BIOLOGICAL PROPERTIES OF KNITTED FABRICS USED IN POST-BURN SCAR REHABILITATION

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1. Introduction

Every year approximately 1% of population suffers various types of burns. The biggest group of patients is children aged 2 to 4 [1]. Burns are defined as tissue damage due to high temperature. Depending on the temperature level a human organism is subjected to and time of contact, the tissue damage will either be local or systemic.

Unfortunately, in many cases after the post-burn wound is healed, complications follow, which later result in hypertrophic scars and joint contractures. Hypertrophic scars are mainly caused by factors such as increased blood supply, elevated development of fibroblasts, elevated collagen deposition, and chronic edema when all the factors are considered. Further, if the redness of the scar does not subside in the course of two months in all those cases and the scar itself becomes harder, one has to take into account the creation of keloid. Keloids have nodular structure which is a result of boosted metabolism. Its growth is stimulated by microphages and fibroblasts which provoke the growth of collagen fibers. The mentioned complications are distressing due to the fact that they impede the fitness of mobility organs and, additionally, disfigure the patient. There are many scientific researches related to wound healing, creation of hypertrophic scars, keloids, and contractures along with their methods of treatment, e.g. [2–7].

Abstract:

Compression therapy along with the use of compression materials is one of the main prevention methods against scars and keloids. Compression knitted fabrics must be characterized by parameters that enabling to reaching class I compression (16–24 mmHg). When constant pressure higher than capillary vessels pressure is applied to scars, it will have a negative influence on formation of keloids and significantly prevents their hypertrophy. Long-term pressure causes tissue hypoxia, slowed-down metabolism, and reduction of the amount of fibroblasts. One of key elements of compression therapy is choosing the required knitted fabric with necessary structure and physicomechanical parameters as well as designing methodology based on Laplace law, which will ensure the application of desired value of single-unit pressure on post-burn scar. Apart from physicomechanical parameters, a medical device, such as compression garment, must be characterized by high level of biocompatibility. An added value in terms of functional parameters is the antibacterial action of the product. It was obtained from the fibers used which contain a compound in their matrix and out of which silver ions are released. Additionally, the antibacterial action was also obtained via impregnation of the knitted fabric in RUČO-BAC AGP which contains silver. In the course of the research presented in the article, compression fabric was designed with a special construction—a knitted fabric comprising two layers. The first layer, which is in direct contact with human skin, is manufactured out of a yarn with conductive-diffusive characteristics. The second layer is made of microfibers which keep the moisture out of skin surface and also diffuse it to the outside. The following article describes the final stage of developing a compression garment aiding the external treatment process; the mentioned stage is related to biological tests such as microbiological contamination, cytotoxicity, sensitization, and irritation. The article closes a series of publications presented by the Authors in bibliography. It also presents the antibacterial activity tests done on the developed double-layer knitted fabric enriched with silver. The obtained results suggest that the fabric may be used not only in compression therapy, but also in the field of cosmetics and aesthetics.

Keywords:

compression therapy, scar rehabilitation, compression fabrics, biocompatibility of compression garments, antibacterial fabrics

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One of the methods of prevention against creation of scars and keloids is compression therapy which renders very good results in terms of rehabilitation. The basic requirement set for compression garment is the application of unit pressure on hypertrophic scar. The available literature mentions varying values of recommended pressure between 15 and 30 mmHg [8–10]. The Roczniki Oparzeń informs that the amount of pressure should exceed 20 mmHg (capillary pressure) [8]. The application of constant pressure exceeding capillary vessel pressure has a positive effect on scar forming and prevents its hypertrophy. Long-term compression causes tissue hypoxia, slows down the metabolism, and reduces the number of fibroblasts.

The increased activity of collagenase, whose function is to decompose collagen, causes a significant reduction of the scar. There are also many alternative therapies, such as laser therapy, cryotherapy, etc. However, compression therapy is considered to be the most effective method. Modern medical techniques and procedures explicitly dictate that not only an effective treatment of burn wounds is necessary, but also it is prerequisite to design the healing process in such way to to minimize the occurrence of keloids.

One of key elements of compression therapy is choosing the required knitted fabric with necessary properties obtained from its structure, physicomechanical parameters, and designing methodology based on Laplace law [11–18], which will ensure application of desired value of single-unit pressure on post-burn scar.

According to the regulations dated 20.05.2010 and regulations related to class I medical devices, the knitted fabrics used for manufacturing Codopress Premium compression garments were subjected to the following biological tests: microbiological contamination, cytotoxicity, irritation, and sensitization. Moreover, the double-layer fabric undergoes antimicrobial activity tests and the effectiveness of construction is evaluated in the context of moisture transport.

2. Experimental part

2.1 Materials and characteristics

Three types of compression garments knitted fabrics were chosen for the tests:

- Liebaert weft commercial knitted fabric by Liebaert Marcel NM of 250 g/m² intended for manufacturing compression garments in the form of gloves. The fabric is made of two types of yarns: the polyamide one (65%) and polyurethane one (35%). The fabric has the OEKO-TEX certificate (Figure 1).

- Penn warp commercial, four-guide bar knitted fabric by Penn Textile Solutions GmbH of 230 g/m² intended for compression garments other than gloves. The fabric is made of two types of yarns: the polyamide one (56%) and polyurethane one (44%). The fabric has the OEKO-TEX certificate (Figure 2).

- double-layer Qskin weft knitted fabric with microfibers and silver ions of 336 g/m². The construction of the double-layer knitted fabric was described in patent application [19] (Figure 3)

The first layer of the knitted fabric is made of yarn with conductive-diffusive properties, which makes direct contact with the body. Its main function is to remove and transport moisture away from the body, both in liquid and in vapor form. The second layer, which does not have any direct contact with skin, is made of microfibers. Its function is to keep the moisture away from the body and also to diffuse it to the outside. Furthermore, the Q-skin polyamide yarn was used to give the fabric its antibacterial properties, which in its matrix has a compound, out of which silver ions with antibacterial properties are released. Moreover, the material was impregnated as a measure to enhance the antibacterial properties of the fabric. The process was carried out by adding from 0.5 to 2 g/l of milk-white suspension of RUCO-BAC AGP by Rudolf GmbH. The washing process was conducted at 30°C, and later the fabric was subjected to drying.

2.2 Methods

According to PN-EN ISO 10993-1 standard, the medical devices which are in contact with human skin for more than...
2.2.2. Cytotoxicity

The evaluation of cytotoxicity was carried out on samples of NCTC 929 ATCC mice fibroblasts, according to the PN-EN ISO 10993-5:2009 standard. The chosen growth medium was EMEM by Lonza, batch no. 5MB018, FBS fetal bovine serum by Euroclone, batch no. EUS00116, and antibiotics solution by Lonza, batch no. 16J215309.

The tests were carried out with diffusion method in agar medium according to PN-EN ISO 10993-5:2009 standard. The negative and positive controls were applied. The previously prepared cells were evenly distributed on agar growth medium. Next, the cells were incubated at 37 °C±1 in the presence of air or carbon dioxide. After appropriate preparation, the agar medium with cells was placed on knitted fabric sample. The samples were incubated during 24–72 hours. Further, the cells were subjected to various tests in order to evaluate the cytotoxicity.

2.2.3. Irritation and sensitization

The irritation and sensitization level tests of the fabric were carried out according to PN-EN ISO 10993-10 2015-02 standard. The tests of each one of three fabrics were performed on 3 rabbits, New Zealand breed males weighing between 3.43 and 4.28 kg. The rabbits were kept in standard environment conditions. The skin on rabbits’ backs was depilated mechanically and treated with 70% ethanol. For the next seven consecutive days, the animals were subjected to application of two pieces of fabric (size:2.5 x 2.5 cm) moistured with injected water on both sides on animals’ backs. The samples were secured against removal with compression dressing. As negative control, hygroscopic gauze was used in the same way.

The application site was evaluated after 1, 24, 48, and 72 h after removal of dressings. The evaluation considered severity of erythema and edema according to the ISO scale.

2.2.4. Antibacterial activity tests

Since the silver is added in one of the knitted fabrics, this knitted fabric was also tested in terms of antibacterial activity according to ISO 20743:2013 standard.

2.2.5. Preparation of test samples

In our method, 6 samples of antibacterial fabric were used for the test. As control group, sterile gauze was used, out of which 6 control samples were prepared. Thus prepared samples were placed in separate sterile containers and sterilized with UV light for 30 min.

The preparation of bacterial suspension was carried out according to PN-EN ISO 20743:2013. The following bacterial strains were used:

- *Staphylococcus aureus* ATCC 6538
- *Klebsiella pneumoniae* ATCC 4352
Out of each mentioned strain, a suspension was created of density varying from $1.0 \times 10^5$ to $3.0 \times 10^5$ CFU/ml. The entry with each of mentioned bacterial strains was carried out separately.

### 2.2.6. Implementation and incubation

The prepared test samples were administered with bacterial suspension using 0.2 ml of the suspension per each sample. The suspension was transferred to the sample with a pipette. After administering the bacteria directly to each of 6 control samples, and 6 samples with antibacterial finishing 20 ml of neutralizer was added and then mixed. Six test samples (3 control samples—without antibacterial finishing and 3 test samples—with antibacterial finishing) were planted on TSA growth medium in dilutions varying from $10^0$ to $10^{-4}$ in order to establish the initial amount of bacteria per sample. After the incubation again 20 ml of neutralizer was added to the other six samples and then mixed. Next, the samples were planted on TSA growth medium in dilutions varying from $10^0$ to $10^{-4}$ in order to establish the final amount of bacteria per sample. The calculations were carried out separately for each sample, using the formulas given in PN-EN ISO 201743:2013 and the antibacterial activity of the tested breathable knitted fabric with silver was established based on these calculations.

### 2.2.7. Impregnation

Additional works were carried out to increase the amount of silver in the knitted fabric. Based on the lack of antibacterial activity, the fabric was impregnated during 20 min of antibacterial wash in RUCO-BAC AGP at the temperature $T=40^\circ$C. Next, the knitted fabric was subjected to 10 cycles of washing. The antibacterial properties tests were carried out on *Staphylococcus aureus* ATCC 6538 with the ASTM E2149-13a research method—incubation time 24 h at room temperature.

### 2.2.8. Wicking evaluation

The evaluation of the moisture transport in the double-layer knitted fabrics was assessed by means of the drop test. From a height of 4 cm, 0.1 ml of the colored aqueous test solution was dropped into of the product with a pipette.

### Table 1. Calculation from (g/100 cm²) to (cfu/g)

| Knitted fabric | Surface mass of the fabric (g/m²) | Amount of bacteria per 100 cm² (cfu/100 cm²) | Amount of bacteria per 1 g (cfu/g) |
|---------------|----------------------------------|---------------------------------------------|----------------------------------|
| Liebaert      | 250                              | 90                                          | 36.0                             |
| Penn          | 230                              | 86                                          | 37.4                             |
| Qskin         | 336                              | 147                                         | 35.5                             |

The highest amount of aerobic microorganisms was present in Qskin knitted fabric with silver, while the lowest amount of microorganisms was observed in commercially purchased Penn and Liebaert knitted fabrics.

The microbiological test results were obtained for the surface area of 100 cm². By considering the nature of the product and the acceptance criteria expressed in cfu/g of the product, the following calculations were made. After the recalculation of total amount of aerobic microorganisms from (cfu/100 cm²) to (cfu/g), it can be stated that the microbiological contamination present on the fabrics is within the criteria established by Pharmacopoeia. It can also be stated that the obtained results fulfill the established criteria and so the knitted fabrics are safe for use.
3.2. Cytotoxicity

The tested cytotoxicity was evaluated according to the description stated in the following table (Table 2).

The interpretation of test results shows that only some cells in the sample were disfigured or degenerated, which means that the sample induces low levels of toxicity. The final results confirmed that the tested samples do not have cytotoxic effects on human skin.

3.3. Irritation and sensitization

The tests showed that the evaluated fabrics after 7 days of application do not result in any cutaneous changes. The skin which is in contact with knitted fabrics was white, smooth, and elastic, and was no different from the skin under control samples. National Medicines Institute stated that the fabrics do not evoke irritation. Bearing in mind that rabbit skin is much more sensitive than human skin, the risk of skin irritation after the use of knitted fabrics in humans is considered insignificant.

3.4. Antibacterial activity test

The tests were performed on the fabric impregnated with RUCO-BAC AGP antibacterial product used during 10 min in a wash at a temperature T=40°C. Then, the knitted fabric was subjected to 10 cycles of washing. The results for antibacterial Qskin knitted fabric after 10 min impregnation with a product containing Ag are presented in Tables 3 and 4.

The results showed that the antibacterial activity of the tested sample at the level are A= −1.0 for S. aureus and A= −0.2 for K. pneumonia and the values indicated the lack of bactericidal activity. Therefore, the tests of antibacterial activity of double-layer knitted fabric with silver ions showed that the fabric is not antibacterial.

As a consequence, additional measures were taken aimed at increasing the amount of silver in the knitted fabric. Taking into consideration the lack of bactericidal action, the knitted fabric was impregnated during 20 min in a wash at a temperature T=40°C with the same antibacterial product—RUCO-BAC AGP. Then, the fabric was subjected to 10 cycles of washing.

The antibacterial activity tests showed that—contrary to the 10 min treatment— the knitted fabric gained antibacterial properties after 20 min impregnation with RUCO-BAC AGP.

The antibacterial action of double-layer Qskin fabric after impregnation was maintained and stayed at an even level after 10 washing cycles. The tests showed that the product maintained its antibacterial parameters on Staphylococcus aureus at above 99% (Table 5).

| Grade | Reactivity | Description of reactivity within the tested area |
|-------|------------|-------------------------------------------------|
| 0     | None       | Lack of discernible zone around or below the sample |
| 1     | Low        | Some disfigured or degenerated cells in the sample |
| 2     | Medium     | Zone limited to the area occupied by the sample |
| 3     | Moderate   | Zone exceeding the sample up to 1.0 cm |
| 4     | High       | Zone exceeding the sample for more than 1.0 cm |

### Table 3. Antibacterial activity values for double-layer Qskin weft fabric with silver

| Bacteria | Staphylococcus aureus ATCC 6538 | Klebsiella pneumoniae ATCC 4352 |
|----------|---------------------------------|---------------------------------|
| Inoculum concentration (CFU/ml) | 3.0 x 10⁵                         | 2.7 x 10⁵                         |
| Extreme differences for three control product samples (log) | | |
| 0 h | 24 h | 0 h | 24 h |
| 0.1 | 0.18 | 0.09 | 0.15 |
| F growth value (F=logCₗ₁-logCₗ₀) | 3.06 | 3.42 |
| G growth value (G=logTₗ₁-logTₗ₀) | 4.06 | 3.62 |
| Antibacterial activity value (A=F-G) | −1.0 | −0.2 |

Symbols: Cₗ₀ — Amount of bacteria in control sample after incubation; Cₗ₁ — Amount of bacteria in control sample before incubation, Tₗ₀ — Amount of bacteria in sample of product with antibacterial finishing after incubation, Tₗ₁ — Amount of bacteria in sample of product with antibacterial finishing before incubation.
with the use of special compression garments has a positive effect on patients' quality of life. However, such products must fulfill the normative requirements also in terms of biological parameters. In compression therapy, one of the most important elements is adequately chosen knitted fabric which, owing to its structure, ensures the safety in use and constant compression on the scar.

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3.5. Wicking evaluation

The drop test results are presented in Figure 5, where the flow of drops on the fabric can be observed after water has been instilled on it. The internal layer with drop applied (1) is made of classic yarns with bacteriostatic properties, while the external layer (2) is made of microfiber yarns.

Based on the function with which the double-layer fabric with enhanced biophysical properties should fulfill, the instilled drop should diffuse into the external layer of the fabric where the liquid should immediately spread in the layer. The photos of the double-layer fabric were obtained after approximately 60 s after instillation shows that there were no significant changes in the size of spots on both sides of the fabric. The conducted drop flow tests on double-layer fabric showed that the fabrics made of internal conductive-diffusive layer and made of external microfiber fibers are characterized with the high ability of transporting liquid from the internal layer to the external one.

4. CONCLUSIONS

The conducted tests confirmed that all tested knitted fabrics fulfill the requirements of PN-EN ISO 10993-1 and can be used as a material for making the compression medical devices. The double-layer Qskin knitted fabric with silver ions, owing to its construction, ensures optimal diffusion of moisture to the outside of the product which may positively influence the patients' comfort of use. The impregnation of the mentioned fabric with RUČO-BAC AGP ensures the desired antibacterial properties of the material, which have a positive effect on functional values of the product.

Post-burn scars are one of the most complicated and hard-to-heal wounds. Their rehabilitation with compression therapy with the use of special compression garments has a positive effect on patients' quality of life. However, such products must fulfill the normative requirements also in terms of biological parameters. In compression therapy, one of the most important elements is adequately chosen knitted fabric which, owing to its structure, ensures the safety in use and constant compression on the scar.

Table 5. Antibacterial properties of double-layer fabric

| Test type                  | Staphylococcus aureus reduction (%) |
|----------------------------|------------------------------------|
| After 10 washing cycles    | 99.63                              |
| Control sample             | No bacteria reduction              |

Figure 5. Flow of drops on the Qskin fabric: 1—The internal layer with drop applied made of classic yarns with bacteriostatic properties; 2—The external layer made of microfiber yarns.

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