In vitro evaluation of the bio-activity of different fabrics for underwear against *Lactobacillus acidophilus, Staphylococcus epidermidis, Staphylococcus aureus* and *Candida albicans*

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Background: fabrics could exert antimicrobial activity against different micro organisms, depending on the addition of antimicrobial molecules to the textile. Silver ions, Triclosan Sanitized T99-19 are used. Recently the AEGIS AEM5772/5 has been linked to a silk fabric (Dermasilk), showing antimicrobial activity. The aim of this study was the, in vitro, comparative evaluation of the microbe-cidal capacity of 10 different fabrics (used in contact with the skin) against 4 different microbes that can be saprophytes or pathogens in the human epidermis.
Methods: the following micro organisms have been used: *Lactobacillus acidophilus* (ATCC11975), *Staphylococcus epidermidis* (ATCC 1228), *Staphylococcus aureus* (ATCC 700698 – methycillin resistant) and *Candida albicans* (ATCC 10261). All the isolates were grown in TSB medium and a final dilution of 1,0 x 108/ml was made in 0.2 M PBS. The antimicrobial activity was evaluated by using a modification of the "Dynamic shake flask test" methods as reported by the standard ASTM E2149-01 and expressed as percent reduction of the initial microbial load.
Results: the quantitative evaluation of the antimicrobial activity showed that all the fabrics studied exerted an anti microbe activity ranging from 18% to 100% over 24 hours of incubation. Most of the textiles showed a marked increase of the antimicrobial activity that correlated with the incubation time, reaching the highest reduction rate after a time variable between 6 and 22 hours of incubation. The only material that showed to exert its maximum antimicrobial activity in a short time (within the first 60 minutes) was Dermasilk. The evaluation of the antimicrobial activity released by fabrics after 24 hours of incubation in PBS, demonstrated that all the textiles released variable levels of antimicrobial molecules in the incubation medium, with the only exception of the pure cotton and Dermasilk (Pure silk,100% fibroin, lacking sericin, treated with AEGIS AEM5772/5).
Conclusion: all the fabrics evaluated had a microbe killing capacity when in strict contact with the micro organisms in a warm and humid environment. *This killing activity was released in various degree in the incubation medium by all the fabrics but Dermasilk, that showed no release in the environment. This fact could raise concern about the insurgence of cutaneous allergy or damage*. In addition Dermasilk's antimicrobial activity was very fast, being the highest level reached within 1 hour of incubation.

Aim of the study

The interaction between textile with antimicrobial properties and the microbes located onto the surface of skin and mucosa may results in a persistent modification of the normal microbial ecology of the body surfaces. This may be caused in particular by fabrics that can release antimicrobial molecules, giving raise to an accumulation of these antimicrobial substances. The aim of the present study was to evaluate and compare, in vitro, the microbe-cidal effect of 10 different fabrics against four different micro organisms: *Lactobacillus acidophilus, Staphylococcus epidermidis, Staphylococcus aureus* and *Candida albicans.*

Conclusions and Comments

The results of this study demonstrated that different textile fabric could have a large variety of antimicrobial activity against germs that are commonly identified as saprophytic or pathogenic micro organisms onto skin and genital mucosa in both male and female, when evaluated following the ASTM-E2149-01 test (after 24 hours of incubation).

Quantitative evaluation of the antimicrobial activity of textiles. All the textile showed a medium high level of antimicrobial activity against *L. acidophilus, S.epidermidis, S.aureus* and *C. albicans* with the only exception of that identified with n. 9. This last textile fabric showed only a very low (3,38%) inhibitory effect against *L. acidophilus*.

Quantitative evaluation of the antimicrobial activity over time.
The antimicrobial activity of fabric textile is dependent upon time with some difference between the different material evaluated. In particular, most of the textiles showed a marked increase of the antimicrobial activity that correlated with the incubation time, reaching the highest reduction rate after a time variable between 6 and 22 hours of incubation.

The only material that showed to exert its maximum antimicrobial activity in a short time (i.e. within the first 60 minutes of incubation) was Dermasilk.

These differences in the antimicrobial activities over the time are likely due to a different structure of the materials evaluated. It is possible to speculate that these differences are due to a different mechanism of action: those materials that required a longer period of incubation are likely to release the antimicrobial in the medium. On the other hand, Dermasilk is likely to exert its activity only by getting in strict contact with the micro organisms, since the activity its self reached the highest level within 60 minutes of incubation, clearly suggesting that the this material has a minimal release of antimicrobial substance in the environment, under the experimental condition used.

Moreover a general consideration based on these findings is that the vast majority of the so called antimicrobial textiles, with the only exception of Dermasilk, exert their functions by releasing the active molecules in the environment where these substance can accumulate. This fact is likely to provoke a marked modification in the microbial ecology of the body district (e.g. the genital area). The consequent variation in the resident microbial flora may be the cause of additional infections, allergies and tissues damage.

Table 1: Tested Bio-functional underwear available in the market

1. DERMASMART: polyester with Silver ions
2. PLATATEX: 50% cotton, 42% polyamide, 8% silver
3. PADYCARE: 82% polyamide, 18% lycra with, 20% Silver filaments
4. ENVICON: cotton with Silver filaments
5. SANITIZED T99-19: polyamide with T 99-19
6. CRABYON: chitosan and viscose
7. TRICLOSAN/SANITIZED: polyamide with TRICLOSAN (SANITIZED)
8. DERMASILK: pure silk (100% fibroine, without sericine) with AEM 5772/5
9. COTTON: 100% without antimicrobial treatment
10. ECZEMACLOTHING: 100% cotton with Silver ions

Materials and methods

Strains used and growing conditions. In these experiments the following micro organisms have been used: *Lactobacillus acidophilus* (ATCC11975), *Staphylococcus epidermidis* (ATCC 1228), *Staphylococcus aureus* (ATCC 700698 – methycillin resistant) and *Candida albicans* (ATCC 10261). All the strains were thawed from a frozen stock (stored at -80°C in individual appropriate storage vials) and streaked onto 5% horse blood agar plates in order to ensure colonies isolation. After 48 hours of incubation under aerobic atmosphere at 37°C one individual colony was picked up with a sterile plastic loop and transferred to a tube containing 5 ml of Trypticase soy broth medium. Individual tubes were further incubated for additional 24 hours and the concentration of the micro organisms in each tube was checked by a standard McFarland density evaluation. A final dilution of each organism to 1,0 x 10^8/ml was made in 0.2 M phosphate buffered saline (PBS) (pH 7.2). This suspension was immediately used for the experiments described in this report. The tested fabric textiles used in this study are reported in Table 1 in the previous page .

Anti micro organism activity of fabric textile – Dynamic shake flask test (ASTM E2149-01). This test was performed in two different ways, as follows.

Quantitative evaluation of the antimicrobial activity of textiles.

The antimicrobial activity of each individual textile was evaluated as follows: 2 grams of each textile were cut in small pieces and the whole amount was inserted in sterile flasks containing 50 ml of PBS. Each flask was inoculated with a final concentration of 1-6 x 10^5 CFU/ml of each micro organism and 10 μliters of suspension was taken and used (see below for the counting procedure) to assess the initial load of micro organism (defined as T0 load and expressed as CFU/ml). Flasks were then incubated under gentle shaking aerobic conditions for 24 hours at 37°C. At the end of this incubation, 10 μliter of each individual micro organism suspension (defined as T24 load and expressed as CFU/ml) was plated onto agar plates as described above at point 1.1. After 48 hours of incubation the number of colonies present onto each individual plate was counted and annotated in order to asses the load of viable micro organisms in each suspension. As a control, for each individual micro organism evaluated, an identically prepared suspension lacking any textile fabric inside was incubated and counted as above reported. Each experiment was made in duplicate and the results are the mean of each individual evaluation.

Quantitative evaluation of the antimicrobial activity over time.

In order to evaluate the antimicrobial activity of selected textiles over the time the following experiments have been performed. The above reported dynamic shake flask test has been performed with the slight modification. Following the inoculum of the micro organism in each individual flask, a 10 microliter samples was immediately taken (T0).Subsequently identical additional samples were taken after 30, 60, 120 and then every 240 minutes up to 26 hours.

Individual samples were plates onto appropriate agar plates in order to asses the number of viable organism, as reported above. Results are showed in the graphs in the next page.

Antimicrobial activity. This parameter was calculated as follow for each individual textiles/micro organism:

(T0-T24)/T0 x100 = % reduction of viable micro organism load

Quantitative evaluation of the antimicrobial activity released by textiles after 24 hours of incubation in PBS.

In order to asses the release of antimicrobial molecule from textiles incubated in the presence of a “water-saline” physiological environment, 3 grams of each textile were cut and incubated in the presence of 75 ml of PBS under aerobic shaking conditions up to 26 hours at 37°C. At the end of the incubation time, 50 ml of each solution obtained from individual textile were removed and inoculated with 1 x 10^5/ml of each micro organism. Incubation and counting was performed as above reported.

Results are shown in the table showed below.

Antimicrobial activity released by textiles

Quantitive evaluation after 24 hours of incubation in PBS

Textiles	Antimicrobial agent	% reduction			
		Lactobacillus acidophilus (ATCC 11975)	Staphylococcus epidermidis (ATCC 1228)	Staphylococcus aureus (ATCC 700698)	Candida albicans (ATCC 10261)
DERMASMART	Ag^+ ions	100,0	98,11	100,0	100,0
ECZEMACLOTHING	Ag^+ ions	100,0	96,07	98,38	86,84
PLATATEX	Pure Ag	94,82	100,0	100,0	100,0
PADYCARE	Ag filaments	96,66	100,0	95,00	100,0
ENVICON	Ag fibers	100,0	100,0	100,0	100,0
SANITIZED T 99-19	T 99-19	96,72	98,0	96,36	56,09
TRICLOSAN/SANITIZED	Triclosan	96,36	98,07	100,0	17,64
CRABYON	Chitosan	98,33	100,0	100,0	34,37
DERMASILK	AEM 5772/5	1,69	0	0	0
COTTON	-	0	0	0	6,25
Negative control (no textile)	-	0	0	0	0

Results. The results of the study are reported in the graphs below.

Quantitative evaluation of the antimicrobial activity over time