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1. INTRODUCTION

The major contributor of development and progression in dental caries is the demineralization of dental hard tissue caused by acid produced by dental plaque. Nano-hydroxyapatite (nHap) has an excellent ability to promote remineralization but no antibacterial property. The current study, we evaluated the remineralization ability and inhibitory effect on the regrowth of cariogenic biofilms of disaggregate nHap (dnHAP).

2. METHODS

Biofilm regeneration models of single-species biofilms, cross-kingdom biofilms and saliva-derived microcosm biofilms were established in vitro. Disaggregated nHAP were applied to biofilms for 30 minutes three times within 48 hours. The metabolic activity, lactic acid, biofilm structure, biomass and virulence factors expression were determined. Remineralization capacity was analyzed by transverse microradiography (TMR). The biofilm composition was analyzed by 16S rRNA gene sequencing.

3. RESULTS

A. Materials characterization

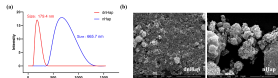


Fig 1. (a) Material particle size by DLS. (b) Morphology of the material observed by SEM after interaction with biofilms.

B. Inhibition of metabolic activity and lactic acid production

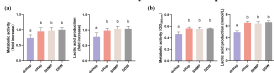


Fig 2. (a) Single-species biofilm of *S. mutans* UA159. (b) Cross-kingdom biofilm of *S. mutans* and *C. albicans*.

C. Inhibition of glucan synthesis and Gtf expression

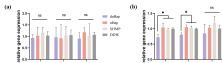


Fig 3. (a) Single-species biofilm of *S. mutans* UA159. (b) Cross-kingdom biofilm of *S. mutans* and *C. albicans*.

4. DISCUSSION

Conclusions:

- dnHap presented better ability to promote remineralization.
- dnHap resulted in a reduction in metabolic activity, lactic acid production and exopolysaccharides synthesis of regrown biofilms.

5. REFERENCES

1. Lamont, R.J., H. Koo and G. Hajishengallis, The oral microbiota: dynamic communities and host interactions. *Nat Rev Microbiol*, 2018. 16(12): p. 745-759.
2. Liao, W., et al., The effect of disaggregated nano-hydroxyapatite on oral biofilm in vitro. *Dent Mater*, 2020. 36(7): p. e207-e216.

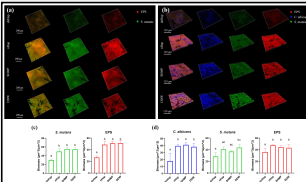


Fig 4. Effects of dnHap on biofilms structure in regrown biofilms. (a) CLSM imaging of single-species biofilm and (b) Cross-kingdom biofilm. (c) Biomass analysis of single-species biofilm and (d) Cross-kingdom biofilm.

D. Inhibition of demineralization

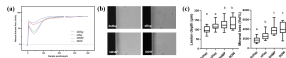


Fig 5. dnHap inhibits the demineralization of *S. mutans* biofilm (a) Mineral content of enamel varies with depth; (b) Representative imaging of TMR; (c) Lesion depth & Mineral loss.

E. Saliva-derived biofilm composition analyzed

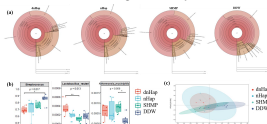


Fig 6. Regulation effects of dnHap on microecology of salivary biofilm (a) Krona species composition diagram. (b) Relative abundance of different bacterial communities. (c) Assessment of microbial diversity using Principal Co-ordinate Analysis (PCoA).

- dnHap did not disturb oral microbiome diversity, while reducing the proportion of *S. mutans* and promoting the growth of certain probiotics

Limitations:

- The inhibitory mechanism remain largely unknown.
- In vivo studies are needed to further evaluate its effectiveness.

6. ACKNOWLEDGEMENT

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