Antimicrobial, Demineralization inhibition potential and Staining effects of Nano Silver-Fluoride

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Introduction

- Silver diamine fluoride (SDF) has emerged as an effective agent in caries arrest and prevention. However, due to its side effect of unaesthetic staining, other anti-caries agents have been developed.
- As SDF has some side effects, it is necessary to develop some alternative biocompatible, antimicrobial, demineralization inhibition potential materials. Recent preclinical and clinical studies have found that Silver Fluoride-Potassium Iodide (SDF-KI) and Nano-Silver-Fluoride (NSF) could be effective alternatives, as they overcome the clinical drawbacks of SDF.
- **This study aims to compare the *in vitro* antimicrobial activities, demineralization inhibition potential and staining effects of NSF, SDF/KI and SDF.**

Methods

Three silver fluoride-based anticariogenic materials were tested: Advantage Arrest® (SDF 38%, Elevate Oral Care, USA), SDF/KI (SDI Limited, Australia) and NSF (AgNPs, Chitosan, and Fluoride, Nano Research Elements, India).

A. The antimicrobial activity against *Streptococcus mutans (S.mutans)* UA159 *and Candida albicans (C.albicans)* SC5314 was assessed using the agar diffusion assay, colony forming units and minimum inhibitory concentration assay. For the agar diffusion assay Chlorhexidine was used as positive control group; the negative control group received no treatment. For Colonies Forming Unit (CFU) and Minimum Inhibitory Concentration (MIC) assays, SDF was used as positive control and Phosphate Buffered Saline (PBS) was negative control.

B. For the Colony Forming Unit (CFU) assay, bacterial cultures were diluted in a 10-fold serial dilution series. For the Minimum Inhibitory Concentration (MIC) assay, anticariogenic agents were diluted to determine the MIC using a 10-fold dilution series: 1:10, 1:100, and 1:1000.

C. The demineralization inhibition potential was assessed using bovine bone discs. 12 bovine discs were used and the discs were submerged in a demineralizing acid solution consisting of 0.115 ml acetic acid and 19.9 ml H_2O, buffered to pH 4.0 using KOH. Microhardness Vickers test was used to assess the demineralization inhibition potential. Microhardness was measured at baseline, after 72 hours demineralization, and after applying the anticariogenic tested agents at 4 weeks. All measurements of the samples in each group were assessed using the Micro Vickers Microhardness Tester Model N0. 900-390 under a 200g (1.962 Newtons) load for 15 seconds. Microhardness percentage was determined by: % microhardness equals 100 (post-treatment -post lesion values/baseline - post lesion measurements).

D. For staining effects, photographs were taken at baseline, after 72 hours demineralization and 4 weeks after application of agents. Established parameters were used to standardize image acquisition: ISO 100, F22 aperture, and TTL flash mode at maximum power.

The one-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to compare the *in vitro* effectiveness of the anticariogenic agents tested

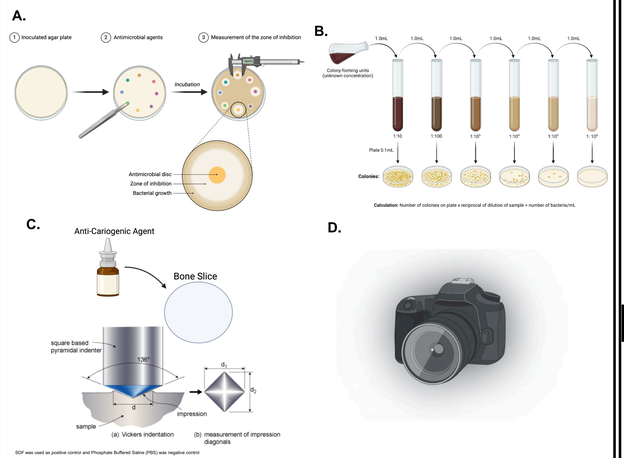

Figure 1. **A.** Representative image of agar diffusion assay for antimicrobial assessment **B.** Representative image of serial dilution for CFU assay. C. Representative image of specimens used for demineralization inhibition potential. D. Representative image of Microhardness test.

Results

Antimicrobial Properties of Anticariogenic Agents/Agar Diffusion assay

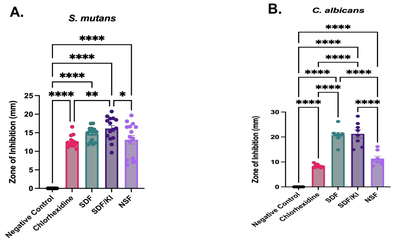

Figure 2. Antibacterial effects on disk diffusion assay (n=12). **A.** Graph showing significance between tested materials on antimicrobial properties against *S. mutans* **B.** Graph showing significance between tested materials on antimicrobial properties against *C. albicans*. Data are mean ± SEM.*P<0.05; **P<0.01; ***P<0.001; ***P<0.001

Antimicrobial Properties of Anticariogenic agents, CFU, and MIC Assays

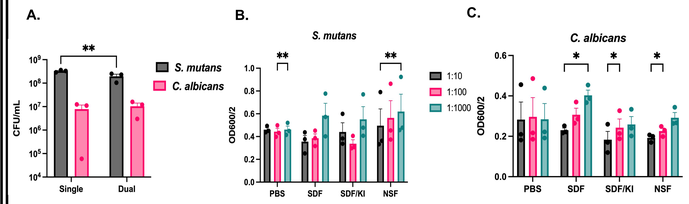

Figure 3. Antimicrobial properties of anticariogenic agents (n=3). **A.** CFU assay demonstrated no bacterial growth in presence of the tested agents. **B.** MIC assay on *S. mutans* at dilutions 1:10, 1:100, and 1:1000. **C.** MIC assay on *C. albicans* at dilutions 1:10, 1:100, and 1:1000. Data are mean ± SEM.*P<0.05; **P<0.01; ***P<0.001; ***P<0.001

Staining Effects of Tested Anti-Cariogenic Agents

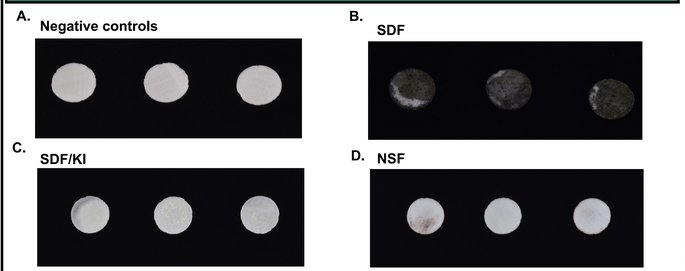

Figure 4. Photos of staining effects on bovine bone discs samples 4 weeks after applying the agent (n=3). **A**. Negative controls; **B.** Silver Diamine Fluoride (SDF); **C.**SDF/KI; **D.** Nano Silver fluoride (NSF).

Demineralization inhibition potential

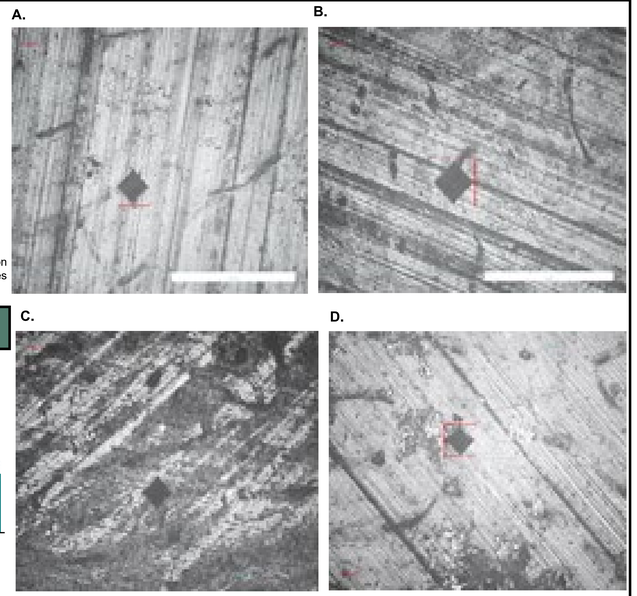

Figure 5. Metallographic microscope photos of bone disc samples under Microhardness Vickers testing (n=18). **A.** Baseline representation of samples pre-treatment. **B** Indent on specimen after 72 hours of acid demineralization used as control **C.** Indent on specimen after 4 weeks application of Nano Silver fluoride (NSF) **D.** Indent on specimen after 4 weeks application of SDF/KI **E.** Indent on specimen after 4 weeks application of Silver diamine fluoride (SDF). **F.** Comparison of bone discs treated with anticariogenic agents. Data are mean ± SEM.*P<0.05; **P<0.01; ***P<0.001; ***P<0.001

Conclusions & References

Conclusions:

1. SDF and SDF/KI exhibited the strongest antimicrobial activity against *S. mutans*, and *C. albicans*
2. SDF demonstrated high antimicrobial activity and demineralization inhibition potential; although the black staining is an undesired effect.
3. SDF/KI is demonstrated as an effective alterative to SDF due to it's high antimicrobial activity, high microhardness and limited staining after application. However, it is a two-bottle system which would require more chair time and patient compliance.
4. Although the NSF used in the present study is not as effective as the other two silver fluoride agents in the antimicrobial effects against *S. mutans* and *C. albicans*, its easy application and the non-staining benefit of NSF warrants further modification for better antimicrobial and demineralization inhibition potential effects.

References