GHENT UNIVERSITY

LASER TREATMENT OF BIOFILMS TO IMPROVE THE EFFECT OF ANTIBIOTIC TREATMENT

BIOMARKED, a consortium of research laboratories of Ghent University, is seeking licensing partners.

Introduction

Biofilms are consortia of micro-organisms that form on various (a)biotic surfaces and are implicated in persistent infections, such as in chronic wounds and dental root canals. One of the typical properties of biofilm cells is their decreased sensitivity to antimicrobial agents (AMA) as compared to non-adherent cells. One of the important reasons for antimicrobial resistance of biofilms is hindered penetration of AMA through biofilms. There is still need for good systems and methods for disrupting or altering microbiological films to enhance the diffusion, and therefore the efficacy of AMA to treat biofilm implicated infections.

To interfere with the biofilm structure, one approach is to treat the biofilms with pharmacological compounds. Those are, however, often organism-dependent so that physical approaches to destabilize the structural integrity of biofilms are considered to be attractive alternatives as they are more generally applicable. Ultrasound therapy is one option that is being considered, but clinical applicability remains uncertain as detrimental tissue damage and bleeding can occur. Therefore, more refined physical biofilm disturbance methods are currently of interest.

Technology

Researchers at Ghent University identified a method for disrupting biofilms related to the field of healthcare and biofouling. They used a combination of laser treatment with light sensitive metallic nanoparticles to form local shock waves that can disturb a biofilm's structure and organization. The nanoparticles are first added to the biofilm, after which irradiation is provided with short but intense laser pulses. Upon absorption of the laser light, the nanoparticles quickly heat up, causing the water layer that is in contact with the nanoparticles to evaporate. This in turn results in the formation of quickly expanding water vapour nanobubbles (VNB) around the nanoparticles. The emergence and collapse of those water VNB produces local shock waves that increase the space between bacterial cells in the biofilm. This treatment of biofilms with laser-induced water VNB ensures a substantially enhanced penetration of AMA even deep into the dense biofilm cell clusters, resulting in a significantly enhanced treatment efficacy up to several orders of magnitude.

It was demonstrated that biofilms of both Gram-negative (Burkholderia multivorans, Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus) bacteria can be loaded with cationic gold nanoparticles and that subsequent laser illumination resulted in the formation of VNB inside the biofilms. For all types of biofilms studied, the combined use of VNB and the antibiotic tobramycin had a greater effect than tobramycin alone. Indeed, the use of laser-induced VNB significantly increased the effect of tobramycin between 25 and up to 3000 times, depending on the organism and treatment conditions. In a follow-up study it was additionally shown that also the efficacy of disinfectants can be enhanced in those biofilms in which there is hindered diffusion for these compounds.

Taken together, these findings demonstrate the potential of laser-induced VNB as a new approach to disrupt biofilms of a broad range of organisms, resulting in improved antibiotic diffusion and more effective biofilm eradication.

Applications

- Disruption of biofilms in the field of healthcare and biofouling
- could be applied to biofilm infections in wounds or dental root canals

Advantages

+ the dense structure of microbiological films can be altered, allowing better penetration of antimicrobial agents deep into the microbiological films, such as those present in chronic wound infections.
+ there is no or limited diffusion of heat so that aspecific effects on healthy tissue caused by heating can be avoided
+ even though laser-induced VNB can alter the biofilm structure, individual cells are not released from the biofilm by this treatment, which minimizes the risk of systemic spread of the infection
+ the method is generally applicable, independent of the composition of the biofilm or the presence of multiple microbial species within the same biofilm.
+ mechanical disturbance of the structure occurs uniformly through the 3-dimensional biofilm such that the anti-microbiotic products can enter deeply into the biofilm.
+ the vapour bubble size can be tuned using the laser intensity and thereby the degree in which the biofilm is disturbed or disrupted can be tuned, e.g. if one would need to loosen the film rather than disrupting it.

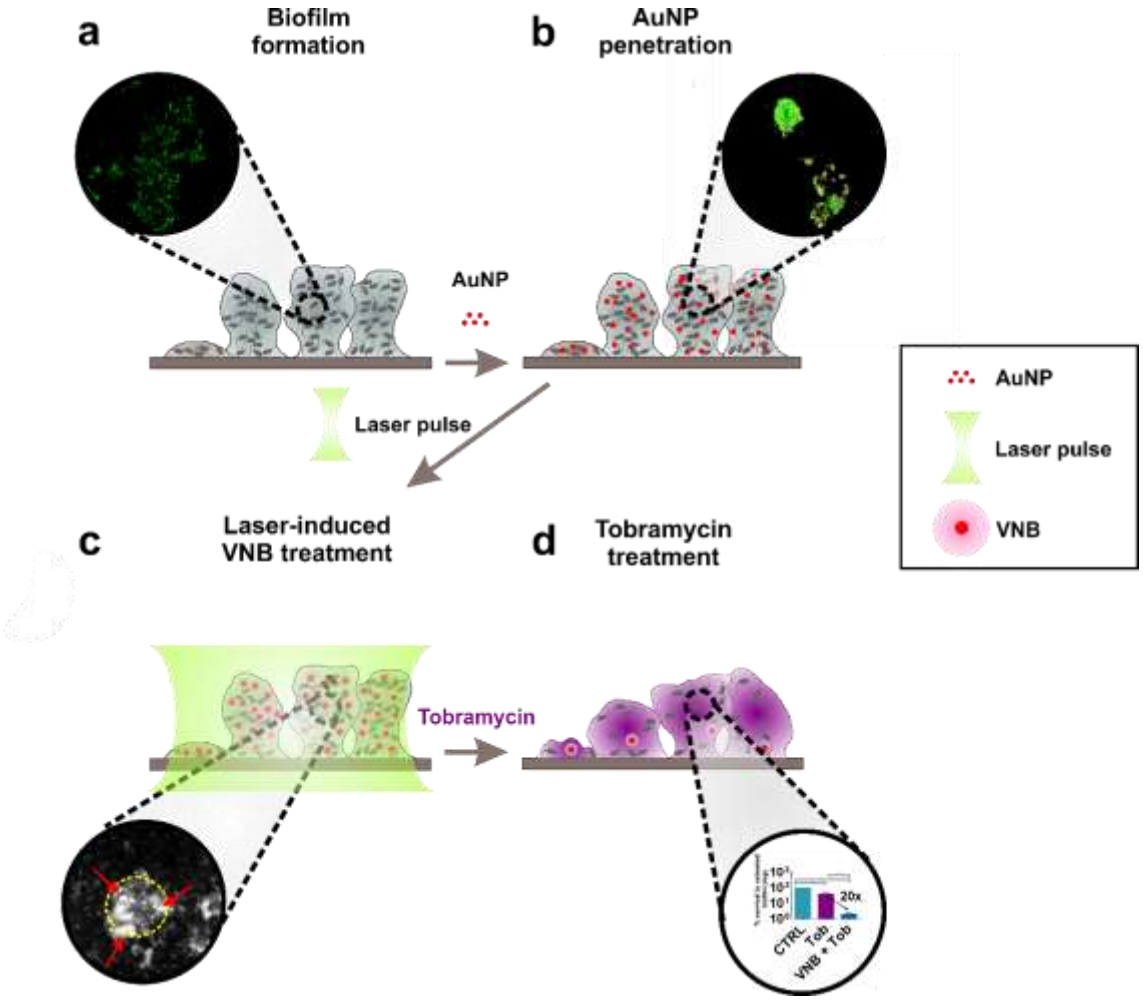

Fig. 1 Schematic overview of the experimental protocol. a) Formation of a 24-h bacterial biofilm in vitro on a glass surface. b) Penetration of gold nanoparticles (AuNP) through the biofilm. c) After absorption of an intense nanosecond laser pulse, vapour nanobubbles emerge around the AuNP. d) The mechanical force of vapour nanobubbles (VNB) creates more space between the cells allowing better penetration of antimicrobial agents.

State of development

In vitro proof-of-concepts were obtained with both Gram-negative (Burkholderia multivorans, P. aeruginosa) and Gram-positive (S. aureus) biofilms, showing that cationic gold NP can penetrate deep into these biofilms and that laser-induced VNB can substantially disturb their structural integrity. Effectivity of tobramycin and disinfectants that are used in the treatment of wound infections or dental root canals were shown to be increased up to more than 3 orders of magnitude depending on the organism and treatment conditions. In an vivo Caenorhabditis elegans model there is negligible toxicity of the AuNP at the concentrations used.

Partnership

Ghent University is seeking a licensing partner.

Intellectual property

Patent application in PCT phase: DISRUPTION OR ALTERATION OF MICROBIOLOGICAL FILMS WO2017009039 Priority Date 14 July 2015

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Keywords

biofilms, infections, antimicrobial resistance, antibiotics, disinfectants, laser treatment, gold nanoparticles, nanomedicine, vapor nanobubbles

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