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

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Introduction

- Antimicrobial photodynamic therapy (aPDT) as an adjunctive disinfection technique revealed several limitations. (1)
- aPDT uses nontoxic dyes called photosensitizers (P.S.s) that can be excited by harmless visible light to produce cytotoxic reactive oxygen species (ROS). (1)
- Regarding the photosensitizers, hydrophobicity and degradation susceptibility impair the bacterial interactions, resulting in poor photosensitizer penetrability into thick and mature oral biofilms. (2)
- Targeting strategies to break the biofilm barrier or enhance the infiltration of antibacterial agents are being increasingly explored. Therefore, the rational design of photosensitizer-based nanoplatforms to overcome the above key obstacles for achieving potent aPDT is of great significance for managing oral infections. (3)
- Here, we conjugated toluidine blue ortho (TBO) and superparamagnetic iron oxide nanoparticles (SPIONs) into a microemulsion, named (MagTBO).

Objectives

This report evaluated the hypothesis that aPDT via the MagTBO nanoplatform, guided by magnetic force, can effectively inhibit thick and mature *S. mutans* biofilms grown for 5 and 10-day.

Materials & Methods

- SPIONs were synthesized via chemical co-precipitation(4) and characterized using a transmission electron microscope (TEM, FEI Tecnai T20, Hillsboro, OR, USA).
- The MagTBO microemulsion containing 2.5 wt.% of SPIONs and 100 μg/mL of TBO was prepared via the high ultrasonication method. (5)
- The 2.5% MagTBO microemulsion was composed of water, eucalyptus oil, polysorbate 20, and glycerol.
- The cytotoxicity of the microemulsion was assessed using human gingival fibroblast.
- The thermodynamic stability was evaluated using freeze-thaw and centrifuge stress tests.
- Root dentin slabs (3×3×1 mm) were prepared, polished, and subjected to Vicker's microhardness value (25 g load for 10 s).
- Then, the dentin slabs were subjected to a CDFF bioreactor for biofilm formation.
- **Statistical Analysis:** Data were analyzed using one-way ANOVA and Tukey tests.

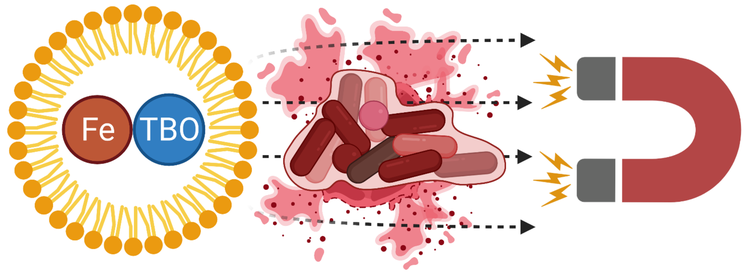

Figure 1. A schematic drawing of the synthesized 2.5% MagTBO microemulsion demonstrates its antibacterial action against oral biofilms using the magnetic field force to attract the microemulsion through the biofilm layers.

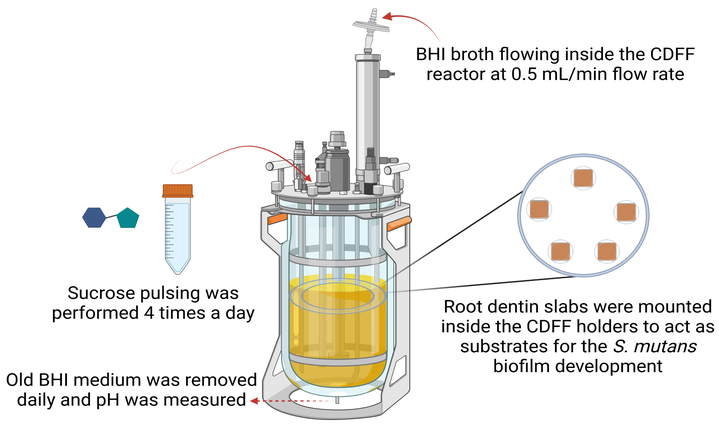

Figure 2. A schematic drawing of the dynamic caries model demonstrates the CDFF reactor's design. The CDFF container having the BHI media is connected to a peristaltic pump to continuously provide fresh BHI media at a flow rate of 0.5 mL/min. The dentin slabs are mounted inside the CDFF holder to allow biofilm development over the slabs.

Results

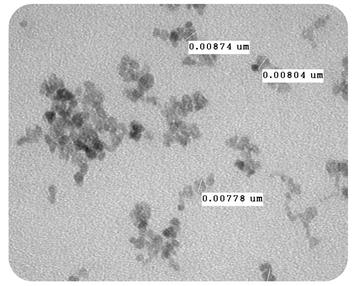

Figure 3. The mean size of the SPIONs was 8.15 nm. The 2.5% MagTBO microemulsion was designed successfully with excellent thermodynamic stability as no phase separation.

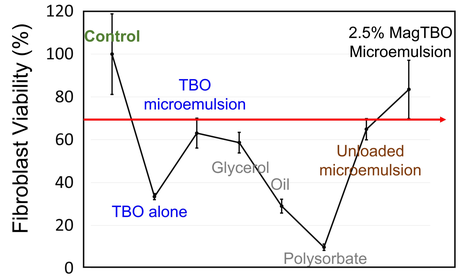

Figure 4. The 2.5% MagTBO microemulsion showed good biocompatibility, higher than TBO alone. The cell growth promotion induced by SPIONs could be achieved by reducing the intracellular H_2O_2, opposing the cytotoxic effect of TBO.

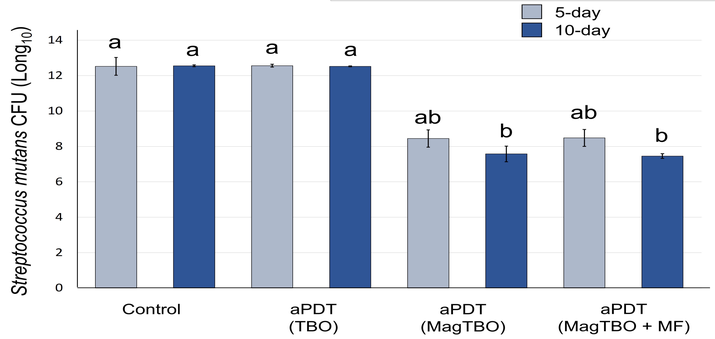

Figure 5. aPDT using TBO alone was ineffective in inhibiting the 5 and 10-day *S. mutans* biofilm ($p > 0.05$). However, aPDT using the MagTBO microemulsion resulted in 4 to 5-log reduction compared to the control ($p < 0.001$).

Conclusion

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.

ACKNOWLEDGMENTS

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