Antibiofilm Effects of Peroxide and Reactive Oxygen: Chemistry and Microbiology

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INTRODUCTION

Biofilm in Dentures

Bacteria grow on surfaces in the form of a biofilm, a complex structure encased in an extracellular matrix of polysaccharides, proteins, nucleic acids among other metabolites[1].

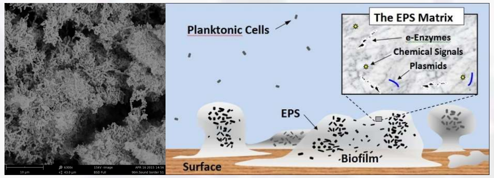


Figure 1: Right *Streptococcus mutans* 48 hour biofilm Left schematic view of a biofilm

Dental plaque is the biofilm that colonizes our mouth cavity. Dentures due to their prolonged use and durability tend to accumulate plaque (biofilm), become stained, and in some extreme cases calculus (an hardened form of plaque, very hard to remove) can be formed[2]. The biofilm found in dentures has the same general composition as the dental biofilm but higher numbers of *Candida albicans*[3].

Poor denture hygiene and the presence of Candida albicans in the mouth cavity has been linked with complications such as denturestomatitis and candidiasis among others[4].

Concerning denture cleaning the ideal system needs to be able to possess good bleaching capabilities and be an excellent antimicrobial agent.

Reactive Oxygen Species

Reactive oxygen species (ROS) such as H_2O_2 and singlet oxygen are important mechanisms in host defence, to mediate inflammation and participate in the killing of microorganisms. by targeting structures in the microbial cell, such as thiol groups, metal centres, protein tyrosines, DNA and lipids[5]. The range of targets and being environmentally friendly make ROS an ideal antimicrobial agent.

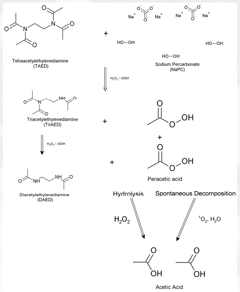


Figure 2: Lifecycle of peracetic acid from generation to decomposition.

Peracetic acid is also seen as an excellent antimicrobial agent it possesses a similar antimicrobial activity as H_2O_2 but at much lower concentrations, approximately 0.3% in comparison to 10 to 30% needed for H_2O_2. Resistance of bacteria towards peracetic acid has not yet been observed[6].

EXPERIMENTAL

Aims and Objectives

The aims and objectives of this work are to study the chemistry behind the generation of ROS within a formulation to contribute towards the optimization of existing formulations that rely on the TAED/ NaPC to produce ROS *in situ;*

Observe the biofilm removal effects of such formulations on oral biofilms.

Methods

Polident3 (denture cleanser) was used to observe the chemical reactions behind the generation of ROS within a formulation. The TAED /H_2O_2 system present will be studied alongside key ingredients and its capability to generate ROS analysed using **[1]H-NMR** and **fluorimetry assays**. (Figure 3).

Subsequently some of the antimicrobial properties of the ROS produced were tested against *Streptococcus mutans* and *Candida albicans* biofilms (figure 4)

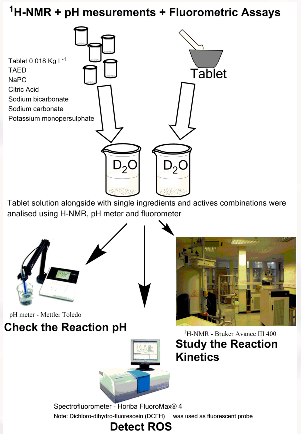


Figure 3: Schematic of H-NMR, pH measurements and Fluorometric assays

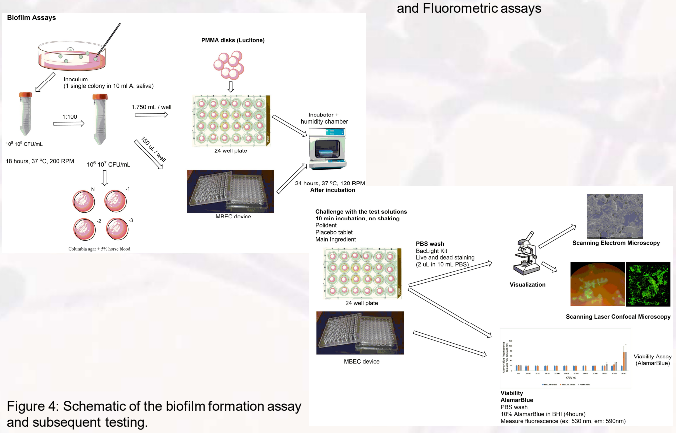


Figure 4: Schematic of the biofilm formation assay and subsequent testing.

RESULTS

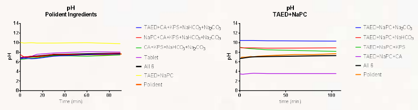


Figure 5: pH measurements, the pH of single ingredients and combinations of ingredients. (TAED-Tetraacetylethylenediamine; NaPC-Sodium percarbonate, CA-Citric acid; KPS-Potassium monopersulfate)

pH plays an important role in the ROS generation, the optimal pH for Peracetic acid decomposition lies around 5.5 – 8.2

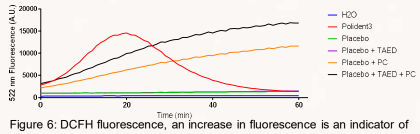


Figure 6: DCFH fluorescence, an increase in fluorescence is an indicator of the presence of ROS (TAED-Tetraacetylethylenediamine; PC-Sodium percarbonate,)

The fluorometric assays allowed us to determine the generation of ROS on the Polident tablet solution and the placebo tablet solution.

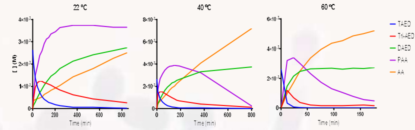
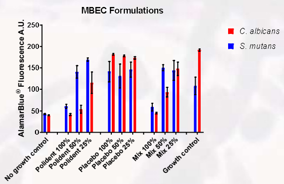
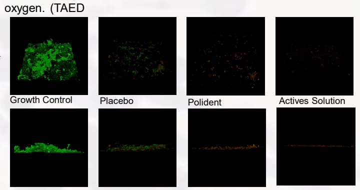


Figure 7: H-NMR kinetic study. Left 22 °C, middle 40 °C and right 60 °C. The increase in temperature increased the reactions speeds including the conversion of peracetic acid into acetic acid and singlet oxygen. (TAED

The H-NMR studies showed that the reaction rates increased with temperature. By applying the ahrrenius law the activation energy for acetic acid formation was found to be 67.8 Kj.mol[-1].





SLCM imaging of dual species biofilm (*C. albicans and S. mutans*) after 10 minutes treatment with Polident, placebo tablet and simplified formulation

The MBEC and SCLM results showed that ROS are essential for biofilm removal within this type of formulation. Polident solution and main actives solution partially removed the biofilm and the majority of remaining cells were dead (red) while the placebo tablet that does not generate ROS removed some of the biofilm but the remaining cells were alive (green).

CONCLUSIONS

- HNMR and fluorometric studies will enable us to study and develop novel formulations.
- The optimal pH for ROS generation within this system was found to be between 7-8.
- The reaction rates increased with the temperature.
- Polident3 and the simplified formulation (Mix) showed strong antimicrobial properties, although the pathogen *C. albicans* was more resistant than the other organisms.
- The generation of ROS plays a vital role in the effective killing and subsequent removal of biofilm.

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