Effects of Singlet Oxygen on Streptococcus mutans

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Abstract

Periodontal disease affects over 700 million people worldwide. Currently, there are various approaches used to treat periodontal disease such as tooth scaling. While this method effectively reduces the microbial density in the periodontal pockets, it is not able to eliminate pathogenic bacteria. In the experiments conducted for this project, our main goal was to observe the effects that singlet oxygen had on the bacterial strain Streptococcus mutans—a facultative anaerobe that is known for initiating dental caries. Singlet oxygen is a highly reactive oxygen species that has the ability to kill bacteria.

Experimental Approach

The singlet oxygen was generated by irradiating a superhydrophobic film (SH film) containing a photosensitizer with red LED light. Superhydrophobic film samples were immersed in a UV-vis cuvette filled with a controlled number of bacteria and irradiated while being constantly mixed. Two irradiation times were used to identify the time interval required to achieve a five log reduction in bacteria per milliliter. For each irradiation time, two superhydrophobic film samples were run in duplicate, along with an untreated sample and two controls: one with the superhydrophobic film but with no light; and one with no superhydrophobic film, but with light. A five-log reduction was observed by counting colony-forming units (CFUs) after a 30-minute irradiation time. Our eventual goal is to treat periodontal disease, and other diseases, by using singlet oxygen.

Results

CFU conversion to bacteria per mL: CFU is converted to bacteria per mL by multiplying the CFU count by the dilution factor, then by the amount pipetted into the plate.

15 MINUTE IRRADIATION
➢ The graph shows the plates observed for each sample calculated for bacteria per mL;
➢ We observed a 3 log reduction from our untreated L-S- sample of 1.2*10⁴ to an average of 1.2*10¹⁵ bacteria per mL;
➢ L-S (no light but has film) had an average of 1.2*10⁴ bacteria per mL;
➢ L-S+ Controls (light but no film) had an average of 1.2*10⁴ bacteria per mL;
➢ Both of our L-S+ Samples show consistent results and promising reproducibility.

30 MINUTE IRRADIATION
➢ L-S+ samples showed a 10⁵ log reduction from the untreated L-S- sample of 1.2*10⁴ to an average of 1.2*10⁴⁴ bacteria per mL;
➢ L-S (no light but with film) had an average of 8.5*10⁴ bacteria per mL;
➢ L-S+ (no film but with light) had an average of 1.3*10⁴ bacteria per mL.
➢ From these findings we observed that time is instrumental to showcasing the efficacy of the SH film.

Sample Key
L+ with LED light
S+ with Superhydrophobic film
L- no LED light
S- no Superhydrophobic film

Absorbance Comparisons

We took absorbances to possibly observe a correlation between the time of irradiation and the increase of absorbance due to cell fragmentation (cell death). Using this quantitative and observable method to compare the absorbances with the amount of time they were treated, we were able to measure the absorbance before treatment and after treatment. This would have enabled us to confirm our hypothesis and further enhance our testing to demonstrate the efficacy of the superhydrophobic film.

For our 15 minute treatment, we were able to achieve a 3 log difference between our controls and our treated samples;
From our data for the 30-minute irradiation treatment, we were able to successfully produce a 5 log reduction in bacteria per mL;
Therefore, we were able to successfully prove our hypothesis with the 30 minute irradiation treatment. Because a bacterial reduction was also achieved at the 15 minute irradiation, we have good reason to believe that the SH film is an effective instrument when used in conjunction with sufficient time as well as an LED light to kill Streptococcus mutans.

References


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