Novel stannous fluoride dentifrice stabilized with amino acid glycine neutralizes toxins as demonstrated in *in-vitro* cell recovery and biofilm penetration model

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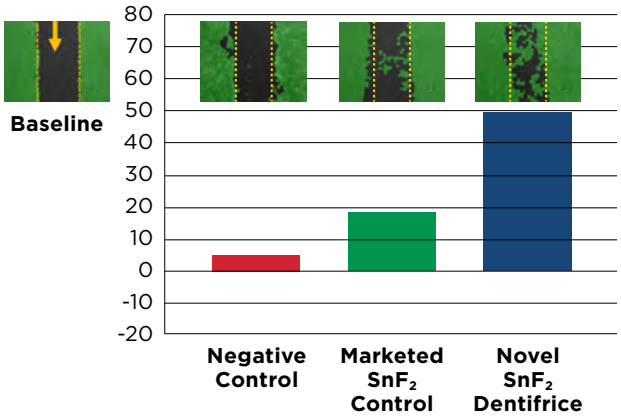
KEY FINDINGS

- The novel 0.454% stannous fluoride (SnF_2) dentifrice with the amino acid glycine as a stabilizer significantly accelerated gingival cell recovery rate compared to a negative and marketed SnF_2 control. See Figure 1.
- The novel SnF_2 formulation also increased uptake of stannous (Sn) penetration into the dental biofilm versus a positive SnF_2 control (Figure 2), and increased lipopolysaccharide (LPS) neutralization compared to a positive and negative control (Figure 3).

Figure 1. Cell recovery % in a simulated gingival wound healing model.*
More green in middle section indicates greater cell recovery.



* Negative control < marketed SnF_2 control < Novel SnF_2 dentifrice, $P<0.05$

Figure 2. Cross-section visualization of Sn penetration in the *in-situ* biofilm.
Brighter color indicates greater Sn penetration.

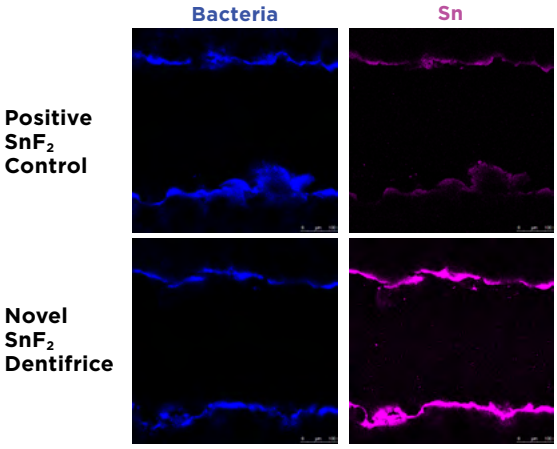
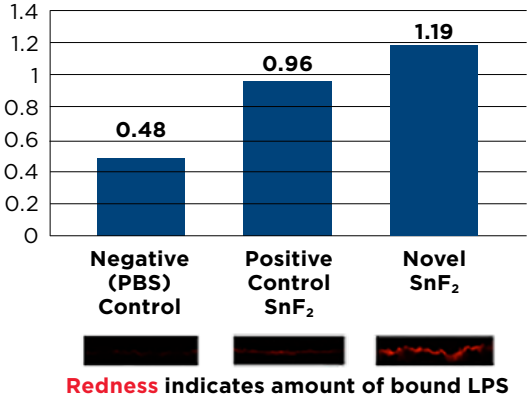


Figure 3. LPS Neutralization Index (Bound LPS normalized by bacterial amount).**
Higher number indicates better LPS neutralization.



** Negative (PBS) Control < Positive Control < Novel SnF_2 Dentifrice, $P<0.05$

OBJECTIVE

To assess *in-vitro* a novel SnF_2 dentifrice with the amino acid glycine as a stabilizer for its efficiency to accelerate gingival cell recovery rate, penetrate plaque biofilm and neutralize LPS, an endotoxin that initiates the inflammatory response.

METHODS

Gingival wound healing simulation migration assay

Human gingival epithelial cells were seeded ($2.1x10^4$ cells) into a 6-well plate and were incubated at 37°C with 5% CO_2 until cell monolayer coverage was formed. A center wound simulation (~500µm) was created manually by scraping the middle area with a sterilized 1 mL pipette tip, and the well was briefly rinsed with PBS to remove cell debris. The cells were then treated with fresh medium containing 0.5% FBS and 1% test product with 50ppm *P. gingivalis* LPS. Images were obtained at 0 and 72 h using an Olympus® IX71 digital SLR camera. The area into which the cells migrated was measured using Wimasis® WimScratch software.

Ex-vivo biofilm penetration and LPS neutralization

These studies were based on a single *ex-vivo* treatment of dentifrice on an undisturbed 48 hour *in-situ* dental biofilm.[1,2] The use of fluorescence staining method combined with oral biofilm model can evaluate overall formulation impact of the Sn delivery and bioavailability in naturally-grown human biofilm.[3,4,5]

To visualize toothpastes' efficacies, two commercial fluorescence probes, Syto-9 Green Fluorescent Nucleic Acid Stain and BODIPY® TR Cadaverine Stain (Molecular Probes®, Eugene, Oregon, USA), and a Sn-specific probe containing a rhodamine B group[4] were used as a staining method to track Sn in the biofilm and LPS binding efficiency.

The following procedures were conducted (Figure 4):

1. Generation and collection of undisturbed 48-hour-old plaque biofilm
2. Treatment of toothpaste and fluorescence staining (Table 1)
3. Confocal laser scanning microscopy (CLSM) observation
4. Image acquisition and data analysis

LPS Neutralization Index was calculated based on bound LPS amount normalized by *in-situ* biofilm bacterial amount.

Figure 4. Overview of test method

Table 1. Treatment details

Treatment	SnF_2 (%w/w)	Manufacturer
Negative control (Figure 1), no dentifrice, LPS (0 ppm Sn)	0	n/a
Negative control (Figure 3), phosphate buffer solution (PBS, 0 ppm Sn)	0	Sigma Aldrich
Marketed SnF_2 control (Figure 1, Colgate Total^{SF})	0.454	Colgate-Palmolive
Positive SnF_2 control (Figures 2 & 3, Crest® PRO-HEALTH™)	0.454	Procter & Gamble
Novel SnF_2 formulation stabilized with amino acid glycine (Crest® Gum Restore™)	0.454	Procter & Gamble

CLINICAL COMMENT

SnF_2 has been used for decades in dentifrice due to its proven antibacterial properties, including the control of bacterial growth and reduction of bacterial toxins (e.g. LPS) in plaque. Since the bacteria in biofilms are 10–1000 times more resistant to antibacterial agents, the penetration ability of agents into the biofilm is fundamental for their efficacy. The formulation design of SnF_2 dentifrices is critical as other ingredients in the formula can enhance or hinder stabilization of SnF_2, impacting its penetration efficiency and LPS neutralization. Thus, dentifrices containing the same level of SnF_2 may have different levels of SnF_2 stability, delivery and/or bioavailability based on the formulation. This research evaluated a novel SnF_2 dentifrice with the amino acid glycine as a stabilizer (Crest® Gum Restore™). Results show this formulation provides increased biofilm penetration and LPS neutralization efficacy, enhancing gingival cell recovery in a simulated wound healing model. Collectively, these data indicate Crest® Gum Restore™ is a significant advancement in dentifrice technology to improve patients' gingival health.

1. Zaure-Arite et al. *J Dent Res* 2001;80:1436-1440.
2. Wood et al. *J Dent Res* 2000;79:21-27.
3. Xiang et al. *Am J Dent* 2018;31:53-60.
4. Lan et al. *Analyst* 2014;139(20):5223-9.
5. Huggins et al. *Am J Dent*, 2016;321-327.