Toluidine Blue Ortho @ Magnetic Photosensitizing Nanoplatform: Antibiofilm Effect against Thick Constant-depth-film-fermenter Biofilms

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Statement of Purpose: Antimicrobial photodynamic therapy (aPDT) as an adjunctive disinfection technique revealed several limitations. Regarding the photosensitizers, hydrophobicity and degradation susceptibility impair the bacterial interactions. Poor photosensitizer penetrability into thick and mature oral biofilms concerning the clinical application. Here, we conjugated toluidine blue ortho (TBO) and superparamagnetic iron oxide nanoparticles (SPIONs) into a microemulsion. The antibiofilm performance of the designed nanoplatform, named MagTBO, was challenged against difficult-to-kill, thick, mature biofilms. For that, we grow biofilms with a well-defined thickness to match the thickness of clinically occurring biofilms in a constant-depth-film-fermenter(CDFF). The antibacterial effect of the designed MagTBO nanoplatform was investigated against *Streptococcus mutans* biofilms grown for 5 and 10 days using the constant-depth film fermenter (CDFF) dynamic model. **Title:** Toluidine Blue Ortho @ Magnetic Photosensitizing Nanoplatform: Antibiofilm Effect against Thick Constant-depth-film-fermenter biofilms. **Materials & Methods:** SPIONs were synthesized via chemical co-precipitation (Sun et al., 2017) and characterized using a transmission electron microscope (TEM, FEI Tecnai T20, Hillsboro, OR, USA). Then, the MagTBO microemulsion containing 2.5 wt.% of SPIONs and 100 µg/mL of TBO was prepared to utilize the high ultrasonication method (Rout et al., 2016). The 2.5% MagTBO microemulsion was composed of water, eucalyptus oil (Spectrum, New Brunswick, NJ, USA), polysorbate 20 (AmericanBio, Canton, MA, USA), and glycerol. The cytotoxicity of the MagTBO microemulsion was assessed using human gingival fibroblast, and the thermodynamic stability was evaluated using freeze-thaw and centrifuge stress tests. For the biofilm assay, root dentin slabs (3×3×1 mm) were prepared, polished, and based on their Vicker's microhardness value (25 g load for 10 s). In a constant-depth-film-fermenter, biofilms are grown on the bottom of wells with set depths, while a scraper blade removes biofilm growing above the wells. The dentin slabs were mounted inside the CDFF holders and recessed to a depth of 300 µm to develop biofilms. Brain-heart infusion (BHI) broth was used to grow the biofilm inside the CDFF reactor (University of Wales, Cardiff) at a flow rate of 0.5 mL/min (Cenci et al., 2009). Sucrose pulsing was performed 4 times a day to maintain a final concentration of 2% sucrose within the growth medium. The pH was evaluated daily. Following 5 and 10 days of biofilm development, the dentin slabs were removed subjected to aPDT treatment using TBO alone, 2.5% MagTBO, and 2.5% MagTBO with a magnetic field (neodymium magnet; 1T). Slabs with no treatment were used as a control. Following the biofilm assay, the microhardness was re-evaluated. Data were analyzed using one-way ANOVA and Tukey tests. **Results:** The mean size of the SPIONs was 8.15 nm. The 2.5% MagTBO microemulsion was designed successfully with excellent thermodynamic stability as no phase separation was observed following the stress tests. Besides, the 2.5% MagTBO microemulsion showed good biocompatibility, higher than TBO alone when they were exposed to gingival fibroblasts. In the biofilm assay, aPDT using TBO alone was ineffective in inhibiting the 5 and 10-day *S. mutans* biofilm, compared to the control ($p > 0.05$). However, aPDT using the MagTBO microemulsion resulted in 4 to 5-log reduction compared to the control ($p < 0.001$). No difference was observed in bacterial reduction when the magnetic field was applied. The mean pH value through the 10-day biofilm challenge was 4.51±0.14. At the 10-day biofilm challenge, dentin's microhardness was reduced significantly by more than 90%, with no difference between the groups.

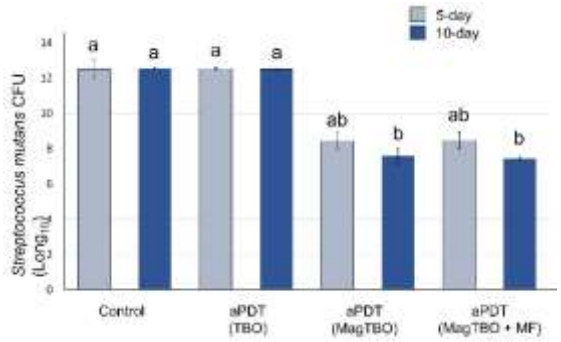

Figure 1. The colony-forming units (CFUs) of *Streptococcus mutans* following different aPDT treatments. aPDT using the 2.5% MagTBO microemulsion inhibited the biofilm by 4 to 5-log compared to aPDT using TBO alone.

Conclusions: Using microemulsion as a photosensitizer carrier and SPIONs as a navigation system demonstrated a potent antibacterial effect against *S. mutans*, the leading pathogen in dental caries. This nanoplatform significantly improved the aPDT antibiofilm properties and enhanced the TBO's biocompatibility and stability.

- Patent pending application related to the use of the MagTBO photosensitizer nanoplatform.