Evaluation of the Penetration Kinetics and Antimicrobial Efficacy of an Alcohol-free Mouthrinse (Listerine Zero) Using *Streptococcus mutans* Biofilms

TAKENAKA Shoji, OSUMI Tatsuya, WAKAMATSU Rika,
TERAO Yutaka\(^1\), OHISHI Hayato\(^2\) and OKIJI Takashi

Division of Cariology, Operative Dentistry and Endodontics, Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences
\(^1\)Division of Microbiology and Infectious Disease, Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences
\(^2\)Division of Anatomy and Cell Biology of the Hard Tissue, Department of Tissue Regeneration and Reconstruction, Niigata University Graduate School of Medical and Dental Sciences

Abstract

Purpose: Listerine mouthrinse has been reported to show a rapid membrane-damaging effect on biofilm bacteria. One drawback of Listerine, however, is that it may be irritative to oral mucosa due to the ethanol contained as a solvent. The aim of this study was to evaluate the penetration kinetics and antimicrobial effects of a new alcohol-free essential oil mouthwash (Listerine Zero), which has been developed to lower the irritative action of Listerine.

Methods: Listerine Zero (Z), Listerine Flesmohtin (F) and a chlorhexidine gluconate-containing mouthrinse (Peridex; P) were investigated as test mouthrinses, and a buffer was used as a negative control. *Streptococcus mutans* biofilms were grown on glass-based dishes for 24 h under anaerobic conditions. Penetration kinetics of the mouthrinses were analyzed with time-lapse confocal laser scanning microscopy where the fluorescence loss of calcium-AM-stained biofilms was monitored after exposure to mouthrinse. Antimicrobial effects of the mouthrinses were also compared using live/dead staining and plate counting.

Results: The maximum biofilm thickness developed in this study was approximately 32 μm. The time required to reach 50% of the initial fluorescence intensity (T50) and biofilm thickness exhibited a high correlation coefficient. P showed significantly smaller penetration velocity compared with the others, whereas there was no significant difference between Z and F.

The live/dead staining analysis after 30 s exposure revealed that propidium iodide (a dead cell marker)-positive percentage was 99.8±0.1% for Z and F and 41.4±5.9% for P. When viable cell counts after 30 s of mouthrinse exposure were determined by plate counting, all of the mouthrinses caused a significant reduction compared with the control. F and Z caused significantly smaller counts than P, whereas there was no significant difference between Z and F.

Conclusion: Listerine Zero was as effective as Listerine Flesmohtin in penetration property and antimicrobial effect against *S. mutans* biofilms of less than 32 μm thick. Both of these mouthrinses also showed significantly superior penetration property and antimicrobial effect than the chlorhexidine gluconate-containing mouthrinse.

Key words: Mouthrinse, Biofilm, Penetration kinetics, Confocal laser scanning microscopy