Theaflavins, tea extract from Camellia sinensis, are rapid and effective dentin collagen stabilizer

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Statement of Purpose: The degradation of collagen in the demineralized dentin layer is one of the major causes for the failure of dentin-adhesive bond[1]. Acid etching of dentin as well as the presence of oral bacteria activates the otherwise dormant host-derived matrix metalloproteinases (MMPs), further increasing the risk of collagen degradation[2]. To solve this issue, a lot of collagen cross-linking agents have been studied to increase the dentin collagen's mechanical and biological stabilities. Theaflavins (theaflavin and its mono/di gallates, TFs), a tea extract, are reported to have multi-biological functions such as anti-inflammatory, and anti-cariogenic activities[3,4].To explore if TFs could work as a high efficient collaging cross-linker and enhance the stability of demineralized dentin, we investigated the capability of TFs in protecting demineralized dentin layer against collagenase digestion under clinically relevant settings, in comparison with the established collagen cross-linker proanthocyanidins (PAs)[5].

Methods: Tea extract from Camellia sinensis (≥80% theaflavin and theaflavin gallates) and collagenase (type I, from Clostridium histolyticum, ≥125U/mg) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Grape seed extract (≥95% Proanthocyanidins) was generously donated by Mega Natural (Madera, CA, USA). All buffer solutions (PBS, TESCA) were prepared according to general protocols. Dentin films (5 mm×5 mm×10 μm) were sectioned from sound human molars. The films were demineralized in 10% H_3PO_4 for 30 min and then treated by TFs (0.4%, 2.0%) or PAs (0.4%, 2.0%) in 90/10 (v/v) PBS/ethanol for 30 s. After rinsing, air-drying and Fourier-transform infrared spectroscopy (FTIR, Spectrum One, PerkinElmer, Waltham, MA) examination, the treated films were digested in 0.1% collagenase in TESCA buffer at 37°C for 1 h. The weight loss (WL) of the films during the digestion was measured by weighing the dried films before and after the digestion. The digestion solutions were then subjected to hydroxyproline assay to quantify the hydroxyproline release (H) from each milligram of demineralized dentin. The in-situ study was performed on dentin slabs (6 mm×1.5 mm×2 mm), which were etched by Scotchbond™ Universal Etchant (3M ESPE, Seefeld, Germany) for 15 s, primed with similar TFs or PAs solutions for 30 s, then digested for 1 h. The demineralized dentin layers were visualized by scanning electron microscopy (SEM, Philips XL 30, Eindhoven, Netherlands).

Results: FTIR spectra indicated that TFs could be bound to the dentin collagen like PAs via 30s of priming. The increase in absorption at 1150 cm^{-1} of dentin collagen treated by TFs was attributed to the C-O-C stretching of TFs bonded to collagen. In addition, a red shift of the absorption near 1400 cm^{-1} was observed in the dentin collagen treated by TFs, an important evidence of collagen-TFs interaction. After digestion in 0.1% collagenase for 1 h, untreated demineralized dentin films were completely digested (Fig.1), while the films treated by 0.4% TFs exhibited dramatically decreased degree of digestion (WL=21.0±4.2%, H=23.0±3.0 μg/mg), even lower than 0.4% PAs (WL=31.9±3.1%, H=30.6±3.8 μg/mg). At the concentration of 2.0%, TFs offered nearly full protection (WL=4.3±3.0%, H=1.8±0.4 μg/mg) to the films, which was similar to 2.0% PAs (WL=4.7±3.7%, H=3.4±0.1 μg/mg). The in-situ 30 s priming of etched dentin with 2.0% TFs provided full protection to demineralized dentin against digestion (like Pas), which was revealed by SEM. The thickness and morphology of demineralized dentin layers treated by 2.0% TFs or PAs had no significant change after digestion, while the untreated layers totally disappeared after digestion.

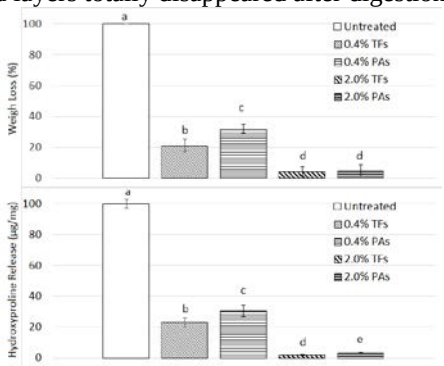

Figure 1. Weight loss and hydroxyproline release of dentin films after digestion (n=6, bars labeled with the same letters are statistically equivalent.)

Conclusions: TFs from Camellia sinensis had high affinity to dentin collagen. Via intermolecular interactions, TFs rapidly stabilized the collagen as PAs. The quantitative study revealed the efficiency of TFs was even higher than PAs in protecting dentin collagen. Facile 30 s priming with 2.0% TFs made the demineralized dentin nearly 100% inert to the extreme digestion. Thus TFs could be a promising dentin cross-linker to enhance the bond strength and life span of dental restorations. The future work will be focused on the long term cross-linking stability of TFs, in comparison with PAs and other chemical cross-linkers such as glutaraldehyde.

References:

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