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Development of Coating for Incorporation of Beneficial Spores on Hospital Textiles

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Abstract
Hospital-acquired infections are a growing problem in hospitalized patient safety. and hospital textiles are reported as a significant source of these nosocomial pathogen. Contact with these textiles leads most often to the contamination and cross-contamination of the hospitalized patient and hospital staff. However, the common antimicrobial agents used in the production of antimicrobial textiles are proving to be a concern in terms of toxicity and antimicrobial resistance. Bacillus spores can be a good solution to combat pathogenic bacteria. In the present study, Bacillus spores were used in the coating of polyester fabrics. Afterwards the growth and viability of the beneficial bacteria applied on fabrics were monitored using the agar plate method. Besides this, the applicability to the fabric was evaluated on the basis of contact angle measurements, abrasion resistance and tensile tests. Major findings included that it is possible to incorporate Bacillus spores in coated fabrics and these can behave like q reservoir for beneficial bacteria.

Key words: *hospital-acquired infections, Bacillus spores, coated fabrics, hospital textiles, beneficial bacteria.*

Introduction

Hospital-acquired infections (HAI) are a growing world-wide problem which causes a high incidence of morbidity and mortality among hospitalised patients [1]. According to the reports of Centers for Disease Control and Prevention (CDC), approximately 722000 HAI cases arise annually, with nearly ten percentage of these resulting in death in the USA [2, 3]. In Europe, the incidence rate of HAI has been estimated as double that, causing 148000 deaths per year [4]. With increasing HAI caused by antibiotic resistant pathogens, the number of patients suffering from morbidity and mortality is sharply increasing [5]. Antibiotic resistance results from the interaction of the pathogens of patients with the hospital environment. Textile materials are an important part of hospital environments as they are common in different healthcare facilities and find application, amongst others, in uniforms, privacy curtains, bed linens and patient apparels. Contaminated textiles are excellent substrates for bacterial growth under appropriate conditions [6-8], and they may contain 10^6-10^8 Colony Forming Unit (CFU) pathogens per m^2. These pathogens can survive on contaminated textiles for up to 3 months. Patients and hospital staff are at high risk of getting infected by contacting these textiles [9-11]. In order to prevent the growth of pathogens on textiles, different antimicrobial agents like triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether), quaternary ammonium compounds (QACs), polyhexamethylene biguanide (PHMB), Polyhexamethylene Guanidine Sulphanilate (PHMG), and metallic salts are used during textile finishing processes. However, there are certain concerns regarding the toxicity and resistance of common antimicrobial agents, most of which work according to the leaching mechanism [12-15]. This leaching, related to the amount, causes health and environmental problems. It is notable that large proportions of the biocides added are washed out of the textile, and hence enter the environment. The most frequently used biocides do not degrade at all or do so slowly in the environment [16, 17]. Besides that, most hospital pathogens tend to develop resistance against traditional antimicrobial agents such as triclosan, QACs and silver [18-20]. Recent developments in biotechnology based on biological substrates such as enzymes and microorganisms have received attention and become a solution for antimicrobial resistance. There is a large range of microorganisms with probiotic properties, but the most common belong to lactic acid or Bacillus bacteria. Most of them have antimicrobial activity or antagonistic ability to inhibit pathogenic microorganisms. Although their efficacy mechanism cannot be totally understood yet, it is supposed to be related to the combination of their antagonistic capacity and antimicrobial ability. The antagonistic capability of Bacillus spores against other bacteria may be due to the competitive exclusion and production of organic acids [21, 22]. Besides that, they exhibit antimicrobial activity by producing antimicrobial substances like enzymes and peptides [23, 24]. Fabrics incorporated with beneficial spores can be good solutions, working as a reservoir for beneficial spores. When the fabric has enough humidity and temperature, in other words, when it meets body heat (when the incubation temperature is 31-32 °C for 24-48 h) and human liquids such as sweat, blood etc, a certain number of beneficial spores turn into beneficial bacteria on the textile surface to protect it from the contamination of pathogens with antagonistic/antimicrobial activity. Vandini and colleagues (2014) revealed that microbial cleaning with Bacillus spores, as part of cleaning products, reduces the number of infection related pathogens in the hospitals [25]. In another study done by Caselli et al. in 2016, the impact of microbial-based cleaning products containing Bacillus spores on the reduction of antibiotic resistant pathogens was investigated. According to their results, the cleaners were not only effective in counteracting the growth of several pathogens, they also did not cause any drug-resistant pathogen population, but rather lowered the already existing resistances [26]. The research herein aimed to develop a proper strategy to attach Bacillus spores to hospital textiles to prevent the growth of nosocomial bacteria in the hospital environment.

Experimental

Materials

PET fabric, provided by FOV Fabrics AB, was used in the experiments. The fabric consisted of polyester microfibres which were reinforced with carbon fibres in both the warp and weft directions. This type of fabric was designed for hospital textiles to obtain more durability. Carbon fibres were placed every 25 picks in the warp and every 23 picks in the weft direction. The density of the fabric was

Table 1. Coating paste composition in weight, %.

Samples	Composition in wt., %						
	Samples without cross linker				Samples with cross linker		
	P0	C0	C1	C2	C3	C4	C5
Permax 232 (Binder)	–	50	50	50	50	50	50
Tubivis DL (Thickener)	–	0.5	0.5	0.5	0.5	0.5	0.5
Distilled water	–	49.5	47	24.5	48.5	46	23.5
Tubassist Fix 157 W (Cross linker)	–	–	–	–	1	1	1
Spore Suspension	–	–	2.5	25	–	2.5	25

Table 2. Classification of bacterial growth on surfaces.

Classification	Bacteria count (CFU/surface)
Heavy growth	>Log 3
Weak growth	Log 1-2
Very weak growth	Log 1
No growth	<Log 1

55 ends/cm in the warp direction and 40 picks/cm in the weft direction, and the weight of the fabric was 145g/m^2. A Binder Permax 232^{TM} (from Lubrizol Advanced Materials', USA), Cross linker Tubasist Fix 157W (from CHT Bezema, Switzerland) and thickener – Tubivis DL 600 (from CHT Bezema, Switzerland) were used for preparing a proper coating to entrap *Bacillus* spores and attach them to the fabrics. The suspension of *Bacillus* spores was provided by Innu Science.

Preparation of coating pastes

As shown in ***Table 1***, different paste formulations, with and without a cross linker, were used in producing coated fabrics. In order to obtain a homogeneous paste, a thickening agent was primarily dissolved in distilled water and then a binder was added, which was then stirred for 30 min at room temperature. Afterwards a spore suspension was added at different percentages (2.5 and 25%) and stirred for 5 min more. A cross linker was added, and the mixture was stirred for 5 min just before pad-batch coating for samples with the cross linker. PET fabrics were padded with coating paste on a laboratory-padding mangle (Ernst Benz & Co), squeezed under cylinders with a load of 14 kg/cm, and run at a speed of 2.0 m/min to obtain a 70-80% liquid pick up. The coated samples were dried for 15 min at 80 °C and then cured for 5 min at 150 °C in a laboratory dryer/oven. Afterwards all cured fabrics were washed in warm water (37-38 °C) for 5 min and rinsed twice in cold water. They were hung on a rack and left to dry overnight. The dried samples were weighted and compared to the weight of untreated fabrics.

Characterization

Viability tests were performed according to the agar plate method in order to evaluate the microbial growth of the probiotics incorporated on the coated fabrics. Prior to the viability tests, all specimens of a size of 5 x 5 cm were heated for 1 h at 75 °C to sterilise/clean them from any other bacterial contamination. Then the samples were put on a tryptic soy agar (TSA) plate in 4.5 ml of 1% triphenyl tetrazolium chloride (TTC) solution to fully cover the samples. After incubation of the petri plates for 48 h at 30 °C, the growth of bacteria on the samples was visually evaluated and classified according to ***Table 2***.

An *Attension Theta* (Biolin Scientific AB, Sweden) optical tensiometer equipped with a high-speed camera was used to measure the contact angle of water according to the ASTM D7334 – 08 standard in order to determine the wettability of the coated samples. The mean value of the static contact angle for the duration of the standing water on the sample surfaces was measured with a sessile drop of approximately 3.0 µl volume. Tensile properties of the coated fabrics were measured using Tensolab (Mesdan S.p.A, Italy) according to the ISO 13934-1:2013 standard in order to determine their applicability. Five dry specimens in both the warp and weft directions were prepared and placed between clips at a 100 mm gauge length. The samples were drawn at a rate of 10 mm/min until they broke. A Martindale abrader (SDL Atlas, USA) was used to determine the abrasion resistance of the samples against a wool abrader fabric in accordance with the ISO 5470-2 standard. A force of 9 kN was applied for each sample type for a set of five test specimens. Visual evaluation of the damage and breakage of fibres and searching for pilling were made at 1000, 2000, 5000, 10000, and 15000 rubs under bright lighting conditions.

Results and discussion

Viability of the beneficial bacteria on PET fabrics

Pad coating was used as an application method to entrap beneficial spores in the coating paste and incorporate them in polyester fabrics. The agar plate method was undertaken to determine the viability of the beneficial bacteria after the coating process. Viability test results of the C0 fabrics (with and without a cross linker) were as before the samples were tested. The results of the samples are summarised in ***Table 3***.

According to these results, all samples exhibited a certain amount of growth, more than 1000 CFU/surface. Recent studies have mainly focused on the encapsulation of beneficial bacteria/spores into textile fibres using electro-spinning or melt-spinning, achieving high viability results [27-29]. Therefore the coating process is a promising approach to incorporate beneficial spores in fabrics as the process conditions (temperature and pressure) can be modified easily and the bacteria/spores can be easily embedded in viscous paste and attached to fabrics. Two different paste contents were tried in this study to determine the effect of the content on the viability of the beneficial bacteria applied. The results showed that the addition of the cross linkers did not cause any changes in the viability. However, the cross linker slightly increased the washing durability of the samples. Despite the cross linkers, the washing durability of the spores was not so high. After 10 washes, the coatings of all samples were already gone

Wettability results of the fabrics

The water contact angle of the samples as a measure of their wettability were determined. The contact angle results of untreated polyester fabric exhibited a high wettability as it is produced with microfibres. Regarding the contact angle results of the coated fabrics, they revealed high angles indicating a low wettability (***Figure 1***). Thus it can be concluded in general that coating process increases the hydrophobic property of the coated fabric surfaces. The samples with cross linker exhibited higher water contact angles than the others. Also the *Bacillus* spores incorporated in the fabric can growth on

these hydrophobic surfaces. This is important because previous studies showed that there is a significant relationship between wettability and bacterial adhesion and differs according to bacteria types. Some bacteria such as *S. aureus* and *E. Coli* have affinity towards hydrophilic surfaces; some others such as *S. epidermidis* have affinity to hydrophobic surfaces [30].

Abrasion resistance of the fabrics

Abrasion resistant of the samples was tested to determine their durability of the coating. The obtained results showed that the reference fabrics gave the worst results and specimen breakdown occurred after 15000 rubs with broken fibres in warp and weft direction. The coated fabrics didn't reveal broken fibres in both directions after 15000 rubs but the coating intensity decreased. The abrasion resistance of the woven fabrics can be improved significantly by coating process by filling the gaps between yarns and covering the surface of the yarns [31].

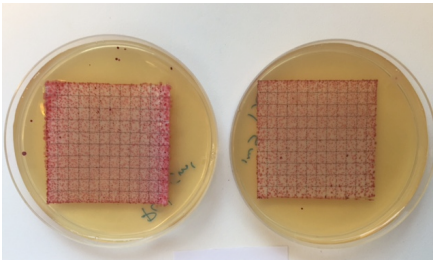
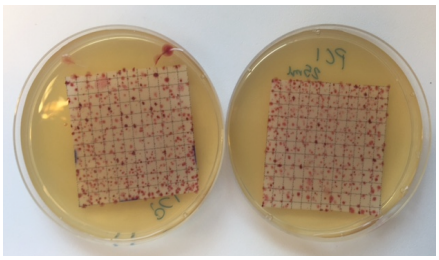
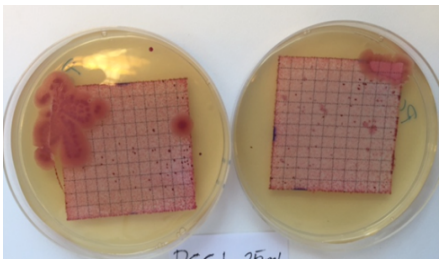
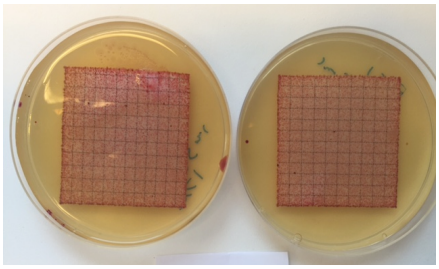
Mechanical properties of the fabrics

The mechanical properties of the coated and uncoated fabrics were evaluated in both weft and warp directions. Coated samples exhibits different tensile behaviors than uncoated fabrics and normally coated process leads increase in modulus by filling up the spaces between threads with coating pastes [32]. However, depending on the nature of fabric and stability to different process conditions, it may alter as well. As summarized in ***Table 4***, coated samples require insignificantly ($p > 0.05$) lower force at breakage and significantly higher elongation in warp direction when compared to uncoated reference sample whereas, coated samples require significantly ($p < 0.05$) higher force at break and exhibit correspondingly lower elongation in weft direction. These results are highly related to the structure of the fabrics as it has higher density in warp direction and coating paste cannot find enough space to go through.

Conclusions

Usage of beneficial spore coated fabrics in the hospital textiles may become an efficient tool for preventing HAIs which is strictly related to spreading of nosocomial pathogens. In the present study, the aim was to develop a strategy to attach beneficial spores on the fabrics to use them in the hospital environment for combating pathogens and the significant-

Table 3. *Viability of beneficial bacteria on the coated fabrics.*

Samples		Agar plates	Bacteria amount (CFU)	Bacterial growth
Without Cross Linker	C1		>10000	Heavy growth
	C2		> 1000	Growth
With Cross Linker	C4		>10000	Heavy growth
	C5		> 10000	Heavy growth

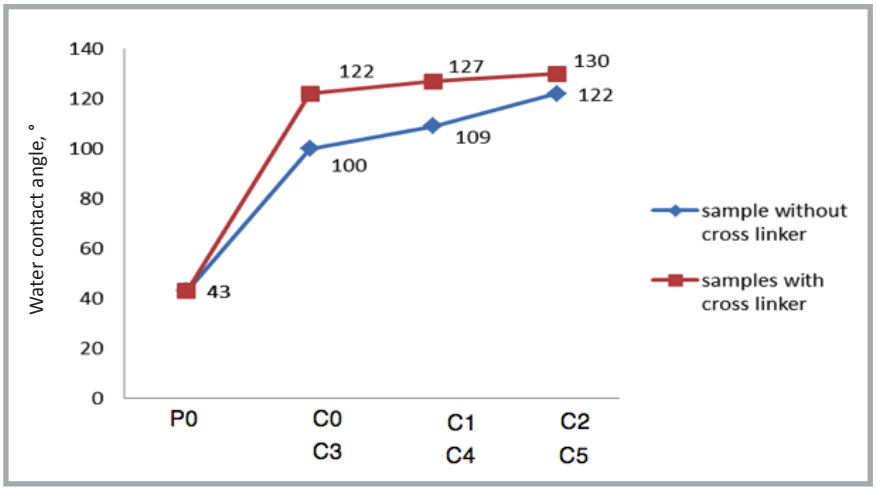


Figure 1. *Water contact angles of the samples.*

Table 4. *Mechanical properties of the samples.*

Samples		Weft		Warp	
		Strength, N	Elongation, %	Strength, N	Elongation, %
Reference	P0	1010	38	1450	36
Without cross linker	C0	1200	38	1401	44
	C1	1290	35	1393	42
	C2	1174	36	1395	38
With cross linker	C3	1200	36	1392	38
	C4	1215	35	1400	39
	C5	1185	36	1400	38

ly high viability of beneficial bacteria on the textiles was achieved by coating them with beneficial spores. Based on data obtained from this study, a further study is underway which will focus on competition tests to determine the inhibition mechanism of coated fabrics with beneficial spores more detailed against common pathogens caused HAI. With the incorporation of beneficial spores in the woven fabrics, these fabrics become a reservoir for beneficial bacteria and may provide an antagonistic/antimicrobial activity against the contamination of pathogens. Usage of these fabrics in bed linens or uniforms may reduce the infections causing by nosocomial pathogens.

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