Highly Efficient and Safe Biofilm Removal from Contaminated Implants using MnO_2 Nanosheet-doped Diatom Microbubbler

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Background

Dental implant has been considered as the most effective clinical approach to restore structures and functions of lost teeth for the last 40 years. Due to the advances in material science and evidence-based clinical practices, success rates of dental implant have steadily increased by the day. However, still there are numerous complications which can lead to the implant failure and thus significant deterioration of oral health. Peri-implantitis, defined as a bacterial infection-mediated pathologic condition of tissue around dental implant, is the most common biological complication of dental implant.

We hypothesized that diatom microbubbler (DM) can be the answer for such demands. DM, first reported by Kong H. et al., is a cylinder-shaped silica diatom with hollow center containing manganese oxide (MnO_2) nanosheets. Manganese oxide (MnO_2)-doped silica diatom microparticles were investigated as a new method for treating peri-implantitis, one of the main causes of dental implant failure. The microparticles can generate oxygen gas microbubbles through catalysis in hydrogen peroxide solution and move randomly, propelled by driving forces, resulting in a mechanical self-cleansing effect. This study aimed to compare the decontamination achieved using the proposed method with that achieved using conventional chemical agents in terms of their effectiveness at removing biofilms formed on the surface of titanium. There has been no study of cell response on the titanium surfaces decontaminated with DM or examine biocompatibility of it.

Materials & Methods

The degree of adhesion, proliferation, and osteogenic differentiation of MG63 cells to the decontaminated disc surfaces were examined. X-ray photoelectron spectroscopy was used to determine the chemical composition of the titanium on the surface of the 3% H_2O_2 and DM, and the change of roughness was examined by SEM and CLSM. To verify the biocompatibility of the DMs, cytotoxicity tests on MG63 cells, toluidine blue staining tests on mouse tongues, and tissue specimen observation using microscopy were performed. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to observe the shape of the particles and for elemental component analysis. Two different types (machined surface; and sand blasted, large grit, and acid-etched surface) of titanium discs were prepared to test the effects of DM. *Porphyromonas gingivalis* (*P. gingivalis*) was cultured on the disc surfaces for 48 h. The discs contaminated with biofilms were treated with phosphate-buffered saline (PBS), 0.12% chlorhexidine gluconate (CHX), 3% hydrogen peroxide (H_2O_2), PBS+4 mg/mL of DM (PBS+DM), and 3% H_2O_2+4 mg/mL of DM (H_2O_2+DM) for 2 min and then rinsed again with PBS.

The biofilm removal effect and survival rate of the remaining bacteria were analyzed by SEM images, confocal laser-scanning microscopy (CLSM), and wheat germ agglutinin-8. The lipopolysaccharide (LPS) endotoxins in the remaining biofilms were quantified and analyzed. It was tested whether the biofilms on the surfaces of implants extracted from patients with severe peri-implantitis could be effectively removed. In the H_2O_2+DM group, the *P. gingivalis* biofilms formed on the titanium disc surface were effectively removed, particularly in comparison with the other groups. Osteoblast-like MG63 cells were successfully attached and proliferated and underwent osteogenic differentiation on the decontaminated surfaces in the H_2O_2+DM group. Treatment with H_2O_2 and DM did not alter the original physicochemical properties of implant surfaces. In experiments to verify biocompatibility and safety, DM was not toxic to MG63 cells up to 10 mg/mL, and there was no detrimental effect at the tissue level when applied with 3% H_2O_2. The amount of LPS endotoxin was significantly reduced only in the H_2O_2+DM group. Multi-species biofilms on the surfaces of implants extracted from patients were effectively removed by treatment with 3% H_2O_2 and DM.

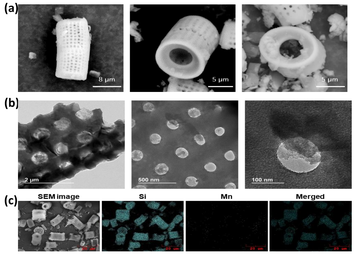

Fig. 1. Microscopy Images of the MnO_2-diatom microbubblers. (a) SEM images of the MnO_2-diatom microbubblers. (b) TEM images of the MnO_2-diatom microbubblers. (c) Elemental mapping images showing the MnO_2 nanosheets on the diatom surfaces.

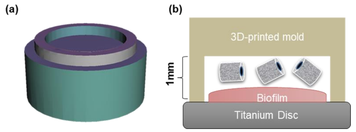

Fig. 2. Images of experimental scaffold design and its use in a biofilm removal experiment. (a) Experimental scaffold designed through a CAD program. (b) Schematic illustration of biofilm removal experiment. A *P. gingivalis* biofilm generated on an SLA titanium disc was placed upside-down on a 3D-printed plastic mold with a 1 mm gap and treated with a chemical agent for 2 min.

Results

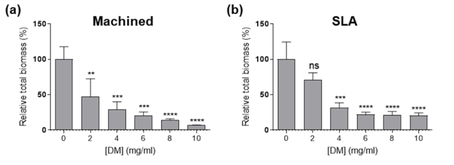

Fig. 3. Biofilm (*P. gingivalis*) removal efficiency according to DM concentration. (a) After the concentration-dependent treatment of DM/H_2O_2, relative total biomass remaining on the machined surface titanium disc and the (b) SLA titanium disc was measured by crystal violet staining (n=4). ns: not significant (p > 0.05), **: p < 0.01, ***: p < 0.001, ****: p < 0.0001

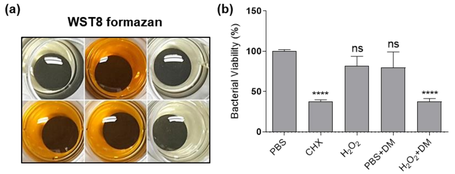

Fig. 4. Bacterial viability assay after decontamination treatment. (a) Yellow WST8 formazan indicates viable bacteria. (b) Absorbance of WST8 formazan solutions at 405 nm in Fig. 8a are quantified (n=4). ns: not significant (p > 0.05), ****: p < 0.0001

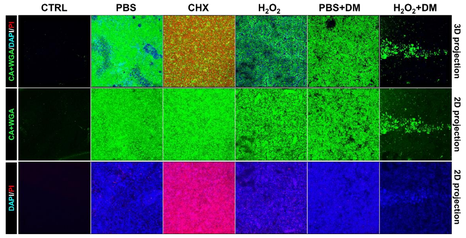

Fig. 5. Visualization of remaining biofilms on the SLA surfaces after decontamination. Remaining biofilm extracellular polymeric substances (EPSs) and viable bacteria are separately stained and visualized after each treatment. Biofilm EPSs are stained with concanavalin A (CA) and wheat germ agglutinin (WGA). Both live and dead bacteria are stained with 4′,6-diamidino-2-phenylindole (DAPI), and dead bacteria are stained with propidium iodide (PI). scale bar = 200 μm.

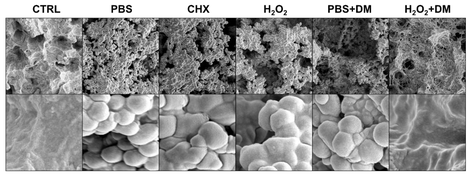

Fig. 6. SEM images of titanium discs after each decontamination treatment at 5,000× magnification (upper row) and 50,000× magnification (lower row).

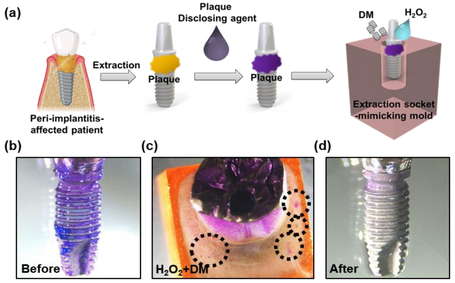

Fig. 7. Effect of DM and H_2O_2 co-treatment on multi-species biofilms of implant fixtures removed from patients with severe peri-implantitis. (a) Implant fixture put in the poly-methyl methacrylate (PMMA) mold made to secure a 1-mL gap and treated with DMs and H_2O_2 for 2 min. (b) Biofilms stained with disclosing agent appeared purple. (c) During the decontamination process, purple biofilm fragments came off from the implant surface (black dotted circles). (d) After decontamination, most of the biofilm was removed from the implant surface. (e) Biofilms stained on the implant surfaces were photographed, and the ratio of the area was calculated before and after treatment (n=6). ns: not significant (p > 0.05), ****: p < 0.0001

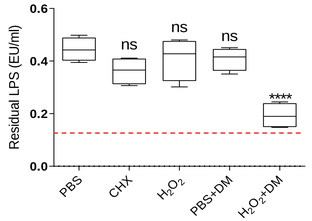

Fig. 8. The amount of lipopolysaccharide (LPS) endotoxin remaining on the titanium disc after each treatment was quantified (n=4). The detection limit is indicated by the red dotted line. ns: not significant (p > 0.05), ****: p < 0.0001

Conclusion

We have developed diatom microparticles fortified with MnO_2 nanosheets that can efficiently and safely decontaminate implant fixtures. Co-treatment with H_2O_2 and DMs effectively cleared both *P. gingivalis* and multi-species biofilms on titanium surfaces within 2 min. For *P. gingivalis* biofilms, the decontamination efficiency of the H_2O_2+DM group was superior to that of conventional methods, such as CHX or H_2O alone. Osteoblast-like MG63 cells were successfully attached and proliferated and underwent osteogenic differentiation on the decontaminated surfaces in the H_2O_2+DM group. Treatment with H_2O_2 and DM did not alter the original physicochemical properties of implant surfaces. In experiments to verify biocompatibility and safety, DM was not toxic to MG63 cells up to 10 mg/mL, and there was no detrimental effect at the tissue level when applied with 3% H_2O_2.

Our new therapeutic approach using H_2O_2 and DM for peri-implantitis treatment is far more effective and regenerative compared with conventional antiseptic approaches, suggesting that it is a promising alternative for resolving current challenges in the treatment of peri-implantitis.

Acknowledgements

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