"Advances in combating antimicrobial resistance of materials via laser based techniques"

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1. Introduction

Antimicrobial surfaces are valuable in medical, and industrial applications, whereas contamination with pathogens and biofouling may cause a severe risk to human well-being. Conventional approaches used for bacteria treatment comprise coating the surface with antibiotics, and application of coating containing microbicidal polymers, or metal nanoparticles. Nevertheless, such coatings have a rather short lifetime and those raise concerns related to leaching and degradation percentage of the developed coating. Therefore, there is a considerable interest in designing persistent and non-leaching bacteria - free surfaces. To acquire an antimicrobial surface, we merge micro and nanoscale structuration of mirror polished stainless steel surfaces by ultra - short laser irradiation and subsequent deposition of magnetron sputtered layer of Ag and Cu. Laser surface patterning (LSP), represents an alternative approach to a variety of surface modification methods, and it has been successfully applied for antimicrobial applications to enhance the resistance of the materials surfaces against bacterial attachment.

The use of surface texturation and incorporation of additional antimicrobial agents of Ag and Cu demonstrated good results in view of most prominent bactericidal effect against *Staphylococcus aureus, Candida albicans, Pseudomonas Aeruginosa* and *Escherichia coli*. More expressive results were obtained for the Laser structured/Cu surfaces. The laser texturation and the deposition of magnetron sputtered layers of antimicrobial material are environmentally - safe processes that are applicable to a broad range of materials. This combined approach is appropriate for studies of bacteria-surface interactions, and could provide possibilities for future antimicrobial applications in everyday use.

2. Femtosecond laser patterning and magnetron post-modification of the stainless steel

Laser induced surface texturing was obtained by ablating the surface of mirror polished cold rolled stainless steel samples (2.5 x 2.5cm, 1mm-thickness). The radiation from a commercial Ti: Sapphire femtosecond laser source (Solstice ACE, Spectra-Physics) was focused to a spot of 25μm. The central laser wavelength is 800nnm laser, pulse duration of 70fs, and 1 kHz repetition rate. Maximum output power of 6W. Two types of textures

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were designed, one which contain only nanometric structures in the form of laser induced periodic surface structures (LIPSS), and one which possess micro and nanostructures (hierarchical).

The film deposition was performed by magnetron sputtering with pure Ag and pure Cu onto the surface of mirror polished and laser structured stainless steel. The working gas was argon at a pressure of p (Ar) ≈ 0.67 Pa in a constant flow mode (13-14 sccm) at room temperature. The final film thickness was established to the 500 nm.

3. Antimicrobial studies

To evaluate the antimicrobial properties of the laser modified surfaces, *S. aureus* ATCC 6538P at specified optical density was prepared from overnight grown LB agar plates and placed onto two types of laser patterned surfaces (LIPSS and hierarchical structures). 25 µl of bacterial suspension was pipetted onto 2.5 x 2.5 cm surfaces, covered with plastic 2 x 2 cm foil and incubated in a closed vessel for 4 and 6 h. After the incubation period, bacteria were washed off from surfaces by introducing the surface to 10 ml of SCDLP medium in 50 ml centrifuge tube and by vortexing the tube over 30 sec. the wash-off in SCDLP medium was seeded on LB agar medium and bacterial number was counted after 24 h of growth at 37ºC. Results showed that already after 4 h of exposure, more than 2 log less bacteria could be retrieved from the hierarchical surfaces than from control and after 6 h of exposure, no bacteria could be washed off from hierarchical surfaces.

A qualitative assessment of antimicrobial efficacy under semi-dry conditions was conducted, utilizing the following strains: *S. aureus* ATCC 6538P, *P. aeruginosa* ATCC 15442, *E. coli* ATCC 8739, and *C. albicans* DSM11225. Briefly, suspensions of each strain with defined optical density (OD) were prepared from overnight cultures, and subsequently plated on PC-agar plates to create microbial lawns. Following air-drying, laser patterned samples (LIPSS structures) were applied onto the bacterial lawns in triplicate and the plates were incubated at room temperature. After 2 hours, the samples were carefully removed, and the plates were further incubated overnight at 37°C to allow for the growth of viable cells. In the next day, pictures of each plate were taken to quantify colony-forming units (CFUs) for the areas in contact with the samples.

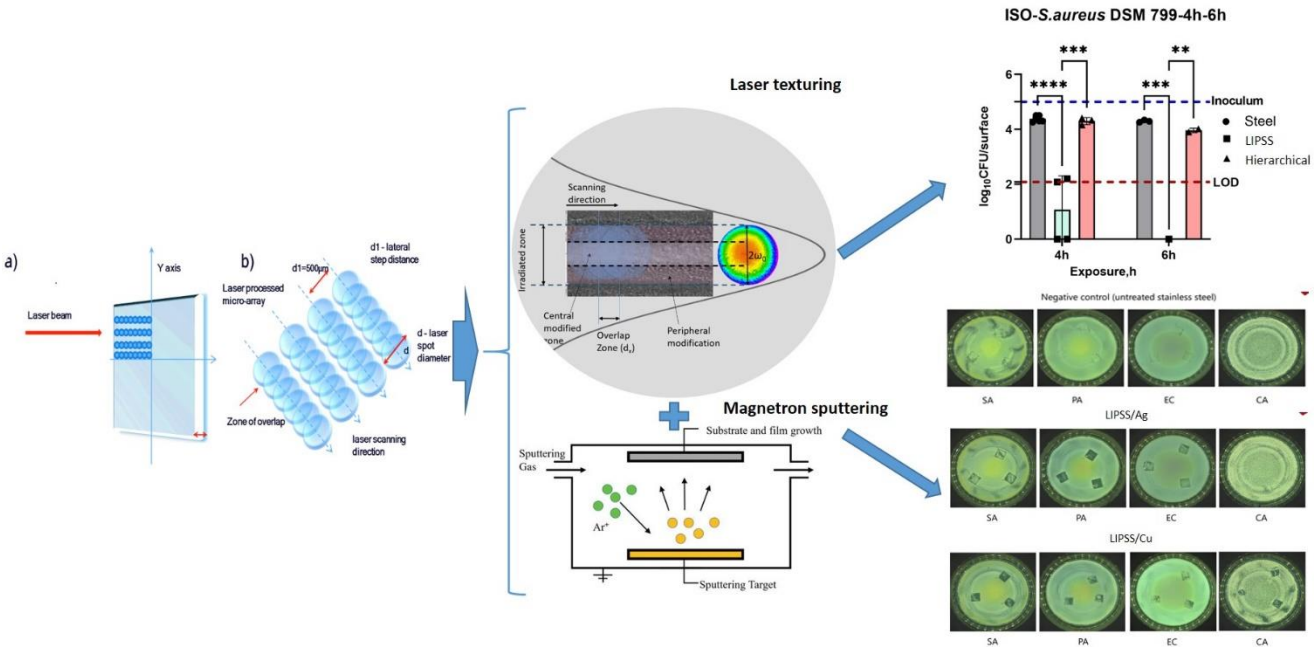

Figure 1: Effects of femtosecond laser texturing and and postmodification with magnetron sputtering and visualisation of bacterial assays and screening touch test performed on laser structured (in the form of LIPSS) magnetron sputtered samples with Ag and Cu. SA : S. aureus ATCC 6538P, PA: P. aeruginosa ATCC 15442, EC: E. coli, ATCC 8739, CA: C. albicans DSM11225

3. Discussion

Two approaches are known, that affect surface antimicrobial properties. One is the deposition of surface-repellent layers of chemical substances, and the other is to creation of an environment that acts as activating antimicrobial characteristics by creating contact between the surface and bacteria cells. The current study evaluated the combination of laser patterning with post-modification of the surface by magnetron sputtering. We first produced a set of diverse test textures (LIPSS and hierarchical structures) by femtosecond irradiation and the selected LIPSS and combined LIPSS/micro-column structures to analyse the effect of local stress imposed by the structures on the disruption of the bacterial cell membrane. After performing a bacterial viability test via a log10 CFU on laser patterned and mirror polished surface, we monitored a decrease of bacterial cell signal, with respect to control SS, and also concerning the surfaces that contain only nanometric (LIPSS) structures. The signal dropped drastically after 6h of exposition for the hierarchical structures. The performed screening test (Screen TT) only on laser-textured surfaces showed a limited effect. However, after exposition to laser structured in the form of LIPSS/magnetron sputtered samples, improved antimicrobial behaviour was clearly seen for Ag sputtered samples from the strains *S. aureus* ATCC 6538P, *P. aeruginosa* ATCC 15442, *E. coli* ATCC 8739. The LIPSS/magnetron sputtered Cu samples exhibit similar behaviour.

4. Conclusions

In summary, we have created a combined treatment approach by comprising femtosecond laser surface processing (using LIPSS and combined micro- and nano texturation) and magnetron deposition deposition to generate enhanced bactericidal surfaces. The results only from non-sputtered samples demonstrate good antimicrobial effect. The interaction between the bacteria cells and the laser structured magnetron

sputtered surfaces, have a synergistic effect simultaneously generating mechanical disruption due to the developed nanometric surface roughness, on the bacteria, and additionally adding the influence of known antimicrobial agents as Ag and Cu.

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5. References

[1] K. Schwibbert, A. M. Richter, J. Krüger, J. Bonse, "Laser-Textured Surfaces: A Way to Control Biofilm Formation?", Laser Photonics Rev.2024,18, 2300753, DOI: 10.1002/lpor.202300753

[2] I. Gnilitskyi, S. Rymar , O. Iungin, O. Vyshnevskyy, P. Parisse [g], G. Potters , A. V. Zayats and O. Moshynets, "Femtosecond laser modified metal surfaces alter biofilm architecture and reduce bacterial biofilm formation", Nanoscale Adv., 2023, 5, 6659, DOI: 10.1039/d3na00599b