Characterization of Novel Antimicrobial Agent for Endodontic Applications
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Endodontic Infections
- Endodontic infections arise from the entrance of microbes into the root canal system and persist regardless of the type of treatment, affecting 39% of the global population over time in their lives. [1,2]
- In the US alone, there are more than 20 million cases of endodontic treatment annually, over 6 million of these treatments for endodontic infections. [3]
- Untreated or recurrent endodontic infections can be painful and have a direct impact on daily life and cause abscesses, cellulitis, and periodontal inflammatory responses. [4,5] More severe, these infections have the potential enter the bloodstream through the root canal to cause chronic diseases. [6,7]
- The infection-causing microbes include bacteria and fungi of various genera including Lactobacillus, Actinomyces, Streptococcus, Staphylococcus, Enterococcus, Entamoeba, and Candida. [8,9]

Antimicrobial Properties
- Calcium hydroxide, and CASA were each deposited into the wells of separate agar plates containing tested microbes: Candida albicans SC5314, Lactobacillus salivarius 17141, Streptococcus sanguis 19587, Streptococcus gordonii 482C (Staphylococcus aureus 29235, Escherichia coli 29282, and Enterococcus faecalis 19453).
- Larger zones of inhibition indicate greater antimicrobial effect.
- Average Zones of Inhibition for CASA and Calcium Hydroxide

Cytotoxicity Assay
- On the initial day, DSPEC (24 h) and CASA or calcium hydroxide were added to cell solutions.
- After 24 hours of incubation, DSPEC or CASA were counted, and additional 0.2 ml of 0.25 mg/ml of CASA, and calcium hydroxide were added into cell solutions.
- Following another 24 hours of incubation, DSPEC were counted.

Viability Staining: Precipitate and Supernatant
- Because CASA separates into a precipitate and supernatant, the antimicrobial properties of each component were investigated. Increasing amounts of CASA precipitate or supernatant were added to Brain Heart Infusion Broth containing either Enterococcus faecalis or Candida albicans. Then, the ratio of dead to live microbes was determined using fluorescence microscopy.

Discussion
- CASA produced significantly larger zones of inhibition compared to those of Ca(OH)₂ suggesting that CASA is the more efficacious antimicrobial.
- Results of the cytotoxicity assay suggest Ca(OH)₂ is also toxic to DPSC whereas CASA is non-cytotoxic to DSPEC, a promising result for potential clinical applications.
- Morphological analysis of DSPEC with fluorescent microscope staining indicated both CASA does not affect DPSC morphology, as nucleus sizes of the measured DSPEC exposed to each compound were not statistically different from the control. Viability staining of CASA precipitate and supernatant affirm that the antimicrobial properties of CASA are derived from the synthesized compound and not excess acid in the supernatant.
- Dose-dependency testing with S. aureus and C. albicans indicate that CASA is dose-dependent with larger concentrations of CASA resulting in greater bactericidal efficacy. E. coli on agar plates containing the highest concentration of sodium salicylate suggest that the sodium salicylate is bactericidal-static. In contrast, the lack of bacterial growth exposed to the highest concentration of CASA suggests that CASA is bactericidal. For future endodontic applications, appropriate clinical concentrations must be carefully determined such that CASA kills all bacteria within the root canal, thus preventing endodontic infection.
- XRD patterns of three-day-old and newly synthesized CASA are similar, but old CASA has smaller peak intensities, the variation likely due to powder orientation or variation in salicylic acid and calcium hydroxide measurements in CASA synthesis. Thus, CASA largely retains its crystalline structure over a three day period and does not need to be synthesized just before application.

Conclusion
- The biocompatibility and antimicrobial properties of CASA suggest it is a more efficacious antimicrobial agent than calcium hydroxide, which is currently used in endodontics. Furthermore, CASA, which is dosage dependent, can kill all bacteria found within the root canal owing to its bacterial-lytic properties.

Selected References