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Survival of human-associated microbes on clean room textiles

Master´s thesis

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science (MSc)
in „Molekulare Mikrobiologie”

Institute of Environmental Biotechnology
Graz University of Technology

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October 2010

Acknowledgement

First of all I would like to thank my advisors Univ.-Prof. Dipl.-Biol. Dr.rer.nat. Gabriele Berg and Dr.rer.nat. Stefan Liebminger for their intellectual support and their prolific suggestions during this master´s thesis.

Thanks also to Dr. Massimiliano Cardinale for his advice on and assistance with the confocal laser scanning microscope.

Special thanks to the Research Center Pharmaceutical Engineering GmbH (Graz, Austria), and to the project partners from Ortner Clean Room Technology GmbH (Villach, Austria) and Dastex Clean Room Accessories GmbH & Co. KG (Muggensturm, Germany) for the possibility to write this thesis.

I am very grateful to all my colleagues at the institute of environmental biotechnology of the Graz University of Technology.

Most importantly, I would like to thank my whole family and above all my parents for their support throughout my study.

Abstract

This study attends to the development of innovative concepts for personal locks in the clean room technology. The change of employees into and out of a clean room is very time-consuming and cost-intensive. To move between different clean room classes, employees in clean room facilities always have to change their entire garment. The aim of this project was the development of a personal airlock including a clean room suite, which allows the passage of different clean room classes and safety zones without changing the clothing. Furthermore, a cross-contamination or objectionable carryover of bacteria and particles should be prevented. To fulfill this objective, a combination of different strategies, e. g. the development of new textiles and fabrics, new alternative decontamination methods, optimization of the air flow in the lock, was applied. In addition to classic methods (e.g. UV irradiation), new decontamination methods and new pretreatments of textiles and fabrics were determined in this study to obtain a secure and long lasting decontamination effect on clean-room garment.

Kurzfassung

Die Arbeit beschäftigt sich mit der Entwicklung innovativer Konzepte für Personenschleusen in der Reinraumtechnik. Der Schleusenprozess für Personen in der Reinraumtechnik ist, wie er heute praktiziert wird, sehr zeit- und kostenintensiv. Die Mitarbeiter müssen bei jedem Wechsel der Reinraumklassen die gesamte Reinraumbekleidung wechseln. Ziel dieses Projektes ist es, das Betreten von mehreren Reinraumklassen ohne einen Umkleidezyklus zu ermöglichen. Dabei soll die größtmögliche Sicherheit gegenüber Kreuzkontaminationen beibehalten werden, welche die Anforderung an Sicherheitsvorkehrungen für die entsprechende Reinraumklasse erfüllt. Eine kombinierte Strategie aus der Entwicklung von neuen Materialen und Stoffen, neuen effektiven Entkeimungsverfahren, Analyse der Luftströme im Schleusensystem, etc. soll zum gewünschten Erfolg führen. In dieser Arbeit wurden zusätzlich zu den herkömmlichen Methoden (z.B. UV Bestrahlung) neue Dekontaminationsmethoden getestet.

List of Contents

List of abbreviations

C	celsius
C.a.	*Candida albicans*
CLSM	confocal laser scanning microscopy
ca.	circa
cm	centimeter
cfu	colony forming units
deion.	deionisiert
dest.	distille
g	gravity
Eppi	Eppendorf reaction vessel
et al.	et alteri
etc.	et cetera
°	degree
Fig.	figure
g/L	grams per liter
gram-	gram negative (gram.neg) bacteria
gram+	gram positive (gram.pos) bacteria
h	hour
L	liter
m	meter
MB	methylene blue
MO	microorganisms
µ	micro
m	milli
min	minute
M	molar; mol/liter
NA	nutrient agar
n	nano
ONC	over night culture
pH	pH-value
%	percentage
RCPE	Research Center Pharmaceutical Engineering
rpm	rounds per minute

s	second
S.a.	*Staphyloccus aureus*
S.m.	*Stenotrophomonas maltophilia*
Tab.	table
TBO	toluidine blue
TiO_2	Titaniumdioxide
UV	ultraviolett
U	unit
v	volume
w	weight

1 Introduction

1.1 Innovative concepts for clean room technology

This master´s thesis attends to the development of innovative concepts for personal locks in the clean room technology. The passage of employees into and out of a clean room is nowadays very time-consuming and cost-intensive. On Research Center Pharmaceutical Engineering GmbH (RCPE; www.rcpe.at), a new lock system is developed in collaboration with industrial partners Ortner Clean Room Technology GmbH (www.ortner-group.com) and Dastex Clean Room Accessories GmbH & Co. KG (www.dastex.de). This new lock system contains a clean room suite, which allows the passage of different clean room classes and safety zones without changing the clothing.

Humans are the major source of contamination in a clean room (Eudy, 2003). This is the reason why clean room garments are absolutely essential for protection of the product and for intention to hinder particles from the wearer´s body to contaminate the clean room environment. A clean room is a work area in which the air quality, humidity and temperature are highly regulated in order to protect sensitive equipment from contamination. Clean rooms are typically involving industries such as pharmaceuticals, medical engineering, the optical industry, electronics and the food industry frequently require products to be manufactured to extremely high standards of purity and quality. In a clean room, contamination may result from different factors including germs and particles from outdoor air, germs and particles generated by the employees´ garments and by the employees´ physical activity. The measure values of the air quality of a clean room are described in the European Union's GMP guidelines (Tab. 1). These guidelines for Europe standards have come into operation on the 1. January 1997 and propose specific recommended limits for microbial contamination for each class (A-, B-, C-, D-class) of clean rooms.

Tab. 1: Clean room classification in pharmaceutical production (GMP).
Clean rooms are classified according to the number and size of particles permitted per volume of air. For the manufacture of sterile medicinal products normally four grades can be distinguished. **Grade A:** The local zone for high risk operations, e.g. filling zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally such conditions are provided by a laminar air flow work station. Laminar air flow systems should provide a homogeneous air speed of 0.45 m/s +/- 20% (guidance value) at the working position. **Grade B**: In case of aseptic preparation and filling, the background environment for grade A zone. **Grades C and D:** Clean rooms for carrying out less critical stages in the manufacture of sterile products. (Source: Pharmaceutical Clean Room Classification)

Maximum permitted particles/m^3 equal to or above

Grade	At rest		In operation	
	≥ 0.5 µm	≥ 5 µm	≥ 0.5 µm	≥ 5 µm
A	3520	20	3520	20
B	3520	20	352000	2900
C	352000	2900	3520000	29000
D	3520000	29000	not defined	not defined

For daily work in clean rooms, the wearing of appropriate clean room clothing for employees is significant. The requirements for clean room textile primarily depend on the security level of the applicable clean room environment. Currently, the exchange of personnel from one standard to the next higher one, e.g. from standard C to standard B, is time consuming and cost intensive (Fig. 1). The time needed to move from C to B can reach up to 30 minutes for changing clothes. For three or four cycles of changing the loss of time is about 2.5 hours on one working day.

In nature complex structures of habitats with organisms more or less adapted to its environment occur. Due to human activities different artificial habitats were established, which somehow correlate to ecological niches. One of these artificial ecological niches is the clean room environment, which forms an ecosystem with extreme a-biotic conditions, where microorganisms can survive. With new methods from the broad field of molecular ecology it was proven that clean rooms host a very complex community of diverse microorganisms. Existing studies deal mainly with cultivable microorganisms in clean rooms, which cover just about 3% of overall microorganisms (La Duc et al., 2007; Moissl et al., 2007). In these

studies, molecular techniques were partly applied in clean rooms, however, where spacecrafts were assembled. Thereby, it was found that clean rooms are colonized by specific, phylogenetic diverse and often extremophil association of microorganisms. These results are in contrast to earlier studies, where mainly human associated microorganisms were found. For clean rooms that serve the pharmaceutical production, however, there are no studies available.

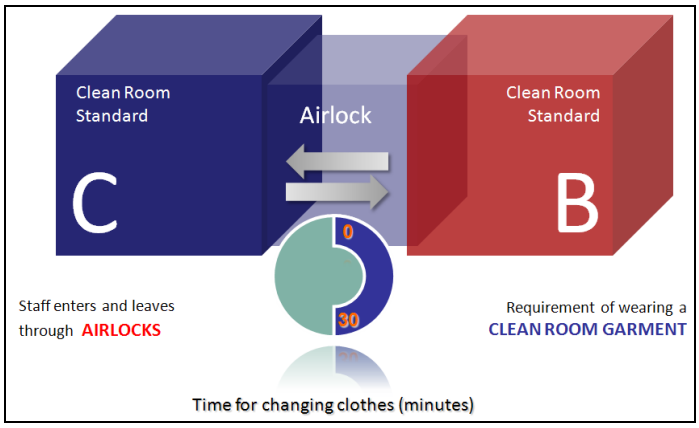

Fig. 1: Expenditure of time in an airlock. Clean rooms are classified according to the number and size of particles permitted per volume of air. To avoid cross contamination from a lower standard to a higher, for example from standard C to standard B, those areas are separated by so called airlocks. Besides those airlocks clean room personnel have to wear special clean room garment adjusted to the prevailing standard. The time needed to move from C to B can reach up to 30 minutes for changing clothes.

Clean room garments should meet several requirements. In addition to water vapor permeability, abrasion resistance and comfort, the particle and germ retention is the main objective. These abilities must be guaranteed to give consideration to the high demands of the various standards of clean rooms. A big problem for a clean room garment is that microorganisms are among the textile fibers (Fig. 2) and so they are inaccessible to current methods of sterilization. Therefore, it is still necessary to change the clean room clothing when entering into a different standard of a clean room. New methods have to be found to treat the protective clothing that a change in a different class of contamination is possible.

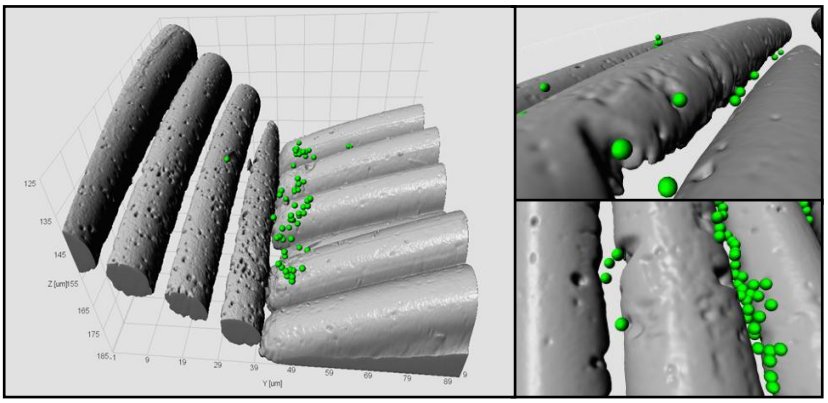

Fig. 2: 3D Reconstruction of polyester fibers (grey) colonized by GFP-marked *Staphylococcus epidermidis* 1457 (green). Software derived texturing of the surface with the associated bacteria. Fibres will allow bacterial cells to attach or hide to the notches and so they are inaccessible to current methods of sterilization. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

Sterile clean room textiles are passed to the carrier. Now, this sterility should be generated and maintained by new innovative concepts from the combination of innovative clean room textiles and lock systems. In the course of recent investigations established processes, e.g. sterilization by UV irradiation, were evaluated. In this study, further examinations like treatment/pre-treatment of clean room textiles with disinfectants, pre-treatment with titanium dioxide (TiO_2) or a light activated disinfection were conducted.

Ultraviolet (UV) light is an electromagnetic radiation and is used as a common method of sterilization. UV light is classified into three types, ultraviolet A (UVA, 400-320 nm), ultraviolet B (UVB, 320-280), and ultraviolet C (UVC, 280-100 nm), depending on its wavelength. The peak wavelengths of 264 nm and 185 nm are the most lethal to a living organism's DNA. UV light at this wavelength leads to dimerisation of adjacent thymine molecules on DNA (Li et al., 2007). An accumulation of these defects causes an inhibition of DNA replication and furthermore to inactivation of cells. Treatment with UVC lamps for killing microorganisms offers a great potential in airlocks, but it also shows the limitations of this method on the effectiveness at the clean room garment and on the protection of employees.

The application of disinfectants to kill microorganisms is another method that was tested in this thesis. These commercially available disinfectants were Sterillium® hand disinfectant and Dr. Beckmann® Disinfectant Hygiene Spray. These disinfectants have already been tested for their skin compatibility and therefore they are not dangerous for employees. The

treatment or pre-treatment of clean room textiles with disinfectant should reduce the number of microorganisms. Thus, a change in a different clean room class can be possible.

Antimicrobial materials are now in many hygienic areas in use, e.g. in germ-reducing surgical textiles, special coatings in refrigerators or antimicrobial surfaces in the food industry. The term “antimicrobial activity” includes all fields of effectiveness, which inhibit the growth of microorganisms, prevent microbial colonization or kill microorganisms. In the case of antimicrobial surfaces, this effect is always limited to the surface that prevents colonization of microorganisms. In this study, clean room textiles were pre-treated with titanium dioxide (TiO_2) to get an antimicrobial effect. Titanium dioxide is able to develop a strong antimicrobial activity. This antimicrobial effect of titanium dioxide on surfaces and in solution prevents the growth of bacteria, yeast, viruses, algae, mold and other microorganisms (Muranyi et al., 2009; Degussa, 2005). Furthermore, it is non-toxic, inexpensive and of high chemical stability. These advantages can be used on clean room textiles as an additionally protection.

Another method that has been tested in this thesis is a light activated disinfection. Light activated disinfection, generally known as photodynamic therapy, is a method which uses photosensitizing agents and light. A range of dyes may be used as photosensitizers for this method. Dyes are activated by light of defined wavelength and develop an antimicrobial effect against a variety of pathogens that includes wild antibiotic resistant gram positive bacteria, gram negative bacteria, viruses, yeasts and protozoa (Jori & Brown, 2004; Procaccini et al., 2004; Wainwright, 2004; Usacheva et.al., 2001; Raab, 1900). The basic principle is the activation of a dye with light of definitely wavelengths, which generates reactive oxygen species (ROS). These reactive oxygen species damage the cell wall of microorganisms and result in cell death (Hamblin & Hasan, 2004). The most commonly used dyes are methylene blue and toluidine blue.

Methylene blue is used in the chemical, dyeing and medicine work and was first synthesized in 1876 by BASF. The dye absorbs light in the range of 530-700 nm, the absorption maximum is at 670 nm. Especially in medicine, methylene blue is used for a large number of applications from the treatment of wounds to the fight against cancer cells and tumors (Ochsner, 1997).

Toluidine blue absorbs light in the range of 560-700 nm and is also used in dyeing and medicine work. The most commonly application is for staining of tissue affected with cancer

(Onofre et al., 2001). This dye is particularly constituted for early diagnosis (Siddiqui et al., 2006) and for treatment (Epstein et al., 1997) for oral cancer.

In addition to methylene blue and toluidine blue, other dyes are known to inhibit the microorganisms in their growth (e.g. rose bengal). Meanwhile, some studies are reported about the effectiveness of lethal photosensitization of bacteria *in vitro* and *in vivo* (Martinetto et al., 1986; Venezio et al., 1985).

In this study, the method was used directly on clean room garments with the objective to kill the adhering microorganisms. In contrast to UV radiation, which requires additional protection to the employee, this radiation with light of defined wavelength is not a danger for employees. In addition, light activated disinfection has a number of advantages in chemical disinfectants. It is an effective and highly cost saving method with no noted negative side effects. An application of this method is intended for the airlock to pass different clean room standards without changing the clothes.

1.2 Pathogens

1.2.1 *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive spherical bacterium and is part of the family of Staphylococcaceae (formerly mentioned Micrococcaceae). In addition, it is a facultative anaerob, coagulase-positiv, immotile and non-sporulating bacterium (Hahn et al, 2009). The name derives from the Greek term “Staphyle”, which refers to the grape-bearing in microscopic preparations. *S. aureus* was first mentioned in 1878 in pus (Hahn et al, 2009).

S. aureus is a very common pathogen causing nosocomial infections. Frequency and variety of diseases caused of this pathogen are a major problem for health care. More than twenty species of *Staphylococcus* are described in Bergey's Manual 2003, but only *Staphylococcus epidermidis* and *Staphylococcus aureus* are pregnant in their interactions with humans. In 25% of the population, *S. aureus* permanently settled the nasal mucosa, 50% are colonized temporarily (Gordeon & Lowy 2008; Laupland et al., 2003; Lindsay, 1977). It was observed that *S. aureus* can penetrate into endothelial cells (Ogawa et al., 1985), epithelial cells (Almeida et al., 1996), fibroblasts (Sinha et al., 1999) and osteoblasts (Bayles et al., 1998; Hudson et al., 1995). Furthermore, *S. aureus* caused 70% of all wound infections, 60% of all osteomyelitis, 40% of all vascular graft infections, up to 30% of all cases sepsis and endocarditis (Hahn et al., 2009).

In pathogenesis, many virulence factors act together. In addition, some strains constitute specific toxins that are responsible for diarrhea with vomiting, for the Toxic-Shock-Syndrome (TSS) or for the Staphylococcal-Scalded-Skin-Syndrome (SSSS). Thus, now some virulence factors are described.

Cell wall bounded virulence factors (Hahn et al., 2009):

Clumping factor (C.F.). The Clumping Factor form *S. aureus* is a cell wall bounded protein that acts as a receptor for fibrinogen. This clumping factor mediates the binding of staphylococci to fibrinogen in injured tissues, on medical implants and catheters. Although, fibrinogen are converted to fibrin and fibrin chains emerge. This leads to clumping of blood plasma and subsequently to abscesses.

Protein A. Most of the *S. aureus* strains generate Protein A, which is associated with the peptidoglycan layer. The binding of Protein A to Fc-fragments of immunoglobulins (generally IgG subclasses) disposes that immunoglobulins are not able to bind to the Fc-receptor of phagocytes. Thus, Protein A avoids opsonization and phagocytosis.

Extracellular virulence factors (Hahn et al., 2009):

Free Coagulase. This protein has no enzyme activity on its own. After binding on prothrombin, this complex acts proteolytic. This complex causes directly the conversion of fibrinogen into fibrin. Thus, Free Coagulase has the same function as the clumping factor. The coagulase is responsible for the characteristic property of *S. aureus* to produce localized abscesses and lesions.

Staphylokinase. The effect of this enzyme is producing plasmin (synonym: fibrinolysin). It allows the batch-wise spread of this virulence factor in infected tissue.
DNase. The thermostable nuclease cleaves DNA and RNA. Thus, *S. aureus* will be facilitated into the tissue. The enzyme DNase has also an important diagnostic significance because it occurs only in *S. aureus* and in a few species of coagulase negative staphylococci.

Haemolysin. Staphylococci produce various haemolysins, including *α*-, *β*-, *γ*-, *δ*-haemolysins (or -toxins). They damage the cell membrane of e.g. erythrocytes, endothelial cells, phagocytes and so they prevent phagocytosis.

Toxic-Shock-Syndrome-Toxin-1 (TSS-1). This toxin is a superantigen and is produced by individual strains in an aerobic environment and by magnesium deficiency. The superantigen effects a polyclonal CD4-T-cell activation with uncoordinated release of TNF-and IL-2. This causes the Toxic-Shock-Syndrome-Toxin-1.

Exfoliatine. The Exfoliatines A and B cause the damage of desmosomes. Within the epidermis, the stratum corneum solves from the stratum granulosum and bubbles are generated, which are characteristic for the Staphylococcal-Scalded-Skin-Syndrome (SSSS). Nowadays, this life-threatening disease occurs rarely. Untreated, the disease often is still lethal.

1.2.2 *Stenotrophomonas maltophilia*

Stenotrophomonas maltophilia is a gram-negative, obligate aerobic, non-sporulating bacterium, motile due to polar flagella and is the sole member of the genus *Stenotrophomonas* (Stenos, Greek: narrow; trophos, Greek: one who feeds; monas, Greek: a unit, monad; i.e., a unit feeding on few substrates; and malt, Old English: malt; philos, Greek: friend; i.e., a friend of malt) (Denton & Kerr, 1998). *S. maltophilia* was previously described as *Pseudomonas maltophilia* (Hugh & Ryschenkow, 1960) and later *Xanthomonas maltophilia* (Swings et al., 1981). The genus was finally proposed in 1993 by Palleroni and Bradbury (Palleroni & Bradbury, 1993).

The genus currently comprises eight species (Ryan et al., 2010). *S. maltophilia* is a ubiquitous free-living bacterium that is commonly found in a variety of environments and geographical regions, including Antarctica (Vazquez et al., 2000), and occupies ecological niches both outside and inside hospitals (Denton & Kerr, 1998). The bacterium has been isolated in various aquatic environments, e.g. in rivers, lakes, drinking- and waste water (Denton & Kerr, 1998; Hernandez-Duquino & Rosenberg, 1987). In addition, the bacterium has been isolated in soil, plants, rhizosphere and food (Ryan et al., 2010; Denton & Kerr, 1998; Juffs, 1973). *S. maltophilia* is an uncommon pathogen in humans and has been detected in hospitals, air dampers, haemodialysis liquids, infusion liquids and antiseptical solutions (Suilen et al., 1999). In hospital the germ has great importance in nosocomial sources, e.g. in dialysis machines, blood-sampling tubes or in deionized-water dispensers (Denton & Kerr, 1998). Denton and Kerr report the isolation of *S. maltophilia* in different clinical infections, e.g. bacteremia, endocarditis, respiratory tract infection, central nervous system infection, ophthalmologic infection, urinary tract infection, skin and soft tissue infection, bone and joint infection and gastrointestinal infection. The respiratory tract of hospital patients is the most common site of isolation of *S. maltophilia* of clinical infections. The dangerousness of this bacterium is the existing resistance to many currently available broad-spectrum antimicrobial agents. Berg et.al. (1999) give an account that environmental strains of *S. maltophilia* are highly resistant to antibiotics. Phenotypically the strains show variability that does not correlate significantly with their source of isolation (Minkwitz & Berg, 2000; Berg et al.; 1999). A new study reports that low mutation frequencies were particularly frequent among environmental *S. maltophilia* strains (58.3%), whereas hypermutators were only found among clinical isolates (Turrientes et al., 2010). These results indicate that clinical environments might select bacterial populations with high mutation frequencies.

1.2.3 *Candida albicans*

Candida albicans is facultative pathogenic, ubiquitous yeast that belongs to the family of yeast-like fungi (Ascomycota). The yeast-like fungus was first described in 1853 by C. Robin as "*Oidium albican*" (Hahn et al., 2005). *C. albicans* is sensitive to desiccation and therefore it is only detectable in a humid environment (Hahn et al., 2005). Furthermore, *Candida ssp*. only colonize the human host and do not exist anywhere else in nature. Morphologically, *C. albicans* is a polymorphic opportunistic fungus that particularly colonizes the gastrointestinal mucosa and the oral mucosa as commensales of warm-blooded animals and humans (Hahn et al., 1999, Schaller, 2006). The percentage of colonized persons in population is very high, e.g. 50% of the people have *C. albicans* in their oral mucosa (Lynch, 1994).

Candida ssp. produce and excrete enzymes like proteinase and lipase that enable the microorganism to penetrate blood vessels and mucosa barriers. Acidic asparticproteases (SAPs) are probably responsible for pathogenesis role as destruction pathogenesis of immunoglobulin and complement, degradation of mucus as an invasion barrier and for nitrogen release by degradation of host proteins. The adherence property is probably mediated by the SAPs. Various extracellular lipases and phospholipases are also associated with increased virulence (Hahn et al., 2005).

Yeast-like fungal infections have become a predominant problem over the last 25 years (Martin et al., 2003). It is the most common mycosis pathogen in humans and accompanying pathogen in AIDS. Fungal infections caused by *Candida* species are called candidosis. The disease affects the skin or mucosa (e.g. oral cavity, esophagus or vagina), so it is known as thrush. Other diseases and infections caused by *C. albicans* are candida onychomycosis, candida esophagitis, urinary tract infections, pneumonia, peritonitis, endocarditic, meningitis, catheter infection and candida sepsis (Hahn et al., 2005).

2 Material & Methods

2.1 Material

Textiles and media, which were applied for microbial work were either autoclaved for 20 min at 121°C before use to avoid contamination. All media were prepared with deionized water.

2.1.1 Manufacturing certificate

If not specifically mentioned otherwise, all chemicals and culture media were obtained from the following companies: Bitplane AG (Zurich, Switzerland), Dastex (Muggenstum, Germany), Fermentas (St. Leon-Rot, Germany), Fluka (Buchs, Switzerland), Invitrogen (Lofer, Austria), Lactan (Graz, Austria), Leica Microsystems (Wetzlar, Germany), Merck (Darmstadt, Germany), MP Biomedicals (Eschwege, Germany), Sifin (Berlin, Germany), Peqlab (Erlangen, Germany), Promega (Mannheim, Germany), Riedel-de Haën GmbH (Seelze, Germany), Roche (Basle, Switzerland), Roth (Karlsruhe, Germany), Qiagen (Wien, Austria), Sigma-Aldrich (St. Louis, USA).

2.1.2 Nutrient Media and Chemicals

Liquid media: Caso Media (TSB)

30.0 g/L	Caso Media

Solid media: NA-media

4.0 g/L	Peptone
2.4 g/L	Beef extract
	pH 7.0 ± 0.2
12.0 g/L	Agar

Buffer: PBS

4.0 g/L	Sodium chloride (NaCl)
7.0 g/L	Disodium hydrogen phosphate (Na_2HPO_4)
3.0 g/L	Potassium dihydrogen phosphate (KH_2PO_4)
	pH 7.1 ± 0.2

Washing solution

-PBS

2.1.3 Pathogens

Staphylococcus aureus ATCC 25923 (gram+)

Stenotrophomonas maltophilia DSM 50170 (gram-)

Candida albicans H5 (yeast)

The bacteria strains were inoculated in 5 ml of nutrient media for an ONC under agitation at 37°C. Next day, cell suspension was transferred into 150 ml of the same nutrient solution and was incubated under agitation at 37°C to an OD_{600} of 0.5-0.7 (exponential phase). Yeast was inoculated in 10 ml of nutrient media for an ONC under agitation at 30°C. Next day, strain suspension also was transferred into 150 ml of the same nutrient solution and was incubated under agitation at 30°C to an OD_{600} of 0.5-0.7 (exponential phase).

2.1.4 Clean room garment

Clean room garments are the most important filter between human and product. Humans are the biggest source of contamination in clean rooms. This is the reason why high quality clean room garments are absolutely essential for protection of the product. The textiles used in the studies were produced by the clean room clothing company "dastex" (source: www.dastex.de) and are listed in Tab 2.

Tab. 2: Used clean room textiles for examination. Source: www.dastex.de

Clean room textiles	
Fig. 3a	ION-NOSTAT VI.2
Fig. 3b	ION-NOSTAT V
Fig. 3c	Red knitted fabric with silver ion fiber

Fig. 3: clean room textiles. a) ION-NOSTAT VI.2. b) ION-NOSTAT V. c) Red knitted fabric with silver ion fiber.

Criterion		ION-NOSTAT VI.2
Fabric	HA	98 % Polyester + 2 % Carbon
Dispersion of the conductive fibre	HA	warp & weft by intervals of 5 mm
Conductive material / yarn	HA	Carbon-Fibre in "Sandwich-Construction" Carbon interim Polyester fibre
Weave	HA	Twill 3/2 (Scale of 2:1)
Weight	ITV	approx. 113 g/m²
Air permeability (DIN 53887) at 200 Pa (l/min x dm²)	ITV	29.2
Water vapour diffusion resistance 35 °C 40 % r.F. 10^{-3} m² mbar/V	FIH	28.3
As a result the **wearing comfort** is		very good
Particle retention capacity for airborne particles Duration: 60 min Crude gas concentration: 25 mg/m³	ITV	0.5 µm, approx. 97 % 5.0 µm, approx. 98 %
Electrostatic behaviour a) Charge tendency (valuation) b) Discharge velocity (valuation)	ITV	in warp & weft direction: low in warp & weft direction: very high
Surface resistance (DIN 54345)	HA	10^7 – 10^9 Ohms
Abrasion tendency Test method Martindale (valuation)	ITV	good to very good

Fig. 4: Information about the fabric ION-NOSTAT VI.2. ITV: the data was derived from the Institute for textile technology, Denkendorf after 50 decontaminations. **FIH**: the data was derived from Hohenstein Institute after 50 decontaminations. **HA**: the data was derives from the manufacturer. Source: www.dastex.de

The fabric ION-NOSTAT VI.2 was additionally tested and certified by the Hohenstein Institute for breathability. The Certification number is 06.4.5535.

2.1.5 Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) is a valuable tool for obtaining high resolution images and 3-D reconstructions (Pawley JB, 2006). CLSM provides the possibility to locate attached bacteria directly on the clean room textile and to follow growth and survival of different species. With specific live/dead measurements (LIVE/DEAD® BacLight™, Molecular Probes) the effect of alternative sterilization methods, which were tested in the conducted experiments, can be followed. A Leica TCS SPE, equipped with solid state laser and UV laser, was used to acquire the Z-stacks with two to three channels sequentially activated. Laser intensity and photomultiplier settings were optimized for every channel and for every stack, in order to enhance the output image. Image analysis and three-dimensional modeling were performed with the software Imaris 7.0 (Bitplane AG, Switzerland).

2.1.6 LIVE/DEAD® Bacterial Viability Kit (BacLight™)

The vital fluorescent double staining is nowadays an accepted method and is used to distinguish between living and dead cells (Auschill et al., 2001; Netuschil et al., 1998). For examination on microscope, textile pieces were placed in a staining solution for 15 minutes. This epifluorescence staining method using the LIVE/DEAD® Bacterial Viability Kit (BacLight™) is able to show viable (green cells) and dead (red cells) bacteria. This Bacterial Viability Kit employs two nucleic acid stains, green-fluorescent SYTO® 9 stain and red-fluorescent propidium iodide stain. These stains differ in their ability to penetrate healthy bacterial cells. When used alone, SYTO® 9 stain labels both live and dead bacteria. In contrast, propidium iodide penetrates only bacteria with damaged membranes, reducing SYTO® 9 fluorescence when both dyes are present. Thus, live bacteria with intact membranes fluoresce green, while dead bacteria with damaged membranes fluoresce red (Fig. 5).

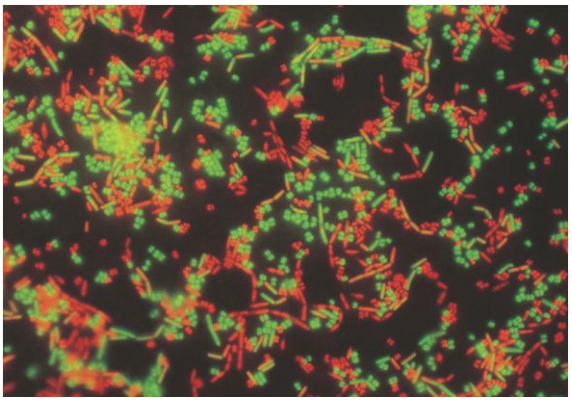

Fig. 5: CLSM analytic: Viability of a mixture of *Micrococcus luteus* and *Bacillus cereus* assessed using reagents in the LIVE/DEAD® BacLight™ Bacterial Viability Kits. © invitrogen.com

2.1.7 UV-lamp

The UV lamp, which was used in the experiments, is a P/N UVC 2016-2N from “vita seed”.

2.1.8 Disinfectant

For the experiments, two different disinfectants were used.

2.7.1. Sterillium® hand disinfectant

2.7.2. Dr. Beckmann® Disinfectant Hygiene Spray

2.1.9 Titanium dioxide

Titanium dioxide (also known as Titanium(IV)oxide, C.I. Pigment White 6, Rutile (Greenwood N et.al. 1984), Anatase or titania) is a naturally occurring oxide of titanium with the chemical formula “TiO_2”. In experiments, TiO_2 was used by the company Riedel-de Haen GmbH.

2.1.10 Dyestuffs

2.1.10.1 Methylene blue

Methylene blue (3,7-(Dimethylamino)phenothiazin-5-ium Chloride) is a green fine crystalline powder and a very commonly used dye. The International Nonproprietary Name (INN) of methylene blue is Methylthioninium chloride.

In experiments, methylene blue was used by the company Merck GesmbH.

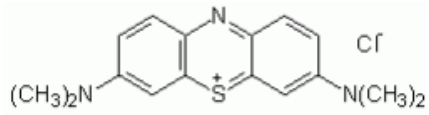

Fig. 6: Chemical structure of methylene blue. © Merck KGaA, Darmstadt, Germany, customer.service@merckchem.co.uk, 2010

2.1.10.2 Toluidine blue

The International Nonproprietary Name (INN) of toluidine blue is tolonium chloride. Toluidine blue is a blue cationic (basic) dye used in biology and medicine. In experiments, toluidine blue was used by the company Sigma-Aldrich.

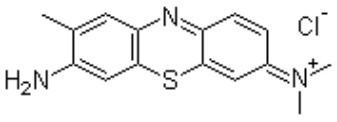

Fig. 7: Chemical structure of toluidine blue. Copyright 2010 © chemBlink, Online Database of Chemicals.

2.1.11 Lamps

2.1.11.1 Black light lamp

For light activated disinfection, a black light lamp (370 nm) was used.

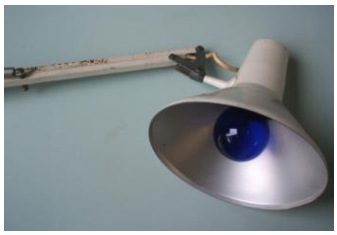

Fig. 8: Black light lamp typ: 75 W / E-27.

2.1.11.2 Laser

The microscope „Leica TCS SPE“ is equipped with a solid state laser (532nm, 15 mW). Source: www.leica-microsystems.com

2.1.11.3 Mercury lamp

The microscope „Leica TCS SPE“ is equipped with a mercury lamp (515-560 nm). Source: www.leica-microsystems.com

2.1.11.4 LED-1

LED-1 is a high power bulb with 38 LEDs (620-640 nm; colour red).

2.1.11.5 LED-2

LED-2 is a low-voltage-LED bulb with 16 LEDs. (620-640 nm; colour red).

2.2 Methods

2.2.1 Adhesion of human skin bacteria on clean room garments

The implementation was made by self-experiment with ten test persons. The clean room textiles, which were used in this study, are listed in Tab 2. Textile pieces of 1x1 cm were fixed on the left and right wrist with a patch and were worn for five hours. Then the textile pieces were investigated with CLSM. In the test, the cell number could not be determined because only one layer could be considered.

2.2.2 UV-treatment of inoculated clean room textiles

In the following experiments, the behaviour of *S. aureus*, *S. maltophilia* and of *C. albicans* were tested on textile pieces (1x1 cm). Care has been taken on the attachment and survival of microorganisms. 3 µl cell culture (10^6 CFU ml^{-1}) were pipetted on textile pieces for each experiment. After incubation, the textile pieces were treated differently with UV.

Tab. 3: Methods - UV treatment

UV treatment
Textile pieces without treatment
Textile pieces with UV treatment → different irradiation time
Change in the textile structure after UV irradiation
Sterile probes

The textiles were analyzed with CLSM and wipe test. For wipe test, textile pieces of 1x1 cm were pressed on petri dishes containing NA. Finally they were incubated at 37°C for 24 or 48 hours.

2.2.3 Pre-treatment of textiles with disinfectants

The growth behaviour of *S. aureus*, *S. maltophilia* and of *C. albicans* was tested on textile pieces (1x1 cm). 3 µl cell culture were pipetted on the textile pieces and after incubation they were dipped in Sterillium® hand disinfectant and in Dr. Beckmann® Disinfectant Hygiene Spray. Textile pieces were also pre-treated with these two disinfectants (Tab. 4). Therefore, all textile pieces were shortly dipped into the disinfectants and then dried. After drying 3 µl cell culture were pipetted on the pre-treated textile pieces for each experiment. Finally, the textiles were analyzed with CLSM.

Tab. 4: Methods – Pre-treatment of textiles with two different disinfectants

Pre-treatment of textiles with two different disinfectants
MO on textile (different time) → treated with disinfectants
Pre-treatment of textile with disinfectants (different time) → MO

2.2.4 Retention capacity of clean room textile

The experiment was performed with a washing test. As control, 3 µl cell culture of *S. aureus* and S*. maltophilia* were pipetted in 1 ml PBS. 100 µl of this solution were plated on nutrient media and incubated at 37°C for 24 or 48 hours. For washing test, 3 µl cell culture were pipetted on a textile piece (1x1 cm). After incubation, the textile piece was taken into an Eppi with 1 ml PBS in it. The Eppi was shaken for 30 min. During this washing process, textile pieces in Eppis were agitated at regular intervals. Finally, 100 µl supernatant of the solution were plated out in different suitable 10-fold serial dilutions on nutrient media and incubated at 37°C for 24 or 48 hours. All plating was done in duplicates. After the incubation period, colonies were counted and the colony forming unit (Puck, Marcus 1956) (cfu) per sample were determined. In addition, the textiles were dried analyzed with CLSM.

Tab. 5: Methods - Experimental procedure for washing test.

Plating
3 µl cell culture in 1 ml PBS → 100 µl were plated out (control)
3 µl on textile → wash in 1 ml PBS for 30 min → 100 µl of supernatant were plated out
CLSM
3 µl on textile → CLSM (control)
3 µl on textile → wash in 1 ml PBS for 30 min → dry → CLSM

2.2.5 Pre-treatment of textiles with Titanium dioxide

Titanium dioxide (TiO_2) is able to develop a strong antimicrobial activity (photocatalytic self-cleaning) upon irradiation. For the experiments, textile pieces were pre-treated with TiO_2. Therefore, all textile pieces were dipped into a suspension with TiO_2 (0.1 mg/ml) and then dried. After drying, 3 µl cell culture of *S. aureus*, *S. maltophilia* and *C. albicans* were pipetted on the pre-treated textile pieces for each experiment. Finally, the textiles were analyzed on microscope (CLSM).

2.2.6 Light activated disinfection

The aim of this experiment is to pretreat clean room textiles with photosensitive dyes methylene blue (MB) and toluidine blue (TBO), which are activated by light to developed an antimicrobial effect against a variety of microorganisms.

Thus, textile pieces were dipped into an aqueous solution of methylene blue (1 µg/ml) or toluidine blue (1 µg/ml) and then dried. The dyes are atoxic and can be used in very low concentrations (1 µg/ml). After drying 3 µl of *S. aureus* (gram+), *S. maltophilia* (gram-) and *C. albicans* (yeast) were pipetted on textile pieces (1x1 cm). By activation with light of a certain wavelength (different irradiation times), singlet oxygen species (radicals) are formed. These radicals damage the cell wall of microorganisms and results into death of the cells. The irradiation was conducted with a black light lamp (370nm), a mercury lamp (515-560 nm) and with a laser (532nm, 15 mW).

In these experiments the effect of MB and TBO were tested with plating and directly on textile through wipe tests.

For wipe test, textile pieces were pressed onto the agar plate and incubated at 37°C for 24 or 48 hours. For the determination of viable cells and cell counting, a mix of 50 µl cell culture and 50 µl dye (MO or TBO) was pipetted in a microtiter plate. After irradiation the mix was plated on an agar plate and incubated at 37°C for 24 or 48 hours. All experiments are listed in chapter 3.6.

3 Results

3.1 Adhesion of human skin bacteria on clean room textiles

Humans are the biggest source of contamination in clean rooms. Thus, the aim of this study was detection of bacteria on three different clean room textiles after wearing. One of the preferred areas for bacterial contamination, the wrist, was examined. The implementation was made by self-experiment with ten test persons. Finally the textiles were analyzed with CLSM. One of the three textiles contains silver ion fibers, which should suppress the attachment of microorganism.

The used clean room textiles for examination were ION-NOSTAT VI.2 (Fig. 9), ION-NOSTAT V (Fig. 10) and a red knitted fabric with silver ion fiber (Fig. 11).

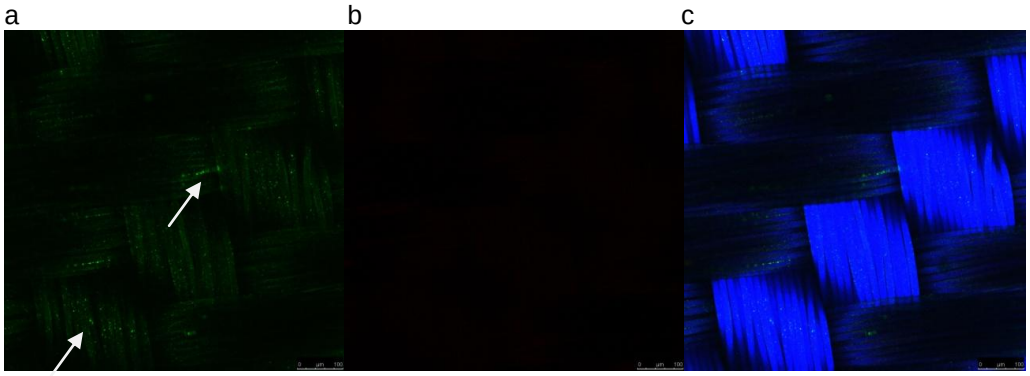

Fig. 9: CLSM analysis of clean room textile ION-NOSTAT VI.2 after wearing for 5 hours (self-experiment). a) viable cells (green). b) dead cells (red). c) overlay of a and b. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

On the clean room textile ION-NOSTAT VI.2 of Fig. 9, living microorganisms (arrows) were barely detectable and dead microorganisms were not visible. ION-NOSTAT VI.2 fibers are not equipped with any antimicrobial properties. Microorganisms attach to the fibers but will not be killed.

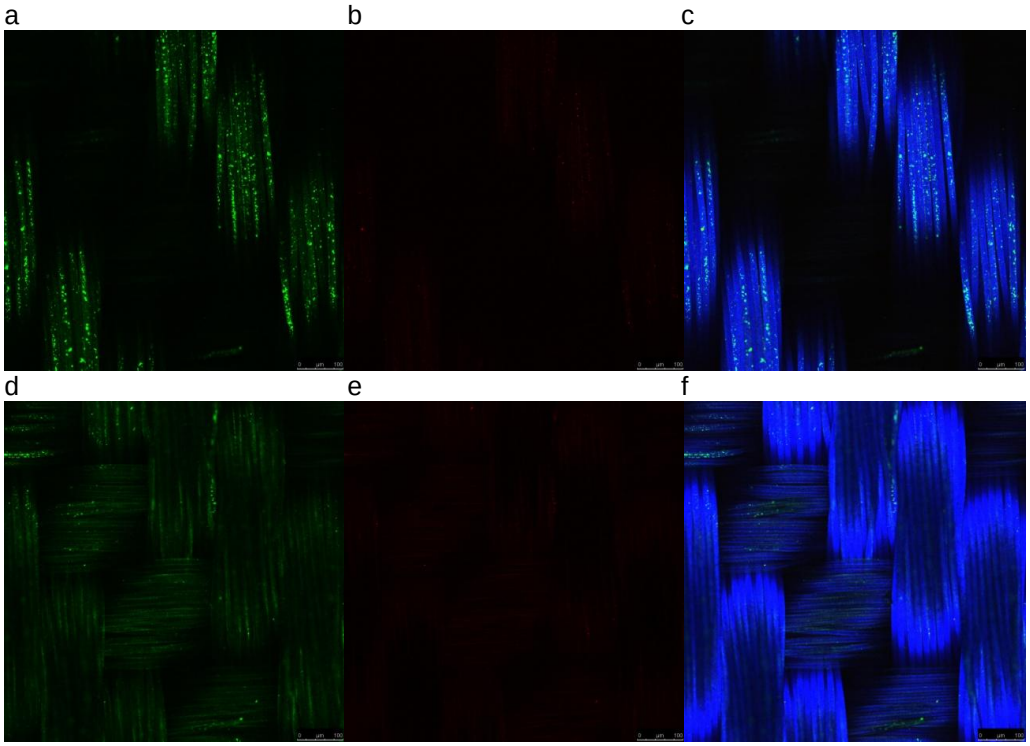

Fig. 10: CLSM analysis of the upper layer (a,b,c) and the lower layer (d,e,f) of ION-NOSTAT V after wearing for 5 hours (self-experiment). a,d) viable cells (green). b,e) dead cells (red). c,f) overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

Fig. 10 shows a fiber containing silver ions. So microorganisms will not be able to attach on this special textile or will be killed shortly after adhering to the fiber matrix. As shown in the Fig. 10a, however, this is not the case. Living microorganisms are visible. This implements that first microorganism may attach to the fiber and killing of them is not happening. It is also important to compare upper layer with lower layer of this textile (same position on textile). On the upper layer, which was worn directly on the skin, you can see viable cells between the fibers. In comparison, the lower layer clearly indicates significantly fewer microorganisms. The detection of microorganism on the lower layer of the textile piece proves the passage of microbes through the textile. As for the upper layer no significant killing by the silver ion blended fiber was detectable.

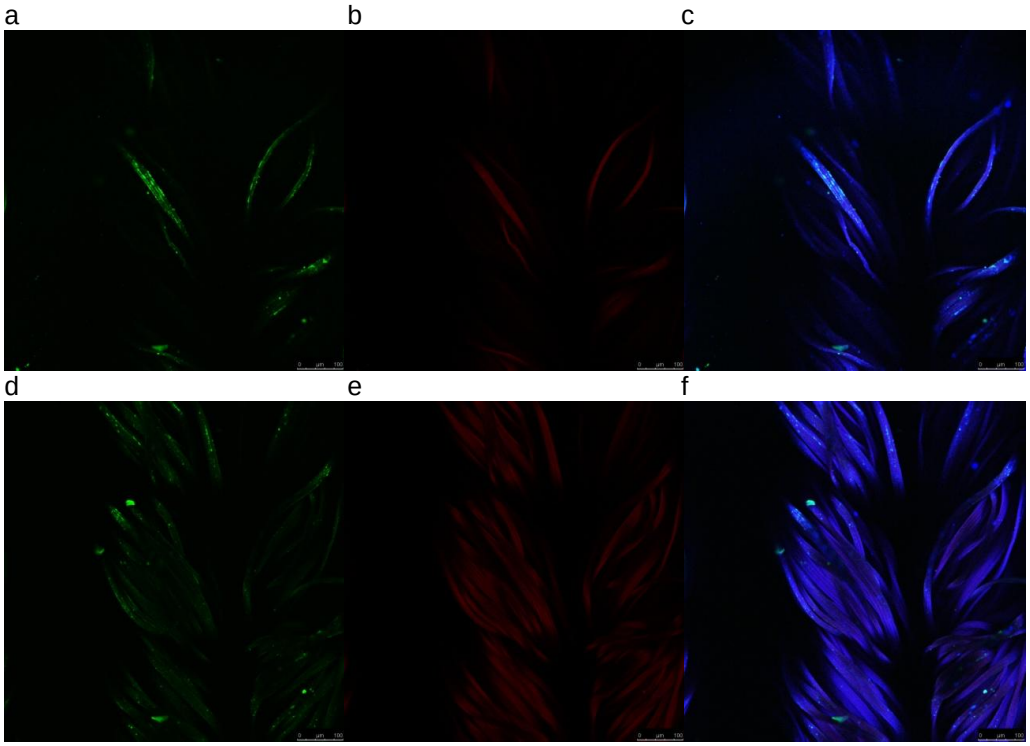

Fig. 11: CLSM analysis of the upper layer (a,b,c) and the lower layer (d,e,f) of red knitted fabric with silver ion fibers after wearing for 5 hours (self-experiment). a,d) viable cells (green). b,e) dead cells (red). c,f) overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

A second antimicrobial textile based on silver ions was tested. Fig. 11 indicates the same results which were observed on ION-NOSTAT V. This textile of Fig. 11 contains silver ion fibers. The same result was observed on ION-NOSTAT V. In comparison to the upper layer, on the lower layer it was also found less colonization. On the upper layer, which was worn directly on the skin, viable cells in thin channelling are detected. These thin channelling are characteristic for this red knitted fabric and they provide a perfect niche for colonization by human skin associated bacteria.

3.2 UV-treatment of inoculated clean room textiles

3.2.1 UV irradiation of inoculated textile pieces

UV light is used for the sterilization of surfaces. The aim of this study was to evaluate the potential of this sterilization technique for the reduction of living microbes on clean room textiles. Clean room textiles were previously incubated with *S. aureus*, *S. maltophilia* and *C. albicans* cell cultures. Afterwards samples were irradiated with UV light. A significant reduction of bacteria should be achieved. The background of these investigations was to observe the behavior of microorganisms on textile pieces. The textile pieces were analyzed with CLSM and wipe test.

In the following experiments, the behaviour of *S. aureus*, *S. maltophilia* and of *C. albicans* were tested on textile pieces (1x1 cm). The setting up for wipe test and CLSM are listed in table 6, 7, 8 and 9. The results of wipe test and CLSM are presented from Fig. 12 to 22.

Tab. 6: Setting up for UV-treatment - wipe test: Differential treatment and incubation of textile pieces, which were previously incubated with the microorganisms (MO) *S. aureus*, *S. maltophilia* and *C. albicans*.

Treatment	Incubation time	Distance to lamp	Textile side	Figure
MO untreated			upper side	12a
MO untreated			under side	12d
MO → UV- irradiated	5 min	33 cm	upper side	12b
MO → UV- irradiated	5 min	33 cm	under side	12e
MO → UV- irradiated	10 min	33 cm	upper side	12c
MO → UV- irradiated	10 min	33 cm	under side	12f

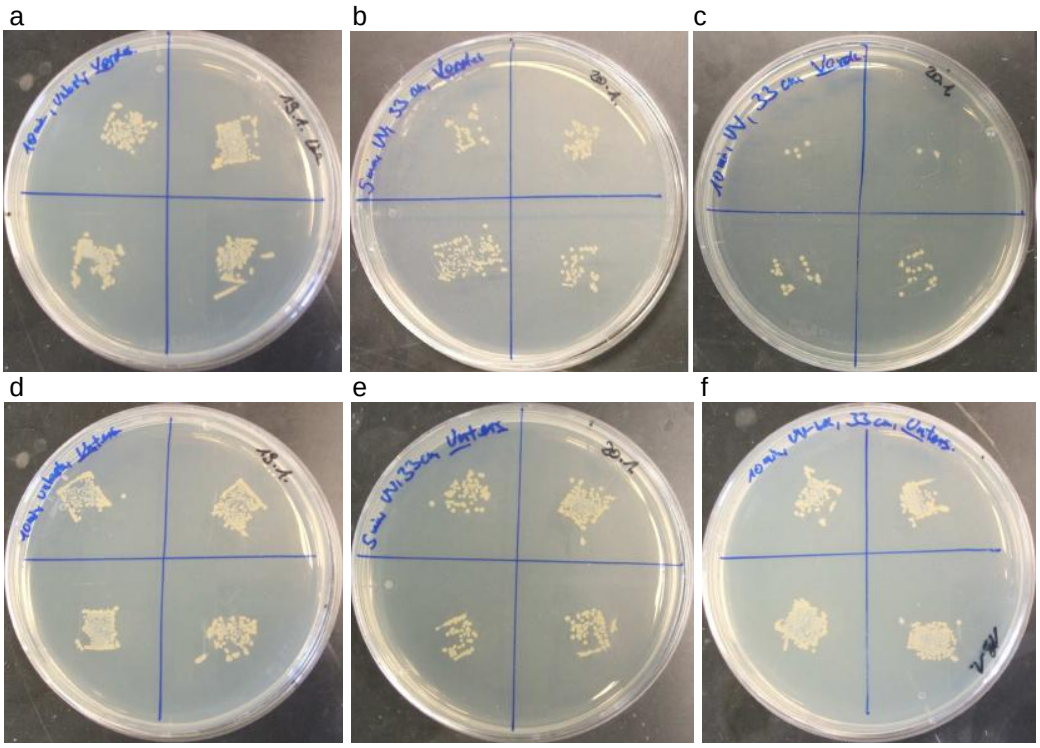

Fig. 12: UV-treatment of textile pieces with *S. aureus* – wipe test. Textile pieces were examined with wipe test. The plates were incubated at 37°C for 48 hours. a) textile without treatment, upper side. b) 5 min UV irradiation, upper side. c) 10 min UV irradiation, upper side. d) textile without treatment, under side. e) 5 min UV irradiation, under side. f) 10 min UV irradiation, under site.

As shown in Fig. 12, a reduction of germs on the upper side of textiles was detectable after UV irradiation. The best effect was observed after 10 min UV irradiation. On the undersides of all textiles no reduction of bacteria was observed, because these sides were not exposed to the UV light. The same results as for *S. aureus* were also observed for *S. maltophilia* and *C. albicans* (data not shown). For *C. albicans* no growth could be detected on the under-sides of textile pieces, which were previously incubated with *C. albicans*. It is expected that yeasts are too big for penetrating through the clean room fabric. This should be attested with CLSM.

Tab. 7: Setting up for UV-treatment - CLSM and *S. aureus*: Differential treatment and incubation of textile pieces, which were previously incubated with *S.aureus* (S.a.).

Treatment	Time of treatment	Textile side	Figure
S.a. → untreated		upper side	13 a-c
S.a. → untreated		under side	13 d-f
S.a. → UV- irradiated	5 min	upper side	14 a-c
S.a. → UV- irradiated	5 min	under side	14 d-f
S.a. → UV- irradiated	10 min	upper side	15 a-c
S.a. → UV- irradiated	10 min	under side	15 d-f

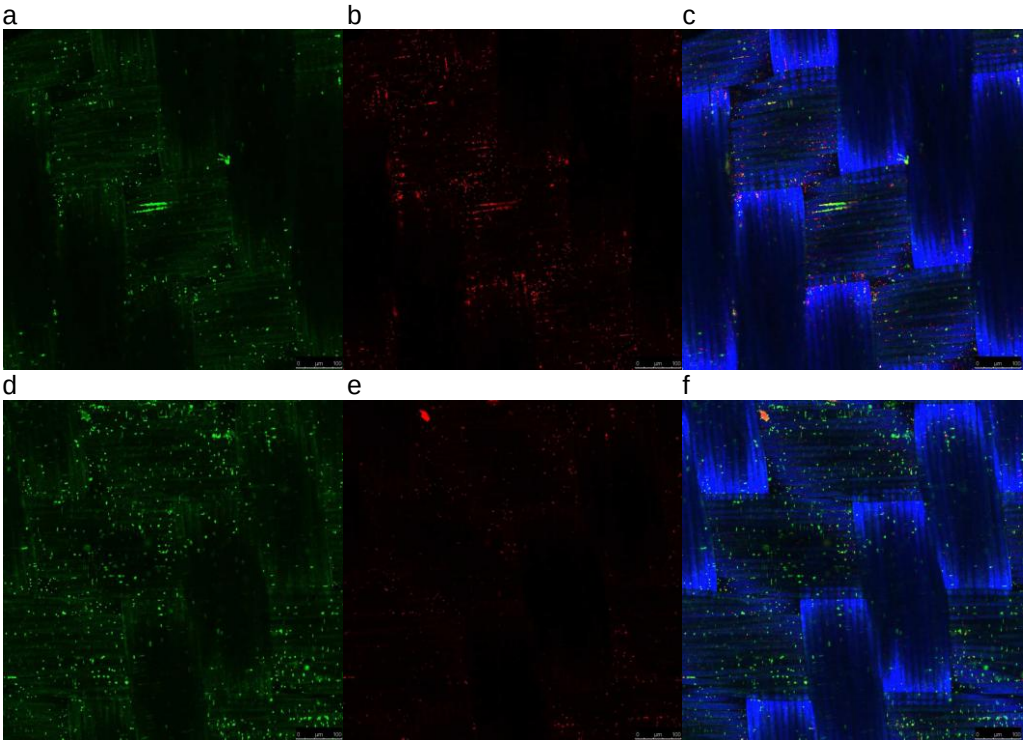

Fig. 13: CLSM analysis of untreated textile pieces incubated with *S. aureus*. a) textile without treatment, viable cells (green), upper side. b) textile without treatment, dead cells (red), upper side. c) textile without treatment, overlay, upper side. d) textile without treatment, viable cells (green), under side. e) textile without treatment, dead cells (red), under side. f) textile without treatment, overlay, under side. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

Fig. 13 shows the results for untreated textile pieces which were incubated with *S. aureus* and analyzed on CLSM. On both sides, upper and under side, living microorganisms were visible. Cells were applied on the textile sample by using 3 µL of a growing cell culture. Cells were brought to one side of the sample. The results of CLSM analytic prove that microbes may passage the fiber matrix and were detectable on the underside of the textile sample. UV light only can kill microbes on the upper layer of surfaces. Cells not exposed to UV irradiation will not be affected by this sterilization technique.

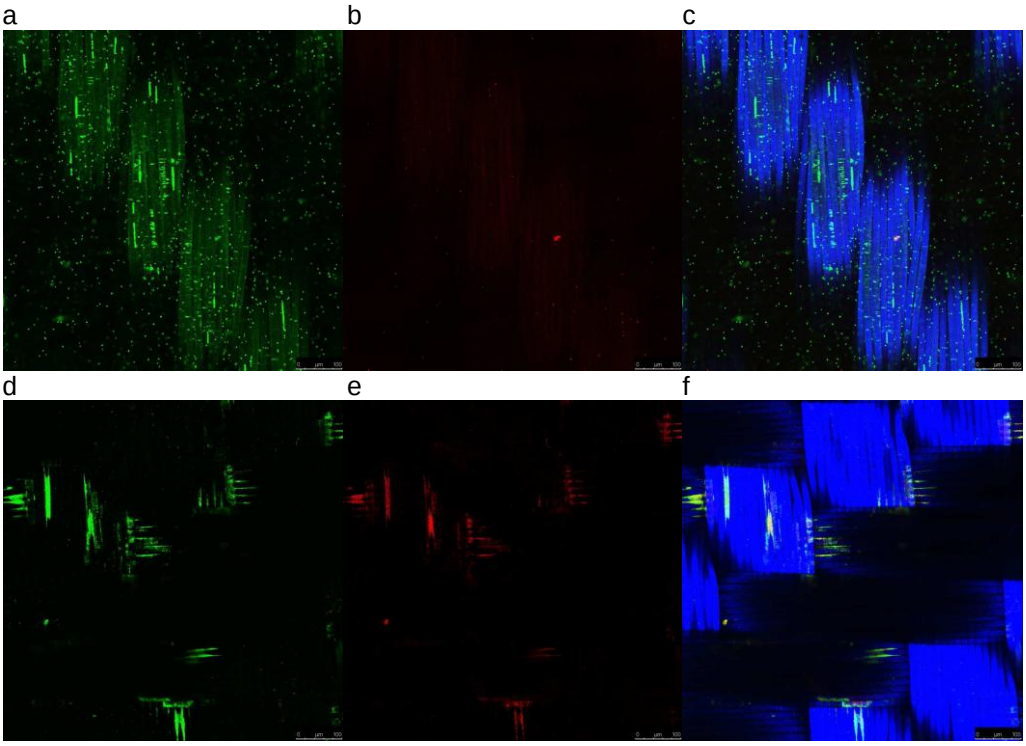

Fig. 14: CLSM analysis of 5 min UV irradiation of textile pieces incubated with *S. aureus*. a) viable cells (green), upper side. b) dead cells (red), upper side. c) overlay, upper side. d) viable cells (green), under side. e) dead cells (red), under side. f) overlay, under side. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

Textile pieces incubated with bacterial cells (*S. aureus*) were treated with UV light for 5 minutes (Fig. 14). On both sides, upper and under side, living microorganisms are visible. Five minutes of irradiation show no significant effect on a reduction of microorganisms.

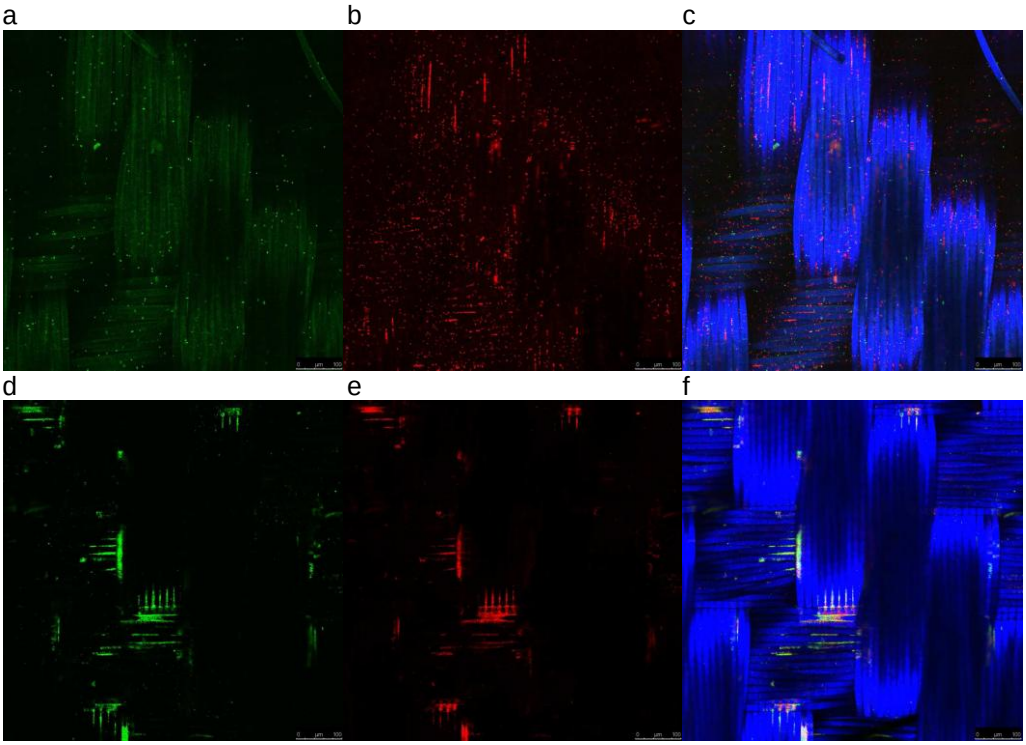

Fig. 15: CLSM analysis of 10 min UV irradiation of textile pieces incubated with *S. aureus*. a) viable cells (green), upper side. b) dead cells (red), upper side. c) overlay, upper side. d) viable cells (green), under side. e) dead cells (red), under side. f) overlay, under side. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

The results for 5 minutes of irradiation showed no significant reduction of cell growth. Therefore incubated textile samples were irradiated for 10 minutes (Fig. 15). A reduction of germs on the upper side of textiles was detectable after 10 min UV irradiation. On the underside of the textile, living microorganisms are visible (d), because these sides were not exposed to UV-light. As mentioned above, cells which are not directly exposed to UV irradiation were not affected by this method.

Tab. 8: Setting up for UV-treatment - CLSM and *S. maltophilia*: Differential treatment and incubation of textile pieces, which were previously incubated with *S. maltophilia* (S.m.).

Treatment	Time of treatment	Textile side	Figure
S.m. → untreated		upper side	16 a-c
S.m.→ untreated		under side	16 d-f
S.m. → UV- irradiated	5 min	upper side	17 a-c
S.m. → UV- irradiated	5 min	under side	17 d-f
S.m. → UV- irradiated	10 min	upper side	18 a-c
S.m. → UV- irradiated	10 min	under side	18 d-f

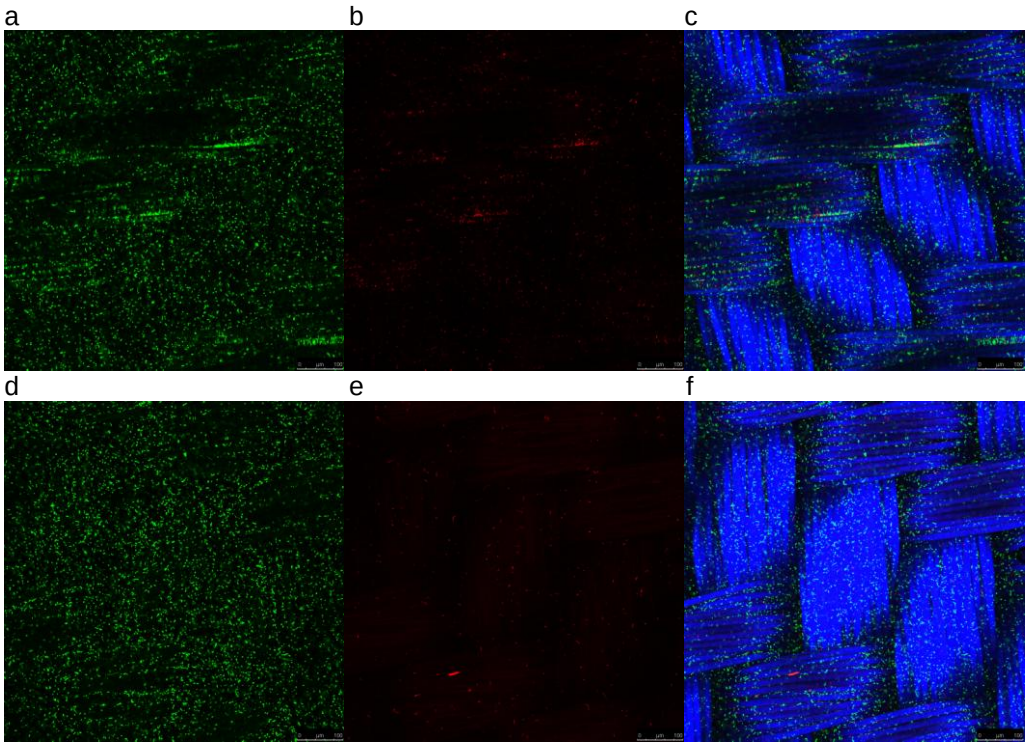

Fig. 16: CLSM analysis of untreated textile pieces incubated with *S. maltophilia*. a) textile without treatment, viable cells (green), upper side. b) textile without treatment, dead cells (red), upper side. c) textile without treatment, overlay, upper side. d) textile without treatment, viable cells (green), under side. e) textile without treatment, dead cells (red), under side. f) textile without treatment, overlay, under side. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

Textile pieces were incubated with *S. maltophilia* (Fig. 16). Both, the upper and the under layer were laced with bacterial cells. As for S. aureus cells spread completely on the surface of the textile but preferably in the niches of the textile matrix which reduce the point of action of UV treatment.

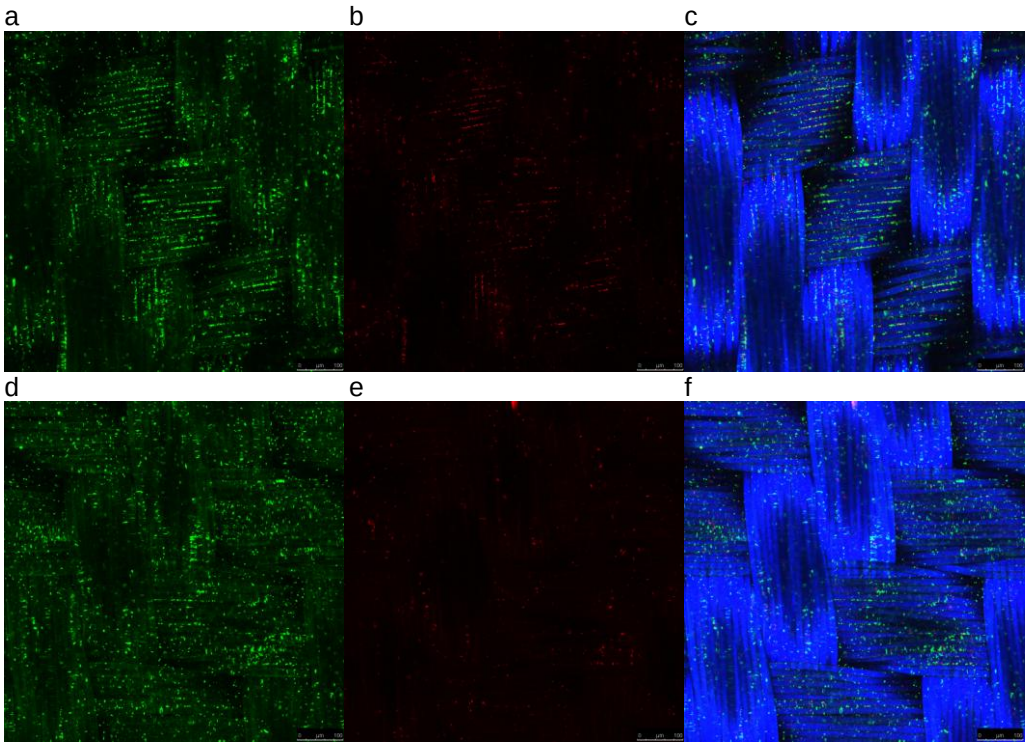

Fig. 17: CLSM analysis of 5 min UV irradiation of textile pieces incubated with *S. maltophilia.* a) viable cells (green), upper side. b) dead cells (red), upper side. c) overlay, upper side. d) viable cells (green), under side. e) dead cells (red), under side. f) overlay, under side. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

Textile pieces were also incubated with *S. maltophilia* and finally 5 min irradiated with an UV-light (Fig. 17). The same result as by *S. aureus* was achieved. On both sides, upper and under side, living microorganisms are visible. There was no visible reduction of cells on the surface of the textile. Five minutes of irradiation show no effect on a reduction of microorganisms.

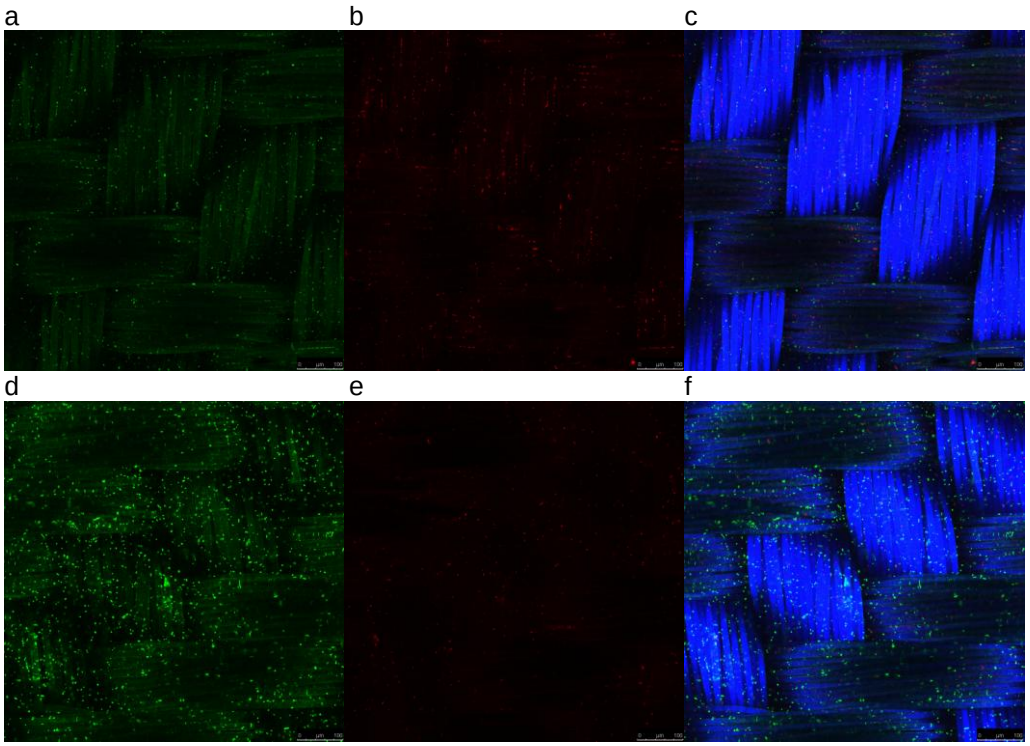

Fig. 18: CLSM analysis of 10 min UV irradiation of textile pieces incubated with *S. maltophilia.* a) viable cells (green), upper side. b) dead cells (red), upper side. c) overlay, upper side. d) viable cells (green), under side. e) dead cells (red), under side. f) overlay, under side. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

Textile pieces were incubated with *S. maltophilia* and finally 10 min irradiated with an UV-lamp (Fig. 18). The same result as by *S. aureus* was also achieved on the upper side. The reduction of germs was only detectable on the upper site of the textile piece after 10 min of UV irradiation. On the underside of the textile living microorganisms are visible (d) in comparison to the upper side (a), because the under side was not exposed to UV-light.

Tab. 9: Setting up for UV-treatment - CLSM and *C. albicans*: Differential treatment and incubation of textile pieces, which were previously incubated with *C. albicans* (C.a.).

Treatment	Time of treatment	Textile side	Figure
C.a. → untreated		upper side	19
C.a. → UV- irradiated	5 min	upper side	20
C.a. → UV- irradiated	10 min	upper side	21
C.a. → untreated		under side	22

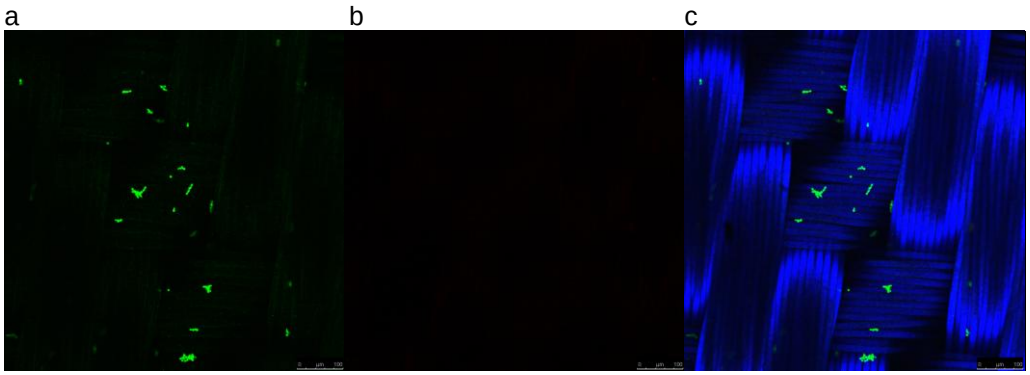

Fig. 19: CLSM analysis of untreated textile pieces incubated with *C. albicans.* a) textile without treatment, viable cells (green), upper side. b) textile without treatment, dead cells (red), upper side. c) textile without treatment, overlay, upper side Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

UV irradiation experiments were also done for *C. albicans* (Fig. 19) First, textile pieces were incubated with *C. albicans* and directly analyzed on CLSM without UV treatment.
On the upper side, living microorganisms are visible. As shown before, *C. albicans* cells are not able to pass the textile matrix, so no cells were detectable on the under site of the textile.

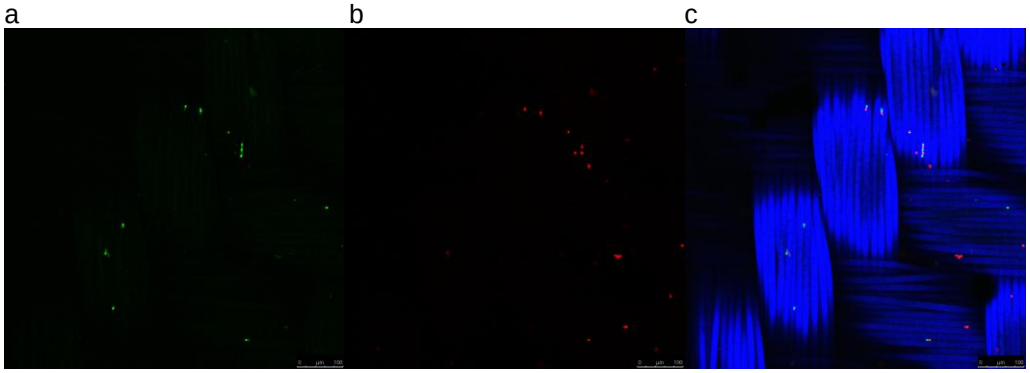

Fig. 20: CLSM analysis of 5 min UV irradiation of textile pieces incubated with *C. albicans*. a) viable cells (green), upper side. b) dead cells (red), upper side. c) overlay, upper side. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

Fig. 20, textile pieces were incubated with *C. albicans* and finally 5 min irradiated with an UV-lamp (Fig. 20). After UV irradiation living and dead microorganisms were visible. Five minutes of irradiation already show an effect of a reduction of microorganisms.

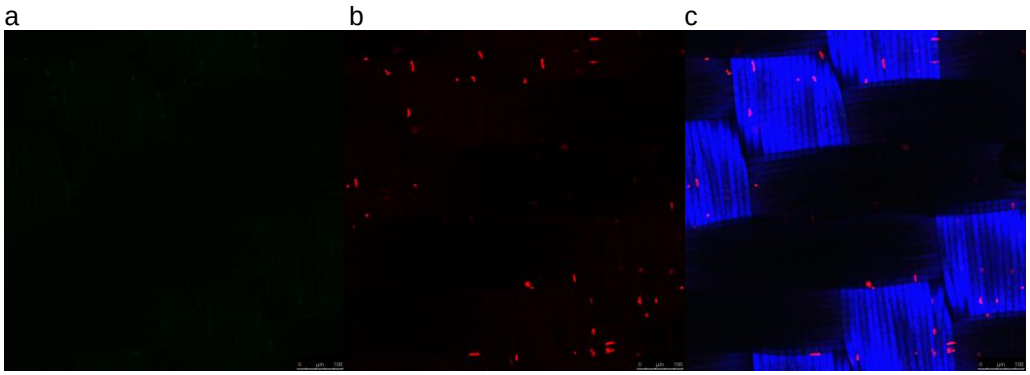

Fig. 21: CLSM analysis of 10 min UV irradiation of textile pieces (upper side) incubated with *C. albicans*. a) viable cells (green), upper side. b) dead cells (red), upper side. c) overlay, upper side. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

Textile pieces (upper side) were incubated with *C. albicans* and finally 10 min irradiated with an UV-lamp (Fig. 21). After 10 min UV irradiation only dead cells are visible on the upper side.

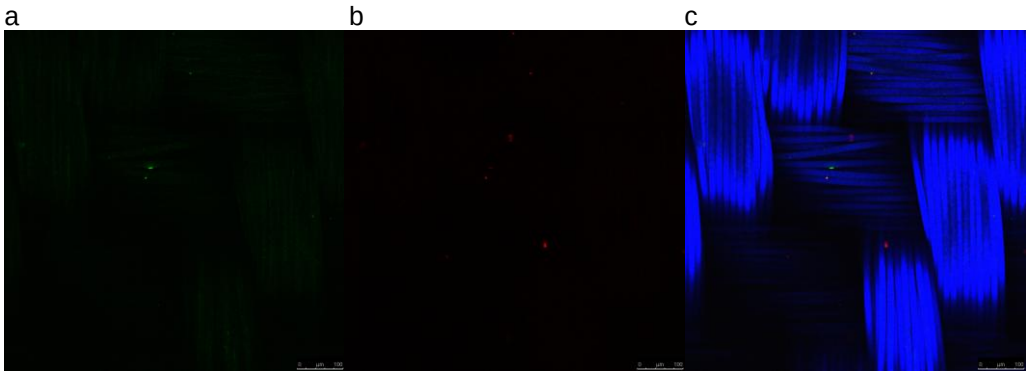

Fig. 22: CLSM analysis of 10 min UV irradiation of textile pieces (under side) incubated with *C. albicans*. a) viable cells (green), under side. b) dead cells (red), under side. c) overlay, under side. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

In Fig. 22, textile pieces (under side) were incubated with *C. albicans* and finally 10 min irradiated with an UV-lamp. No microorganisms could be detected on the under sides of textile pieces. This indicated that yeasts are too big for penetrating through the clean room fabrics.

The following images present the comparison of CLSM and wipe test after UV-treatment of *S. aureus* incubated textile pieces.

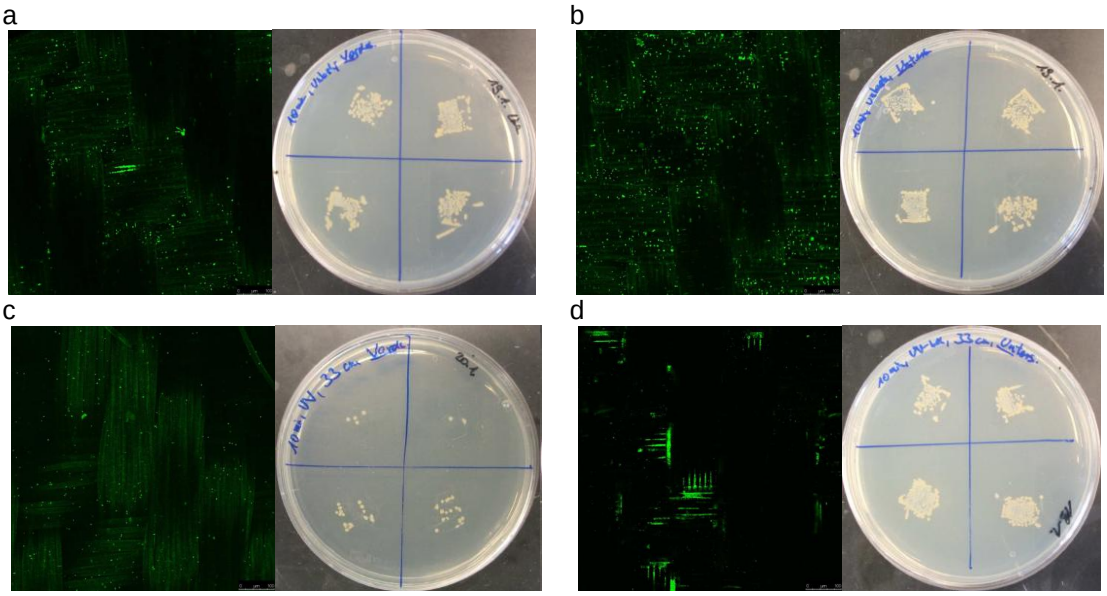

Fig. 23: Comparison of CLSM and wipe test after UV-treatment. a) *S. aureus* → textile without treatment, upper side (show figure 13a and 12a). b) *S .a*ureus → textile without treatment, under side (show figure 13d and 12d). c) *S. aureus* → 10 min UV irradiation, upper side (show figure 15a and 12c). d) *S. aureus* → 10 min UV irradiation, under side (show figure 15d and 12f). Scale bar 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

Fig. 23 shows a reduction of microorganisms on the upper side of textiles which was detectable after 10 min UV irradiation. On the under side of the textile living microorganisms are visible (d) in comparison to the upper side (c), because the under side was not exposed to UV-light.

3.2.2 Change in the textile structure after UV irradiation

UV radiation is very powerful and can affect the surface of the polyester. A change in the surface functionalities of especially clean room textiles might reduce the durability of the clean room garment. Subsequent experiments indicate a change in the surface structure of the textile fiber after UV irradiation.

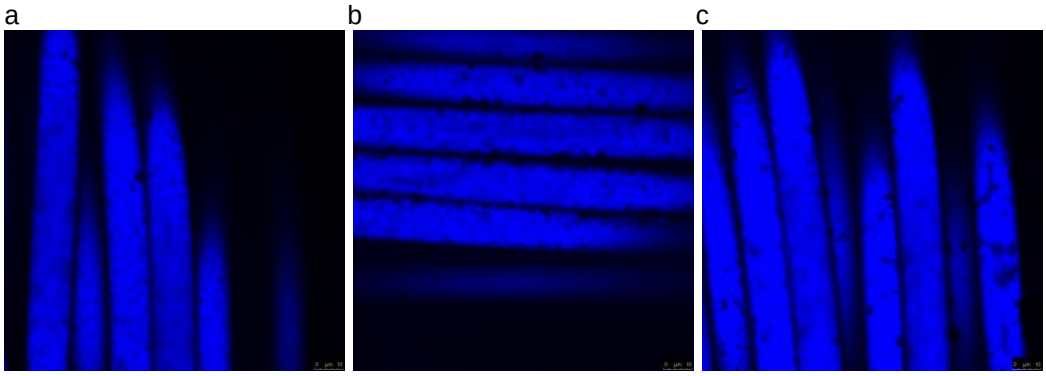

Fig. 24: Change in the textile structure (blue) after UV irradiation analyzed with CLSM. a) untreated textile. b) textile structure after 10 min UV irradiation. c) textile structure after 20 min UV irradiation. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

Fibers treated with UV light for 10 minutes (Fig. 24) showed an increase of the black spots on and between the fibers (blue) which were clearly identified as pinholes. These pinholes are a perfect niche for bacteria. In these recesses microorganisms can accumulate and thus they are not directly exposed to UV radiation as well. Fig. 24c indicates that the fiber changed slightly after UV-irradiation and more holes or craters were visible after the treatment with UV light.

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3.2.3 Sterile samples

The sterility of the textile pieces was conducted with wipe test and with CLSM. Sterile samples were important for all experiments to except contamination and to ensure their veracity.

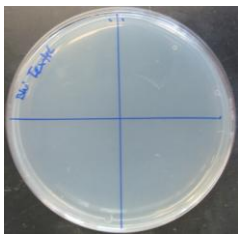

Fig. 25: Sterile sample of textile examined with wipe test. The plate was incubated for 48 hours.

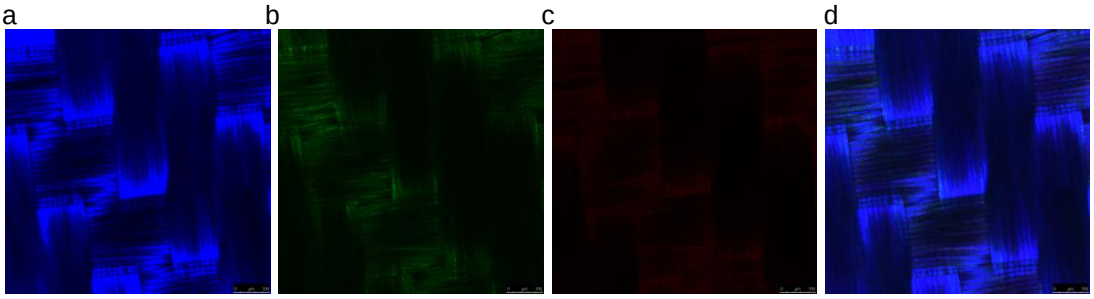

Fig. 26: Sterile sample of textile examined with CLSM. a) textile. b) green-fluorescent SYTO® 9. c) red-fluorescent propidium iodide. d) overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

Both wipe test and CLSM proved the sterility of the sterile samples (Fig. 25 and 26). This test was also a comparison of the traditional wipe test with CLSM analytics. Wipe test can only detect cells, which are on the very upper layer of the textile sample. Cells which are between the fibers or attached to small niches will not be present on the agar plate. Therefore staining of the bacterial cells with fluorescent dyes and observation with CLSM will give a profound insight in the complex interaction of bacteria with textile structures.

3.3 Treatment/Pre-treatment of textiles with disinfectants

The aim of this study was to treat and pre-treat clean room textiles with two different disinfectants, which were previously incubated with a *S. aureus*, *S. maltophilia* and *C. albicans* cell culture. The used disinfectants were Sterillium® hand disinfectant (STE) and Dr. Beckmann® Disinfectant Hygiene Spray (Bec). Both are commercially available and tested for application on the human skin. The disinfectant was applied prior to incubation with the pathogen and dried. The idea behind is a possible pre-treatment of textiles with antimicrobials which should give the clean room garment an antimicrobial function.

3.3.1 Treatment of textiles with disinfectants

In the following experiments textile pieces were treated with disinfectants. The used microorganisms were *S. aureus*, *S. maltophilia* and *C. albicans*. Finally, the textile pieces were analyzed with CLSM and with wipe test. As shown in Tab. 10 and 11 different modes of treatment were chosen.

Tab. 10: Differential treatment of textile pieces with disinfectants – wipe test. Differential treatment and incubation of textile pieces, which were previously incubated with *S.aureus* (S.a.).

Treatment/Pretratment	Incubation Time *S.a.*	Figure
S.a. → STE	5 min	27b
S.a. → STE	10 min	27c
S.a. → Bec	5 min	28b
S.a. → Bec	10 min	28c

Tab. 11: Differential treatment of textile pieces with disinfectants – CLSM. Differential treatment and incubation of textile pieces, which were previously incubated with *S.aureus* (S.a.).

Treatment/Pretratment	Incubation Time *S.a.*	Figure
S.a. → STE	5 min	29 a-c
S.a. → STE	10 min	29 d-f
S.a. → Bec	5 min	30 a-c
S.a. → Bec	10 min	30 d-f

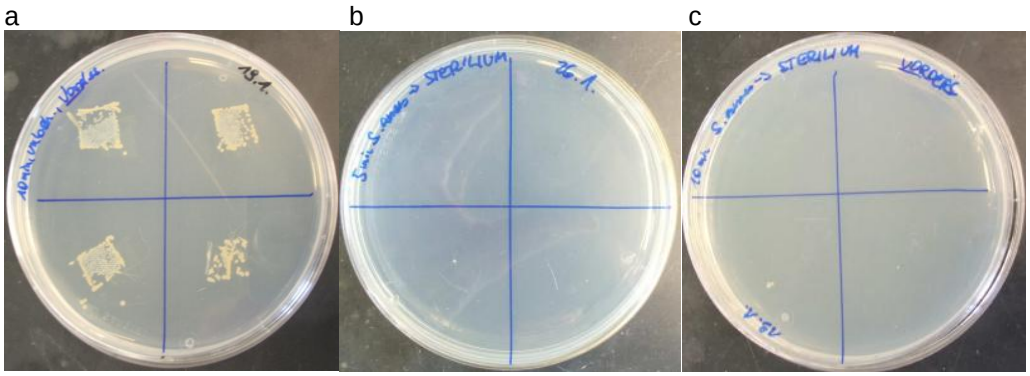

Fig. 27: Textiles were different treated with Sterillium and *S. aureus* - wipe test. The plates were incubated for 48 hours. a) 3 µl *S. aureus* on textile → control. b) 5 min incubation 3 µl *S. aureus* on textile → afterwards treatment with Sterillium. c) 10 min incubation of 3 µl *S. aureus* on textile → afterwards treatment with Sterillium.

Textile pieces were incubated with a defined volume of a growing culture of *S. aureus* and afterwards treated with Sterillium. After a certain incubation time, textile pieces were pressed on agar plate and incubated (Fig. 27). The results for the treatment with Sterillium show one growing colony after 5 min and two colonies after 10 min of incubation with *S. aureus*.

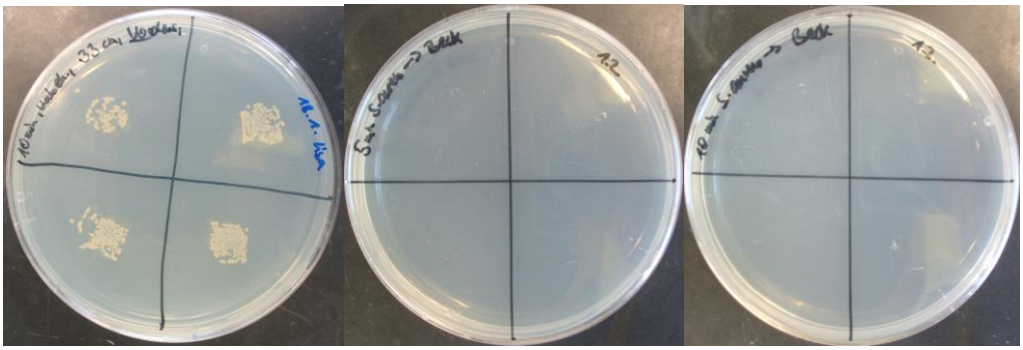

Fig. 28: Textiles were different treated with Dr. Beckman hygiene spray (Bec) and *S. aureus* - wipe test. The plates were incubated for 48 hours. a) 3 µl *S. aureus* on textile → control. b) 5 min incubation 3 µl *S. aureus* on textile → afterwards treatment with Bec. c) 10 min incubation of 3 µl *S. aureus* on textile → afterwards treatment with Bec.

Same results were achieved for the treatment of the textile pieces with Dr. Beckman hygiene spray. Textile pieces were analysed with wipe test. After incubation with *S. aureus* cell culture, textile pieces were treated with Dr. Beckman hygiene spray. There was no growing colony detectable after 5 min and 10 min incubation of *S. aureus*.

Wipe test is used for the testing of microbial contamination of surfaces. On complex surfaces like the matrix of a textile piece bacteria will attach very tightly to this surface and will not be detected by wipe test. Therefore the staining of bacterial cells with fluorescent dies will give us a more precise insight (Fig. 29). After treatment of textile pieces with Sterillium, which were inoculated with 3 µl of *S. aureus*, viable (a,d) and dead (b,e) cells were visible after fluorescent staining. As it was expected only a fraction of the total number of the cells on the surface of the textile piece was covered by wipe test. This could also be shown for textile pieces treated with Dr. Beckmann Disinfectant Hygiene Spray (Fig. 30). After treatment with Dr. Beckmann Disinfectant Hygiene Spray, a germ-reducing effect was observed in all experiments. The same results as per *S. aureus* were also shown by *S. maltophilia* and by *C. albicans* (data not shown).

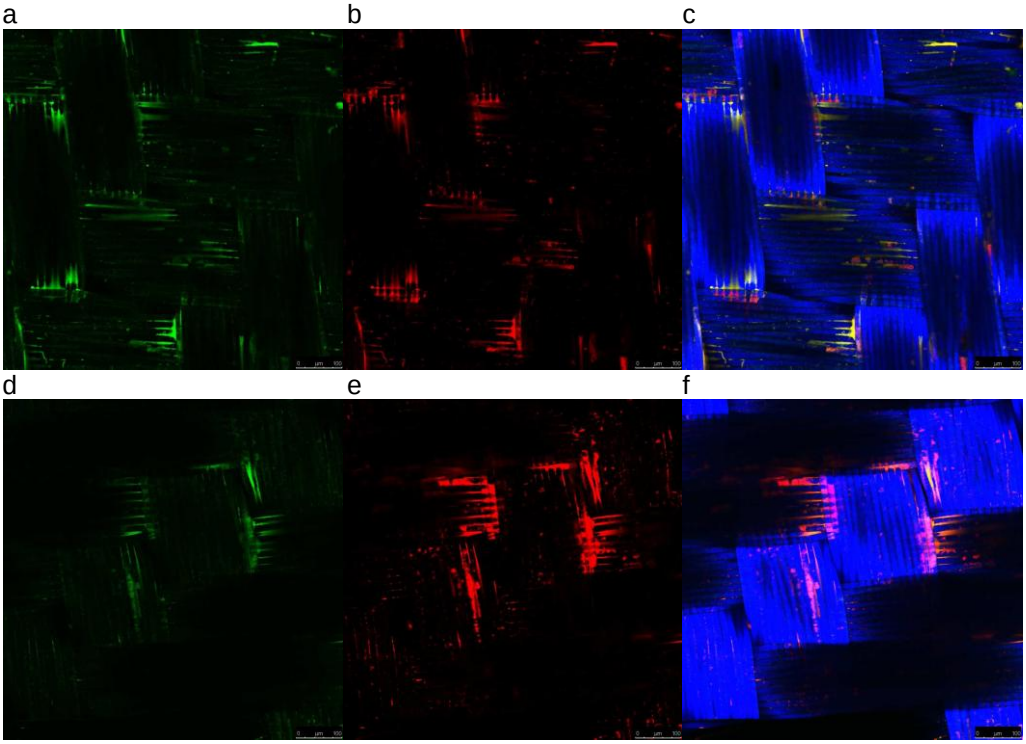

Fig. 29: Textiles were different treated with Sterillium and *S. aureus* - CLSM analysis. After incubation of *S. aureus*, textiles were treated with disinfectant Sterillium. a) 5 min incubation of *S. aureus*, afterwards treatment with Sterillium. Viable cells (green). b) 5 min incubation of *S. aureus*, afterwards treatment with Sterillium. Dead cells (red). c) 5 min incubation of *S. aureus*, afterwards treatment with Sterillium. Overlay. d) 10 min incubation of *S. aureus*, afterwards treatment with Sterillium. Viable cells (green). e) 10 min incubation of *S. aureus*, afterwards treatment with Sterillium. Dead cells (red). f) 10 min incubation of *S. aureus*, afterwards treatment with Sterillium. Overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

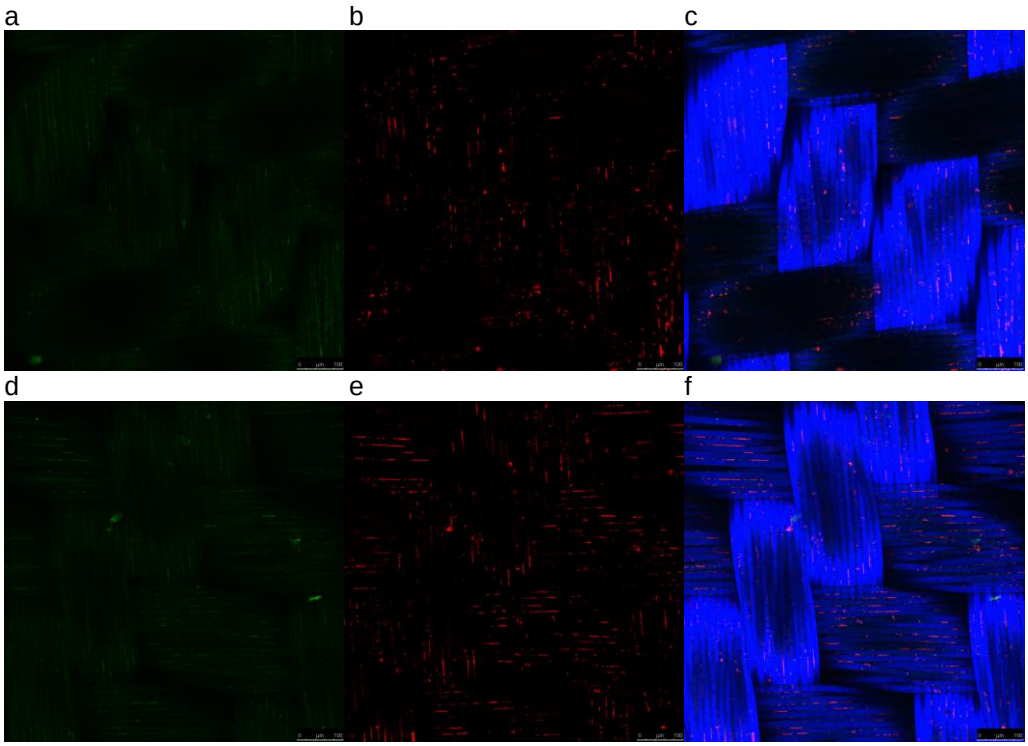

Fig. 30: Textiles were different treated with Dr. Beckmann Disinfectant Hygiene Spray and *S. aureus* – CLSM analysis. After incubation of *S. aureus*, textiles were treated with Dr. Beckmann Disinfectant Hygiene Spray. a) 5 min incubation of *S. aureus*, afterwards treatment with Dr. Beckmann. Viable cells (green). b) 5 min incubation of *S. aureus*, afterwards treatment with Dr. Beckmann. Dead cells (red). c) 5 min incubation of *S. aureus*, afterwards treatment with Dr. Beckmann. Overlay. d) 10 min incubation of *S. aureus*, afterwards treatment with Dr. Beckmann. Viable cells (green). e) 10 min incubation of *S. aureus*, afterwards treatment with Dr. Beckmann. Dead cells (red). f) 10 min incubation of *S. aureus*, afterwards treatment with Dr. Beckmann. Overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

3.3.2 Pre-treatment of textiles with disinfectants

Disinfectants reduce the cell growth of bacteria on textiles when applied after the incubation of the microbes on the surface of the textile. This wet application is the suggested way of application of these disinfectants. We were interested if an antimicrobial effect is still given when the substances of the chosen disinfectant are dried and no wet interaction is given. In the following experiments textile pieces were different pre-treated with disinfectants and textile pieces were completely dried. The used microorganisms were *S. aureus*, *S. maltophilia* and *C. albicans*. Finally, the textile pieces were analyzed with CLSM and with wipe test.

Tab. 12: Pre-treatment and incubation of textiles with Sterillium and *S. aureus*. Settings up for CLSM.

Incubation Time STE	Incubation Time *S.a.*	Figure
30 min → *S.a.*		31a
1 h → *S.a.*		31d
2 h → *S.a.*		31g
1 h → *S.a.*	20 h	32a
2 h → *S.a.*	20 h	32d
24 h → *S.a.*		32g
24 h → *S.a.*	20 h	32j

In Fig. 31, textile pieces were pre-treated (30 min, 1h, 2h) with Sterillium and afterwards incubated with *S. aureus*. After an incubation time of 30 min (STE) and growth limiting effect is visible but viable cells are still detectable. After one hour of incubation hardly any growing cell was visible and for 24 hours a total reduction of microorganism was observed.

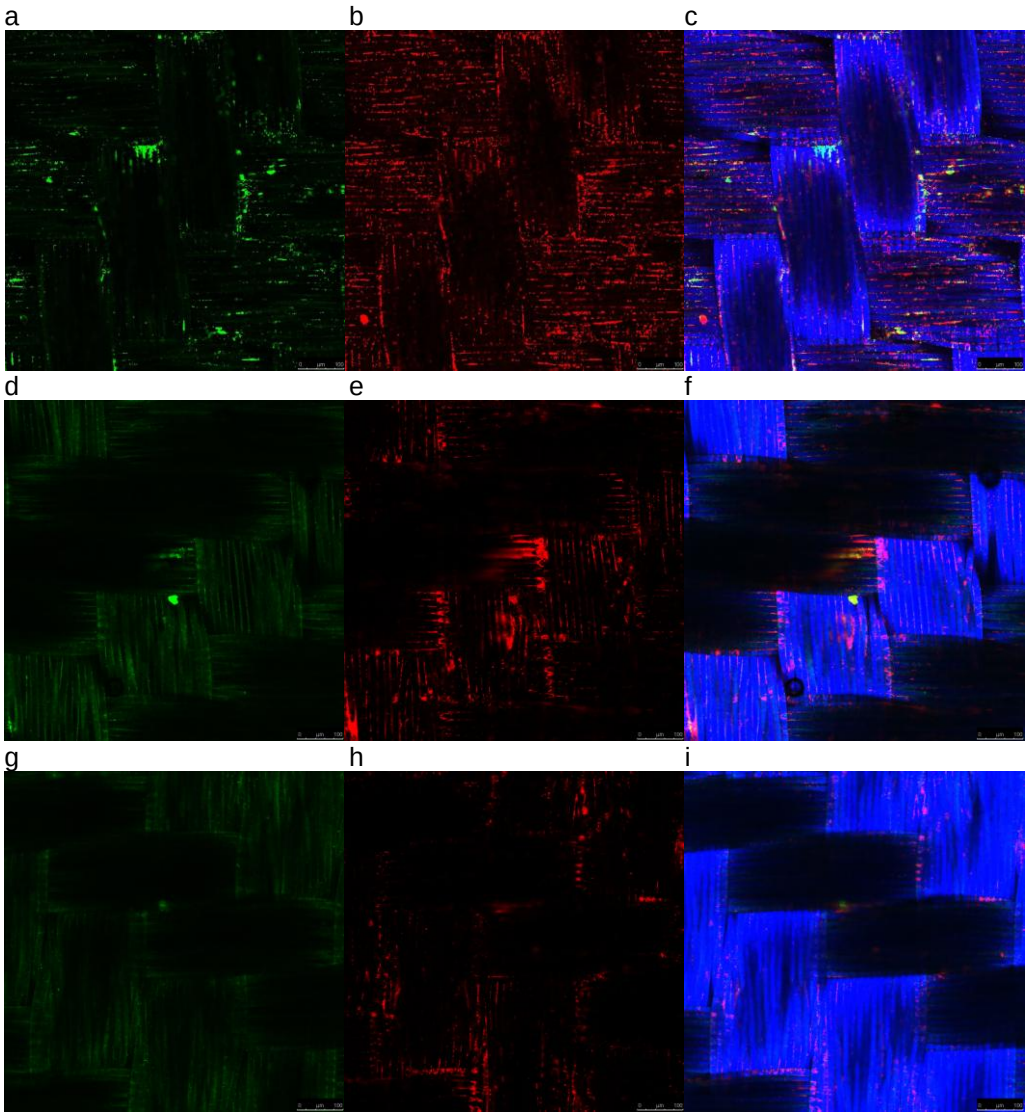

Fig. 31: Textiles were different pre-treated (30 min, 1h, 2h) with Sterillium and *S. aureus* - CLSM analysis. a) 30 min incubation of STE, afterwards treatment with *S. aureus*. Viable cells (green). b) 30 min incubation of STE, afterwards treatment with *S. aureus*. Dead cells (red). c) 30 min incubation of STE, afterwards treatment with *S. aureus*. Overlay. d) 1 h incubation of STE, afterwards treatment with *S. aureus*. Viable cells (green). e) 1 h incubation of STE, afterwards treatment with *S. aureus*. Dead cells (red). f) 1 h incubation of STE, afterwards treatment with *S. aureus*. Overlay. g) 2 h incubation of STE, afterwards treatment with *S. aureus*. Viable cells (green). h) 2 h incubation of STE, afterwards treatment with *S. aureus*. Dead cells (red). i) 2 h incubation of STE, afterwards treatment with *S. aureus*. Overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

A second experiment was performed where textile pieces were pre-treated with Sterillium for defined period of time. Cells were incubated for 20 to 24 hours (Fig. 32). For all samples an almost total reduction of microbial growth was detectable.

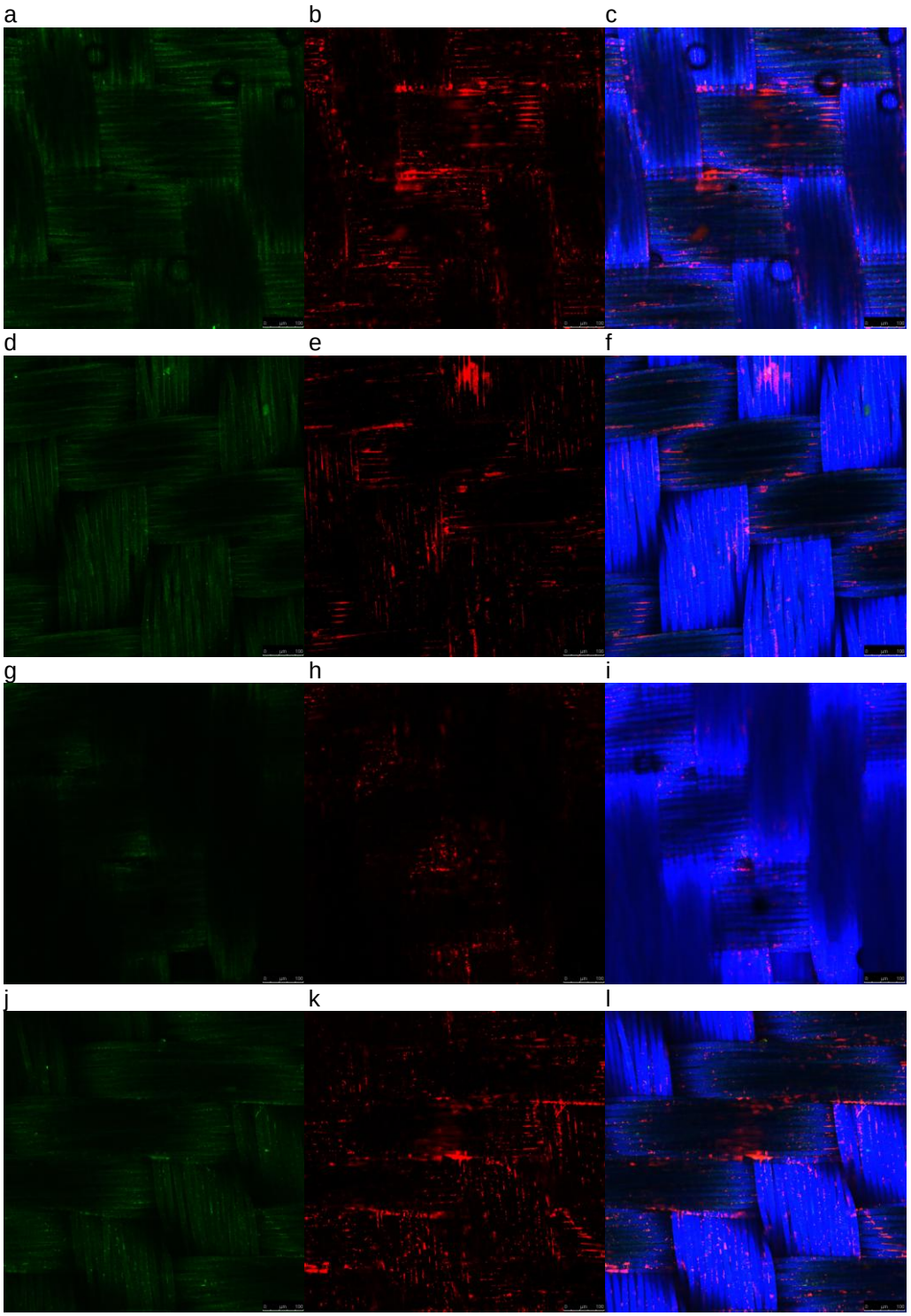

Fig. 32: Textiles were different pre-treated (1h, 2h, 24h) with Sterillium and *S. aureus* - CLSM analysis. a) 1 h Sterillium → *S.aureus* 20 h Incubation. Viable cells (green). b) 1 h Sterillium → *S.aureus* 20 h Incubation. Dead cells (red). c) 1 h Sterillium → *S.aureus* 20 h Incubation. Overlay. d) 2 h Sterillium → *S.aureus* 20 h Incubation. Viable cells (green). e) 2 h Sterillium → *S.aureus* 20 h Incubation. Dead cells (red). f) 2 h Sterillium → *S.aureus* 20 h Incubation. Overlay. g) 24 h Sterillium → *S.aureus*. Viable cells (green). h) 24 h Sterillium → *S.aureus*. Dead cells (red). i) 24 h Sterillium → *S.aureus*. Overlay. j) 24 h Sterillium → *S.aureus* 20 h incubation. Viable cells (green). k) 24 h

Sterillium → *S.aureus* 20 h incubation. Dead cells (red). l) 24 h Sterillium → *S.aureus* 20 h incubation. Overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

The same experimental setting was chosen for Dr. Beckmann Disinfectant Hygiene Spray and *S. aureus* (Fig. 33). The chosen disinfectant showed a slight better antimicrobial effect after drying on the textile piece in comparison to Sterillium. Incubation periods of 30 min to 2 hours proved a germ-reducing effect for all experiments after pre-treatment with Dr. Beckmann Disinfectant Hygiene Spray.

Tab. 13: Pre-treatment and incubation of textiles with Dr. Beckmann Disinfectant Hygiene Spray (Bec) and *S. aureus*. Settings up for CLSM.

Incubation Time Bec	Incubation Time *S.a.*	Figure
30 min → *S.a.*		33a
1 h → *S.a.*		33d
2 h → *S.a.*		33g
1 h → *S.a.*	20 h	34a
2 h → *S.a.*	20 h	34d
24 h → *S.a.*		34g
24 h → *S.a.*	20 h	34j

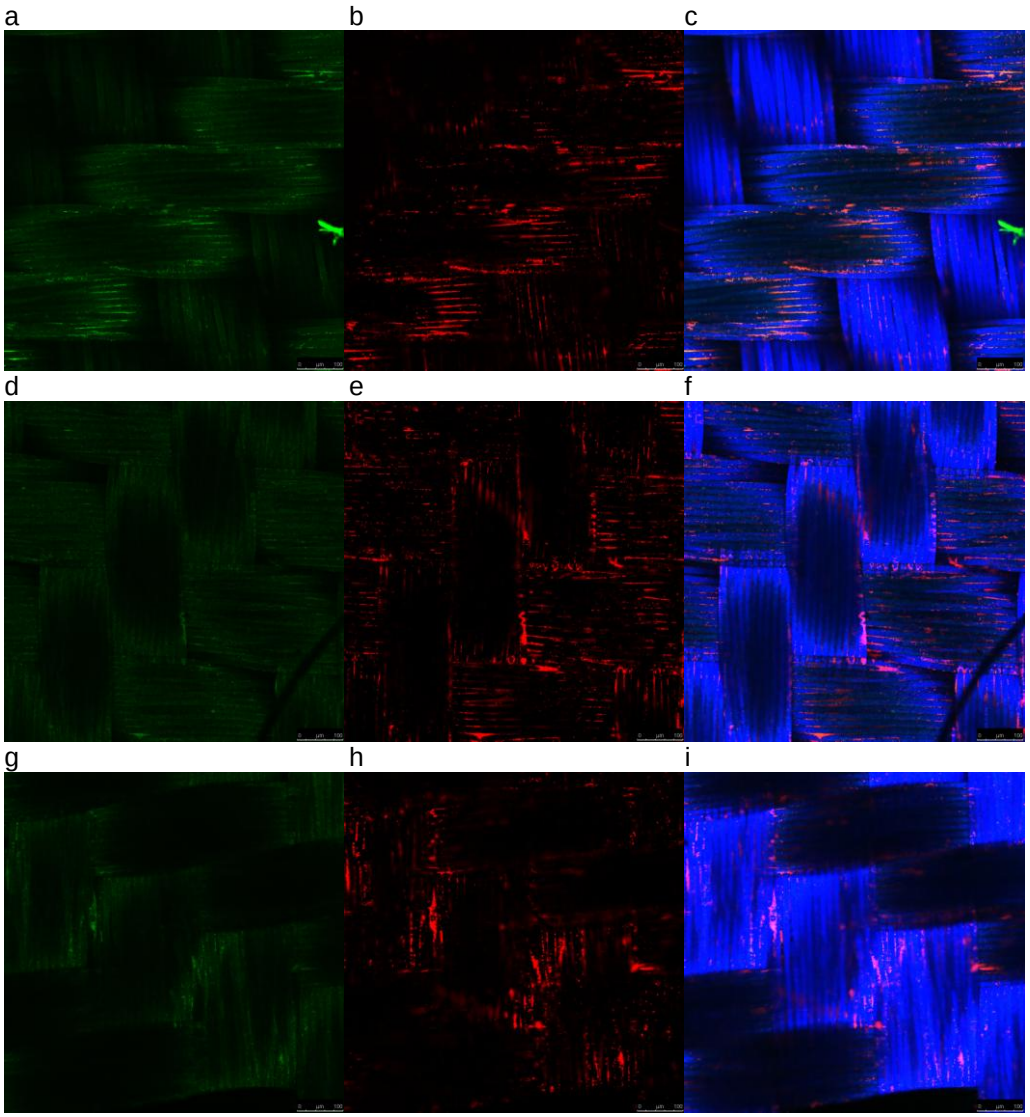

Fig. 33: Textiles were different pre-treated (30 min, 1h, 2h) with Dr. Beckmann Disinfectant Hygiene Spray and *S. aureus* - CLSM analysis. a) 30 min incubation of Dr. Beckmann, afterwards treatment with *S. aureus*. Viable cells (green). b) 30 min incubation of Dr. Beckmann, afterwards treatment with *S. aureus*. Dead cells (red). c) 30 min incubation of Dr. Beckmann, afterwards treatment with *S. aureus*. Overlay. d) 1 h incubation of Dr. Beckmann, afterwards treatment with *S. aureus*. Viable cells (green). e) 1 h incubation of Dr. Beckmann, afterwards treatment with *S. aureus*. Dead cells (red). f) 1 h incubation of Dr. Beckmann, afterwards treatment with *S. aureus*. Overlay. g) 2 h incubation of Dr. Beckmann, afterwards treatment with *S. aureus*. Viable cells (green). h) 2 h incubation of Dr. Beckmann, afterwards treatment with *S. aureus*. Dead cells (red). i) 2 h incubation of Dr. Beckmann, afterwards treatment with *S. aureus*. Overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

The results for pre-treated textile samples (Fig. 34) with Dr. Beckmann Disinfectant Hygiene Spray and *S. aureus* showed a reduction of microorganisms after pre-treatment up to 24 hours.

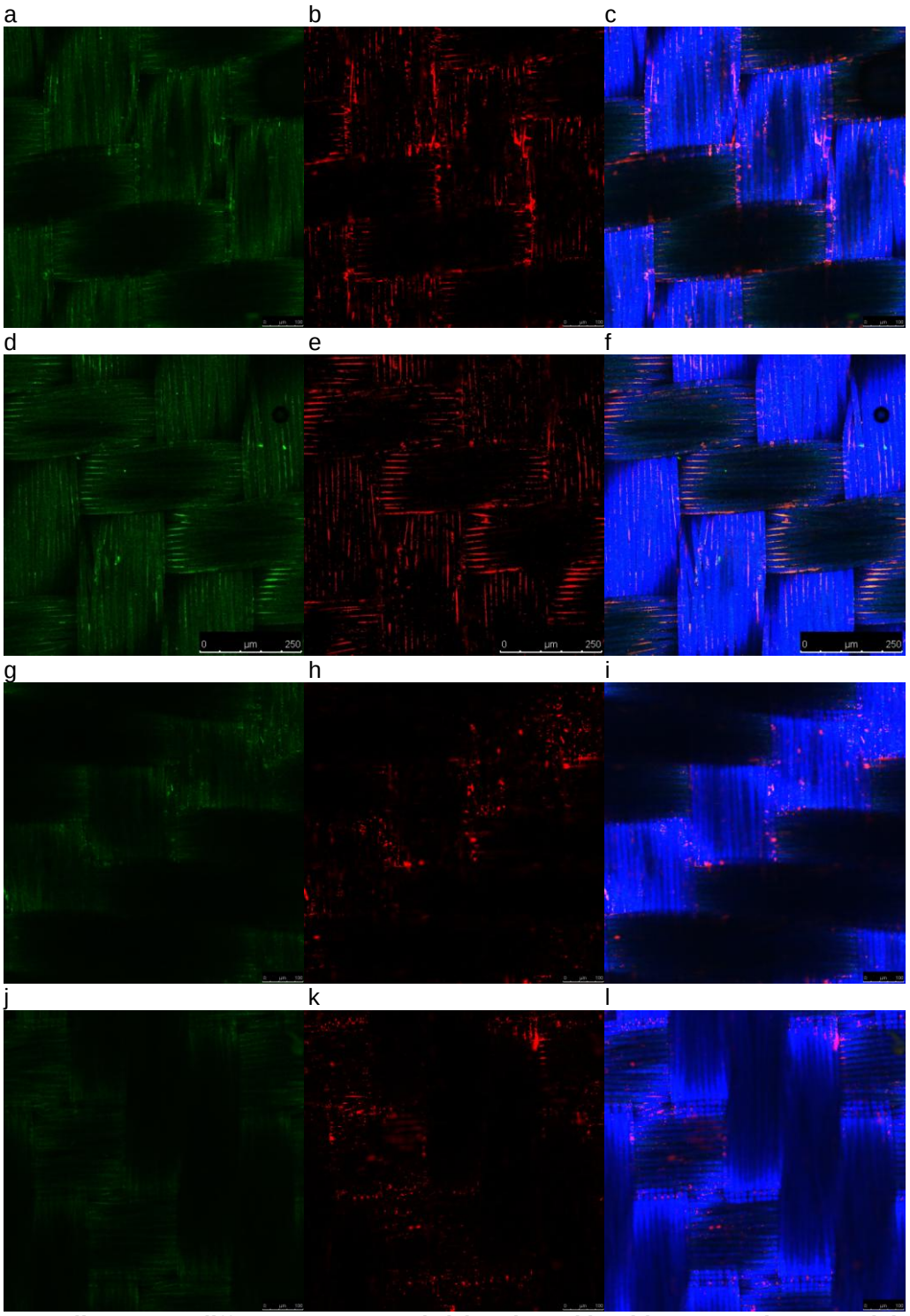

Fig. 34: Textiles were different pre-treated (1h, 2h, 24h) with Dr. Beckmann Disinfectant Hygiene Spray and *S. aureus* - CLSM analysis. a) 1 h Dr. Beckmann → *S.aureus* 20 h Incubation. Viable cells (green). b) 1 h Dr. Beckmann → *S.aureus* 20 h Incubation. Dead cells (red). c) 1 h Dr.

Beckmann → *S.aureus* 20 h Incubation. Overlay. d) 2 h Dr. Beckmann → *S.aureus* 20 h Incubation. Viable cells (green). e) 2 h Dr. Beckmann → *S.aureus* 20 h Incubation. Dead cells (red). f) 2 h Dr. Beckmann → *S.aureus* 20 h Incubation. Overlay. g) 24 h Dr. Beckmann → *S.aureus*. Viable cells (green). h) 24 h Dr. Beckmann → *S.aureus*. Dead cells (red). i) 24 h Dr. Beckmann → *S.aureus*. Overlay. j) 24 h Dr. Beckmann → *S.aureus* 20 h incubation. Viable cells (green). k) 24 h Dr. Beckmann → *S.aureus* 20 h incubation. Dead cells (red). l) 24 h Dr. Beckmann → *S.aureus* 20 h incubation. Overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

The potential antimicrobial effect of disinfectants was also tested for gram negative *S. maltophilia* cells. In Fig. 35, textile pieces were different pre-treated (30 min, 1h, 2h) with Sterillium and *S. maltophilia*. A germ-reducing effect was detected after pre-treatment of textile pieces (30 min, 1h, 2h) with Sterillium.

Tab. 14: Pre-treatment and incubation of textiles with Sterillium and *S. maltophilia*. Settings up for CLSM.

Incubation Time STE	Incubation Time *S.m.*	Figure
30 min → *S.m.*	15 min	35a
1 h → *S.m.*	15 min	35d
2 h → *S.m*.	15 min	35g

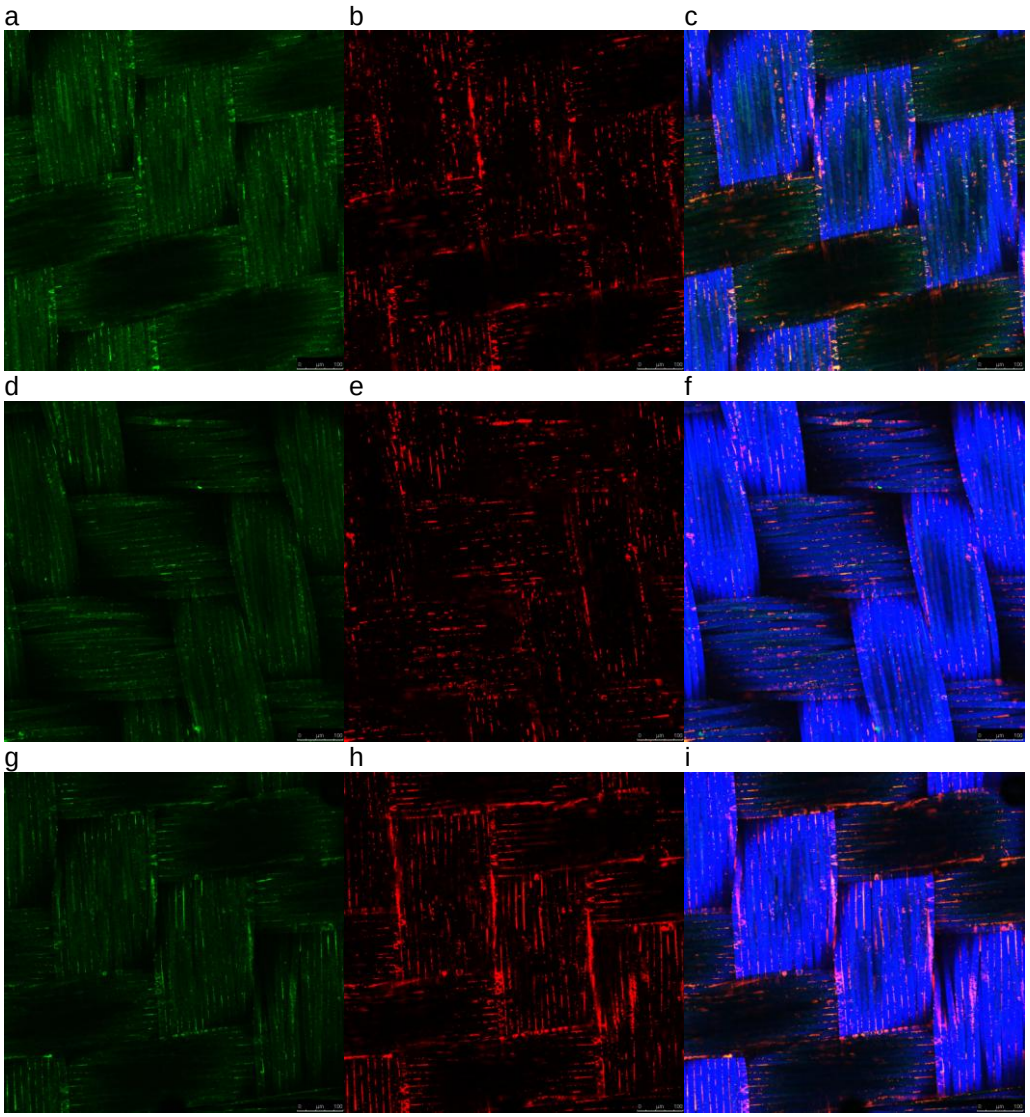

Fig. 35: Textiles were different pre-treated (30 min, 1h, 2h) with Sterillium and with *S. maltophilia* - CLSM analysis.

a) 30 min incubation of STE, afterwards treatment with *S. maltophilia*. Viable cells (green). b) 30 min incubation of STE, afterwards treatment with *S. maltophilia*. Dead cells (red). c) 30 min incubation of STE, afterwards treatment with *S. maltophilia*. Overlay. d) 1 h incubation of STE, afterwards treatment with *S. maltophilia*. Viable cells (green). e) 1 h incubation of STE, afterwards treatment with *S. maltophilia*. Dead cells (red). f) 1 h incubation of STE, afterwards treatment with *S. maltophilia*. Overlay. g) 2 h incubation of STE, afterwards treatment with *S. maltophilia*. Viable cells (green). h) 2 h incubation of STE, afterwards treatment with *S. maltophilia*. Dead cells (red). i) 2 h incubation of STE, afterwards treatment with *S. maltophilia.* Overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

Growth of *S. maltophilia* cells was also reduced after pre-treatment of textile pieces (30 min, 1h, 2h) with Dr. Beckmann Disinfectant Hygiene Spray and incubation for 20 hours.

Tab. 15: Pre-treatment and incubation of textiles with Dr. Beckmann Disinfectant Hygiene Spray (Bec) and *S. maltophilia*. Settings up for CLSM.

Incubation Time Bec	Incubation Time *S.m.*	Figure
30 min → *S.m.*	15 min	36a
1 h → *S.m.*	15 min	36d
2 h → *S.m*.	15 min	36g

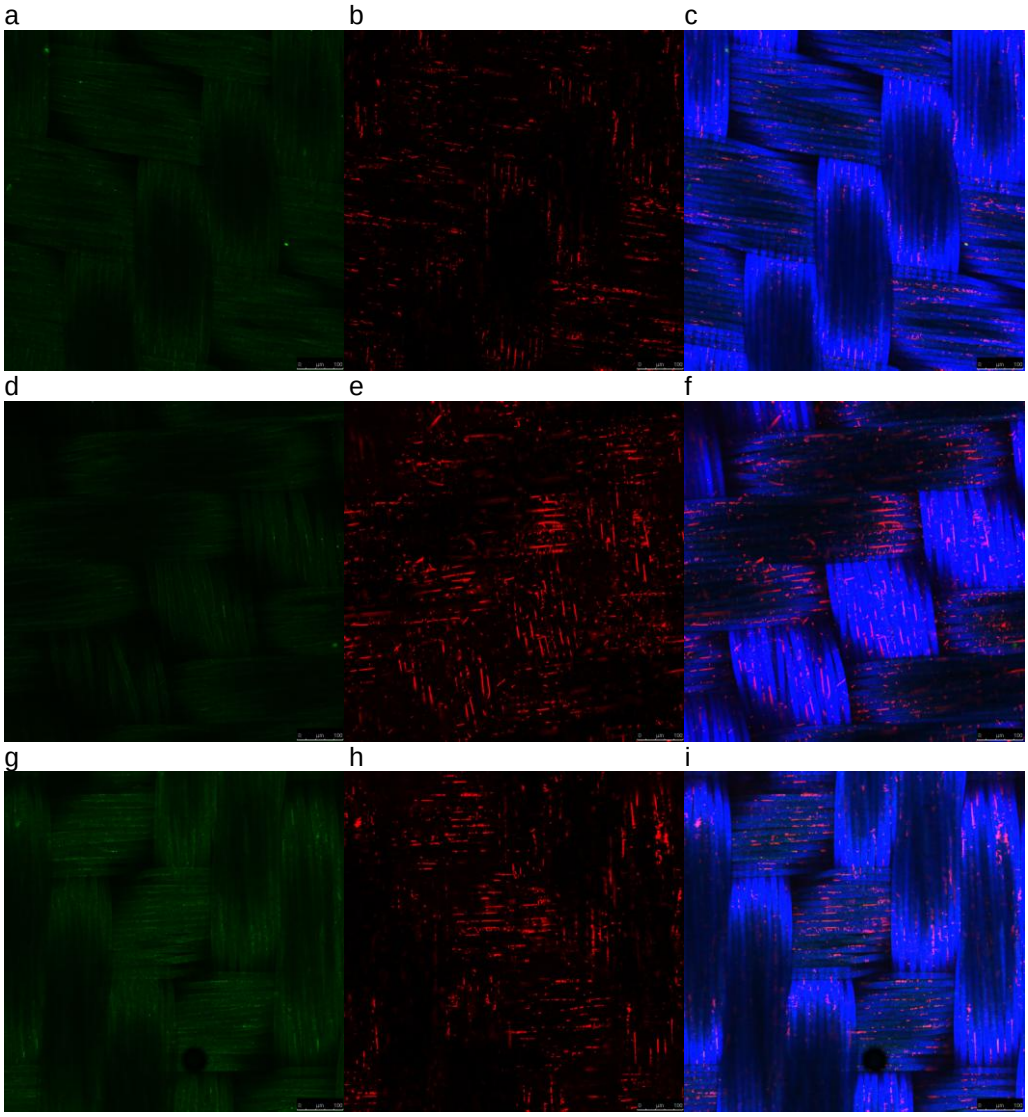

Fig. 36: Textiles were different pre-treated (30 min, 1h, 2h) with Dr. Beckmann Disinfectant Hygiene Spray and *S. maltophilia* - CLSM analysis.

a) 30 min incubation of Dr. Beckmann, afterwards treatment with *S. maltophilia*. Viable cells (green). b) 30 min incubation of Dr. Beckmann, afterwards treatment with *S. maltophilia*. Dead cells (red). c) 30 min incubation of Dr. Beckmann, afterwards treatment with *S. maltophilia*. Overlay. d) 1 h incubation of Dr. Beckmann, afterwards treatment with *S. maltophilia*. Viable cells (green). e) 1 h incubation of Dr. Beckmann, afterwards treatment with *S. maltophilia*. Dead cells (red). f) 1 h incubation of Dr. Beckmann, afterwards treatment with *S. maltophilia*. Overlay. g) 2 h incubation of Dr. Beckmann, afterwards treatment with *S. maltophilia*. Viable cells (green). h) 2 h incubation of Dr. Beckmann, afterwards treatment with *S. maltophilia*. Dead cells (red). i) 2 h incubation of Dr. Beckmann, afterwards treatment with *S. maltophilia*. Overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

Besides bacterial cells chosen disinfectants were also tested on their antimicrobial efficacy using yeast cells. *C. albicans* was chosen as test organism (Fig. 37). A reduction of *C. albicans* was detected after pre-treatment (30 min, 1h, 2h) with Sterillium.

Tab. 16: Pre-treatment and incubation of textiles with Sterillium and C. albicans. Settings up for CLSM.

Incubation Time STE	Incubation Time *C.a.*	Figure
30 min → *C.a.*	15 min	37a
1 h → *C.a.*	15 min	37d
2 h → *C.a.*	15 min	37g

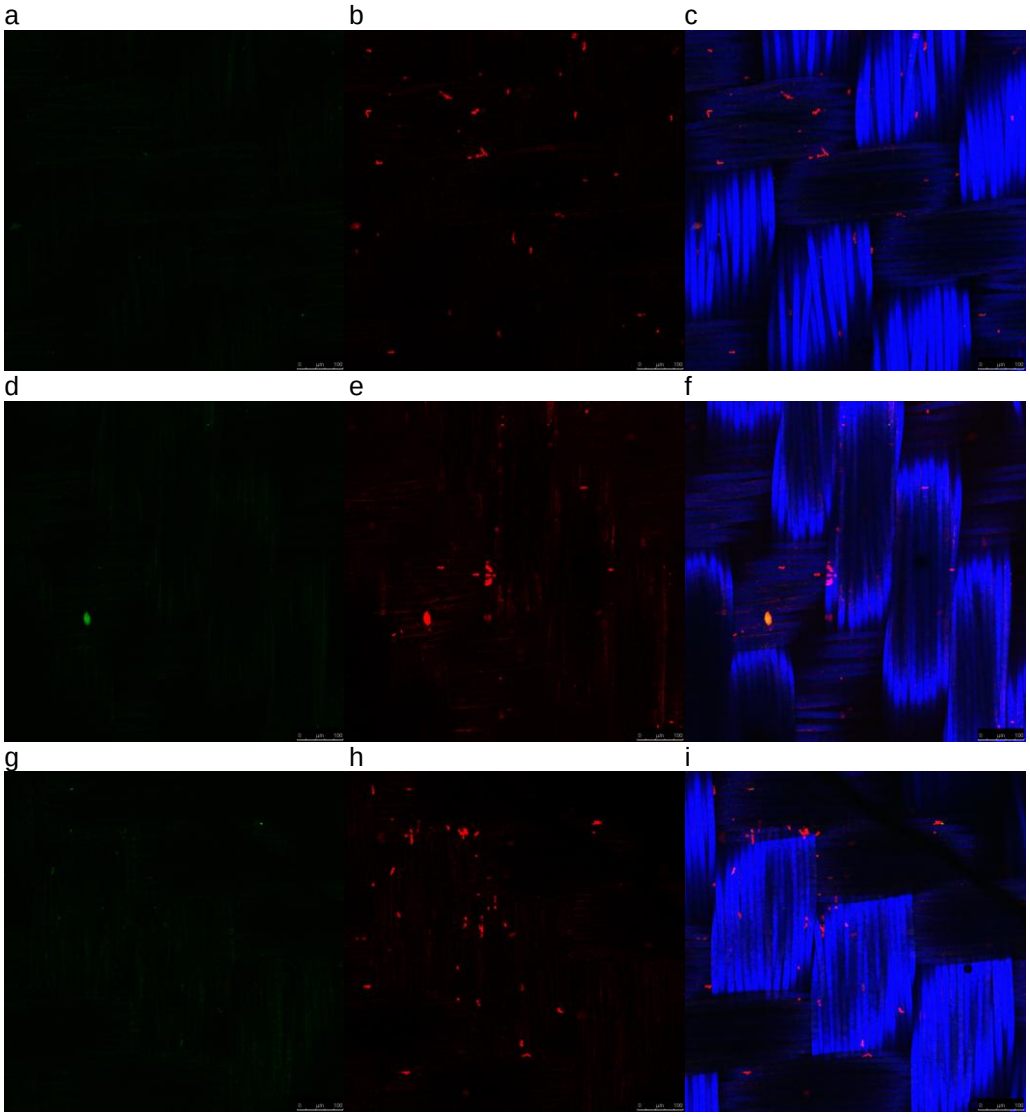

Fig. 37: Textiles were different pre-treated (30 min, 1h, 2h) with Sterillium and *C. albicans* - CLSM analysis.

a) 30 min incubation of STE, afterwards treatment with *C. albicans*. Viable cells (green). b) 30 min incubation of STE, afterwards treatment with *C. albicans*. Dead cells (red). c) 30 min incubation of STE, afterwards treatment with *C. albicans*. Overlay. d) 1 h incubation of STE, afterwards treatment with *C. albicans*. Viable cells (green). e) 1 h incubation of STE, afterwards treatment with *C. albicans*. Dead cells (red). f) 1 h incubation of STE, afterwards treatment with *C. albicans*. Overlay. g) 2 h incubation of STE, afterwards treatment with *C. albicans*. Viable cells (green). h) 2 h incubation of STE, afterwards treatment with *C. albicans*. Dead cells (red). i) 2 h incubation of STE, afterwards treatment with *C. albicans.* Overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

In Fig. 38, textile pieces were pre-treated with Dr. Beckmann Disinfectant Hygiene Spray and *C. albicans*. The same result was observed as with Sterillium. A germ-reducing effect was detected in all experiments by using Dr. Beckmann Disinfectant Hygiene Spray.

Tab. 17: Pre-treatment and incubation of textiles with Dr. Beckmann Disinfectant Hygiene Spray (Bec) and *C. albicans*. Settings up for CLSM.

Incubation Time Bec	Incubation Time *C.a.*	Figure
30 min → *C.a.*	15 min	38a
1 h → *C.a.*	15 min	38d
2 h → *C.a.*	15 min	38g

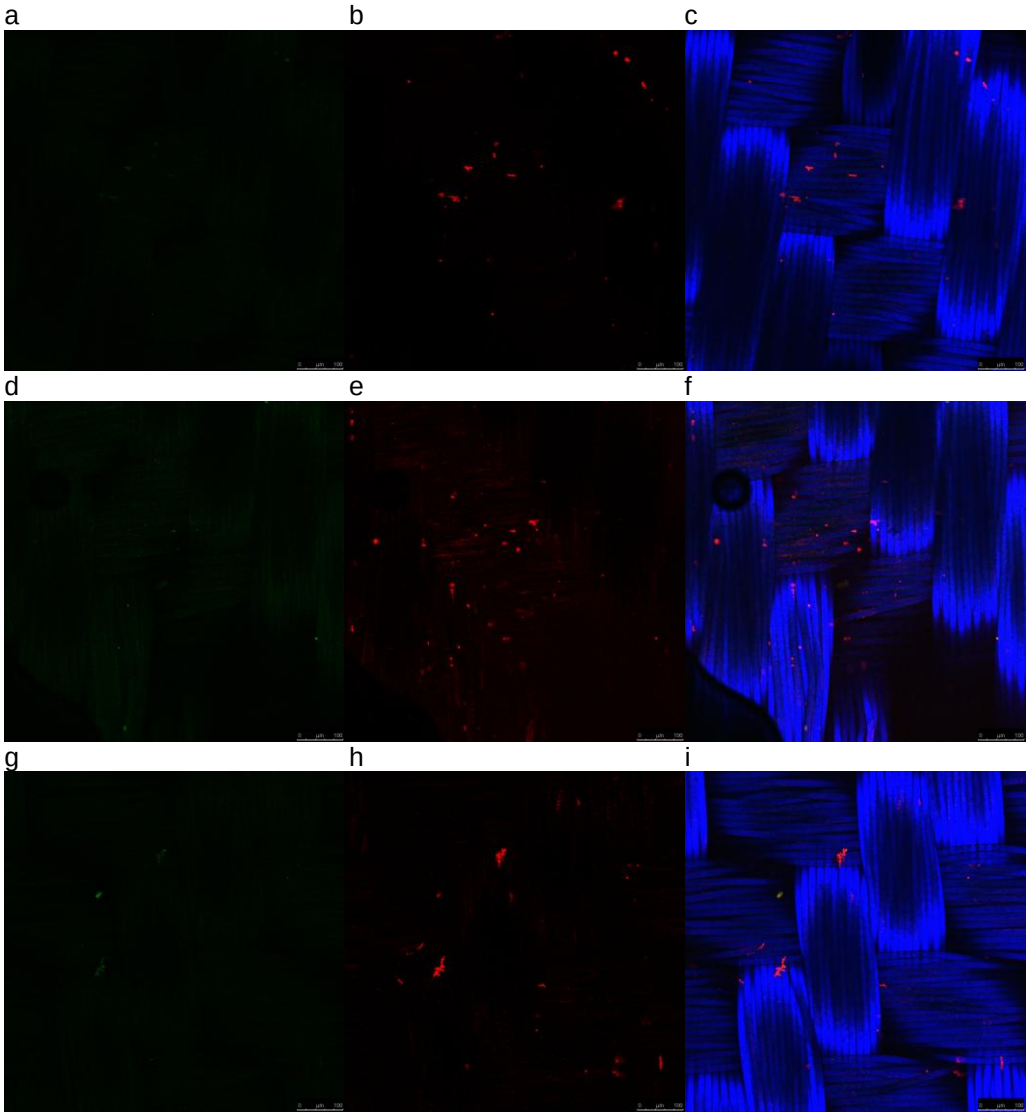

Fig. 38: Textiles were different pre-treated (30 min, 1h, 2h) with Dr. Beckmann Disinfectant Hygiene Spray and *C. albicans* - CLSM analysis.

a) 30 min incubation of Dr. Beckmann, afterwards treatment with *C. albicans*. Viable cells (green). b) 30 min incubation of Dr. Beckmann, afterwards treatment with *C. albicans*. Dead cells (red). c) 30 min incubation of Dr. Beckmann, afterwards treatment with *C. albicans*. Overlay. d) 1 h incubation of Dr. Beckmann, afterwards treatment with *C. albicans*. Viable cells (green). e) 1 h incubation of Dr. Beckmann, afterwards treatment with *C. albicans*. Dead cells (red). f) 1 h incubation of Dr. Beckmann, afterwards treatment with *C. albicans*. Overlay. g) 2 h incubation of Dr. Beckmann, afterwards treatment with *C. albicans*. Viable cells (green). h) 2 h incubation of Dr. Beckmann, afterwards treatment with *C. albicans*. Dead cells (red). i) 2 h incubation of Dr. Beckmann, afterwards treatment with *C. albicans*. Overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

Disinfectants are generally applied in a wet stage when treating surfaces. It was expected that there will be no antimicrobial effect of the chosen formulation when used in a dry stage. As the results show we could reach a very high rate of cell reduction even after drying of the textile pieces. This finding shows that there is a high potential of impregnating textiles with antimicrobial formulation to reach an antimicrobial effect.

3.4 Retention capacity of clean room textile

Clean room textile is intended to hinder particles and microorganisms from the wearer's body to avoid contamination in the environment. Therefore clean room textiles are to be sterilized after washing. In this experiment, a washing test was carried out. As mentioned before the complex matrix of the textile structure offer many niches for the attachment of microbes to the polymeric fibers. After washing, textiles had been investigated for their potential of retention of microorganisms using CLSM and wipe test. The used microorganisms were *S. aureus*, *S. maltophilia* and *C. albicans*.

After inoculation of textile pieces with 3 µl (10^6 CFU ml^{-1}) of a cell suspension of *S. aureus*, the textile pieces were washed for 30 minutes (Fig. 39). Before washing, many viable cells were visible (Fig. 39a). After washing, a significant reduction of viable *S. aureus* cells (Fig. 39d) was detectable. The washing of the textile pieces should remove both viable and dead cells but CLSM shows a big quantity of dead cells (Fig. 39e), which adhere to the textile. These bacterial fragments may be released from the textile as organic particles in the clean room area.

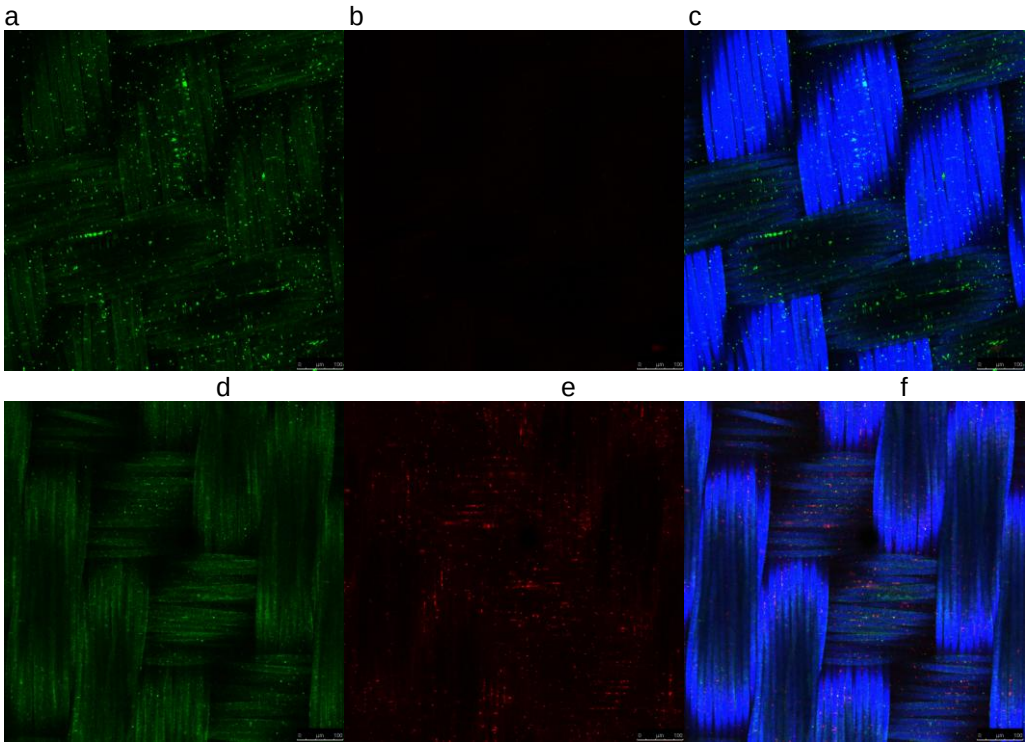

Fig. 39: Washing test analyzed with CLSM (*S. aureus*). a) viable cells (green) before washing. b) dead cells (red) before washing. c) Overlay before washing. d) viable cells (green) after washing. e) dead cells (red) after washing. f) Overlay after washing. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

Fig. 40 shows the comparison of plating the washing solution and CLSM (*S. aureus*). *S. aureus* cells were dramatically killed during the washing procedure but there was no real removement of cell material from the textile surface. What also could be shown was the fact that wipe test did not show all living cells after washing. CLSM shows alive cells after 30 minutes of washing closely attached to the fibers.

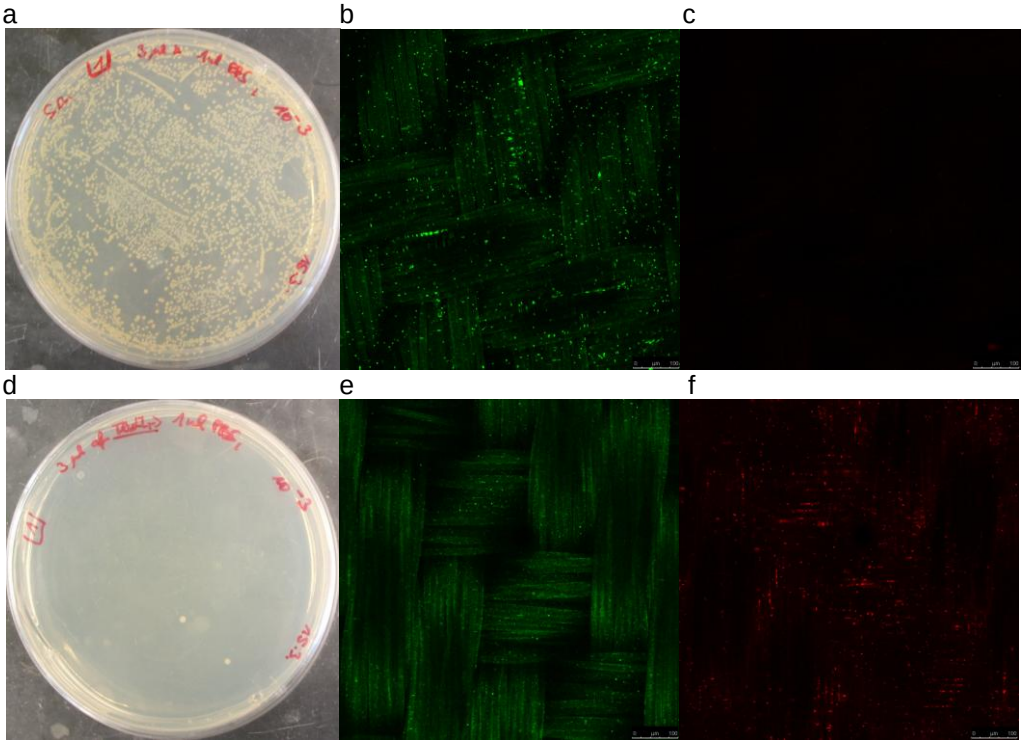

Fig. 40: Comparison of plating and CLSM (*S. aureus*). a) 3 µl *S. aureus* culture were pipetted in 1 ml PBS. 100 µl of the mixture was plated out. b) 3 µl *S. aureus* on textile before washing; viable cells (green). c) 3 µl *S. aureus* on textile before washing; dead cells (red). d) 3 µl *S. aureus* culture were pipetted on textile. After incubation, the textile was washed in 1 ml PBS and 100 µl of the supernatant was plated out. e) 3 µl *S. aureus* on textile after washing; viable cells (green). f) 3 µl *S. aureus* on textile after washing; dead cells (red). Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight^{TM}).

After inoculation of textile pieces with 3 µl (10^6 CFU ml^{-1}) of a cell suspension of *S. maltophilia* (Fig. 41), the textile pieces were washed for 30 minutes (Fig. 41 d-f). The same result as on textile pieces inoculated with *S. aureus* was observed. Before washing, many viable cells were visible (Fig. 41 a). After washing, a big quantity of dead cells (Fig. 41e), which adhere to the textile, was observed.

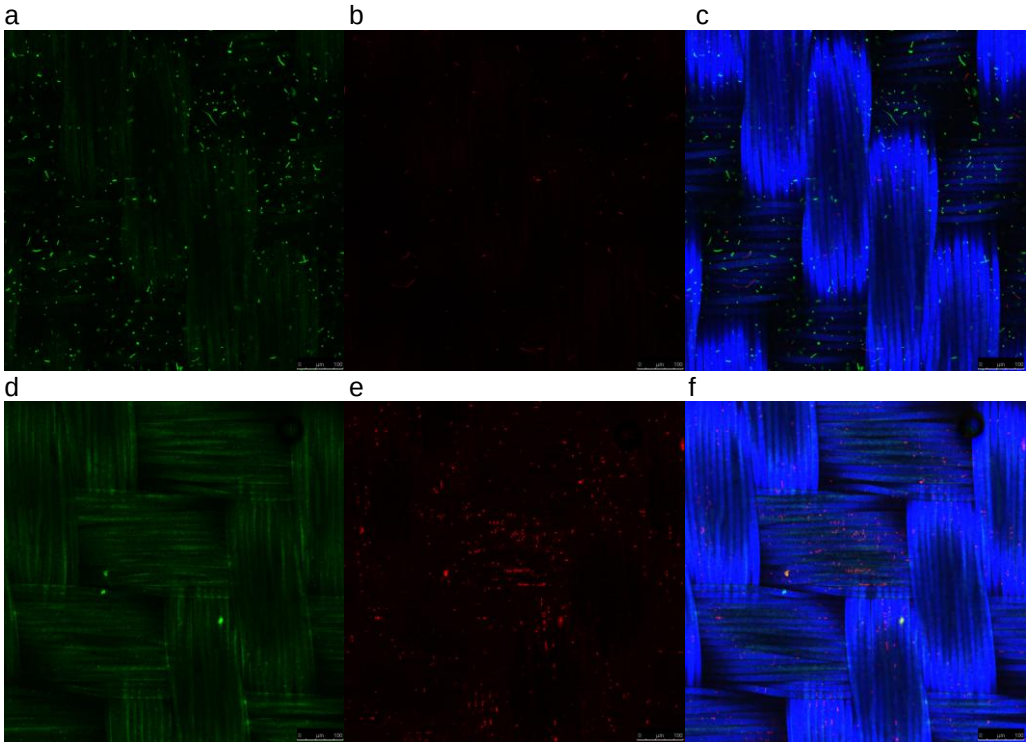

Fig. 41: Washing test analyzed with CLSM (*S. maltophilia*). a) viable cells (green) before washing. b) dead cells (red) before washing. c) Overlay before washing. d) viable cells (green) after washing. e) dead cells (red) after washing. f) Overlay after washing. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

After inoculation with 3 µl of a cell suspension of *C. albicans* (Fig. 42), the textile pieces were washed for 30 minutes (Fig. 42 d-f). The same result as on textile pieces inoculated with *S. aureus* and *S. maltophilia* was observed. After washing, a big quantity of dead cells (Fig. 42e), which adhere to the textile, were observed too.

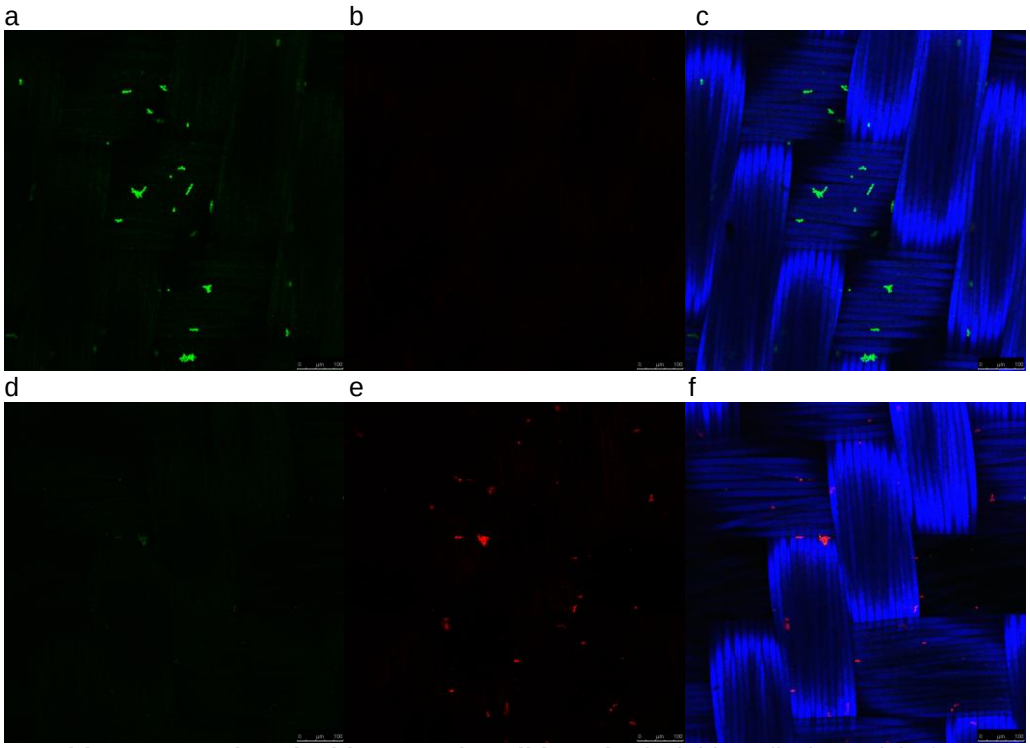

Fig. 42: Washing test analyzed with CLSM (*C. albicans*). a) viable cells (green) before washing. b) dead cells (red) before washing. c) Overlay before washing. d) viable cells (green) after washing. e) dead cells (red) after washing. f) Overlay after washing. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

Tab. 18: Washing test analyzed with plating. The tested microorganisms were *S. aureus*, *S. maltophilia* and *C. albicans*.

Washing test - plating	
Staphylococcus aureus	
3 µl *S. aureus* culture in 1 ml PBS	1.9×10^{9} CFU ml^{-1}
3 µl *S. aureus* culture on textile → in 1 ml PBS → wash for 30 min	2.0×10^{4} CFU ml^{-1}
Stenotrophomonas maltophilia	
3 µl *S. maltophilia* culture in 1 ml PBS	6.4×10^{8} CFU ml^{-1}
3 µl *S. maltophilia* culture on textile → in 1 ml PBS → wash for 30 min	8.0×10^{4} CFU ml^{-1}
Candida albicans	
3 µl *C. albicans* culture in 1 ml PBS	3.5×10^{7} CFU ml^{-1}
µl *C. albicans* culture on textile → in 1 ml PBS → wash for 30 min	2.0×10^{5} CFU ml^{-1}

The results from the washing procedure showed two things. First cell were not removed from the textile surface after 30 minutes of rigid treatment. Cells closely attached to the fibers and could not be released by the harsh washing procedure. These dead particles could be a serious problem for clean room environment. Second once more the CLSM analytics with fluorescent dyes gave a much more profound insight on the situation on the textile surface. Cells attached to the fiber or hidden in niches will not be detected by wipe test. This could be overcome by CLSM.

3.5 Treatment of clean room textiles with TiO_2

Titanium dioxide (TiO_2) is able to develop a strong antimicrobial activity when illuminated with light of a certain wavelength. Titanium dioxide, particularly in the anatase form, is a photocatalyst under ultraviolet (UV) light but also the usage of daylight show cell reducing effect. The aim of this study was to pre-treat textile pieces with a suspension containing titanium dioxide to obtain an antimicrobial property of the clean room textile. The used strains were *S. aureus*, *S. maltophilia* and *C. albicans*. Finally, the textile pieces were analyzed with CLSM.

After inoculation of TiO_2 pretreated textile, pieces were incubated with 3 µl (10^6 CFU ml^{-1}) of a cell suspension of *S. aureus*. Incubated cell pieces were illuminated with daylight for 10 minutes and the textile pieces were analyzed with CLSM (Fig. 43d-f). In comparison to the control (Fig. 43a-c), a big quantity of dead cells (Fig. 40e) was observed. This confirms that titanium dioxide showed a big germ-killing effect even under normal condition not using special light sources.

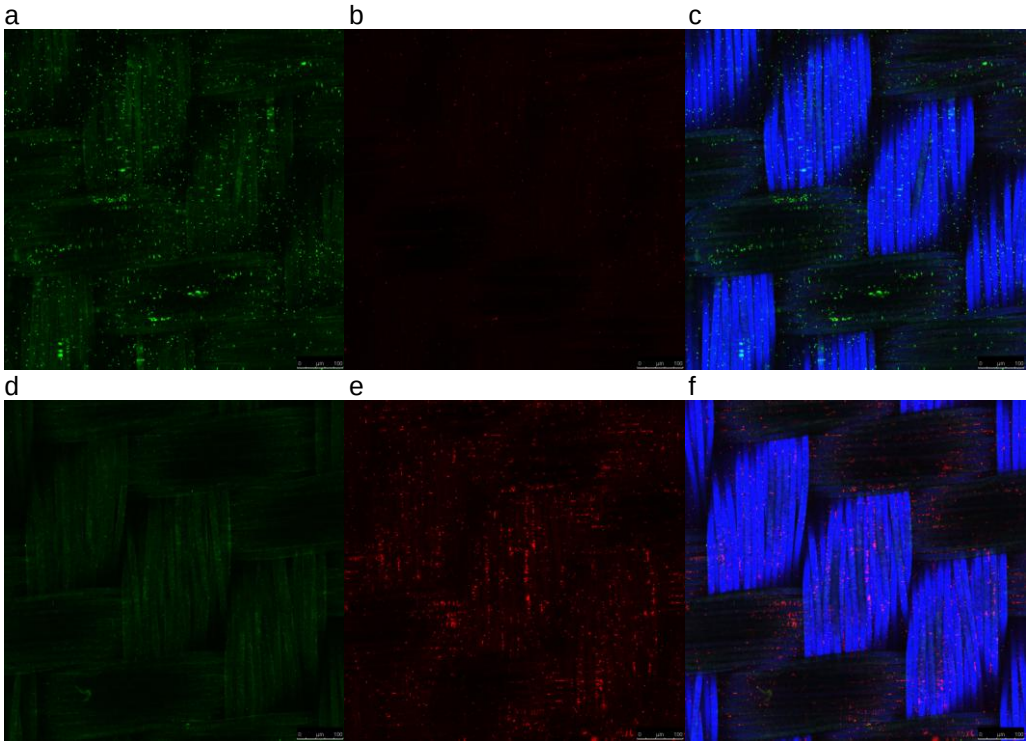

Fig. 43: TiO_2 pretreated clean room textiles inoculated with *S. aureus*. a) control; viable cells (green). b) control; dead cells (red). c) control; overlay. d) pretreated textile; viable cells (green). e) pretreated textile; dead cells (red). f) pretreated textile; overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

Besides *S. aureus* experiments with *S. maltophilia* were carried out. A significant reduction of living cells was found (Fig. 44d) in comparison to the control (Fig. 44a). A high mortality rate of MO (Fig. 44e) was visible.

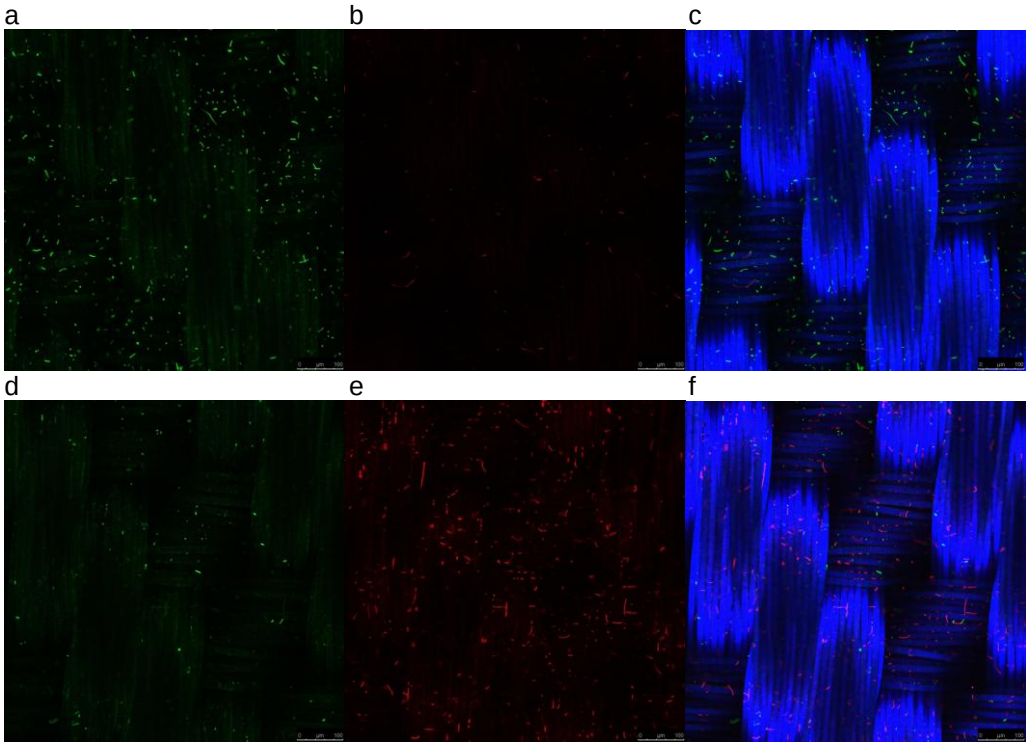

Fig. 44: TiO_2 pretreated clean room textiles inoculated with *S. maltophilia*. a) control; viable cells (green). b) control; dead cells (red). c) control; overlay. d) pretreated textile; viable cells (green). e) pretreated textile; dead cells (red). f) pretreated textile; overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

The antimicrobial effect of TiO_2 on a growing culture of *C. albicans* was tested. After inoculation of TiO_2 pretreated textile pieces with 3 µl of a cell suspension of *C. albicans*, the textile pieces were analyzed with CLSM (Fig. 45d-f). The same result as on textile pieces inoculated with *S. aureus* (Fig. 43) and *S. maltophilia* (Fig. 44) were observed. Pretreated clean room textile pieces showed a very good antimicrobial effect.

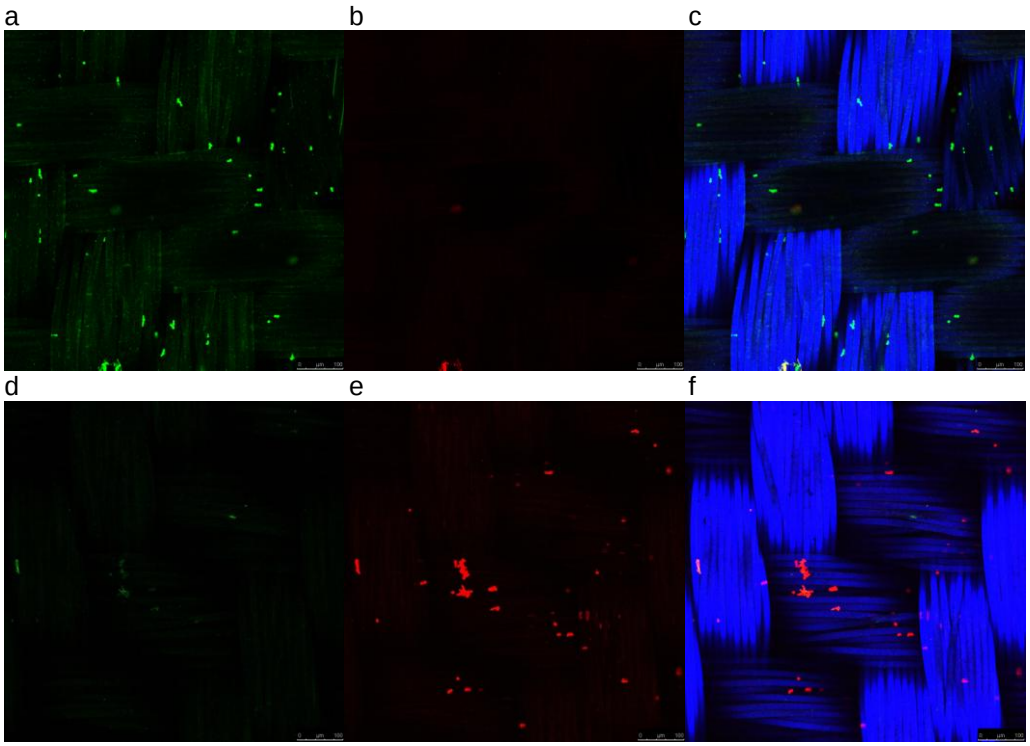

Fig. 45: TiO_2 pretreated clean room textiles inoculated with *C. albicans*. a) control; viable cells (green). b) control; dead cells (red). c) control; overlay. d) pretreated textile; viable cells (green). e) pretreated textile; dead cells (red). f) pretreated textile; overlay. Scale bar: 100 µm. For examination with CLSM, textile pieces were placed in a staining solution (LIVE/DEAD® Bacterial Viability Kit - BacLight™).

These results prove the antimicrobial potential TiO_2 when applied on clean room textiles. The germ killing process occurs under ambient conditions, no direct UV light exposure was needed for the increase of the rate of reaction.

3.6 Light activated disinfection

The aim of this experiment was to pre-treat clean room textiles with photosensitive dyes. They became activated by light and developed an antimicrobial effect. The dyes tested in this experiment were methylene blue (MB) and toluidine blue (TBO).
The dyes absorb light in the range of 530 – 700 nm, the absorption maximum is about 670 nm. The irradiation with light of defined wavelength was conducted with a black light lamp (370nm), a mercury lamp (515-560 nm), a laser (532nm, 15 mW) and with two different LEDs. In this experiment the effect of MB and TBO were tested directly on textile and with plating. The abbreviations for experiments are listed in Tab. 19.

Tab. 19: Abbreviations for experiments.

Abbreviations	Treatment
MB-/L-	without methylene blue / without irradiation
MB-/L+	without methylene blue / irradiation with a mercury lamp (3 min)
MB+/L-	with methylene blue / without irradiation
MB+/L+	with methylene blue / irradiation with a mercury lamp (3 min)
TBO-/L-	without toluidine blue / without irradiation
TBO -/L+	without toluidine blue / irradiation with a mercury lamp (3 min)
TBO +/L-	without toluidine blue / irradiation with a mercury lamp (3 min)
TBO +/L+	with toluidine blue / irradiation with a mercury lamp (3 min)

3.6.1 Growth control after irradiation with a mercury lamp

The following experiments present the results of treatments of *S. aureus*, *S. maltophilia* and *C. albicans* with methylene blue or toluidine blue and irradiation with a mercury lamp. The results were analyzed with wipe test and with plating.

Fig. 46 shows the growth control after treatments of *S. aureus* and *S. maltophilia* with methylene blue and light/mercury lamp for 3 minutes. After irradiation, no colony of *S. aureus*, but two colonies of *S. maltophilia* were detected.

a b

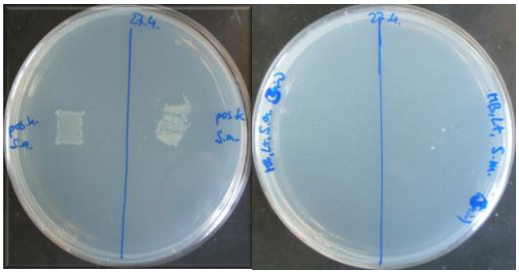

Fig. 46: Growth control after treatments of *S. aureus* and *S. maltophilia* with methylene blue and light/mercury lamp – wipe test. On the left side of the plates of a and b *S. aureus* is represented and on the right side *S. maltophilia* is represented. a) 3 µl cell culture on textile → control MB-/L-. b) 3 h MB incubated on textile → 3 µl cell culture on textile → 3 min irradiation with the mercury lamp (MB+/L+.).

In addition the cell killing effect was also tested for *C. albicans* (Fig. 47). After irradiation a significant reduction of bacteria was detected in all experiments. The results of this method exert a germ-reducing effect after irradiation with light of defined wavelength on textile pieces incubated with *S. aureus* (Fig 46), *S. maltophilia* (Fig. 46) and *C. albicans*.

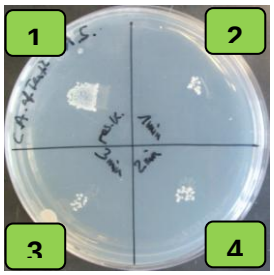

Fig. 47: Growth control after treatment of *C. albicans* with methylene blue and light/mercury lamp – wipe test. 1) 3 µl cell culture on textile → control MB-/L-. 2) One minute irradiation with the mercury lamp (MB+/L+). 3) Three minutes irradiation with the mercury lamp (MB+/L+). 4) Two minutes irradiation with the mercury lamp (MB+/L+).

It is known that toluidine blue also produce reactive oxygen species on illumination with light of a defined wavelength. When illuminated with a mercury lamp (Fig. 48) the results of growth control after treatment of *S. aureus* with toluidine blue showed a very fast reduction of the cell number on the textile piece. The results of all experiments with TBO correlated to all experiments with methylene blue. A significant reduction of microorganism was detected.

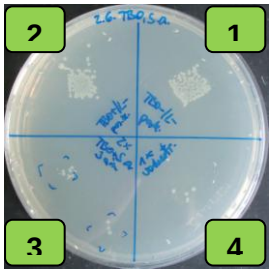

Fig. 48: Growth control after treatment of *S. aureus* with toluidine blue and light/mercury lamp – wipe test. 1) 3 µl cell culture on textile → control TBO-/L-. 2) 3 µl cell culture on textile → control TBO+/L-. 3) 3 h TBO incubated on textile → 3 µl cell culture on textile → 3 min irradiation with the mercury lamp. 4) 3 h TBO incubated on textile → 3 min irradiation with the mercury lamp → 3 µl cell culture on textile → 3 min irradiation with the mercury lamp.

The comparison of the two controls (a and b) in Fig. 49 already showed a slightly reducing of microorganisms because of the effect of the dye methylene blue alone (see results of plate counting in Tab. 20). After treatment of *S. aureus* with methylene blue and irradiation with a mercury lamp an excellent germ-reducing effect was detected. After an irradiation of three minutes, no growth was detected. These results are consistent with those of the wipe tests (Fig. 46). The same results were achieved with the microorganisms *S. maltophilia* and *C. albicans* too (figures not shown). A detailed determination was done with plating and is shown in Tab. 20.

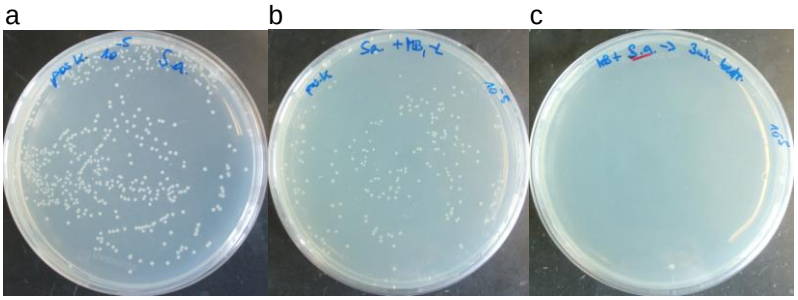

Fig. 49: Growth control after treatment of *S. aureus* with methylene blue and light/mercury lamp – plating. a) 50 µl *S. aureus* (10^{-5}) + 50µl PBS → control MB-/L-. b) 50 µl *S. aureus* (10^{-5}) + 50µl MB → control MB+/L-. c) 50 µl *S. aureus* (10^{-5}) + 50µl MB → 3 min irradiation with the mercury lamp (MB+/L+).

All experiments with toluidine blue (Fig. 50) gave the same result which was achieved as with MB. Even without irradiation a slight reduction of microorganisms was detected by TBO. After an irradiation of three minutes, no growth was detected. The same results were observed with the microorganisms *S. maltophilia* and *C. albicans* (figures not shown). A detailed determination of plating is shown in Tab. 20.

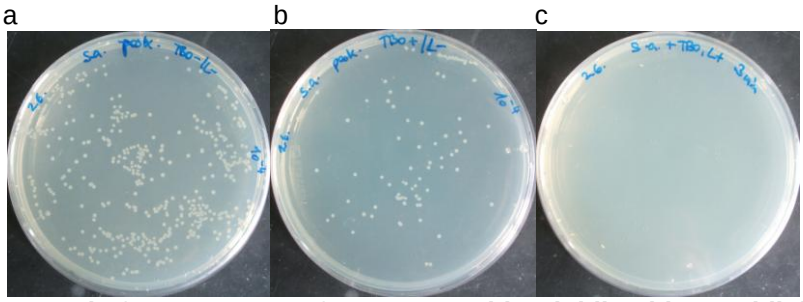

Fig. 50: Growth control after treatment of *S. aureus* with toluidine blue and light/mercury lamp – plating. a) 50 µl *S. aureus* (10^{-5}) + 50µl PBS → control TBO-/L-. b) 50 µl *S. aureus* (10^{-5}) + 50µl MB → control TBO +/L-. c) 50 µl *S. aureus* (10^{-5}) + 50µl TBO → 3 min irradiation with the mercury lamp (TBO +/L+).

Light activated disinfection of surfaces has a very high potential. For the activation of the specific dyes a certain wavelength is necessary. Interestingly we could show that even mercury lamps can activate the production of reactive oxygen species which kill bacteria in a very short period of time. It could be demonstrated that a total killing of microbes could be achieved after 3 minutes of illumination. As mentioned before both dyes have their adsorption maximum between 600 and 700nm. Therefore subsequent experiments will be carried out with light sources of defined wave length.

3.6.2 Growth control after irradiation of *S. aureus* with different lamps

The following experiment demonstrates the growth control after treatment of *S. aureus* with methylene blue with different lamps (blacklight, laserlight and LEDs). The results were analyzed with plating.

Fig. 51 shows the growth control after treatment of *S. aureus* with methylene blue and different lamps. All tested lamps (blacklight, laserlight and LEDs) indicated a germ-reducing effect. After irradiation with blacklight a germ-reducing effect was detected. A detailed determination of plating is shown in Tab. 21.

a b c d e

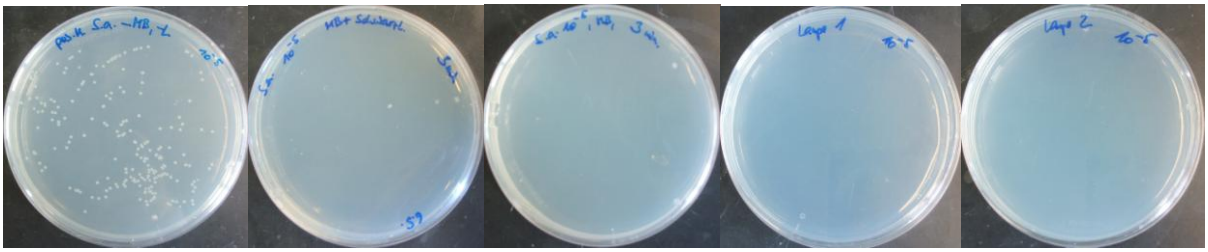

Fig. 51: Growth control after treatment of *S. aureus* with methylene blue and different lamps – plating. a) 50 µl *S. aureus* (10^{-5}) + 50µl PBS → control MB-/L-. b) 50 µl *S. aureus* (10^{-5}) + 50µl MB → 3 min irradiation with the **blacklight** (MB+/L+). c) 50 µl *S. aureus* (10^{-5}) + 50µl MB → 3 min irradiation with **laserlight** (MB+/L+). d) 50 µl *S. aureus* (10^{-5}) + 50µl MB → 2 min irradiation with the **LED-1** (MB+/L+). e) 50 µl *S. aureus* (10^{-5}) + 50µl MB → 2 min irradiation with the **LED-2** (MB+/L+).

3.6.3 Growth control after repeated irradiation of microorganisms

The following experiments present the growth control after treatment of *S. aureus* with MB and TBO and repeated irradiation with a mercury lamp. The results were analyzed with wipe test and with plating.

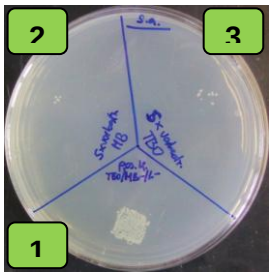

Fig. 52: Growth control after treatment of *S. aureus* with MB/TBO and repeated irradiation with a mercury lamp – wipe test. 1) 3 µl cell culture on textile → control MB-/L-. 2) 3 h **MB** incubated on textile → 5x irradiation of the textile → 3 µl cell culture on textile → 3 min irradiation with a mercury lamp (MB+/L+.) 3) 3 h **TBO** incubated on textile → 5x irradiation of the textile → 3 µl cell culture on textile → 3 min irradiation with a mercury lamp (MB+/L+).

The result of growth control after treatment of *S. aureus* with MB/TBO and repeated irradiation with a mercury lamp (Fig. 52) shows that both dyes (MB and TBO) retain their germ-reducing effect after repeated (five times) irradiation.

a b c d

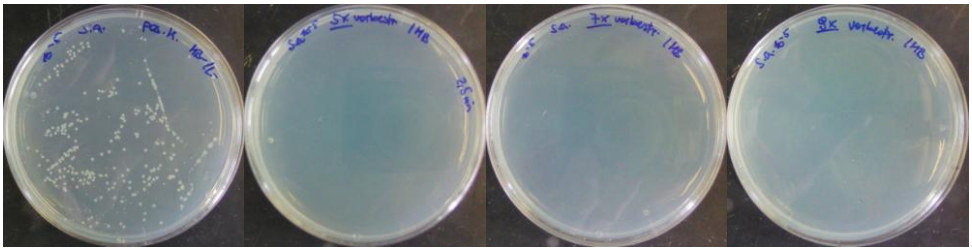

Fig. 53: Growth control after treatment of *S. aureus* with methylene blue and repeated irradiation with a mercury lamp – plating. a) 50 µl *S. aureus* (10^{-5}) + 50µl PBS → control MB-/L-. b) 5x irradiation of 50 µl MB → + 50 µl S. aureus → 3 min irradiation with the mercury lamp (MB+/L+). c) 7x irradiation of 50 µl MB → + 50 µl S. aureus → 3 min irradiation with the mercury lamp (MB+/L+). d) 9x irradiation of 50 µl MB → + 50 µl S. aureus → 3 min irradiation with the mercury lamp (MB+/L+).

Fig. 53 presents the growth control after treatment of *S. aureus* with methylene blue and repeated irradiation with a mercury lamp with plating. The results of the wipe test (Fig. 52) conform to plating. After repeated irradiation (5x, 7x and 9x), a significant reduction of microorganism was detected. This shows that the method can be repeated several times.

Tab. 20: Growth control after treatment with methylene blue/toluidine blue and light/mercury lamp. The tested microorganisms were *S. aureus, S. maltophilia and C. albicans.*

MB+/L+ mercury lamp		
S. aureus (10^{-5})	MB-/L-	2.3×10^{8} CFU ml^{-1}
	MB+/L-	1.7×10^{8} CFU ml^{-1}
	MB+/L+ (3min)	0 CFU ml^{-1}
S. maltophilia (10^{-5})	MB-/L-	4.9×10^{8} CFU ml^{-1}
	MB+/L-	1.1×10^{8} CFU ml^{-1}
	MB+/L+ (3min)	1.0×10^{5} CFU ml^{-1}
C. albicans (10^{-4})	MB-/L-	3.3×10^{7} CFU ml^{-1}
	MB+/L-	3.1×10^{7} CFU ml^{-1}
	MB+/L+ (3min)	2.3×10^{6} CFU ml^{-1}
TBO+/L+ mercury lamp		
S. aureus (10^{-5})	MB-/L-	3.8×10^{7} CFU ml^{-1}
	MB+/L-	8.9×10^{6} CFU ml^{-1}
	MB+/L+ (3min)	0 CFU ml^{-1}
S. maltophilia (10^{-5})	MB-/L-	4.4×10^{8} CFU ml^{-1}
	MB+/L-	1.2×10^{8} CFU ml^{-1}
	MB+/L+ (3min)	1.0×10^{5} CFU ml^{-1}
C. albicans (10^{-4})	MB-/L-	3.4×10^{7} CFU ml^{-1}
	MB+/L-	3.1×10^{7} CFU ml^{-1}
	MB+/L+ (3min)	2.1×10^{6} CFU ml^{-1}

Tab. 21: Growth control after treatment of *S. aureus* with methylene blue and different lamps. The tested lamps were a laser, blacklight lamp, LED1 and LED2.

MB+/L+ Laser	
S. aureus (10^{-5}) - control	2.8 x 10^{8} CFU ml^{-1}
S. aureus (10^{-5}) - 3 min. irradiation	1.0 x 10^{5} CFU ml^{-1}
MB+/L+ Blacklight	
S. aureus (10^{-5}) - control	1.3 x 10^{8} CFU ml^{-1}
S. aureus (10^{-5}) - 3 min. irradiation	1.5 x 10^{7} CFU ml^{-1}
MB+/L+ LED1	
S. aureus (10^{-5}) - control	1.2 x 10^{8} CFU ml^{-1}
S. aureus (10^{-5}) - 2 min. irradiation	1.2 x 10^{7} CFU ml^{-1}
MB+/L+ LED2	
S. aureus (10^{-5}) - control	1.2 x 10^{8} CFU ml^{-1}
S. aureus (10^{-5}) - 2 min. irradiation	2.0 x 10^{6} CFU ml^{-1}

3.6.4 Does the method work without dye?

The following experiment demonstrates the growth control after treatment of *S. aureus* and *S. maltophilia* without methylene blue and with irradiation with a mercury lamp. The results were analyzed with wipe test.

a b

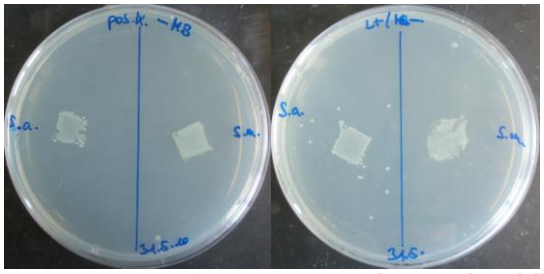

Fig. 54: Growth control after treatment of *S. aureus* and *S. maltophilia* without methylene blue and light/mercury lamp – wipe test. On the left side of the plates of a and b *S. aureus* is represented and on the right side *S. maltophilia* is represented. a) 3 µl cell culture on textile → control MB-/L-. b) 3 µl cell culture on textile → 3 min irradiation with the mercury lamp (MB-/L+.).

Fig. 54 indicates the growth control after treatment of *S. aureus* and *S. maltophilia* without methylene blue and light/mercury lamp. After irradiation, the same growth in a and b was observed. This shows that irradiation alone (MB-/L+) has no effect on a reduction of microorganisms. The method only works with a reactive dye like MB or TBO.

4 Discussion

4.1 Adhesion of human skin bacteria on clean room garments

Humans are the biggest source of contamination in clean rooms. Thus, the aim of this study was detection of bacteria on three different clean room textiles after wearing. The examination utilizing CLSM-analytics, however, shows a clearly result. At all three textiles (ION-NOSTAT VI.2, ION-NOSTAT V and red knitted fabric with silver ion fiber) living bacteria were visible. Especially on the layer of the textiles, which were worn directly on the skin, it was found the closest colonization. On clean room textiles with silver ions, we expect bacteria not to attach themselves to the fiber or should be lethally damaged. The disinfectant properties of silver ions are used in medical appliances, e.g. for wound dressings to treat external infections or used as an antiseptic and disinfectant (Vermeulen et al., 2007). Thus, according to the manufacturer, the silver ion containing clean room textiles should not be colonized with microorganisms after wearing. In our study, this was not the case; our findings indicate that human skin bacteria attach on all textiles after wearing for five hours.

4.2 UV-treatment of inoculated clean room textiles

A significant reduction of bacteria should be achieved after UV irradiation. With wipe test, a reduction of germs on the upper side of textiles was detectable. 5 minutes of UV irradiation had no germ reducing effect. The best effect was observed after 10 min UV irradiation. A longer exposure was not performed, because it is not relevant for our purposes respectively for practice. On the undersides of all textiles exposed to human skin, no reduction of bacteria was observed, because these sides were not exposed to UV light. The same results as for *S. aureus* were also observed for *S. maltophilia* and for *C. albicans*. But no growth could be detected on the under sides of textile pieces, which were previously incubated with *C. albicans*. We suppose that yeast cells are too big for penetrating through the microstructure of the clean room fabric.

With CLSM, more detailed monitoring could be performed. Untreated and UV-irradiated samples showed the same results as it were found with wipe test. In addition, no microorganisms could be detected on the under sides of textile pieces. This confirms that yeasts are too big for penetrating through the clean room fabrics.

The killing of germs on the surface of textiles is very difficult. A major problem is the microstructure of the textile fiber, because the microorganisms can still settle between the fibers and in craters of the fiber surface. In addition, the textile fiber changed slightly after UV-irradiation and more holes or craters were visible. In a nutshell, UV irradiation leads to a significant reduction of bacteria on the upper side. In practice, however, it is not relevant, because this method is very time consuming.

4.3 Pre-treatment of textiles with disinfectants

Pretreatment and treatment of clean room textiles with two different disinfectants lead to a significant reduction of bacteria. According to the wipe test, a reduction of germs was detectable. After an incubation period of 5 min or 10 min of *S. aureus* on textiles and subsequent treatment with Sterillium no or very few colonies were observed. Reduction of bacteria was detected on textiles, which were pretreated with the disinfectants, in comparison to untreated textiles. In the experiments, care has been taken of a drying of disinfectants into the textiles. Thus, a killing effect could be excluded because of residual moisture. This shows that both disinfectants have a toxic effect even in the dry state. On CLSM, the same result was found as with wipe test.

Further experiments were carried out with pre-treatment of the textile pieces with different length of incubation. After 30 min pre-treatment of the textiles, a reduction of germs has already been determined with CLSM. After an incubation period of Sterillium for one or two hours, an almost complete destruction of the microbes could be detected. In addition, textiles were investigated, which were pre-treated with disinfectant, further *S. aureus* were pipetted on it and finally these textiles were incubated for 20 hours. CLSM shows, that on these textiles only a few living germs were visible. As expected, the same results were observed on textiles, which were pretreated with a disinfectant spray by Dr. Beckmann.

Pre-treatment and treatment of clean room textiles with disinfectants are successful methods to kill microorganisms. A germ-reducing effect of *S. aureus*, *S. maltophilia* and *C. albicans* was detected in all experiments after pre-treatment with Sterillium and with Dr. Beckmann Disinfectant Hygiene Spray.

4.4 Retention capacity of clean room textile

Clean room textile is intended to hinder particles and microorganisms from the wearer's body to contaminate the environment. A washing test was carried out as it is usual in industry to check the killing effect of this process. In addition, textile pieces have been investigated for their potential of retention of microorganisms after washing.

In this experiment it was found that *S. aureus*, *S. maltophilia* and *C. albicans* are strongly adhering on textile pieces after 30 min washing under mechanical stress. A very large proportion of inoculated microorganisms remained to the fabric after treatment. A significant decline of viable cells and a big quantity of dead cells, which adhere to the textile, were observed with CLSM. The same result was detected after plating.

This potential could be used to support the killing of these microorganisms.

4.5 Treatment of clean room textiles with Titanium dioxide

As already mentioned in introduction, Titanium dioxide (TiO_2) is able to develop a strong antimicrobial activity. The aim of this study was to pre-treat textile pieces with a suspension containing TiO_2 to obtain an antimicrobial property of the clean room textile. Studies reported, that a TiO_2 film was coated on a poly vinyl chloride (PVC) surface and showed good bacterial anti-adhesion activity following photo-activation and sterilization property under UV irradiation (Lin et al., 2008). In this study, *S. aureus*, *S. maltophilia* and *C. albicans* were inoculated on TiO_2 pretreated textile pieces. After incubation, a significant reduction of living cells was analyzed by CLSM. This effect was already observed without UV irradiation. This result indicates that TiO_2 has an antibacterial and germ reducing effect under visible light. This ability can be advantageous in clean rooms because no specific exposure is necessary. An antimicrobial effect would be given under visible light during an entire working day in a clean room environment.

4.6 Light activated disinfection

Light activated disinfection has a number of advantages in relation to both traditional antibiotic treatment and chemical disinfectants. The photosensitizers, which were used in this study, were methylene blue and toluidine blue. Textile pieces were pre-treated with these photosensitive dyes and finally activated by light of certain wavelength. An antimicrobial effect against a variety of microorganisms was expected. It was reported, that this method can exert an appreciable antimicrobial effect against a range of microbes, including *S. aureus* or *C. albicans* (Saji & Anil, 2007). The results of this study demonstrate that all of the irradiated microorganisms were eradicated to some extent by light in the presence of MB or TBO. Irradiation alone (MB-/L+ or TBO-/L+) had no effect on a reduction of microorganisms.

The pretreated textile pieces were kept in visible light or illuminated for different periods of time using lamps of different wavelength maxima. The results showed that even visible light showed a slight reduction of microorganisms. After an irradiation of three minutes, no growth was detected after plating.

The results of this study also demonstrate that both, methylene blue and toluidine blue, are efficient photosensitizers of gram-positive, gram-negative and yeast using lamps of appropriate wavelength. All tested lamps (black light lamp, mercury lamp, laser and LEDs) show a germ-reducing effect after an irradiation time of only 30 seconds. The best germ-killing effect was detected after an irradiation of 5 minutes. After plating, no colonies of *S. aureus*, *S. maltophilia* and *C. albicans* were observed after 3 min irradiation. Wipe test proved also, that cell growth was inhibited after combined treatment with light and reactive dyes.

It is worth mentioning that a volume of 3 µl cell culture, which was used for wipe test, is not relevant for practice, because such large quantity is not expected in a clean room. With a less number of germs a great killing-effect could already be detected after irradiation of 30 seconds.

Furthermore, it was tested, how often the dyes can be re-activated. The result indicated that both dyes (MB and TBO) retain their germ-reducing effect after repeated (up to 10 times) irradiation. Also a very low concentration of dyes is sufficient to achieve an antimicrobial effect.

The results of all conducted experiments show a significant reduction of microorganisms after irradiation with different lamps. This indicates that this method has great potential. In addition to methylene blue and toluidine blue, there are other dyes, which can broaden the spectrum. Derivatives of methylene blue and other dyes also can be of interest for the use in locks or in the clean room environment. A great challenge will be the incorporation of photosensitizers into materials like clean room textiles. Further experiments are planned regarding the coupling of reactive dyes to synthetic polymers for the production of fibers and antimicrobial clean room clothing.

5 Conclusio

The aim of this project is the development of innovative concepts for personal locks in the clean room technology. Humans are the major source of contamination in a clean room. This is the reason why clean room garments are absolutely essential for protection of the product and for intention to hinder particles from the wearer´s body to contaminate the clean room environment. The contamination may result from different factors including microorganisms and particles from outdoor air, microbes and particles generated by the employees' garments and by the employees' physical activity. The exchange of personnel from one clean room standard to the next higher one, e.g. from standard C to standard B, is time consuming and cost intensive, because the employees have to change their clean room cloth to avoid contamination. In this master´s thesis, different methods of sterilization procedures were conducted to get a germ-reducing effect directly on the clean room textile.

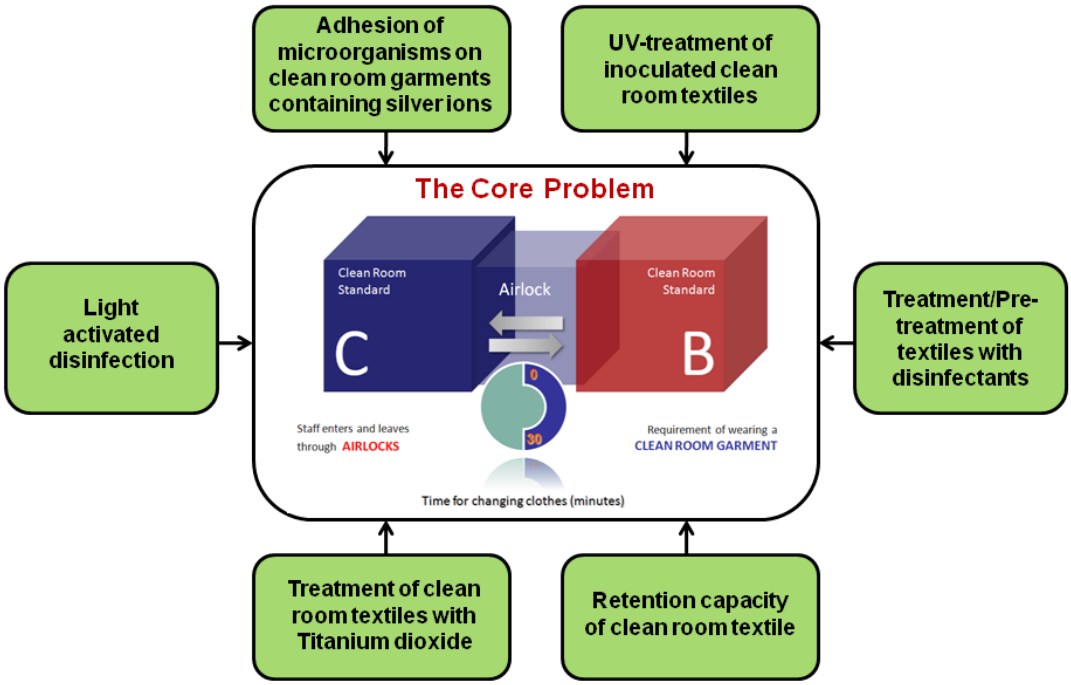

Fig. 55: Innovative concepts for personnel lock in clean room technology. Existing concepts for personnel locks in clean rooms are characterized by cost and time-consuming procedures. Clean room personnel have to change their clean room garment in personnel locks when moving from one clean room class to another. There is a demand of the industries for the design of textiles with new features and concepts for innovative personnel lock systems. Innovation is taking place in the modification of the textile and in the implementation of new sterilization methods (green accentuated). This new methods will allow the passage from one clean room class to another without changing the clean room suite.

A summary of all conducted methods is listed in the following Table, where treatment, efficiency, advantage and disadvantage of each method are shown (Tab. 22).

Tab. 22: Summary of all conducted methods. The Table shows the treatment, efficiency, advantage and disadvantage of each method.

Treatment	Efficiency	Advantage	Disadvantage
Adhesion of microorganisms on clean room garments containing silver ions	Contrary to expectance, no antimicrobial effect of silver ions were observed	Disinfectant properties of silver ions are already used in medical appliances	No significant killing by the silver ion blended fiber was detectable; fabrics provide a perfect niche for colonization by microorganisms
UV-treatment of inoculated clean room textiles	Reduction of microorganisms on the upper side of textiles was detectable after 10 min UV irradiation	UV light kill microbes on the upper layer of surfaces, which were exposed to UV irradiation	No reduction of MO were observed on the under side of textiles; cells not exposed to UV irradiation will not be affected by this sterilization technique; Change in the textile structure after UV irradiation;
Treatment/Pre-treatment of textiles with disinfectants	A complete destruction of the microbes could be detected	A killing effect could be excluded because of residual moisture; disinfectants are commercially available and tested for application on the human skin	Attachment and process of drying of disinfectants on clean room textiles
Retention capacity of clean room textile	Clean room textile hindered microorganisms from the wearer's body to contaminate the environment	A very large proportion of inoculated microorganisms remained to the fabric after a washing test	A few quantity of viable cells, which adhere to the textile, were observed after a washing test
Treatment of clean room textiles with Titanium dioxide	Titanium dioxide is able to develop a strong antimicrobial activity	Titanium dioxide incubated clean room textiles indicated an germ-reducing effect under visible light	No disadvantages of Titanium dioxide were observed
Light activated disinfection	the method exerted an appreciable antimicrobial effect against a range of microbes	All of the irradiated microorganisms were eradicated to some extent by light in the presence of MB or TBO; a great killing-effect could already be detected after irradiation of 30 seconds and after repeated irradiation	Attachment of dyes on the fibers should be optimized

By the combination of different strategies (Fig. 55) from the development of new garments over the evaluation of different sterilization procedures, a concept for producing a new lock system will be created. All conducted experiments, e.g. microbes and particle retention of clean room textiles, antibacterial effect of Titanium dioxide, reactive dyes, etc., should be included in an overall concept. Each part should be studied and optimized of its possibilities and limitations. The aim is the maintenance of sterility of a clean room textile during an entire working day in a clean room environment. Passing through different classes of clean rooms without changing of clothes should be made possible by this concept.

The new lock system is to provide a maximum of safety against cross-contamination, to fulfill all qualifications of safety precautions for the highest clean room standard. This allows a quick change between different clean room classes with one cloth. Furthermore, this leads to cost reduction, time saving and to better working conditions in clean rooms.

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9 Statutory Declaration

Deutsche Fassung:
Beschluss der Curricula-Kommission für Bachelor-, Master- und Diplomstudien vom 10.11.2008
Genehmigung des Senates am 1.12.2008

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