The effect of active components from citrus fruits on dentin MMPs
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Objective

This study was aimed to evaluate the anti-matrix metalloproteinases (MMPs) ability of active components from citrus fruits (hesperetin: Hst, hesperidin: Hsd and naringenin: Nge) and screen the most suitable one to be further used in dentin bonding systems in order to prolong the resin-dentin bonding durability.

Methods

(1) Inactivation effects of citrus flavonoids (Hst, Hsd, Nge) at different concentrations on soluble collagenase were measured using a fluorometric assay.
(2) Matrix-bound endogenous MMPs activity was evaluated via dry mass loss and hydroxyproline (HYP) release of demineralized human dentin. Demineralized dentin beams were pretreated with 500 μg/mL citrus flavonoids for 10 min. Chlorhexidine (CHX) was used as inhibitor control. Beams pretreated with distilled water served as blank control.
(3) Dentin slabs were used for in situ zymography and evaluated under confocal microscopy. Ultrastructure of demineralized collagen fibers was exhibited by Transmission Electron Microscopy (TEM).

Results

Figure 1. Inhibition of soluble collagenase with increasing concentrations of different citrus flavonoids. The extent of inhibition increased in a dose-dependent manner. When the concentration increased to 500 μg/mL, the inhibition of Hst, Hsd or Nge surpassed the 1–10P inhibitor control and reached above 90%

Figure 2. Loss of dry mass for demineralized dentine beams after pretreated by Hst, Hsd or Nge, the percentage of dry mass loss reduced compared with unpretreated group.

Figure 3. Hydroxyproline released from the demineralized dentine beams after 15-day storage. After pretreated by Hst, Hsd or Nge, the amount of HYP releasing from dentine beams reduced compared with unpretreated control group.

Figure 4. Percentage of hydroxyproline stabilization for 15 days.

Figure 5. After storage in incubation media for 15 days, the collagen fibers of control group degraded. The banding of collagen fibrils were unclear or disappeared. The fibrils became thinner and less crossed. However, when demineralized dentin beams were pretreated with CHX, Hst, Hsd or Nge for 10 min, collagen fibrils in dentin matrix were preserved, exhibiting defined architecture of dentin matrix with compact collagen arrangement and intact collagen fibers with clear banding.

Figure 6. In situ zymography for different groups. After 24-h incubation, lower green fluorescence intensity was detected in samples pretreated with Hst, Hsd or Nge, which suggested that the amount of active matrix-bound enzymes was low due to their MMPs inactivation.

Conclusion

Within the limits of this study, it concluded that the citrus flavonoids (Hsd, Hst, Nge) had MMPs inactivation ability. The 7-O-rutinoside group on Hsd or the 4-methoxy group on Hst didn’t influence their anti-MMPs abilities. Hst may be used as an effective substance to prevent collagen degradation within hybrid layer to extend the longevity of resin-dentin bonding.