Supporting Information

Effective Aggregation-Induced Emission sonosensitizer DPA-SCP mediates sonodynamic therapy to remove biofilms from infected root canals.

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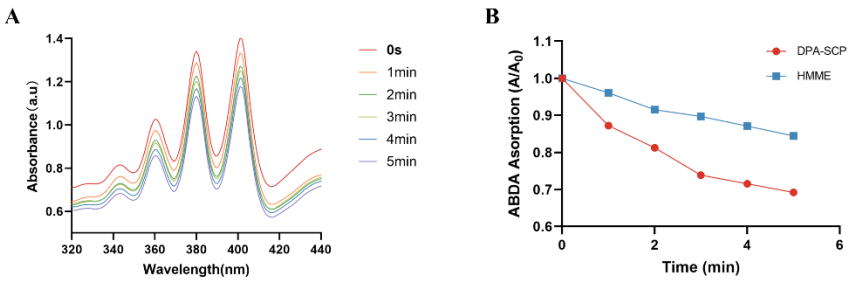

Figure S1. (A) Decrease in absorption at 378 nm of mixed ABDA and HMME solutions in the presence of US. (B) Comparison of ros generation capabilities of DPA-SCP and HMME.

In the present study, the reactive oxygen species (ROS) generation capacity of hematoporphyrin monomethyl ether (HMME) in the presence of 9,10-anthracene bis (methylene) dipropanedioic acid (ABDA) was evaluated in the presence of ultrasound irradiation. This experimental design aims to demonstrate the advantage of DPA-SCP over HMME in terms of ROS generation efficiency by comparison. The experiments were performed by mixing HMME (at a concentration of 25 μg/mL) with ABDA in solution and irradiating the mixture with ultrasound at 1.5 W/cm², 1 MHz, and 50% duty cycle in order to assess ROS generation. The UV-Vis absorption spectra of ABDA at 378 nm were recorded at different time intervals of 0, 1, 2, 3, 4 and 5 min. By measuring the decrease in absorbance of ABDA at 378 nm, it was possible to determine the extent of ABDA depletion by HMME-generated ROS. These results were analyzed in comparison with the data obtained from the DPA-SCP/ABDA mixture, thus validating the efficiency of DPA-SCP in generating ROS (Fig. S1).

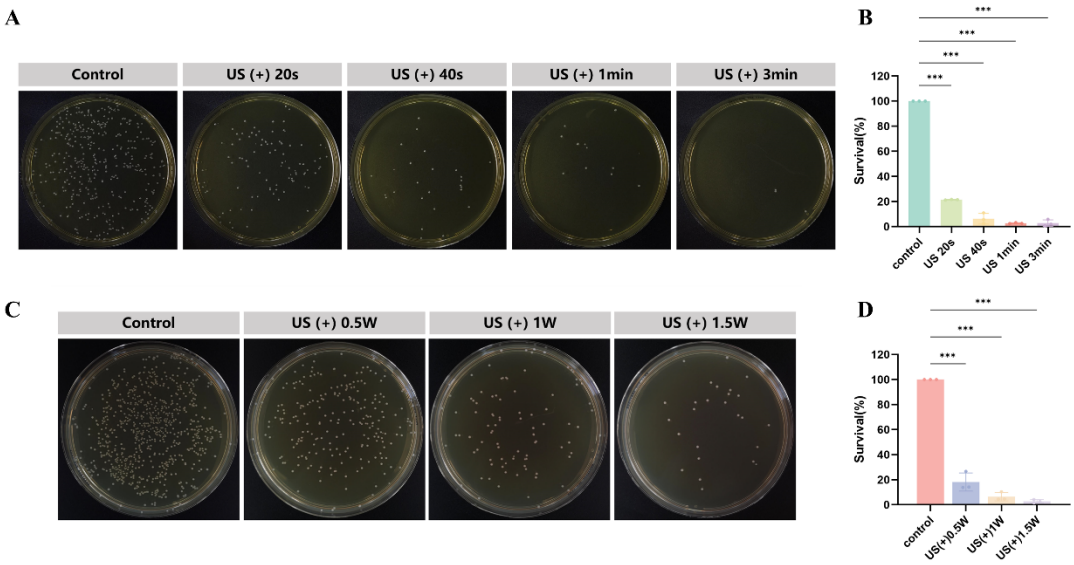

Figure S2. (A) Colony growth and (B) bacterial survival rate of each group after sampling and dilution in the in vitro anti-suspension bacterial assay at DPA-SCP concentration of 75ug/ml and ultrasonic power of 1W/cm2. (C) Growth of colonies and (D) survival rate of bacteria after sampling and dilution in each group of the in vitro anti-suspension bacterial assay when the concentration of DPA-SCP was 75ug/ml and the ultrasonic action time was 40s.

An ultrasound frequency of 1 MHz was used in this study and this selection was based on the following considerations:

The 1 MHz frequency has been widely validated and applied in the field of sonodynamic therapy (SDT) with good tissue penetration and acoustic cavitation effect. What's more, most commercial and laboratory ultrasound devices use 1 MHz frequency, which facilitates the reproducibility of research results and practical applications.[1-5] In addition, the 1 MHz frequency can minimize the damage to the surrounding healthy tissues while maintaining good therapeutic effects.

Figure S2 delineates the optimization procedure for the SDT effect under varying conditions of ultrasound irradiation time (20 s, 40 s, 1 min, and 3 min) and power settings (0.5 W/cm², 1 W/cm², and 1.5 W/cm²). A suspension of *E. faecalis*, at a concentration of 75 μg/mL DPA-SCP, was subjected to specified ultrasound treatments. Post-irradiation, the samples were serially diluted and subsequently plated on BHI agar to allow for the quantification of bacterial colony-forming units (CFU), enabling the assessment of bacterial viability.

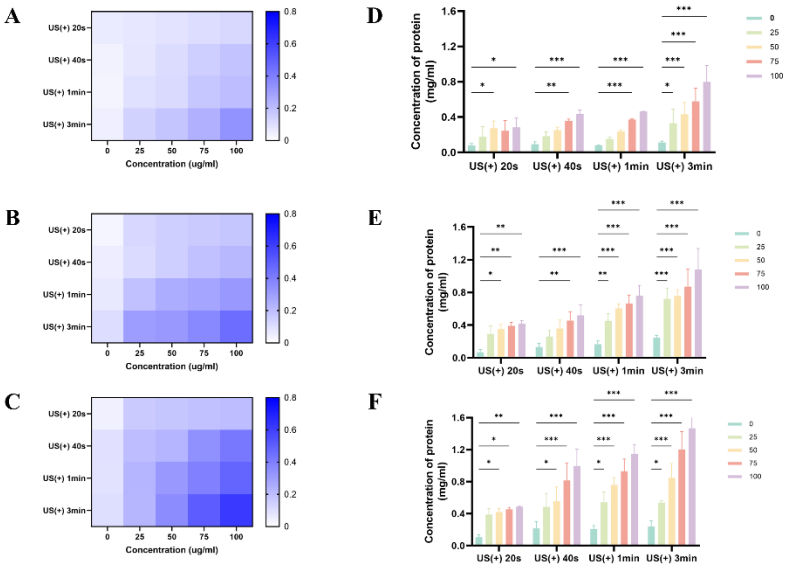

Figure S3. Effects of different concentrations of DPA-SCP and various ultrasonic parameters on cell membrane damage of *E. faecalis*. When the ultrasonic power was 0.5 W/cm² for (A and D), 1 W/cm² for (B and D), and 1.5 W/cm² for (C and E), the protein concentration in each group's supernatant was quantified by Bradford assay.

In order to assess the effect of treatment with different concentrations of DPA-SCP and multiple ultrasound parameters on the extent of cell membrane disruption in *E. faecalis*, protein leakage analysis was performed on treated cells. The purpose of the experiment was to determine the extent

of treatment-induced cell membrane disruption by measuring the amount of protein released after cell membrane rupture. *E. faecalis* suspensions were treated with 0 μg/mL, 25 μg/mL, 50 μg/mL, 75 μg/mL and 100 μg/mL of DPA-SCP, respectively. Subsequently, each suspension was exposed to ultrasonic conditions at 0.5 W/cm², 1 W/cm² and 1.5 W/cm² for 20s, 40s, 1 and 3 min, respectively. After treatment, the samples were centrifuged and the supernatant was collected for protein analysis. Protein concentration in the supernatant was quantified using the Bradford assay. Absorbance was measured at 595 nm to determine the amount of leaked protein, thus indicating the extent of cell membrane disruption. The results showed that the protein concentration in the supernatant increased significantly with increasing DPA-SCP concentration and ultrasound intensity and treatment time (Figure S3). This result clearly shows that DPA-SCP has a significant role in the disruption of *E. faecalis* cell membranes under sonication activation.

Reference

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