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Antimicrobial applications of cold plasma and plasma-activated water (PAW)

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Background: Cold plasma generates a mix of atoms, excited molecules, charged particles, reactive oxygen and nitrogen species (RONS), and UV photons. Many of these reactive species demonstrate antimicrobial activity against bacteria, viruses, fungi and even otherwise resistant biofilms and spores[1]. Biofilms are complex communities of microbial cells that are attached to a living or non-living surface or themselves and are encased within self-produced extracellular polymeric substances (EPS). Resistance through the EPS and community lifestyle make biofilms very hard to eradicate.

It has been shown that a plasma discharge can be made in water to produce plasma-activated water (PAW) that retains the antimicrobial properties. PAW is particularly suitable for the treatment of heat sensitive products or tissue, because it does not cause any etching effects. PAW treatment is environmentally friendly, as it will revert to normal water and will not leave any toxic residues on the treated product.

Methods: Plasma treatment was performed using different plasma bubble reactors (Fig 1). The reactors consisted of acrylic tubes with machined caps on each end with a stainless-steel rod (8mm OD) positioned coaxially inside the tube along its full length as the high voltage electrode. The plasma was generated under atmospheric conditions via electrical discharges supplied by a Leap100 high voltage power supply (PlasmaLeap Technologies, Sydney, Australia) capable of supplying voltages up to 80kV (p-p) at discharge frequencies up to 3000 Hz[2].

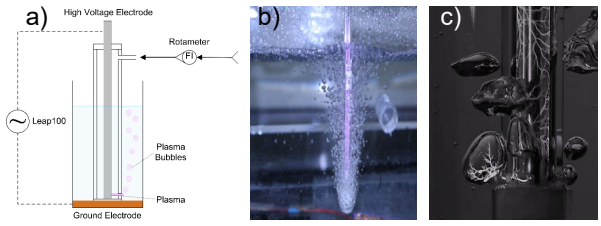

Fig 1: Generalized schematic diagram of the plasma-bubble generator (a), photograph of the reactor in operation (b) and of a discharge in a forming bubble (c)

The tube of the plasma generator was perforated with four 04 µm holes approximately 8mm from the base of the tube which was submerged under the water. Another reactor had a diffuser fitted at the end to maximize air bubble generation. A rotameter provided compressed air at a flow rate of 1L/minute into the plasma reactor. A discharge was formed in bubbles leaving the reactor holes into the water (Fig 1b, c), generating reactive species inside the bubbles which contacted with the water via the large surface area at the bubble/water interface. PAW generated from MilliQ and tap water were compared. The resulting PAW was tested against a range of microorganisms, including planktonic cells and biofilms.

Results: The different plasma reactors were tested for antimicrobial activity against planktonic *Escherichia coli* (Gram negative) and *Listeria innocua* (Gram positive) cells in situ. Both species were effectively eliminated after 10 min of treatment with PAW MilliQ having stronger antibacterial efficiency than PAW tap water. The pH of PAW MilliQ decreased significantly (from 7.1 to 3.3) over the course of the experiment possibly contributing to the higher antibacterial activity.

The PAW reactors were also tested for their suitability to be used as a method of decontamination for chicken meat. Five to fifteen-minutes of plasma-bubble treatments of inoculated chicken skin led to a significant reduction in colony forming units (CFU). Interestingly, the decontamination efficiency is dependent on the plasma discharge frequency with a higher frequency of 2000 Hz leading to a higher CFU reduction compared to a lower reduction at 1000 Hz. An evaluation of the physicochemical properties of the generated PAW revealed increasing conductivity, ozone, nitrite, nitrate, hydroxyl and peroxide concentration with higher frequencies, all contributing to the observed antimicrobial effect.

Moreover, PAW was also demonstrated to be active against *E. coli* biofilms. Biofilms were grown on stainless steel and on glass coupons. Freshly prepared PAW was then added to mature biofilms and incubated for up to 1 h. A 3 log CFU reduction was achieved for both coupon materials (Fig 2a). Fluorescent microscopy using live/dead fluorescent staining (where viable cells appear green and dead cells appear red) revealed that PAW treated biofilms had large amounts of dead cells (Fig. 2 b, c).

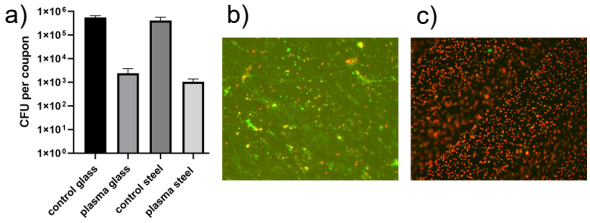

Fig 2: CFU reduction of PAW treated biofilms (a). Live/dead stained images of control (b) and PAW treated biofilms (c).

References:

1. Mai-Prochnow, A. et al. Int. J. of Antimicrob. Agents 43, 508-517 (2014).
2. Scally, L., et al. Materials 11, (2018)