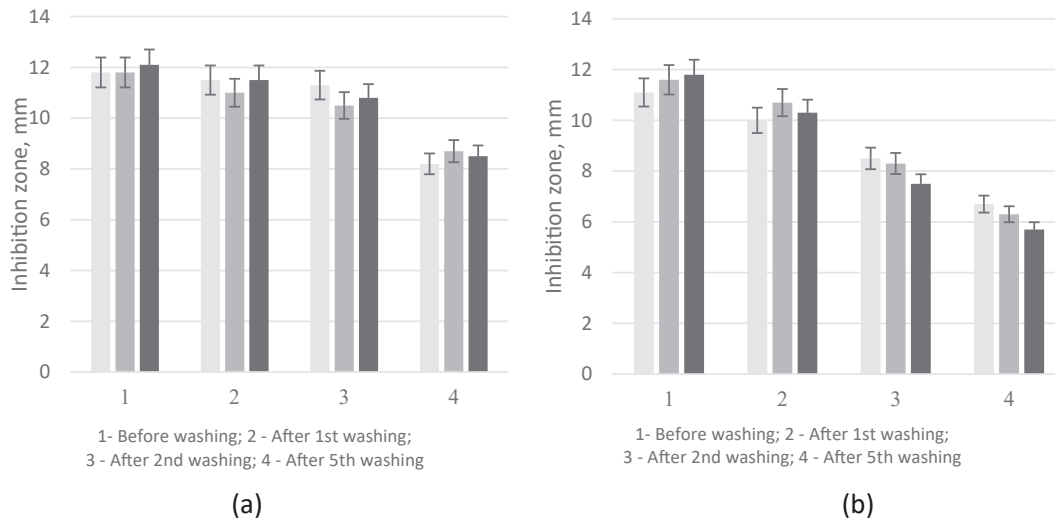


Figure 5: Antimicrobial activity of copper treated knitted fabrics against *S. aureus* bacteria: (a) pattern repeat 1 × 1; (b) pattern repeat 1 × 3: Course density 9 cm⁻¹, 10 cm⁻¹, 11 cm⁻¹.

Despite the literature data on the use of copper nanoparticles and phenyl phenolic compounds in medical products, potential toxicity of the biocidal treated compression covers must be further tested before clinical application.

3.2 Compression properties

It is well known [46] that compression generated by a compression garment depends on the area of the garment. Therefore, it is highly important to find out how repeated washing influences possible compression changes. These changes may have a crucial effect on the healing effectiveness of the amputated stump. If the compression is too

low, the compression treatment does not give the expected result for scar healing. On the other hand, if the compression is too high, this can disrupt scar healing and even cause complications. It is also well known that cotton-based weft knitted fabrics tend to change their dimensions after washing and drying, and usually they shrink, that is, their dimensions and, accordingly, their surface area decreases. Shrinkage values in both lengthwise (wales) and transversal (courses) directions were carefully measured after each washing and drying cycle. The results obtained are presented in Table 3.

The presented results demonstrate that all investigated knitted samples shrunk and the highest shrinkage values were reached in the longitudinal (vertical)

Table 3: Shrinkage after repeated washing and drying cycles.

Sample code		Shrinkage, %				
		Direction	After 1st washing cycle	After 2nd washing cycle	After 3rd washing cycle	After 5th washing cycle
Tuck-miss repeat of the elastomeric yarn 1 × 3						
1 × 3/11	Transversal	-2.75 ± 0.22	-0.56 ± 0.22	-0.11 ± 0.10	0	
	Longitudinal	-7.11 ± 0.67	-1.89 ± 0.42	-0.65 ± 0.11	0	
1 × 3/10	Transversal	-2.88 ± 0.34	-0.67 ± 0.21	-0.21 ± 0.17	0	
	Longitudinal	-7.50 ± 0.56	-1.08 ± 0.30	-0.55 ± 0.19	0	
1 × 3/9	Transversal	-2.56 ± 0.20	-0.67 ± 0.21	-0.15 ± 0.11	0	
	Longitudinal	-7.0 ± 0.56	-1.36 ± 0.26	-0.55 ± 0.13	0	
Tuck-miss repeat of the elastomeric yarn 1 × 1						
1 × 1/11	Transversal	-2.06 ± 0.21	-0.55 ± 0.16	-0.12 ± 0.10	0	
	Longitudinal	-5.05 ± 0.40	-1.04 ± 0.12	-0.85 ± 0.15	0	
1 × 1/10	Transversal	-2.25 ± 0.15	-0.59 ± 0.12	-0.14 ± 0.13	0	
	Longitudinal	-5.50 ± 0.39	-1.08 ± 0.13	-0.80 ± 0.16	0	
1 × 1/9	Transversal	-2.08 ± 0.24	-0.65 ± 0.11	-0.12 ± 0.09	0	
	Longitudinal	-5.25 ± 0.42	-1.60 ± 0.14	-0.54 ± 0.10	0	

direction: approx. -7% for knitted fabrics with 1×3 repeat of the elastomeric yarn and approx. -5% for knitted fabrics with 1×1 repeat of the elastomeric yarn. In the 1×1 repeat knitted structures, tucks of elastomeric inlay yarn are formed on each second needle and are arranged in a chequerboard pattern in the structure. This prevents dimensional changes in the main structure that occur due to moisture and heat during washing. It demonstrates that the knitting pattern can have a significant effect on the dimensional stability of the weft knitted fabrics. However, for the investigated structures, the influence of loop density on shrinkage values was statistically insignificant – changes are in the limits of error. Even more, for compression garments, transverse dimensional stability is more important as compression is usually generated due to the stretch in transversal direction. After the first washing and drying cycle, the investigated knitted structures shrunk in the transversal direction by less than 3% . This is a very good result, as it is accepted that shrinkage of less than $\pm 3\%$ does not significantly affect the properties of the knit. Even more, as shown in Table 3, dimensional changes in transversal direction after the third washing and drying cycle are in the limits of error, and the total shrinkage after 2–3 washings is approximately -3% or less. There were no dimensional changes after the fourth and fifth washing and drying cycle; thus, all knitted samples achieved their stable structure.

Results of a tensile force and compression before washing and after repeated washings are presented in Table 4 and graphically demonstrated in Figure 6.

The presented data demonstrate that compression of knitted covers increased after repeated washings; however, this increase is not critical – approximately 10% . The influence of knitting pattern and loop density on the increase in compression after repeated washing is insignificant, despite the fact that both the knitting pattern and loop density have influence on the compression generated by the knitted cover.

It can also be seen from the results presented that it is possible to determine the design zone with the appropriate structural parameters, in which the knitted cover will maintain compression corresponding to the second compression class according to the standard RAL-GZ 387/1:20,083 [47] even after repeated washing (the zone defined in Figure 8). The results obtained also demonstrate that distribution of the elastomeric inlay yarn in the knitted structure and structural parameters such as loop density have a statistically significant influence on compression level. Knits with shorter elastomeric floats and higher loop density generate higher compression.

Table 4: Tensile force at 30 % elongation and compression before and after repeated washings.

Sample code	Before washing			After 1st washing			After 2nd washing			After 5th washing		
	Tensile force, N	Compression, mmHg		Tensile force, N	Compression, mmHg		Tensile force, N	Compression, mmHg		Tensile force, N	Compression, mmHg	
Tuck-miss repeat of the elastomeric yarn 1×3												
$1 \times 3/11$	5.11	30.09		5.82	34.27		5.98	35.22		6.42	37.81	
$1 \times 3/10$	4.82	28.38		5.29	31.15		5.50	32.40		5.55	32.68	
$1 \times 3/9$	4.18	24.62		4.45	26.21		4.60	27.09		4.71	27.74	
Tuck-miss repeat of the elastomeric yarn 1×1												
$1 \times 1/11$	5.82	34.27		6.07	35.75		6.19	36.45		6.24	36.75	
$1 \times 1/10$	4.59	27.03		4.93	29.03		5.09	29.97		5.34	31.45	
$1 \times 1/9$	4.08	24.03		4.29	25.26		4.44	26.15		4.52	26.62	

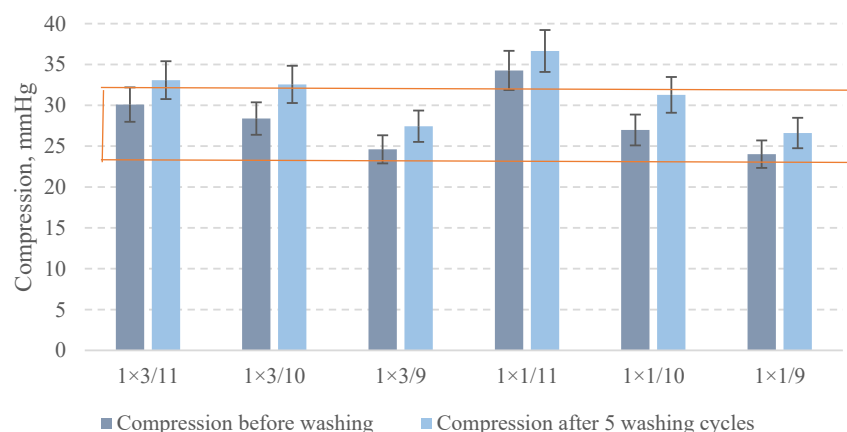


Figure 6: Compression changes after five washing cycles.

4 Conclusions

Wash-resistant antimicrobial active knitted covers for compression treatment of amputated limbs were successfully developed. Research has revealed that the most effective method to ensure the wash-resistant antimicrobial activity of knitted compression covers is surface modification using a phenyl phenolic intensifier in the dye bath, which also allows to combine dyeing and antimicrobial treatment into one process. This treatment method was found to have very good antimicrobial activity against Gram-negative *E. coli* (KMY1T) and Gram-positive *S. aureus* (ATCC25923) bacteria, as well as exceptionally good resistance to repeated washing. An average inhibition zone of approx. 30 mm was formed around the samples against *S. aureus* and 16 mm against *E. coli* bacteria; the inhibition zone remained much more than 10 mm even after five washings. The proposed method of antimicrobial treatment of textile materials is highly promising, given its potential compatibility with other finishing processes, which contributes to saving energy, water and time. An additional advantage is the possibility of applying this treatment to a wide range of raw materials, as well as its high resistance to repeated washing, which provides a long-term antimicrobial effect.

The method of surface modification of knitted compression covers treated with copper sulphate and hydrazine sulphate as a reducing agent, which ensures the formation of a stable antimicrobial activity of the textile substrate, is also worthy of attention. It gives very good and wash resistant activity against *S. aureus* bacteria (with an average inhibition zone of 11.5 mm) and reasonably good activity against *E. coli* bacteria (with an average inhibition zone of 4 mm), which persists after five washes.

The main dimensional changes of compression covers after the first washing were in longitudinal direction. And the total summary shrinkage after repeated washing and drying cycles in the transverse direction was less than -3% (very

good result). Dimensional changes after repeated washings also affected changes in compression, but most of the developed compression knitted covers remained in the same, second, compression class according to the German standard RAL-GZ 387/1:2008 even after multiple washes, which ensures proper compression treatment of amputee limb scars.

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