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COMBAT AND EVALUATION OF MIXED BIOFILMS

Conventional and alternative approaches combating antimicrobial resistance

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DEVELOPMENT OF DIAGNOSTIC AND ANTIMICROBIAL TRIGGERED RELEASE SYSTEMS FOR WOUND DRESSINGS

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Healthcare-associated infections (HAIs) can be acquired in any healthcare facility. Increasing morbidity, prolonged hospital stay, and increased treatment costs are some of the consequences of HAIs. Thus, there is a critical need for novel antimicrobial strategies and products for emerging antibiotic resistance and the search for cutting-edge infection detection and treatment systems detection systems (theranostics). This project aims to create rapid screening systems for theranostic antimicrobial devices and to analyze the effectiveness of novel antimicrobial peptides (AMPs)[1] that can be used in such devices for wound dressings.

A novel set of peptides was designed using artificial intelligence and synthesized, and their antimicrobial activities under physiological conditions were analyzed against planktonic *Staphylococcus aureus* JAR060131 and multidrug-resistant *Acinetobacter baumannii* RUH875. The lethal concentration killing 99.9% of the inoculum (*i.e.* $LC_{99.9}$) of these peptides ranged from 0.94-15 μM in the presence of 50% human plasma. The efficacy of selected lead peptides (*i.e.* AMP-038 and AMP-045) and their retro-inverso (RI) variants were also compared to promising AMPs in the preclinical/clinical phase of development. Moreover, within 2 hours, 60 μM of AMP-038 and 30 μM of AMP-045 showed more than 3-log reduction against biofilm-encased *S. aureus*. Because resistance development is one of the major concerns, we assessed whether *S. aureus* and *A. baumannii* developed resistance to the lead peptides. While the MIC of *S. aureus* for rifampicin, and of *A. baumannii* for ciprofloxacin increased ≥4096-fold and >256-fold, respectively, no significant change in MIC was observed when the strains were cultured in the presence of AMP-038 (-RI) and AMP-045 (-RI) peptides for more than 20 passages.

In conclusion, we showed the antimicrobial and antibiofilm efficacy of the candidate peptides, their lack of resistance development, and the rapid action of these AMPs which contribute to the death of the bacteria within minutes.

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THE EFFECTIVENESS OF PHOTOACTIVE MATERIAL ON MRSA IS NOT AFFECTED BY THE EFFLUX PUMPS

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Staphylococcus aureus is Gram-positive bacteria that cause nosocomial infections and form robust biofilms on medical devices. Mechanisms of resistance against antibacterial drugs, such as efflux pumps, make treatment of staphylococcal infections difficult. This study evaluated the efficacy of polyurethane (PU) hybrid film composed of clay mineral saponite (Sap) modified with octadecyltrimethylammonium (ODTMA) cations and functionalized photoactive compound phloxine B (PhB) [1]. Part of the study analyzed the effect of hybrid material on the expression of the gene *norA* and *norB* encoded staphylococcal efflux pumps.

Experiments were performed with the standard strain *S. aureus* CCM3953 and methicillin-resistant strains *S. aureus* L12 (MRSA) and *S. aureus* S61 (MRSA). Strains were tested to oxacillin, ciprofloxacin, and norfloxacin by E-tests. The strains L12 (MIC>256 µg/mL, MIC>32 µg/mL, *MIC*>256 µg/mL, *respectively)* and S61 (MIC>256 µg/mL, MIC>32 µg/mL, *MIC*>256 µg/mL, *respectively)* were confirmed to be resistant. The strain CCM3953 was sensitive to all tested drugs. The effectiveness of the material with hybrid films was tested before and after irradiation using a green laser (λ = 532 nm, 100 mW, duration of irradiation for 120 s) by counting CFU/mL. Results showed on a log scale: 4.386, 4.058, and 3.394 inhibition after irradiation for CCM3953, S61, and L12, respectively. RT-qPCR (the $2^{\triangle\triangle CT}$ method) confirmed a higher efflux activity for biofilm *cells of* L12 and S61 (7.51 and 10.44 times, respectively) on non-modified PU material compared to the standard strain CCM3953. Strain L12 did not show significant changes in the relative expression of the *norA* gene on material with hybrid films before and after irradiation. Strain S61 showed radically higher gene expression before and after irradiation on tested material. The expression of the *norB* gene was not increased in tested strains.

Results claimed the effectiveness of PU material with photoactive hybrid films containing PhB on MRSA biofilms despite of over-expressed efflux pump NorA.

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ANTIMICROBIAL PHOTODYNAMIC INACTIVATION - A PROMISING APPROACH COMBATING INFECTIOUS PATHOGENS

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The yeasts of *Candida albicans* and *Candida auris*, and methicillin-resistant bacteria of *Staphylococcus aureus* (MRSA) are important human pathogens. They also are important because of resistance and ability to form single or mixed biofilms on medical devices. Searching for alternative options for eradication of resistant microorganisms and their biofilms is a current issue. Antimicrobial photodynamic inactivation (aPDI) is a promising approach based on of the photosensitizer (PS)-mediated and light-induced formation of reactive oxygen species (ROS) causing destruction of microbial cells.

Our research presents the action of aPDI using two PSs, namely methylene blue or phloxine B, to biofilms of above-mentioned microorganisms. Additionally, it provides an analysis of the mode of action as well as the participation of efflux genes involved in resistance to conventional drugs.

For this purpose, different approaches have been involved: determination of viability based on colony forming unit calculation, measurement of ROS generation by luminescence, estimation of change in efflux gene regulation using RT-q PCR, and various microscopies (CLSM, SEM). aPDI efficiently reduced the survival of biofilms formed by all microorganisms concerning selected PS, their concentration, and duration of irradiation. Generally, single biofilms showed a 10 to over 1000-fold reduction in growth, while mixed biofilm of *C. albicans*/*S. aureus* was less sensitive (reduction was 10 to 100 times compared to control). Phloxine B showed excellent aPDI against MRSA biofilm, including that formed on polyurethane nanocomposite with a hybrid film based on clay mineral saponite functionalized with PS. A significant generation of ROS was observed after irradiation of both methylene blue and phloxine B. Microscopy revealed an increased number of dead cells after aPDI (CLSM) and cell disruptions (SEM). All resistant microorganisms manifested overregulation of the efflux genes coding for Cdr proteins (*C. albicans* and *C. auris*) or MFS (the *MDR1* and *NorA* genes for *C. albicans*, *C. auris*, and *S. aureus*, respectively).

Results suggest that the activity of efflux transporters do not significantly affect aPDI, what is an excellent assumption for the use of aPDI in the eradication of resistant microorganisms.

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WHOLE GENOME ANALYSIS OF FOOD RELATED BACTERIA

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Whole-genome sequencing (WGS) based subtyping demonstrates several advantages over traditional subtyping approaches, including enhanced discrimination, prediction of antimicrobial profiles, attribution of transmission sources, and so it shows great potential for microbial food-safety surveillance. In the present study, subtyping based on WGS was used in the monitoring of the *Listeria monocytogenes* strains isolated from food processing plants as well as for characterization potential starter strains of the *Lactobacillaceae* family selected for production of Slovak bryndza cheese.

We analyzed whole genome sequences of 29 *L. monocytogenes* strains isolated from two production plants. MLST and cgMLST were used for analysis of strain relativeness and strains were classified into twelve sequence types. Based on repeating isolation from the sheep farm, twelve *L. monocytogenes* belonging to ST14 were assessed as persistent contaminants. As for the meat factory, we identified persistent strains that corresponded to the sequence types ST9 and ST14. As prophages integrated into bacterial genomes can modify host properties and contribute to stress survival, we detected presence of prophages in sequenced *L. monocytogenes* genomes. Totally 72 prophages were present, the number varied from one to five per strain. According to the genome similarity, the prophages were divided into thirteen groups.

We have also analyzed 16 LAB strains, which belonged to *Lactiplantibacillus plantarum/paraplantarum* (n=6), *Lacticaseibacillus casei/paracasei* (n=5), *Limosilactobacillus fermentum* (n=2), *Levilactobacillus brevis* (n=2) and *Lentilactobacillus parabuchneri* (n=1). Regions containing prophage genes were detected in all LAB strains, but incomplete regions lacking some essential genes were also present.

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ENCAPSULATION OF PHOTOSENSITIZERS IN BACTERIA-RESPONSIVE NANOCARRIERS FOR ON-DEMAND TREATMENT OF WOUND INFECTIONS

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Chronic wound infections are a global concern, with over 6.5 million patients worldwide [1]. The presence of biofilm-forming bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* results in the formation of a protective extracellular polymeric matrix which renders the bacteria resistant to conventional antibiotic treatment. A stimuli-responsive wound dressing which combines the detection of bacterial infection with rapid early treatment provides an opportunity to minimise the development of wounds to a chronic stage by being able to monitor the wound status and preventing such biofilm formation.

Our approach is based on smart bacterial enzyme-labile nanocarriers [2,3]. Here we report on the synthesis of the Food and Drug Administration (FDA)-approved amphiphilic block copolymer poly(ethylene glycol)-*block*-poly(lactic acid) (PEG-*b*-PLA) with varying PLA chain lengths, which can be self-assembled into nano-sized vesicles and micelles (Fig. 1a). The PEG unit provides the stealth function, while the PLA unit results in a stimuli-responsive nano-assembly that degrades in the presence of bacterial protease enzymes. These nanocarriers have been used to encapsulate reporter dyes and therapeutic agents. Dye release upon enzymatic treatment enables a rapid light-on effect, signalling the presence of infection (Fig. 1b). The triggered-release and subsequent irradiation of photosensitizers generates reactive oxygen species which cause oxidative stress and eradicate the bacteria (Fig. 1c).

This novel approach can be incorporated into a stimuli-responsive wound dressing, to enable rapid detection of infection and high localised photosensitizer delivery.

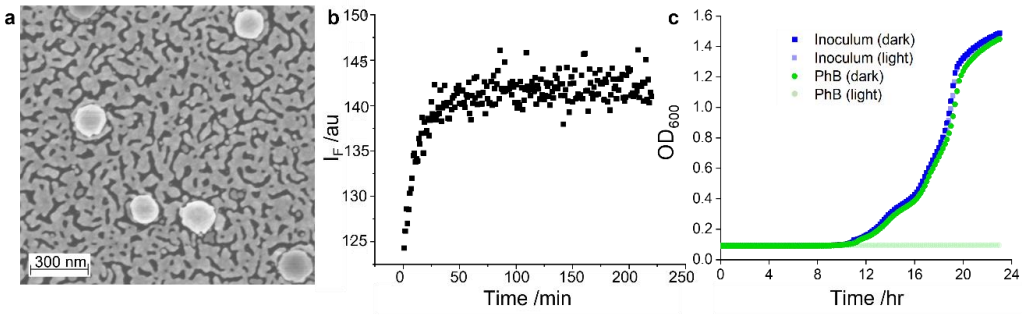

Fig. 1. a) Field emission scanning electron microscopy (FESEM) image of phloxine B-loaded PEG_{114}-*b*-PLA_{400} vesicles, b) Fluorescence intensity increase upon enzyme-triggered release of the photosensitizer phloxine B from PEG_{114}-*b*-PLA_{400} vesicles, c) Effect of phloxine B (250 μg/mL) on *Pseudomonas aeruginosa* (ATCC 19660) growth upon irradiation (10 minutes at 532 nm).

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MICROPLASTICS AND ANTIBIOTIC RESISTANCE

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Microplastics represent a new source of pollution in the environment. In this work, we monitored the influence of selected microplastics on the development of mutations leading to ciprofloxacin resistance in model bacteria *Salmonella* Typhimurium using a modified Ames test. The adsorption capacity of microplastics for the plasmid carrying antibiotic resistance genes (pRS426) was assessed with a simple experiment. The results of modified Ames tests show the greatest effect of microplastics size on the development of ciprofloxacin resistance in *S.* Typhimurium. In case of 0,09 mm microplastics, the ratio of resistance index (RI) was higher than in all tested microplastics except from 5 mg of ABS. In general, 0,09 mm PLA microplastics caused increase in RI ratios in all used concentrations. In case of 0,125 mm microplastics we observed increase in RI ratio only with addition of 5 mg of PET microplastics. Other tested microplastics caused reduction in RI ratios. Values of RI ratios show that the increase in development of mutations induced by microplastics is varying and is mostly dependent on the size of tested microplastics, not the material or the concentration. The highest value was recorded in experiment with concentration 5 mg of 0,09 mm PLA microplastics, where RI increased 2,9-fold against spontaneous resistants. Adsorption experiments with plasmid DNA and microplastics did not show any significant results. The concentrations of DNA have increased in all cases after 20 minutes of incubation and then dropped to original levels. The material and size of microplastics did not influence the adsorption of plasmid DNA.

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PHOTODYNAMIC INACTIVATION AGAINST SINGLE- AND DUAL-SPECIES BIOFILMS: EFFICACY EVALUATION WITH METHYLENE BLUE

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Infections associated with biofilms represent a medical problem, mainly from the point of view of high resistance to treatment. Therefore, the search for alternative methods of biofilm eradication is a great challenge. These approaches also include photodynamic inactivation (PDI).

The effectiveness of PDI was tested on 48-h single- and dual-species biofilms (standard strains Candida albicans SC5314, Staphylococcus aureus CCM3953, resistant clinical isolate C. albicans CCY29-3-164, methicillin-resistant S. aureus 6102/1/2010-MRSA). Different concentrations of methylene blue (MB; 0.25, 0.5; 1 mM) were tested during pre-incubation period with MB (2; 4; 16-h). A red laser (190 mW/cm^2, λ 660 nm, 60 s) was used for irradiation.

The results of PDI efficacy (CFU/ml) differed with respect to the structure of the prokaryotic vs. eukaryotic cells. The most sensitive to PDI were biofilms formed by S. aureus (16-h, 0.25 mM MB) with a reduction of 2.08-log$_{10}$ and 1.6-log$_{10}$ for the standard and MRSA strains, respectively. Inhibition determined for C. albicans corresponded to 0.4-log$_{10}$ and 0.1-log$_{10}$ for the standard and resistant isolates, respectively. For dual biofilms, the reduction after PDI was 1.91-log$_{10}$ and 0.6-log$_{10}$ in the combination of standard strains and clinical isolates of C. albicans and S. aureus, respectively.

UV-Vis spectroscopy proved that the transformation of MB to leucomethylene-blue can affect the photoactivity of MB related to increasing pre-incubation in yeast. Measurement of reactive oxygen species (ROS-Glo™ H_2O_2 Assay) after 2- and 16-h pre-incubation with MB before and after PDI resulted in a significant increase of ROS in biofilms.

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COMPARATIVE GENOMICS OF POTENTIAL THERAPEUTIC BACTERIOPHAGES INFECTING ENTEROBACTERIA

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One of the most critical challenges of contemporary medicine is the continual spread of new antimicrobial resistance mechanisms among important human bacterial pathogens. Presently, the demand for alternative therapies for difficult-to-treat infections, including biofilm associated infections, is steadily rising. Phage therapy as a promising way to achieve this aim is now experiencing its renaissance. This procedure requires well-defined virulent phages which attack pathogenic bacteria in a strain specific manner. Multiplication of therapeutic phages at the site of infection increases the efficiency of bacterial clearance. All this happens without adverse effects on the equilibrium of microbiota or toxicity to the human body and other usual side-effects of antibiotic treatment. Moreover, phages are affected neither by mechanisms of antimicrobial resistance nor by drug interactions, which are common in antibiotic therapy.
Escherichia coli, a member of the family *Enterobacteriaceae*, is a commensal gut bacterium as well as an opportunistic pathogen causing both extra intestinal and intestinal pathologies. It is the most common causative agent of urinary tract infections and *E. coli* ST131 clone resistant to antibiotics represents a big problem. *Cronobacter* and *Enterobacter* are other enterobacterial opportunistic pathogens capable of producing a wide variety of infections.
In the present study we isolated and characterized bacteriophages with broad host specificity against a panel of local *E. coli*, *Cronobacter* and *Enterobacter* strains for the establishment of a national phage bank. Phages with the best properties were combined into a phage cocktail and its antibacterial activity was measured in liquid artificial urine medium.

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CATECHINS AS THE NOVEL STRATEGY IN ENHANCEMENT OF ANTIFUNGAL ACTIVITY OF AZOLES

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Candida glabrata infections caused by drug-resistant strains has been an leading cause of mortality in immunocompromised patients in recent years [1]. Unprecedent rise in frequency of drug-resistant strains and restricted armamentarium of antimycotics has drawn attention to the potential of natural products as antifungals drugs. Promising strategy in combating invasive candidiasis represents the catechins - polyphenolic compounds found in a variety of plants [2]. Aim of this work was to evaluate the effect of catechins on azole antifungal activity in *C. glabrata* laboratory strain, azole- resistant and azole-sensitive clinical isolates. We demonstrated that catechin-hydrate alone had no antifungal activity on *C. glabrata.* However, we showed that combinational use of antifungal azoles and catechin-hydrate enhanced activity of antifungal azoles both in laboratory strain and clinical isolates. Further analysis showed that combinational use of catechin-hydrate and miconazole leads to increased production of reactive oxygen species (ROS). Incubation of *C. glabrata* cells in presence of catechin-hydrate and miconazole affects membrane fluidity. Changes in membrane fluidity was accompanied by changes in activity of *Cg*Cdr1p efflux pump. qRT-PCR analysis showed that catechin-hydrate subvert the induction of *CgCDR1* gene. Taken together, our results demonstrated that catechin-hydrate potentiates the effect of antifungal azoles. The enhanced effect of catechin-hydrate on miconazole antifungal activity may arises from increased production of intracellular ROS, decreased rigidity of plasma membrane and substantial changes in transcriptional and efflux activity of *Cg*Cdr1p membrane transporter. Combinational use of catechins and antifungal azoles could significantly improve our capacity to manage *C. glabrata* infections.

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E. COLI BACTERIOPHAGES USEFUL FOR THERAPY OF URINARY TRACT INFECTIONS

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Urinary tract infections represent one of the most common problems in clinical medicine. Around 150 million people become infected every year. Currently, the most commonly recommended therapy for UTIs are antibiotics. However, with the increasing rates of bacterial resistance, alternative therapies should be considered to reduce the burden of these common infections [1]. Many promising approaches are being developed, phage therapy is one of the alternative treatment options. Phages are viruses that attack bacteria to complete their life cycle [2]. Phage therapy can be defined as the application of strictly virulent phages for the purpose of lysing specific bacterial pathogen which is causing clinical infection [3]. To increase the host spectrum and reduce the likelihood of developing phage-resistant strains of bacteria, cocktails composed of multiple phages are most often used in therapy. The main aim of the presented work was to prepare and characterize a phage cocktail. Bacteriophages were isolated from wastewater. A phage cocktail composed of six phages belonged to *Straboviridae* and *Autographiviridae* families was prepared. The host phage specificity in a panel of 77 clinical strains of *E. coli* was tested, the cocktail infected up to 57% tested strains, mainly the B2 and D phylogroup strains. One-step growth curve and the adsorption of phages to host strains were established, phages showed high adsorption to *E. coli* strains with life cycle from 15 to 30 min and the burst size of 38-212 phages per cell. The acquired results could be used in the preparation of cocktails for UTIs phage therapy.

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THE CONTRIBUTION OF OXIDATIVE STRESS TO THE ANTIFUNGAL EFFECT OF AZOLE COMPOUNDS IN *NEUROSPORA CRASSA*

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Azoles are the most frequent compounds used in treatment of fungal diseases. Little is known about the contribution of oxidative stress to the antifungal activity of azole compounds in *N. crassa*. Oxidative stress in filamentous fungi is defined as an excess production of reactive oxygen species (ROS) relative to antioxidant defense. The damage rate caused by increased oxidative stress is related to antioxidant capacities of the filamentous fungus. The antioxidant defense is a network of enzymatic and nonenzymatic molecules that includes SODs, catalases, the thioredoxin system and the glutathione system [1]. In our work we have observed the expression of the *sod1*, *cat1*, *mrp* and *pcat* genes in the presence of azole compounds. Our results have shown two different physiological stages of the fungus related to the gene expression of *cat1* and *pcat* in the presence of ketoconazole.

As glutathione has an important role in coping the oxidative stress, we evaluated the ability of L-methionine sulfoximine (MSO), an inhibitor of glutathione synthesis to enhace the antifungal activity of azole compounds in *N. crassa* and *Aspergillus fumigatus*. MSO is undergoes rapid phosphorylation by glutamine synthetase producing the active form, methionine sulfoximine phosphate (MSO-P). MSO-P binds essentially irreversibly to the active site in the glutamine synthetase, preventing the entry of the glutamate substrate [2]. Based on FICI we evaluated additive effect between MSO and azoles on *N. crassa* and *A. fumigatus*.

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GREEN TEA CATECHINS AND THEIR ANTIFUNGAL POTENTIAL

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Catechins are natural tea flavonoids known for their antioxidant, anti-inflammatory and antimicrobial properties. There are many studies that show the potential of catechins as scavengers of reactive oxygen species and therefore they protect cells against oxidative stress. On the other hand, catechins can act as prooxidants in higher concentrations; they can increase a production of reactive oxygen species inside the cell and thus have inhibitory activity against filamentous fungi. In our research, we tested the antifungal effect of catechin-hydrate on the model organism *Neurospora crassa* and its inhibitory potential in combination with azole compounds in different concentrations. This experiment was done with a wild strain of *N. crassa* and with a strain with a deletion in *cdr4* gene. As catechins could show prooxidative activity in higher concentrations, we also monitored an expression of genes involved in cellular response to oxidative stress in *N. crassa* in the presence of catechins. This monitoring included the expression of genes encoding antioxidant enzymes - *sod1*, *cat1*, *mrp*, *pcat*, the gene *cdr4* coding for the main azole efflux pump, genes coding for enzymes involved in biosynthesis of ergosterol (*cyp51*, *erg5*, *erg24*, *erg6*) and in plasma membrane remodelation (*lac*, *gsc*, *rta2*, *psd2*). Our goal was to identify an inhibitory activity of catechins against fungal model organism, to find a potential synergistic effect between catechin and certain antifungal drugs and we try to estimate main mechanisms of catechin´s inhibitory activity on the molecular level.

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ANTIMICROBIAL PHOTODYNAMIC THERAPY WITH RU(II)-POLYPYRIDYL NANOCARRIERS FOR TREATING CYSTIC FIBROSIS LUNG INFECTIONS

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Cystic fibrosis (CF) patients suffer from severe chronic lung infections that are caused mainly by the bacterium *Pseudomonas aeruginosa*. Since this highly adaptable pathogen is increasingly resistant to antibiotic treatment, the development of new therapies is of major interest. Antimicrobial photodynamic therapy (aPDT) combines the use of photosensitizers (PS) and light to locally generate reactive oxygen species at the infection site. This technique can be used to locally treat multi-resistant microbial infections. [1,2]

Herein we report on our approach to overcome the key drawbacks of conventional aPDT for future CF treatment. Efficient PS encapsulated in polymeric "stealth" nanocarriers facilitates their delivery through the highly viscous airway mucus and safeguards a high local PS concentration. Stealth-type micelles and vesicles with an outer poly(ethylene glycol (PEG) corona are formed by self-assembly of the block copolymer PEG_{114}-*block*-PLA_X (PLA: poly(lactic acid)), which are loaded with novel Ru-based PS. It is hypothesized that release of the PS triggered via bacterial enzyme-mediated cleavage of the nanocapsules enhances the aPDT efficiency. In particular, PS encapsulation and release, as well as the efficiency of singlet oxygen ($^1O_2$) generation upon irradiation with blue light is studied spectroscopically and with microbiological methods in various media (Fig. 1). This work lays the foundation for the targeted eradication of *Pseudomonas aeruginosa* using aPDT, which possesses potential to be further developed for use in clinical care of CF-patients.

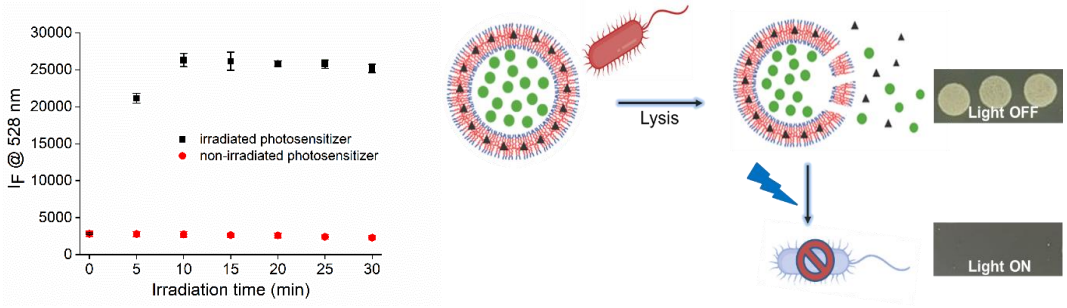

Fig 1. Fluorescence emission of a $^1O_2$ sensing indicator dye as a function of irradiation time (left), the release of PS via cleavage of capsule using a bacterial enzyme, which then kills bacteria upon irradiation (right).

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IN VITRO AND *IN VIVO* PHOTODYNAMIC INACTIVATION OF *CANDIDA ALBICANS-STAPHYLOCOCCUS AUREUS* MIXED BIOFILM

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The complexity of mixed infections and biofilms caused by *Candida albicans* and *Staphylococcus aureus* presents a substantial medical problem. The present work evaluates the antimicrobial effect of photodynamic inactivation (PDI) on mixed biofilm of both pathogens, in combination with the photosensitizer methylene blue (MB) and quorum sensing molecule farnesol (FAR), *in vitro*, and *in vivo* – using a model organism – *Galleria mellonella* larvae (GML). PDI of 48-h single-species or mixed-species biofilms with/without 150 μM FAR was evaluated by counting colony-forming units (CFU)/ml. GML were inoculated with different doses of pathogens to monitor the survival of single/co-species infection. The survival of GML was evaluated and compared between infected/co-infected groups and the groups of individuals treated with either MB, MB irradiated with a red laser (PDT) or with the combination of MB-FAR and PDT. PDI *in vitro* revealed the reduction in CFU/ml in both, single-species and mixed-species biofilms. The optimal inocula for *C. albicans* and *S. aureus* were determined for $2.5x10^5$ and $1x10^6$ cells/larva, respectively. In co-infection, $5x10^4$ and $6x10^5$ cells/larva were tested for *C. albicans* and *S. aureus*, respectively. The highest therapeutic effect was achieved in the group of GML infected with *S. aureus* after application of PDT in combination with 0.5 mM MB and 150 μM FAR resulting in a 50% difference in survival between treated and untreated GML. The results proved antimicrobial effect of PDI *in vitro* and PDT *in vivo*, however, to achieve the optimal effect of PDI/PDT on the mixed biofilm/co-infection, it is necessary to optimize the method.

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ANTIBIOTIC RESISTANT COLIFORM BACTERIA AND ENTEROCOCCI IN FLOURS AND PLANT POWDERS

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In recent years, several alimentary diseases have been connected with consumption or tasting of raw flour and dough[1]. Microbiological quality concern is also raising due to increased consumer demand for alternatives to flour (such plant or insect powders), while some of them can be consumed without prior thermal processing[2]. Thermal untreated food may also represent a microbiological risk connected with the possible presence of bacteria, where it may occur antimicrobial resistance.

According to these facts we have determined the presence of antibiotic resistant coliform bacteria and enterococci in flours and powders from Slovak retails. Our results indicated presence of both total and antibiotic resistant coliform bacteria and enterococci in the flour and powder samples. Lower numbers of total as well as resistant bacteria were detected in wheat flours compared to plant powders. Coliform bacteria isolates were predominantly identified as *Klebsiella* spp. and *Enterobacter* spp. Ampicillin resistance appeared in 97 % of isolates followed by chloramphenicol resistance (22 %) and tetracycline resistance (17 %). The presence of the bla_{SHV} gene was confirmed in 13 % of isolates. The *tetA* and *tetE* genes were present in 25 % of coliform bacteria isolates. Enterococci were less detected compared to coliform bacteria and enterococci were present only in non-cereal flours (powders). Identified antibiotic resistant strains were *E. casseliflavus*, *E. gallinarium* a *E. faecium*. None of these isolates harboured multidrug resistance and all showed normal efflux. Majority of antibiotic resistant isolates belonged to the group of medium biofilm producers.

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A CLOSER LOOK ON CONNECTION OF ANTIBIOTIC RESISTANCE BACTERIA IN THE FOOD CHAIN AND THE ENVIRONMENT

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The food chain may be considered as one of the reservoirs for antibiotic resistant bacteria. Monitoring, prevention and reduction of antimicrobial resistance is needed throughout the whole food chain, in principle from the farm to the fork. The aim of this work was to monitor resistant bacteria and selected resistance phenotypes in different parts of the food chain. Screening has shown the presence of resistant variants in both surface water and sediment samples as well as in ready to eat products. Samples taken from the environment showed high numbers of total and resistant bacteria. There was also observed some correlation between the occurrence of resistance in the water and sediment of the pond compared to the resistance present in samples of intestinal and gill of caught carp. During the fish dissect process, cross-contamination by these bacteria my occur. Occurrence of specific resistance was tied to a particular food establishment in samples of sushi as well as effect of specific sampling time was observed. The numbers found indicate more contamination from the environment of establishment. We have observed multidrug resistant bacteria, as well as different resistance genes, predominantly *vanA* gene.

The eating habits of the population are constantly evolving, and new dietary preferences bring new risks associated not only with the occurrence of foodborne infections. Ready to eat foods, without additional heat treatment, pose highest risk in contend to occurrence of bacterial contamination therewith connected occurrence of antibiotic resistance and resistant genes.

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INTELLIGENT WOUND DRESSING FOR AUTONOMOUS DETECTION AND TREATMENT OF BACTERIAL INFECTIONS

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The presence of bacterial infections in wounds, which are often difficult to detect in an early stage, can significantly impede the healing process, inflicting an increased burden on the patient's well-being and costs on the health system, or even posing a severe risk to human life. To overcome this "black box" status of the wound healing process, an urgent need evolves to develop a system with the ability to diagnose and signal the presence of bacteria at an early stage to autonomously induce an on-demand treatment. Therefore, we are developing polymer matrices for a next-generation smart wound dressing with "sense and treat" features, where we aim to merge diagnostic and therapeutic capabilities in a single medical device. In this work, a copolymer of poly ATM-01 was synthesized by free radical polymerization and via click chemistry in multiple steps. Then this polymer was modified with P1 and X1 respectively, both act as a chromogenic compound and subsequently crosslinked by UV light to yield a network structure. When swollen with water, the resulting hydrogel allows detection of bacterial infections via the release of a yellow and indigo indicator dye by the chromogenic effect of P1 and X1 respectively in response to the bacterial enzymatic environment. Furthermore, the polymer was chemically post-modified with antimicrobial peptides (A1) via click chemistry, and the efficacy of the resulting hydrogel (Poly ATM-02) against different strains of bacteria is currently under investigation. Furthermore, toxicity behavior of hydrogel and its effect on blood coagulation is also being analyzed. The anticipated research is expected to provide a new strategy for autonomous identification and effective treatment of wound infections.

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EVALUATION OF ANTIMICROBIAL PROPERTIES OF BIOACTIVE GLASS

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The presence of pathogenic microrganisms, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Pseudomonas aeruginosa has been identified in 90% of the operated implants and antibiotic resistance is relatively often determined (1). The solution may be the targeted preparation of biomaterials which, in addition to tissue regeneration, would also suppress the growth of micro-organisms. Bioactive glasses (BGs) enriched with therapeutic ions posses antimicrobial activity and could medicate the patient directly at the implantation site, provide not only acute but also long-term protection by gradual control degradation and releasing the active substances. The key role is to identify the appropriate BGs, by selection and use of an adequate microbiological tests. Antibacterial activity is often associated with ion release after immersion in liquid media leading to local physiological changes in the environment (pH, osmolarity)(2). In this work, glasses based on the 45S5 system, and its variants enriched with therapeutic ions such as Cu^{+}, Zn^{2+}, B^{3+} and Sr^{2+} were tested. Frequently used microbiological methods such as modified Kirby-Bauer disc diffusion method and microdilution methods were used to determine antimicrobial effect in direct as well as indirect contact. Representatives of G- and G+ bacteria as well as fungal pathogens *Candida sp*. were tested. The antimicrobial effect of all BGs against all tested microorganisms in indirect contact with the elution extracts (10 mg/mL) was demonstrated, depending on the time of the active substance leaching (1-28 days). In direct contact (Kirby-Bauer agar diffusion method), the tested glasses in concentration 10 mg had an antimicrobial effect against *Candida sp*., specifically *C. parapsilosis*, *C. auris* and most significant against *C. glabrata however* the effect against *C. albicans* was no significant. The marked sensitivity of *C. glabrata* to BGs also open the way to test several clinical isolates and confirming the species susceptibility. The results obtained so far predict the use of BGs as a non-antibiotic alternative treatment of microbial infection associated with implantation interventions.

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THE POSSIBILITIES OF ENHANCING THE ANTIFUNGAL ACTIVITY OF COMMON USED ANTIFUNGALS

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In the era of increasing resistance to antifungal agents, the scientists are looking for new possibilities how to enhance the activity of commonly used antifungal agents. The conventional way lies in searching for new compounds as well as for new specific target sites. However, apart from resistance, high metabolic variability (hence adaptation capacity) of fungi should not be overlooked. By revealing the response of fungal cells during the treatment with antifungals we are able to uncover how the fungal microorganisms cope with toxic compounds. Blocking the adaptive response offers an alternative strategy how to increase the activity of common antifungal agents. Echinocandins were not so frequently used in clinical practice in the past, but rising resistance of fungal cells to azole compounds boosted their application. In our work we were interested in the possibility of enhancing the activity of echinocandins using the combination strategy of two biologically active compounds. We have already uncovered the potential of the 1,4-dihydropyridine-2,3,5-tricarboxylate, an intermediate of nilvadipine synthesis (in our work labelled as derivative H) to increase the activity of azole compounds (fluconazole and voriconazole). Now, we were interested in its ability to enhance the activity of caspofungin. We observed an increased effect of caspofungin in the presence of derivative H on the model fungus *Neurospora crassa* despite the mutation in the hot spot of *fks1* gene.

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CHARACTERIZATION OF PROTEINS WITH SLT DOMAINS AND THEIR PROPERTIES

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The worldwide usage of antibiotics has an adverse effect on rapid spreading of resistance among pathogenic bacteria, therefore, new approaches to combat bacterial infections have been studied. Bacteriophages encode several lytic proteins which are usually highly specific towards host bacteria. These lytic proteins can be used as alternative antimicrobials for elimination of pathogenic bacteria. Most important are endolysins and virion-associated peptidoglycan hydrolases (VAPGHs). In our study, we characterize biological and structural properties of soluble lytic transglycosylase (SLT) protein domain encoded by bacteriophage BFK20. SLT is a part of phage tail, which directly interacts with bacterial cell envelope during infection process. Study of the SLT domain of bacteriophage BFK20 should provide a valuable insight into infection process and properties coupled with degradation of the host cell wall.

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QS MOLECULE FARNESOL AND ITS IMPACT ON MIXED BIOFILMS OF *CANDIDA ALBICANS* AND *STAPHYLOCOCCUS AUREUS*

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In polymicrobial biofilms, QS represents a key process in cell-cell communication. One of the best-characterized QS molecules is the tetraprenoid alcohol farnesol (FAR) naturally produced by *C. albicans*. In a concentration-dependent manner, it plays a central role in biofilm physiology. In this work, the effect of FAR alone and in combination with antibiotics on planktonic *S. aureus* bacteria (MSSA and MRSA) as well as on mixed biofilms of *C. albicans* and *S. aureus* was investigated. In planktonic bacteria, a synergistic effect of FAR with beta-lactams, a moderate effect with kanamycin and no effect with ciprofloxacin was observed. For this experiment, E-assays were used in the presence of two concentrations of FAR (150 μM and 300 μM). The antibiofilm potential of FAR (62.5-1000 μM) was measured by the XTT method in terms of MBIC50. The MBIC50 FAR for *C. albicans*-MSSA1 and *C. albicans*-MRSA2 mixed biofilms were determined to be 125 and 250 μM, respectively. The combination of FAR (300 μM) with OXA (2 mg/ml) tested on mixed biofilms resulted in 80% inhibition compared to 4% inhibition after treatment with the same concentration of OXA alone. Scanning electron microscopy was used to characterize the architecture of biofilms and the effect of studied antimicrobial agents on biofilms. Significantly fewer candidal hyphae were observed in samples treated with FAR (300 μM) and FAR (300 μM/OXA 2 mg/ml). However, little difference was observed between the biofilm treated with FAR and the FAR/OXA combination using microscopy as well as the XTT method. Therefore, FISH has been used as a tool to monitor the activity of microbial cells in biofilms based on ribosome content. Candidate cells were active in all mixed biofilm samples, while bacteria were only partially active in the FAR-treated sample and no activity was observed in the FAR/OXA-treated sample. We may conclude, that FAR acts on several levels. By blocking the hyphae, it prevents the formation of a compact biofilm and at the same time, it increases the sensitivity of MSSA or MRSA to beta-lactam antibiotics.

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NOVEL BIOACTIVE GLASS S53P4 CREAM AS A BACTERICIDAL COATING TO PREVENT BIOMATERIAL-ASSOCIATED INFECTIONS

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Biomaterial associated infection (BAI) is a frequent complication in the use of medical implants, often cause by staphylococci. BAI are generally challenging to treat due to resistance to antibiotics which raises the danger of unsuccessful treatment.

Aim: To investigate the potential of Bioactive Glass (BAG) S53P4 cream, a non-antibiotic antimicrobial agent, to prevent BAI. BAG granules are currently used as bone fillers with osteostimulatory and antimicrobial properties. Application of BAG on biomaterial surfaces may therefore provide a way to prevent BAI. A novel BAG cream has the potential to be applied on implant material surfaces. In this study we compare the bactericidal activity of different BAG formulations against *Staphylococcus aureus*. **Method**: The bactericidal activity of BAG cream, granules and powder against *S. aureus* over 24h was compared using an antimicrobial activity assay. Similarly, the antimicrobial activity of novel BAG cream applied on Titanium Aluminum Niobium (TAN) and Polyether Ether Ketone (PEEK) discs against *S. aureus* was analysed either directly, or after pre-incubation of the discs in medium for 24h. **Results**: The bactericidal activity of BAG cream and powder was found to be higher than BAG granules. Additionally, when BAG cream is applied on TAN and PEEK implant materials, both of the surfaces exhibited bactericidal activity. **Conclusion:** The BAG cream shows promising bactericidal activity and can be applied to different types of materials. To gain deeper understanding of the mechanism of action of BAG, we intend to investigate its impact on pH levels, ion release, and their influence on bacterial killing.

NOVEL ANTIMICROBIAL COATING ON TITANIUM WITH STABLE NON-ANTIBIOTIC QUATERNARY AMMONIUM COMPOUNDS TO PREVENT IMPLANT-ASSOCIATED INFECTION

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Aim: Infection of implanted medical devices (biomaterials), like titanium orthopaedic implants, can have disastrous consequences, including removal of the device. These so-called biomaterial-associated infections (BAI) are mainly caused by *Staphylococcus aureus* and *Staphylococcus epidermidis*. To prevent biofilm formation using a non-antibiotic based strategy, we aimed to develop a novel permanently fixed antimicrobial coating for titanium devices based on stable immobilized quaternary ammonium compounds (QACs).
Method: Medical grade titanium implants were dip-coated in subsequent solutions of hyperbranched polymer, polyethyleneimine and 10 mM sodium iodide, and ethanol. The QAC-coating was characterized, and the antimicrobial activity of the coating was evaluated against *S. aureus* strain JAR060131 and *S. epidermidis* strain ATCC 12228. Lastly, we assessed the *in vivo* antimicrobial activity in a mouse subcutaneous implant infection model with *S. aureus* administered locally on the QAC-coated implants prior to implantation to mimic contamination during surgery.
Results: Detailed material characterization of the titanium samples showed the presence of a homogenous and stable coating layer at the titanium surface. Moreover, the coating successfully killed *S. aureus* and *S. epidermidis in vitro*. The QAC-coating strongly reduced *S. aureus* colonization of the implant surface as well as of the surrounding tissue, with no apparent macroscopic signs of toxicity or inflammation in the peri-implant tissue at 1 and 4 days after implantation.
Conclusions: An antimicrobial coating with stable quaternary ammonium compounds on titanium has been developed which holds promise to prevent BAI. Non-antibiotic-based antimicrobial coatings have great significance in guiding the design of novel antimicrobial coatings in the present, post-antibiotic era.

[#]*These authors contributed equally. This research was financially supported by the Health~Holland/LSH-TKI call 2021-2022, project 25687, NACQAC: 'Novel antimicrobial coatings with stable non-antibiotic Quaternary Ammonium Compounds and photosensitizer technology'.*

DIFFERENT MUTATIONS IN THE *CYP51* GENE – DIFFERENT SUSCEPTIBILITY PROFILES OF *A. FUMIGATUS* STRAINS?

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A. fumigatus is the most common causative agent of serious fungal infections. Even though we inhale several hundred conidia per day without observing health difficulties, conidia in the respiratory tract of immunocompromised people can acquire suitable conditions for germination and growth, that can lead to a wide range of clinical manifestations. Endangered groups include patients with tuberculosis, chronic obstructive pulmonary disease, or oncological diseases. An increase in reported cases of aspergillosis has also been observed in association with the COVID-19. The most severe form is invasive pulmonary aspergillosis, associated with high mortality. In order to avoid fatal consequences, early initiation of effective treatment is necessary. However, the treatment is complicated by the growing incidence of resistant strains.

In our work, we focused on the susceptibility profile of several *A. fumigatus* strains to antifungals used in clinical practice as well as in agriculture. These strains were collected in several Czech hospitals and the isolates originated from patients suffering on various breathing difficulties, and on COVID-19 bronchopneumonia as well. These isolates had confirmed various mutations in the *cyp51* gene, encoding the target enzyme of the azoles. We compared the results with a susceptible strain without mutation in *cyp51*. To evaluate the fitness cost of resistant strains, we exposed the resistant isolates to multiple stressors with different targets. Finally, we compared the virulence of the strains in Galleria mellonella *in vivo* model.

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ANTIMICROBIAL TESTING OF POTENTIAL MATERIALS IN HEALTHCARE

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Non-healing wounds are a serious social and economic problem for patients worldwide [1]. A biopolymer for wound dressing applications, such as hyaluronic acid (HA), has a supportive effect in wound care [2]. In the present study, we focused on the antimicrobial activity of materials from staple microfibers of hyaluronic acid and oxidized starch in combination with acidifying compounds. We detected the diameter of the inhibition zones around the sample. The sensitivity of selected bacteria (Staphylococcus aureus, Enterococcus faecalis, *Escherichia coli*, *Pseudomonas aeruginosa*) and yeast (*Candida albicans*) to the tested materials is also determined.
We used an agar diffusion assay for antimicrobial susceptibility testing. The evaluation of this test is based on the ability of the covering materials to inhibit bacterial growth under and around the sample. The results of this study suggest that HA-starch-based materials with acidifying addition may prove beneficial in minimizing bacterial contamination of wounds.

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CHARACTERIZATION OF VIRULENCE FACTORS OF *STAPHYLOCOCCUS AUREUS* STRAINS AFTER PHOTODYNAMIC INACTIVATION

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Staphylococcus aureus belongs to common commensal microorganisms, frequently colonizing the upper respiratory tract and skin in humans. At the same time, it is one of the most prevalent opportunistic pathogens of clinical importance causing a plethora of infections ranging from mild to life-threatening and hospital-acquired infections. Such pathogenic potential of *S. aureus* is based on arsenal of virulence factors, including toxins and immunomodulatory substances that facilitate host colonization during infection. In this study, we characterized differences in gene expression of selected genes by qPCR and the ultrastructure of *S. aureus* cells by TEM after photodynamic inactivation (PDI) between planktonic and biofilm cells of standard strain CCM 3953 and multi-resistant clinical isolate L18. The most significant differences in gene expression compared to the control were observed in genes from the group of pore-forming toxins (*psma* and *hlg*), proteases (*sspA*) and regulatory proteins (*rsp, agrA* and *mgrA*). Differences were observed in biofilm cells after PDI with an effective concentration of Phloxine B (0.05 mM) as well as in the presence of 0.05 mM Phloxine B without PDI, with a higher level of expression observed in the clinical isolate. The ultrastructure data showed that after PDI with an effective concentration of PhB (0.05 mM), the formation of mesosomes (intracellular membranes of bacteria) occurred in the cells. Comparison of the cell walls (CW) between the standard and clinical strain showed a rough CW in the standard strain in contrast to the clinical isolate. The data provided new insights in characterization of PDI-treated *S. aureus*.

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THE MICROBICIDAL ACTIVITY OF GRAPHENE QUANTUM DOTS AND A GRAPHENE QUANTUM DOT COATING

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One of the most common complications related to implantation of a biomaterial is biomaterial-associated infection (BAI). BAI is predominantly caused by commensal staphylococci, which can become pathogenic in the presence of a biomaterial. Biomaterial surfaces coated with antimicrobials are a strategy for prevention of BAI, but the use of antibiotics is discouraged because of resistance development. Graphene quantum dots (GQD) may provide an alternative for antibiotics. GQD consist of a single layer of carbon atoms in a honeycomb-like structure with photoactivation properties. Upon photoactivation, GQD produce reactive oxygen species which can kill bacteria. The aim of our study was to develop a novel GQD coating with microbicidal activity. First we tested the microbicidal activity of liquid GQD against *S. aureus* and *E. coli* in the minimal bactericial concentration assay. After photo-activation of liquid GQD with 435nm blue light, *S. aureus* appeared to be highly susceptible and was killed by liquid GQD at concentrations as low as 3.13 ug/ml, whereas *E. coli* was not susceptible to concentrations as high as 200 ug/ml. We subsequently tested a novel coating containig these GQD for its microbicidal activity against *S. aureus* and *E. coli* using the Japanese Industrial Standard assay. The coating consisted of alternating layers of GQD and polymer on glass slides. Surprisingly, the GQD coating showed promising bactericidal activity against both *S. aureus* and *E. coli*, as photo-activation of the GQD coating resulted in complete killing of both bacterial strains. Therefore, a GQD coating may be a promising strategy to prevent BAI.

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IDENTIFYING ANTIMICROBIAL PEPTIDES WITH ANTIMICROBIAL AND WOUND HEALING PROPERTIES FOR THE TREATMENT OF CHRONIC WOUNDS

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Wound healing is a complex physiological process aimed towards skin repair after injury. In case of chronic wounds, normal healing is impaired. The conditions become favorable for microbes to colonize and infect the skin. Current decline in effective antibiotic-based treatment due to the resistance crisis demands the need for alternatives. Antimicrobial peptides (AMPs) have aroused great interest as potential next-generation antibiotics. Particularly, AMPs with dual bioactivity of antimicrobial and immunomodulatory functionalities can effectively treat chronic non-healing wounds[1]. They possess antimicrobial activity by membrane disruption of microbes and immune modulation by promoting the cross talk between immune cells, resulting in progression of wound healing in an orderly manner. In the present study, a large set of AMPs are screened for the dual role with a particular interest in their wound healing function. We tested the peptides for their cytotoxity in human fiboblast cells, followed by screening for their role in distinct phases of wound healing including cell migration after wounding and angiogenesis. A robust tool to investigate cell migration is the 2D cell scratch assay, where a mechanical scratch wound is made in a cell monolayer. The assay follows the closure of the wound by cell migration from the wound edges over time. For testing the angiogeneic properties of the peptides, an *in vitro* tube formation assay was performed. Capillary tube-like structures formed by endothelial cells on a matrix was quantified using an imageJ[2] plugin to identify peptides with the best angiogenic propeties. By screening a large set of peptides for their dual bioactivity and identifying the best performing peptides, we aim to contribute to the treatment of chronic infected non-healing wounds.

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NATURAL PEPTIDES PRODUCED BY *TRICHODERMA* SPP. INHIBIT BACTERIAL AND YEAST PATHOGENS WHILE NOT AFFECTING *GALLERIA MELLONELLA* LARVAE

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Short bioactive peptides have a potential to be used as an alternative to treat infections caused by resistant microbial pathogens. Peptaibols are secondary metabolites of peptide nature produced primarily by *Trichoderma* sp. Despite being known for decades, their applicability in the light of current increase in resistance of pathogens to antimicrobials has not been re-evaluated yet. In this work, we analyzed the peptaibols produced by *Trichoderma atroviride* O1 and *Trichoderma harzianum* H1. We isolated crude extracts of peptaibols, where using MALDI-TOF we detected 19-residue (*T. atroviride* O1) and 12-,14-,18-residue peptaibols (*T. harzianum* H1). The production of peptaibols is bound to conidiation and nutrient status. The activity of peptaibol-containing extracts from the peak of production strongly inhibited the growth of methicillin-resistant *Staphylococcus aureus* (MRSA, growth under 10% compared to control) as well as *Candida albicans* SC5341 and clinical isolates of non-*C. albicans* species. In the case of MRSA, the activity of peptaibols was dampened by adding lipoteichoic acid into the cultivation medium, while horse blood serum caused minimal changes. To test cytotoxicity of peptaibols on macroorganisms *in vivo*, we used *Galleria mellonella* larvae. The larvae injected with peptaibol-containing extract from *T. atroviride* O1 displayed survival comparable to the control group. To conclude, our results demonstrate that the peptaibols may have a potential as antimicrobial agents, because of their ability to inhibit bacterial and yeast pathogens, without obvious negative effects on *in vivo* model.

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ANTIMICROBIAL PEPTIDE-FUNCTIONALIZED POLYMER BRUSHES COATED ON TITANIUM ALLOY SURFACES: EVALUATION AGAINST *STAPHYLOCOCCUS AUREUS* BIOFILMS

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Biomaterial-associated infections (BAI) remain the main cause of severe complications in patients in trauma surgery, often requiring prolonged hospitalization and the use of antibiotics. Titanium implants, being employed for decades in trauma surgery, are not exempt from bacterial colonization by opportunistic pathogens. Among them, *Staphylococcus aureus*, being one of the most recurrent bacteria associated to hard-to-treat infections, is able to produce biofilms with high occurrence rate of antibiotic resistance. Surface modification with protein, cell and bacteria repellent ultrathin polymer layers has emerged as promising approach to tackle BAI. In this regard, titanium alloy surfaces were coated with ultrathin and non-fouling homopolymer or diblock copolymer brushes, and subsequently functionalized with various antimicrobial peptides (AMPs)[1].

Titanium alloy surfaces were modified with protein- and cell-repellent poly(N(2-hydoroxypropyl)methacrylamide) (poly(HPMA)) brushes via surface-initiated atom-transfer radical polymerization (ATRP), reversible-deactivation radical polymerization (RAFT) techniques. To further increase their resistance to the bacterial attachment and biofilm formation, poly(HPMA) brushes were functionalized with highly effective AMPs bearing appropriate functionalities, such as azide (-N3) moieties, suitable for "click" chemistry. The presence of poly(HPMA) brushes on titanium surfaces and their successful modification with AMPs was confirmed by X-Ray Photoelectron Spectroscopy (XPS), whereas the assessment of dry poly(HPMA) brush thickness was realized by Spectroscopic Ellipsometry (SE).

The ability of *S. aureus* to form biofilms on the bare and AMP-functionalized poly(HPMA) brush coated surfaces will be evaluated by using the CDC Biofilm Reactor and measuring the quantity of biofilm produced and the viability of the bacteria attached. Moreover, the attachment and viability of osteoblasts on the coatings will be quantified. The strategy presented here aims to propose an experimental approach to identify promising and optimizing biomaterials.

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