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ORIGINAL PAPER

Do multi-use cellulosic textiles provide safe protection against the contamination of sterilized items?

B. Rogina-Car · A. Budimir · V. Turcic · D. Katovic

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Abstract The aim of this study was to determine whether multiple use cellulosic medical textiles (cotton blends, Tencel®) could provide protection against contamination after sterilization, regardless of the barrier system of only qualified materials, as per EN 868-2, used in the process. New methods for testing permeability and durability of the microbial barrier cellulosic textiles were developed. The most resistant endospores of two apathogenic bacteria of the Bacillus genus (*Geobacillus stearothermophilus* and *Bacillus atrophaeus*) were used. Testing was conducted after 1, 10, 20, 30 and 50 washing and sterilization cycles under real hospital conditions of the University Hospital Centre Zagreb. The retention period of the microbial barrier of the diagonally packaged packages (one layer; EN ISO 11607-1:2009) after sterilization was tested after the time period of 1, 2 and 3 months of storage under controlled conditions. Bacterial permeability occurred in cellulosic medical textiles when they were contaminated with an extremely high quantity of aerobe bacterial spores. During the testing of microbial barrier durability, the package remained uncontaminated after 1, 2 and 3 months of storage. Medical cellulose textiles used under real hospital conditions functioned properly as a microbial barrier system after 50 cycles of washing and sterilization and 3 months of storage, as the sterilized content was not contaminated at all; they could be used as a microbe barrier system for packing in sterilization, regardless of the fact that they did not meet the standard EN 868-02:2009 Packaging materials for terminally sterilized medical devices. Part 2: sterilization wrap—requirements and test methods or the International standard, for example EN ISO 11607-1:2009 Packaging for terminally sterilized medical devices, part 1: requirements for materials, sterile barrier systems and packaging systems.

Keywords Cellulose · Multiple use medical textiles · Microbial barrier · Sterilization · *Geobacillus stearothermophilus* · *Bacillus atrophaeus*

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Introduction

Cellulosic textiles are, under suitable conditions of moisture and temperature, an excellent basis for the development of bacterial and fungal growths, which enhances spreading pathogens and causes hospital-acquired infections. In such situations, medical staff is in constant contact with the contaminated textiles and microorganisms are transferred from patients to staff and vice versa. Obviously, contaminated medical textiles are a potential source of microbes and contribute to the transfer of hospital pathogens by endogenous and indirect contacts (Borkow and Gabbay 2008; Stephens-Borg 2008; Fijan and Šoštar-Turk 2012).

The problems concerning evaluating the efficiency of the microbial barrier of medical textiles are described extensively in literature, and they mainly relate to surgical gowns and covers. However, apart from their protective and hygienic functions, medical textiles are used for protective packing of medical material in sterilization. Physical properties of textiles are tested for these purposes as well (i.e. raw material content, thickness, air and water steam permeability).

Liquids can contain microorganisms, which penetrate through textiles in the form of aerosols. Standardized methods for determining barrier efficiency of surgical gowns have been offered in literature, but unfortunately these texts have not been accepted universally. Our research is based on adopting the standardized texts, where most methods used concern the penetration of liquids.

The research of textile barrier properties presented here is based on permeability of liquids (i.e. Mason jar test, penetration of liquid under pressure). The tests use water, synthetic and human blood. Some are qualitative and offer only positive or negative results, while others provide quantitative results (Leonas 1998; Rutala and Weber 2001).

Initially, the primary purpose of surgical gowns was to protect the patient form the medical staff. In 1952, these gowns were made from cotton fabrics (muslin), and it was believed they functioned as a barrier in dry conditions; however, this function was lost in wet conditions. The appearance of human immunodeficiency virus raised the awareness of the need to protect medical staff from the patients, adding greater importance to the microbe barrier property. This discovery led to the research and development of better materials for this unique purpose (Belkin 1980). In 1950s, Beck warned the surgeons that the materials used for surgical gowns did not provide an efficient microbe barrier. During this period, and despite clinical research and progress in textile technology, the community specializing in infection control was faced with numerous unanswered questions about the methods of evaluating the efficiency of the "barrier" the materials used (Belkin 2002a, b). Sterile barriers can be defined as materials located between the sterile and contaminated area, aimed at preventing the microorganism penetrating through them. These kinds of barriers include textile materials used in packaging material for sterilization, surgical gowns, covers and masks. The barriers are widely used; however, generally accepted criteria for their efficiency hardly exist at all (Beck and Carlson 1981; Beck 1963).

Belkin gave an overview of the methods for testing the efficiency of microbe barriers, based on the permeability of liquids. Some of the more significant methods will be mentioned here (Belkin 1988, 2002a, b). In 2002, Lankester and associates developed a method for measuring the penetration of bacteria through surgical gowns during surgery (Lankester et al. 2002).

The Mason jar test involves a jar with a cover containing microorganisms in a liquid medium, covered with a textile sample. The jar is turned over onto a sterile Petri dish, remains in this position for 30 min, while the number of the microorganism passing through is determined.

Laufman's "stress" test evaluates the penetration of liquid through a textile material. The sample is secured to provide a concave form for the hammock. The test liquid, containing microorganisms, is poured into the hammock, and then a print is taken underneath the hanging hammock.

The studies also mention a method for testing blood permeability through the surgical gown during surgery. The apparatus has two bubbles, the lower air bubble presses the water bubble which simulates the surgeon's stomach, and it also presses the test sample of the fibre, resulting in a contact with the blood. Permeability is tested with an absorbing paper (ASTM 1997, 1998; Belkin 1994; Granzow et al. 1998).

The packaging protects the sterilized material against contamination after sterilization and provides efficient protection from microorganisms, particles and solutions, at the same time allowing air sterilization medium

permeability, while each sterilized material has its own duration which relates to the time the sterility is maintained. The sterile material, which is not used immediately after the sterilization process, should be stored, and the period of storage depends on the type of package, transport, storage and handling conditions.

There are almost no papers studying the permeability of the microbe barrier of multiple use cellulosic medical textiles used often as a microbial barrier system for packaging in sterilization. The efficiency of the microbial barrier material for packaging is tested in our work presented here, with the main aim being to establish the time period during which the bacteria do not penetrate the barrier.

The microbial barriers are tested for the purpose of determining whether the applied medical textiles (one layer) can provide safe protection against contamination after sterilization, regardless of the fact that they do not meet the standards EN 868-02:2009 and EN ISO 11607-1:2009.

Method

The microbial barrier system should provide protection against the penetration of microorganisms and maintain sterility of the products from the moment they have been used onwards. The criteria for the microbial barrier systems we tested were: to enable sterilization, to provide a barrier for microorganisms and to maintain sterility (Enko 2009; Bojic-Turcic 1994). The purpose of this study was to test the efficiency of microbial barrier systems of certain cellulosic medical textiles.

Field emission scanning electron microscope (FE SEM, Mira II LMU, Tescan, Brno, Czech Republic) was used for sample analysis. The samples were coated with a conductive Ag/Pt layer and scanned under the conditions of high voltage (HV 10.00 kV).

Tested textiles

Two cellulosic medical textiles and three-layer textile laminate PES/PU/PES used for packaging surgical material in sterilization and in the operation theatres were tested. The properties of the textiles used are shown in Table 1, and their surfaces can be seen in Fig. 1.

Due to the possible contamination by cotton dust and due to microbiological cleanness 100 % cotton fabric were not used in the test. Materials should exhibit acceptable levels of cleanliness, particulate matter and listing to be appropriate for the purpose.

Table 1 Properties of the textiles used

	Raw material content	Weave	Surface mass (g/m^2)
Sample I	PES/cotton 50 %/50 %	Linen	178.6
Sample II	Tencel® 100 %	Bluette 2/1	193.7
Sample III	Three-layer textile laminate PES/PU/PES		216.0

Microorganisms used

The most resistant endospores of two apathogenic bacteria of the Bacilllus genus (*Geobacillus stearothermophilus* and *Bacillus atrophaeus*) were used (Fig. 2).

Testing was conducted after 1, 10, 20, 30 and 50 washing and sterilization cycles, under real hospital conditions of the University Hospital Centre Zagreb. The retention period of the microbial barrier of the diagonally packaged packages (one layer; EN ISO 11607-1:2009) after sterilization was tested after the time period of 1, 2 and 3 months of storage under controlled conditions.

The new method for testing microbe barrier permeability of dry medical textiles intended for packaging in sterilization

A new method for testing and evaluating medical textile barriers was developed for the purpose of this investigation. It was supposed to be used in evaluating textiles used as microbial barrier systems for packaging in sterilization. The efficiency of the microbial barriers of medical textiles was investigated, having in mind their consistency in washing, sterilization and storage periods.

The advantage of the method is was possibility to observe the front and the back side simultaneously, without manipulating the test sample.

A device for testing was constructed, consisting of two stainless steel rings, with the diameter of 10 cm (Fig. 3). They were used to reinforce the test sample, so that the bacterial endospores could be applied to the upper side (front), without contaminating the lower side (back).

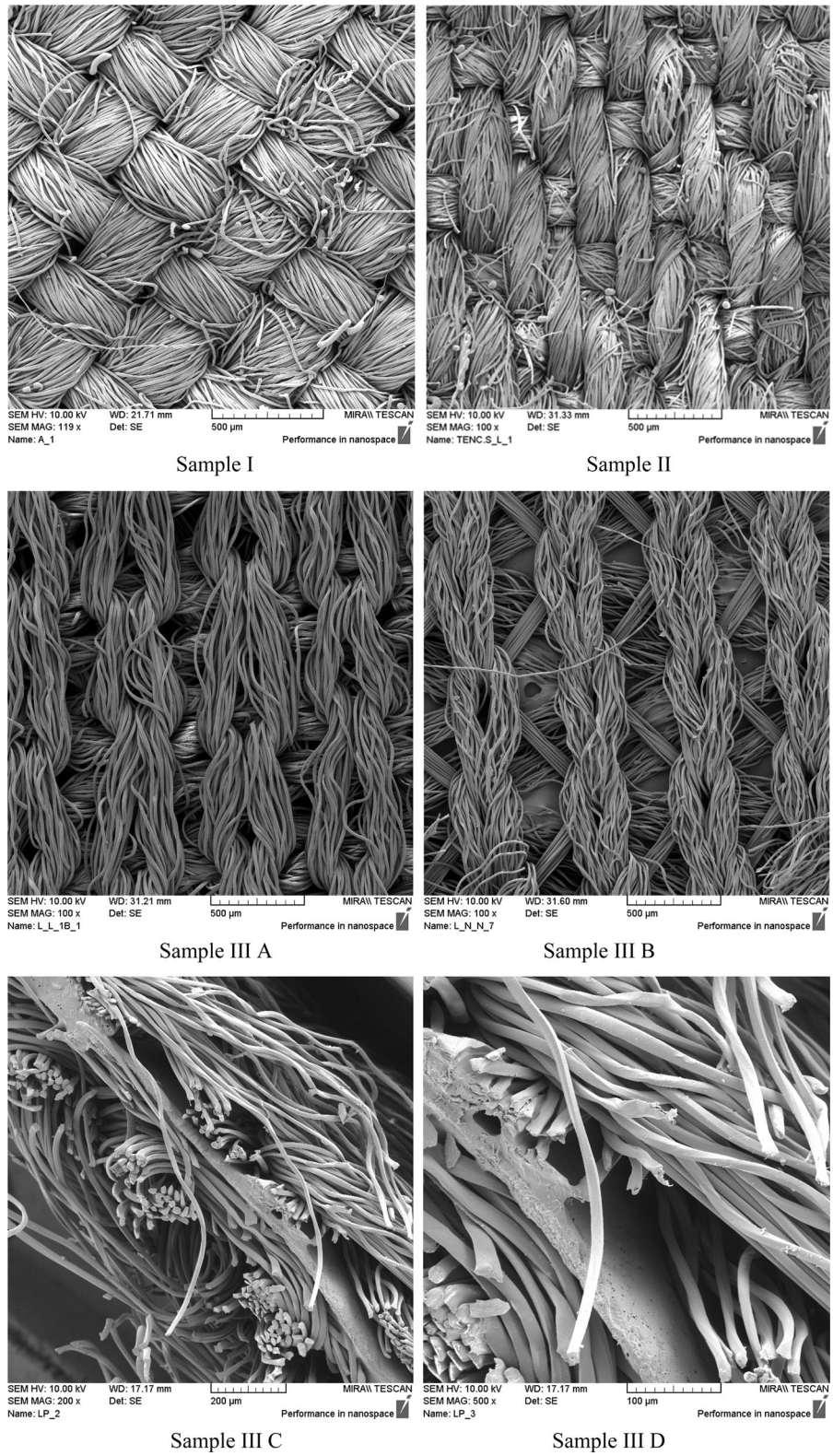

Fig. 1 Overview of the medical textile surface using a scanning electronic microscope (SEM). Sample I 50 % PES/50 % cotton; Sample II 100 % Tencel®; Sample III three-layer textile laminate PES/PU/PES. **a** Upper side (*front*); **b** lower side (*back*); **c**, **d** cross-section of a three-layered textile laminate

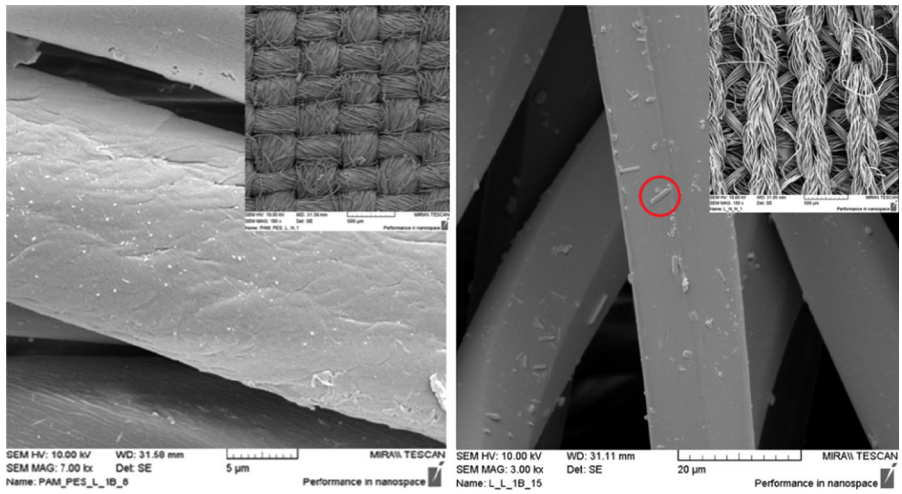

Fig. 2 Bacterial spores and vegetative forms of *G. stearothermophilus* and *Bacillus atrophaeus* recorded on fibre using a SEM, ×7,000 and ×3,000 enhanced

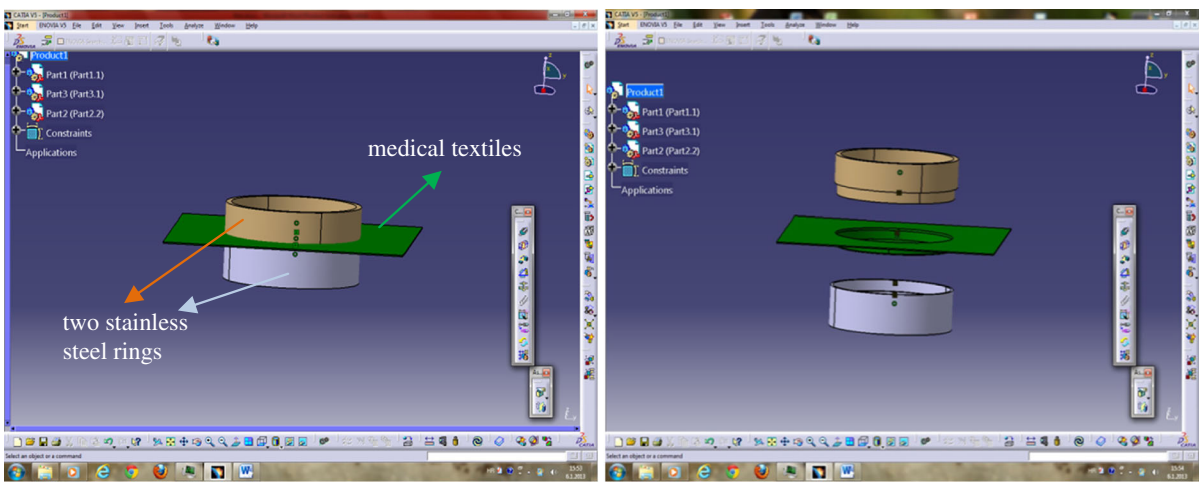

Fig. 3 Constructed and executed device for testing the efficiency of the microbe barrier of medical textiles

The washed sample (22 cm × 22 cm) was fastened through a ring-shaped device for testing the efficiency of the microbial barrier. The construct was put in a see-through package for sterilization and sterilized at 134 °C for 5 min. After the sterilization, the bag with the test sample was opened in a sterile environment.

Using a sterile pincer, the apathogenic bacterial endospores of the Bacillus genus (*G. stearothermophilus* and *B. atrophaeus,* Fig. 4) were placed on the sample test field, following the predetermined order of movements (left-right, up-down and at 45°). After this, the Q-tip of biological indicator was turned, and the procedure repeated by the same order.

24 h incubation followed. A print was taken using a CT3P agar print plate (bioMérieux SA, Marcy I'Etoile, France), first from the back side, and then the front side, with a new plate. After the print had been taken, the print plates were placed in an incubator, at 35 °C for 72 h incubation under atmospheric conditions. The number of bacterial colonies on the front and back side was counted after 72 h.

New method for testing the durability of microbial package barrier after sterilization

The durability of the microbial barrier after sterilization was tested according to the following procedure: the tested samples (22 cm × 22 cm), after certain number of washing cycles, were packed diagonally with gauze, with an absorbing paper placed underneath (Whatman

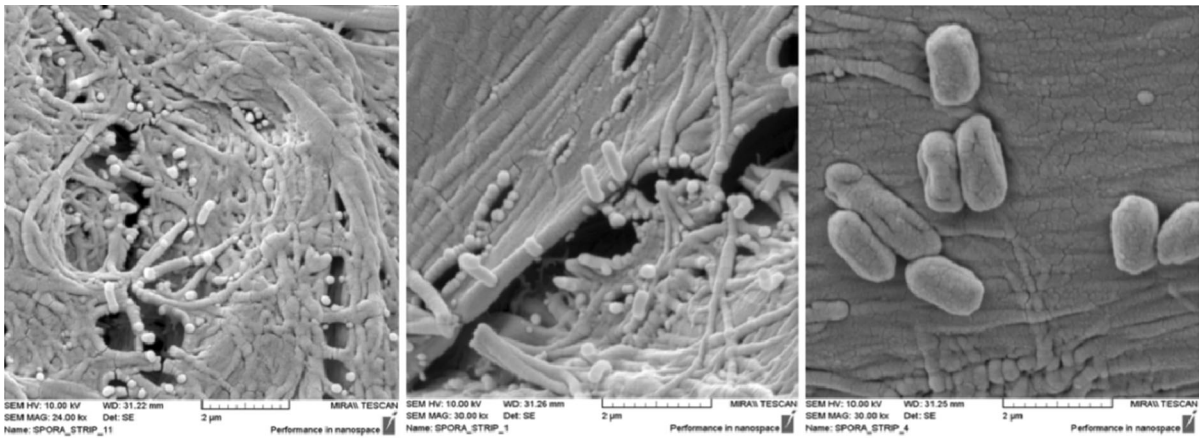

Fig. 4 Bacterial spores *Geobacillus stearothermophilus* and *Bacillus atrophaeus* recorded with SEM, ×24,000 and ×30,000 enhanced

no. 1, 1 cm^2). The packages were sterilized, and then placed in a protected storage under the following microclimate conditions: temperature 15–30 °C, relative humidity 30–60 %. The tested materials were stored on the shelves according to the rule: 25 cm distance from the floor, 45 cm distance from the ceiling and 5 cm distance from the walls. After the period of storage, the packages were taken out from the storage; unwrapped in a sterile environment, and then, using a sterile pincer, an absorbing paper "Whatman no. 1" (1 cm^2) was placed into a test tube with a Brain—Hearth broth. After 48 h of incubation at 35 °C, the changes in the clarity and possible blurriness of the broth were observed. The sterility was additionally checked by fitting on a solid nutrient basis (blood agar). Using a pipette 0.5 ml of Brain—Hearth broth was inoculated on the agar containing 5 % of sheep blood. After 48 h incubation at 35 °C, the number of the bacterial colonies was recorded.

Results

The change in thickness, air permeability during washing and sterilization of the medical textiles, as well as the number of bacterial colonies, were measured in the course of our work. The results were analyzed by using descriptive statistical analysis and are presented in Tables 2, 3 and 4.

The packaging materials used should be suited to and manufactured for the intended packaging and sterilization processes. Suitability is to be determined on the basis of the information provided by the manufacturer. This includes confirmation of conformity with the ISO 11607-1 standard and pertinent sections of the EN 868, parts 2–10 standard series, in respect of microbial impermeability and compatibility with the sterilization process.

Cellulosic textiles used in this study did not meet the above standards. For this reason, the study on the new methods of comparison with cellulose textiles took a three-layer textile laminate that met all the standards.

We performed a regression and correlation analysis to determine the statistical dependence and intensity of such an addiction. There was a negative correlation, meaning that an increase in one variable accompanied the fall of the other and vice versa. Increasing number of washing and sterilization cycles reduced permeability of the microbial barrier and its air permeability (Figs. 5, 6).

Test results for durability, that is, the efficiency of the microbe barrier under controlled conditions (microclimate conditions: temperature 15–30 °C, relative humidity 30–60 %) during the period of 1, 2 and 3 months, after 10, 20, 30 and 50 washing and sterilization cycles, showed that the microorganisms did not grow inside the sterilized packages (N = 360).

Discussion

The packaging protects sterilized material against contamination after sterilization and provides efficient protection from microorganisms, particles and solutions, at the same time allowing air and sterilization

Table 2 Tested sample thickness in mm, according to the ISO 5084:1996 standard

	Sample thickness (mm)					
Samples	0 W+S	1 W+S	10 W+S	20 W+S	30 W+S	50 W+S
Samples I 50 % PES/50 % cotton	0.28	0.32	0.34	0.34	0.33	0.34
Samples II 100 % Tencel®	0.32	0.34	0.36	0.35	0.35	0.34
Samples III Laminate	0.62	0.60	0.71	0.75	0.72	0.78

W+S washing and sterilization

Table 3 Air permeability of the tested medical textiles R (mm/s) according to the EN ISO 9237:2003 standard

Samples	Number of washing and sterilization cycles	R (mm/s)	SD (%)	CV (%)
Samples I	0 W+S	233.9	7.3	6.3
50 % PES/50 % cotton	1 W+S	194.6	5.2	5.3
	10 W+S	184.0	15.1	16.4
	20 W+S	170.3	4.7	5.5
	30 W+S	135.3	3.5	5.2
	50 W+S	131.3	7.2	11.1
Samples II	0 W+S	194.8	4.4	4.5
100 % Tencel®	1 W+S	216.2	6.6	6.1
	10 W+S	221.0	4.7	4.3
	20 W+S	202.4	5.2	5.1
	30 W+S	178.4	3.9	4.4
	50 W+S	168.3	4.6	5.5
Samples III	0 W+S	0	–	–
Laminate	1 W+S	0	–	–
	10 W+S	0	–	–
	20 W+S	0	–	–
	30 W+S	0	–	–
	50 W+S	0	–	–

W+S washing and sterilization, *R (mm/s)* average air permeability (n = 10), *SD* standard deviation, *CV* coefficient variation (%)

Table 4 Test results of microbial barrier permeability for the tested medical textiles after extreme contamination with bacterial spores *Geobacillus stearothermophilus* and *Bacillus atrophaeus*

Samples	The number of washing and sterilization cycles	The average number of bacterial colonies on the front side (CFU)	The average number of bacterial colonies on the back side (CFU)	Ratio (CFU)
Samples I	1 W+S	356	11	32:1
50 % PES/ 50 % cotton	10 W+S	275	14	20:1
	20 W+S	318	9	35:1
	30 W+S	286	7	41:1
	50 W+S	396	2	198:1
Samples II	1 W+S	419	7	60:1
100 % Tencel®	10 W+S	359	8	45:1
	20 W+S	294	2	147:1
	30 W+S	182	3	60:1
	50 W+S	341	2	170:1
Samples III	1 W+S	155	0	–
Laminate	10 W+S	167	0	–
	20 W+S	175	0	–
	30 W+S	132	0	–
	50 W+S	464	0	–

CFU colony forming unit

medium permeability. Each sterilized material has its own life-cycle, corresponding to the time sterility is maintained. The sterile material, which is not used immediately after the sterilization process, should be stored, and the period of storage depends on the type of the package, transport, storage and handling conditions. We have found no method of testing the microbial barrier of multiple use cellulose medical textiles for packaging in sterilization in the available literature. Microbial barrier we used was tested with the aim of determining whether the applied medical textiles (one layer) could provide safe protection against contamination after sterilization, regardless of the fact that the textiles did not meet the standards EN 868-02:2009 packaging materials for terminally sterilized medical devices. Part 2: sterilization wrap—

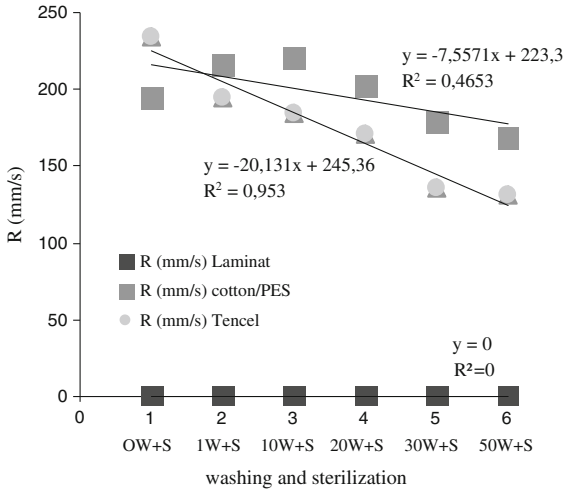

Fig. 5 Regression and correlation analysis of the results for air permeability of the tested medical textiles R (mm/s) after washing and sterilization

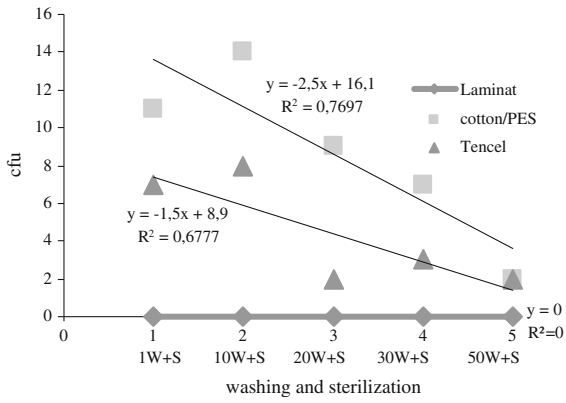

Fig. 6 Regression and correlation analysis of the results for microbial barrier permeability of the tested medical textiles (CFU) after washing and sterilization

requirements and test methods or the International standard for example, EN ISO 11607-1:2009 packaging for terminally sterilized medical devices, part 1: requirements for materials, sterile barrier systems and packaging systems (EN 868-02:2009; EN ISO 11607-1:2009).

Textile materials shrink in washing and sterilization processes as well, resulting in thicker materials (Table 2), that is, the penetration of the bacterial colonies decreases. Sample II exhibited lower air permeability in unwashed state, and it could be assumed that finishing dissolved during washing, allowing greater air permeability after the first washing cycle. In the case of Samples I and II, it was clear that air permeability continually decreased during washing and sterilization procedures (Table 3).

Due to the polyurethane membrane, Sample III was completely impermeable to air penetration. It should be noted that it was, in fact, permeable to the sterilization medium, which provided basic conditions for packaging in sterilization.

The results of testing microbial barrier permeability of medical textiles (Table 4) after extreme contamination with bacterial spores *G. stearothermophilus* and *B. atrophaeus*, showed that Tencel provided better microbe barrier than PES/cotton. The three-layered textile laminate provided full protection against the penetration of microorganisms, that is, an efficient microbial barrier, since not a single bacterial colony was able to penetrate, which was to be expected, as it met all the standards mentioned above.

The results of testing microbial barriers showed that not a single medical textile was contaminated, that is, the microorganisms did not grow inside the sterilized packages after storage period of 1, 2 and 3 months under real controlled conditions.

It was also proved that multiple use cellulose medical textiles, tested in this study, could provide safe protection against contamination after sterilization. With regard to all physical–mechanical influences that the tested samples were subject to during 50 washing cycles and 50 sterilization procedures, all the tested samples exhibited excellent properties of a microbial barrier, and no contamination of the sterilized content inside the package occurred after the storage period of 1, 2, 3 months.

The conclusion was that the tested cellulose textiles and the three-layered textile laminate (one layer/single layer) could be used as a microbial barrier system after sterilization. They provided a safe microbial barrier against contamination for packaging in sterilization, with the term of validity of minimum 3 months and 50 washing cycles and 50 sterilization procedures.

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