Interaction of cold microwave plasma torch with biopolymer surfaces: RONS distribution and decontamination effects

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Interactions of various non-thermal plasma systems with various surfaces have been studied for many decades. But not only due to the increased resistance of microorganism to conventional antimicrobial drugs, special attention is given to different plasma jets or torches interacting with biological surfaces mainly during the last decade [1]. Although numerous studies are available on the diagnostics of selected reactive oxygen and nitrogen species (RONS) like ·OH, ·O, NO, NO_2, O_3 or H_2O_2 in the gaseous phase using optical emission and absorption spectroscopies or laser induced fluorescence [2], information about their distribution on the solid surfaces is still lacking. The presented contribution demonstrates the RONS distribution of the microwave plasma torch on the biopolymer surfaces and compares it with its decontamination effect on selected microorganisms.

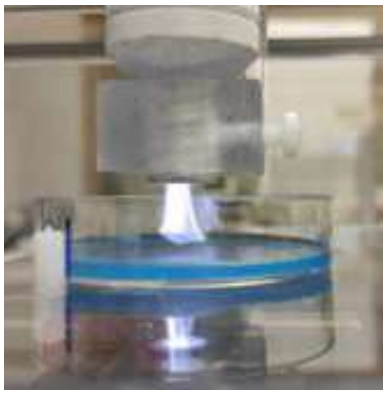

Fig. 1: Plasma treatment of biopolymer containing indigo dye by the microwave plasma torch.

The used plasma system was based on the microwave torch (surfayok) in argon [3]. Plasma was generated at atmospheric pressure with low power from 9 to 15 W, and the argon flow of 5 L/min. The treated biopolymer plates containing reagents for RONS determination or microorganism culture for decontamination effect observation were placed under the plasma torch so that the tip of the active plasma plume was positioned just 1.5 mm above the biopolymer surface ensuring the highest RONS spreading on the whole plate surface (Fig. 1). Petri dishes with the diameter of 50 mm were used for the agar-based biopolymer layer preparation (thickness of 5 mm). Following reagents were embedded into the biopolymer structure in order to determine particular reactive species by their colour change: indigo dye for ozone, potassium iodide with starch for non-specific ROS, and Griess reagent powder kits (Merck) for nitrates and nitrites. To observe decontamination effects visible as an inhibited growth area, bacteria *Escherichia coli*, *Staphylococcus epidermidis*, *Propionibacterium acnes* as well as yeast *Candida glabrata* were chosen. The prepared biopolymer layers were treated by the cold plasma torch at different treatment times and modes (static or scanning).

Formation and surface distribution of ozone during the static microwave plasma torch treatment in different distances of the active plasma tip from the biopolymer surface was evaluated by the indigo dye discoloration. Among three tested distances of 0, 1.5, and 10 mm, 1.5 mm was determined as the most suitable for the uniform static treatment of the whole biopolymer surface in the 50 mm Petri dish. Formation of nitrates and nitrites was confirmed by the brownish or pink colour of the commercial kit, respectively. Their distribution over the biopolymer surface was increased during the static torch treatment time from 30 to 300 seconds (Fig. 2). The same results were achieved for the non-selective ROS effects. The total ROS formation caused oxidation of potassium iodide to iodine which was determined by the blue colour in the reaction with starch. According to the colour intensity related to the RONS concentration, nitrites (pink colour) seemed to be the most effectively produced species in the microwave plasma torch. Comparing the results of RONS distribution during the static microwave

torch treatment to antimicrobial effects on microorganisms evaluated by the size of the inhibited growth area, we can state that they were in good agreement, even for highly resistant bacteria *P. acnes* (Fig. 2). I.e., the size of the inhibited growth area increased with the increasing treatment time and the affected area corresponded to the area of the highest RONS production.

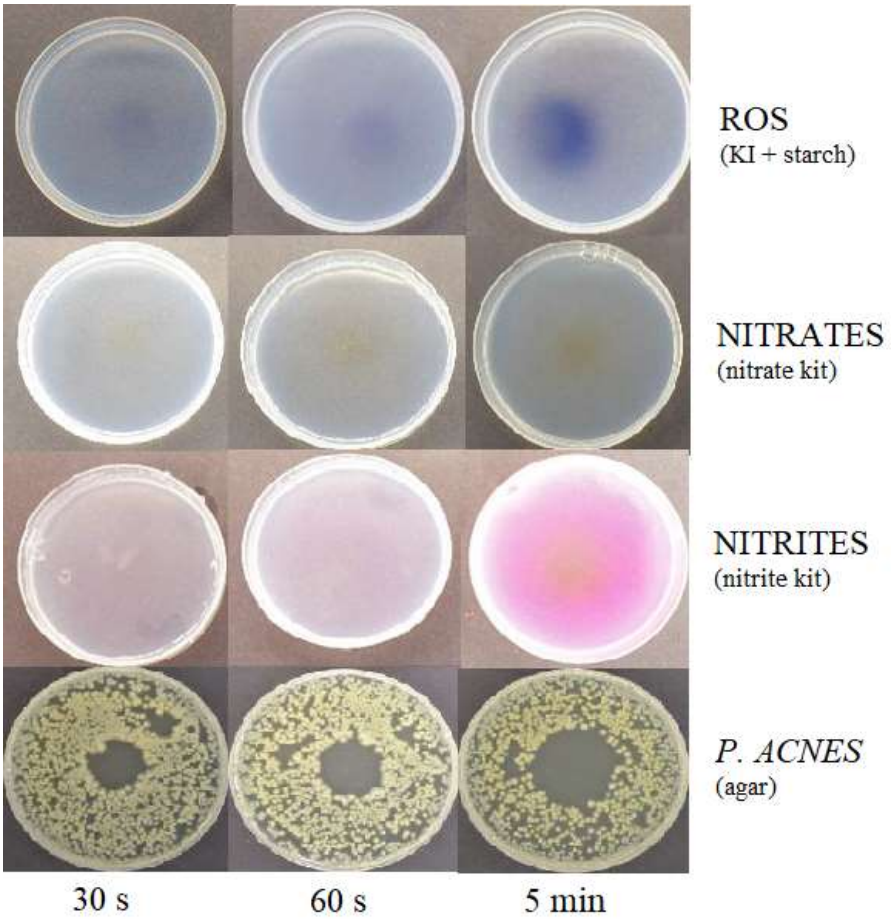

Fig. 2: Demonstration of RONS distribution and decontamination effects observed by inhibited growth area of *Propionibacterium acnes*. Biopolymer containing appropriate reagent kit was treated by the cold microwave torch in argon (power of 9 W; argon flow of 5 L/min) for 30 s, 60 s, and 5 min, respectively.

Besides the static mode of the cold microwave torch, two modified plasma regimes were applied for the biopolymer surface treatment to obtain homogeneous RONS distribution and higher decontamination effects. Employing the scan mode of the plasma torch in a “snake” shape, a uniform distribution of desired species was observed. Moreover, decontamination over 70 % was achieved for bacteria *E. coli* and yeast *C. glabrata* even after one run of such scanning treatment. Contrary to the scan mode, using the lid on the Petri dish during the treatment did not bring the expected impact of the increased RONS formation and antimicrobial decontamination, probably due to the argon accumulation under the lid preventing the supply of the surrounding air.

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