Quantifying Singlet Oxygen Required To Eradicate Oral Pathogens

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Introduction
Challenge: The prevalence of periodontal diseases, such as periodontitis, is widespread, and their advanced forms affect approximately 11% of the global population. Through recent treatment and analysis of periodontal pathologies, it has become evident there is a great need for an innovative treatment with greater capabilities than traditional scaling and root planning alone or the use of antimicrobial agents.

Singlet Oxygen (\(\text{O}_2^*\)) Photodynamic therapy (PDT) has the potential to fulfill this need. PDT is a non-invasive therapeutic modality that can be used to treat various periodontal pathologies. PDT is reliant on the combination of ground state molecular oxygen (\(\text{O}_2\)), a photosensitizer (PS), and light to form reactive oxygen species with cytotoxic action. Specifically, by a Type II photo-process, energy transfer from the PS triplet state to ground state molecular oxygen (\(\text{O}_2\)) occurs, which creates excited state singlet oxygen (\(\text{O}_2^*\)). Singlet oxygen oxidizes many biological molecules and ultimately leads to complete inactivation of target cells. However, when considered as a treatment modality for periodontitis, the hypoxic environment of periodontal pockets limits the effectiveness of PDT treatments.

Methods

Figure C depicts the test setup used. A 669 nm laser diode was attached to a fiber optic pen aligned into a cuvette with 10^5 CFU planktonic cultures of \(P\). gingivalis or uric acid trapping solution for different lengths of time. The superhydrophobic surface responsible for generating the PDT is attached at the end of the fiber optic pen. The printing technique and preparation of the PDMS posts cured with Ce6 sensitizer particles is shown.

Results & Discussion

Figure D demonstrates uric acid degradation, which is directly correlated to singlet oxygen generation. The rate is initially rapid, but slows over time. This may be due to the photobleaching of the chlorin e6 photosensitizer. Based on the uric acid degradation, the greatest yield of singlet oxygen occurred at 60 minutes, but generally began to level out after exposure of 50-60 minutes.

Each experimental group, in both the uric acid and bacterial trials was repeated three times. Values displayed represent an average of those trials with standard deviation indicated. Based on the uric acid degradation, the relationship depicted clearly portrays the linear correlation between singlet oxygen generation and the reduction of \(P\). gingivalis.

Light Only, n=3
Surface Only, n=3
PDT, n=3

Conducting PDT on \(P\). gingivalis depicted the greatest log reduction after 50 minutes of exposure with an approximate log reduction of 2.8. This represents a reduction of approximately 99.9% of the \(P\). gingivalis bacteria.

Differences in log reductions between the 50 and 60 minute group are not significant, suggesting that once the production of singlet oxygen plateaus, so too does the reduction of bacteria during PDT.

Since the production of singlet oxygen is Type I photoprocess and free radicals in a Type II photoprocess (5), which are cytotoxic to cells.

Significance & Current Research

Pre-clinical in vivo trials using this technology are being performed on Wistar rats induced with periodontitis at Harvard Medical School/ Massachusetts General Hospital.

Disease induction is performed using the ligament model followed by a seven day induction period, as seen in Fig. H.

In vivo treatment is being performed at a significantly lower light dose compared to the planktonic cultures to avoid oxidative and heat damage to gums.

PDT is applied on both the buccal and lingual regions, as portrayed in figures I and j, respectively.

While bacterial reductions are important to achieve, the clinical application strongly focuses on evaluating the ability of the therapy to treat the bone resorption and potent immune response triggered by periodontitis.

Future work will focus on clinical human trials using this technology to treat advanced or chronic periodontitis.

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