been linked with some major health problems, such as diabetes, atherosclerosis, heart disease, pregnancy complications, among others. Periodontal disease affects more than 50% of the world's population during their lives, and chronicle periodontitis may affect more than 35% of the adult population. This infection causes a long-standing increase of inflammatory markers, such as C-reactive protein, which is linked with several systemic complications. The oral environment is complex and combines several bacteria and fungi living in an organized structure called biofilm. In order to maintain the balance of the oral system, the antimicrobial treatment of oral infections should selectively destroy pathogens, while keeping the non-pathogenic flora stable. Besides the microflora balance, microbial resistance of oral pathogens has been reported worldwide. In this context, Photodynamic Antimicrobial Therapy (PAT) can be an important adjuvant in the treatment of oral cavity infections. Circumvent biofilm resistance, promoted mostly by the extracellular matrix, is one of the issues that has to be addressed. In clinical practice, PAT in oral cavity is straightforward, since the site of infection is usually localized and not deep, therefore, the photosensitizer can be topically applied and the light source can be directly applied over the areas. Some clinical and experimental studies have been conducted in this field and promising results encourage the pursuit of improvements on PAT parameters, mostly to overcome the biofilm challenge.

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O182

Delivery systems for topical photodynamic antimicrobial chemotherapy

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Light and photosensitizer-mediated killing of many pathogens, termed photodynamic antimicrobial chemotherapy (PACT), has been extensively investigated *in vitro*. A wide range of organisms from the Gram-positive *Staphylococcus aureus* to the Gram-negative *Pseudomonas aeruginosa* have been proven to be susceptible to PACT. Multidrug-resistant strains are just as susceptible to this treatment as their naïve counterparts. Both enveloped and non-enveloped viruses have demonstrated susceptibility *in vitro*, in addition to fungi and protozoa. Significantly, however, only one clinical treatment based on PACT is currently licensed. This presentation provides a comprehensive review of work carried out to date on delivery of photosensitizers for use in PACT, including topical, intranasal and oral/buccal delivery, as well as targeted delivery and photo-antimicrobial surfaces. It is hoped that, through a rational approach to formulation design and subsequent success in small-scale clinical trials, more widespread use will be made of PACT in the clinic, to the benefit of patients worldwide.

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O183

Reduction of endotracheal tube biofilms using antimicrobial photodynamic therapy

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Background: The leading cause of patient death from hospital-acquired infections is pneumonia. Ventilator-associated pneumonia (VAP) is reported to occur in 12—25% of patients who require mechanical ventilation. Patients who develop VAP have a significantly longer need for mechanical ventilation (14 days vs. 5 days), a significantly longer stay in the ICU (11 days vs. 6 days), a significantly longer stay in the hospital (25 days vs. 14 days) and a mortality rate of 24—71%. The cost of treating VAP is conservatively estimated to be $1.8 billion dollars annually. The endotracheal (ET) tube has long been recognized as a major factor in the development of VAP since the microorganisms and biofilm harbored within the ET tube become dislodged during mechanical ventilation and have direct access to the lungs. These life-threatening infections are perpetuated by continuous microbiological seeding from the ET tube biofilms and become difficult to treat due to the propensity of the biofilm microorganisms to develop antibiotic resistance. Antimicrobial photodynamic therapy (aPDT) is a non-antibiotic broad spectrum antimicrobial treatment that has been demonstrated to eradicate antibiotic resistant bacteria and biofilms.

Objective: The objective of this study was to demonstrate the effectiveness of a non-invasive aPDT treatment method of eradicating antibiotic resistant biofilms/microorganisms from ET tubes without removing the tube from the patient or interrupting the ventilator circuit or cycle in an *in vitro* and *ex vivo* model.

Methods: Antibiotic resistant polymicrobial biofilms of *Pseudomonas aeruginosa* and MRSA were grown in ET tubes and treated, under standard ventilator conditions, with a methylene blue photosensitizer and 664 nm non-thermal activating light. Cultures of the lumen of the ET tube were obtained before and after light treatment to determine efficacy of biofilm reduction. An *ex vivo* study of aPDT treated ET tubes from acutely extubated patients was also performed to demonstrate the effectiveness of the treatment on human native grown endotracheal tube biofilms.

Results: The *in vitro* ET tube biofilm study demonstrated that aPDT reduced the ET tube polymicrobial biofilm by >99.9% after a single treatment. The *ex vivo* study demonstrated that 65% of the ET tubes treated obtained complete eradication of the pathogenic organisms after one PDT treatment ($p < 0.05$) and another 15% obtained a significant reduction in the pathogenic organisms.

Conclusions: aPDT can effectively treat polymicrobial antibiotic resistant biofilms in an ET tube both *in vivo* and in extubated human endotracheal tube studies. Human clinical studies are currently underway to assess the safety and efficacy of this treatment on the prevention of VAP.

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O184

Antimicrobial effects of azulene induced by light

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The purpose of this study was to investigate the effect of a polycyclic aromatic hydrocarbon (PAH), the azulene, which is an anti-inflammatory component of chamomile of the family of Asteraceae, as a photosensitiser on the reduction of *Enterococcus faecalis*, *Escherichia coli* and *Candida albicans*. Test tubes with 1 mL of BHI culture medium were incubated with 1 mL of each microorganism culture for 24 h at 37 °C and received 1 mL of the dye (25% m/V) and red laser radiation ($\square = 685$ nm, $P = 50$ mW, $\Delta t = 180$ s, $D = 45$ J/cm^2). Samples were harvested to evaluate the microbial reduction. Survivors were specified by viable counting. All three species were susceptible to photosensitisation using azulene associated to red laser. The maximum activity of azulene was observed against *E. faecalis*, followed by *C. albicans*, and *E. coli*. Data from this study support the hypothesis that the herbal azulene could be used as a photosensitiser to reduce medically critical

Gram-positive and Gram-negative bacteria, and yeast. This finding may be a significant instrument for the treatment of topical infections.

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O185

Chitosan augments photodynamic inactivation of *Candida*

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Introduction: Drug-resistant *Candida* infection became an issue in immune-compromised patients. Antimicrobial photodynamic inactivation (PDI) was shown to be a promising treatment modality for microbial infections. *Candida* was also susceptible to PDI of toluidine blue or methylene blue but related to higher dosages of photosensitizers (PS) or lasers. Both human and *Candida* are eukaryotic and high dose of PS or lasers could be harmful to human cells.

Methods: This study explored the effect of chitosan, a polycationic biopolymer, in increasing the PDI efficacy against *Candida albicans* in planktonic or biofilm states.

Results: Chitosan can augment the PDI mediated by TBO or Ce6 against *C. albicans* with 30-min incubation after PDI. At conditions when PDI could kill the microbe for about 2—4-log scale, an addition of chitosan at as low as 0.25% for 30 min after the PDI could further eradicate *C. albicans* (originally was 10^7 CFU/ml). However, without PDI treatment, chitosan alone did not exert significant antimicrobial activity with 30 min of incubation suggesting that the potentiated effect of chitosan worked after the cell damage induced by PDI. Similar results were also found in fluconazole-resistant strains and the biofilm of *C. albicans*, although higher dosages of PS and irradiation were needed.

Conclusion: The combination of PDI and chitosan was shown to be a promising antimicrobial approach against infectious disease.

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O186

Combination of photodynamic therapy with an antiseptic drug for local antimicrobial therapy

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Introduction: A therapeutic approach for selective and efficient elimination of topical bacterial and fungal infections is being developed based on the combination of a local wound antiseptic with photodynamic therapy (PDT).

Methods: The effectiveness of PDT using the sensitizer toluidine blue O and a local antiseptic as well as combinations of both modalities to kill different clinically relevant pathogens was investigated. In view of potential toxicity of this treatment strategy for host tissue, experiments on ex-vivo organ cultures of pig's ears with surgical wounds were performed using corresponding treatment modalities. Histochemical evaluation of tissue cell vitality was performed on frozen tissue sections by staining with nitro blue tetrazolium chloride.

Results: The combination of TBO—PDT with the antiseptic Octenisept resulted in a highly effective killing of the pathogens based on a synergistic interaction between PDT and the antiseptic drug, thus reducing pathogen count by more than eight orders of magnitude. In *P. aeruginosa*, this effect was assisted by coincubation with EDTA. For Gram-positive bacteria our results indicate that the synergistic interaction between PDT and the antiseptic is based on an increased cellular uptake of TBO mediated by the major components of the antiseptic, octenidine dihydrochloride and phenoxyethanol.

Corresponding experiments on organ cultures of the pig's ear with surgical wounds revealed no cytotoxic effect of the treatment on epidermal or dermal tissue cells, thus indicating good selectivity for microorganisms versus host tissue.

Conclusion: The combination of TBO—PDT with the antiseptic Octenisept may have great potential in reducing microbial load in infected wounds.

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O187

Field trials using chlorophyll derivatives with sunlight for malaria and filaria vectors control in Sub-Saharan Africa

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Introduction/Background: It is well documented that PDT has become a major approach for the control of human parasites and noxious insects. Malaria is a major life-threatening disease, where more than 350 million people are infected and more than one million people die annually due to malaria complications. Lymphatic filariasis is a painful and profoundly disfiguring disease, which infects an estimated 120 million people in tropical and subtropical areas. In this study, field trials using chlorophyll derivatives for the control of two different species of mosquito, namely *Anopheles gambiae* and *Culex pipiens*, at their larval stage, which are the vectors of malaria and filaria respectively, were performed in the infested swamps of Ethiopia, Sudan and Uganda in Sub-Saharan Africa.

Methods: Field investigations were carried out in the sewage routes as well as in the infected swamps. Confocal laser scanning microscope (CLSM) results were used for investigating the concentration and behavior of chlorophyll derivative dynamics and distribution inside the larvae.

Results: It was observed that from 85% to 100% mortality of larvae population was obtained as a result of the different concentrations of chlorophyll (0.1—100 μM). Other biological beneficiary organisms, such as the dragon fly larvae and mosquito predator larvae, which were present in the same treated swamps were not affected (target selectivity).

Conclusion: This work introduces an innovative modality for malaria and filaria vector control, which combines efficiency and low cost with the highest levels of human safety and environmental friendliness.

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O188

Clinical attachment level (CAL) gain in periodontics using photodynamic therapy as adjunct treatment

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Nowadays scaling and root planning (SRP) is a widely used procedure for the treatment of inflammatory periodontal diseases and