# Nano-hydroxyapatite Toothpaste With Hydrolyzed Conchiolin Increases Tooth Enamel Gloss



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#### Objectives

Hydrolyzed conchiolin (HC), derived from proteins promoting biomineralization in shells, is used in cosmetics and shampoos for moisturization, repair and gloss improvement. We tested its ability in toothpaste, in combination with nano-hydroxyapatite (nHAP), to increase enamel gloss.

#### Materials and Methods

#### 1. Preparation of Tooth Specimens

Specimens were prepared from sound extracted human teeth without previous restorative treatment, caries or white-spot lesions, and cleaned using typical dentifrice. After removing the root, the crown was sliced parallel to the tooth axis with a diamond disc. For gloss measurement, test teeth were fixed to acrylic cells with quick self-curing resin (UNIFAST | GC) and analyzed by surface gloss analyzer (SGA, 806H, TRICOR Systems Inc.).

### 2. Adjustment of Gloss

Since initial analysis showed variations in gloss, baseline adjustment of gloss was necessary. Test teeth were treated with a 300-times diluted phosphoric acid etchant (Clearfil, Kuraray-Noritake Dental) for 30 seconds and after washing with distilled water and drying with an air blow, the enamel surface gloss was remeasured. This process was repeated until all test teeth had an adjusted gloss value of approximately 70.

#### 3. Brushing Test

3-1. Test Dentifrices
Test dentifrices of different nHAP concentration were used: control pastes A, B, C, without HC (nHAP content ratio A: B: C = 1: 1.4: 2, n=8), and experimental pastes A+, B+, C+, containing HC (Promois PEARL-P, Seiwa Kasei) (nHAP concentration as for controls, n=8). The pastes were adjusted with water to slurry concentration (25g / 40ml) as a test solution.

#### 3-2. Brushing and Gloss Measurement

The brushing machine used was composed of a constant temperature shaking incubator fitted with a brush portion and a slurry bath containing the test teeth. To limit the effect to only the test solution, the bristle portion of the toothbrush was eliminated, and replaced by felt. The test teeth were fixed in the slurry bath and slurry poured in to soak the test teeth. They were then brushed 9min/day for 6 days (load 100g, amplitude 2cm, speed 100 rpm/min). After each daily brushing, teeth were washed with distilled water, dried with an air blow, and their surface gloss measured, after which they were stored in 100% humidity at 37°C. The difference between the obtained value and adjusted gloss value ( $\triangle G$ ) for each tooth was calculated, and the difference in daily  $\triangle G$  values between experimental and control groups compared. Statistical analysis was performed using Student's t-test.

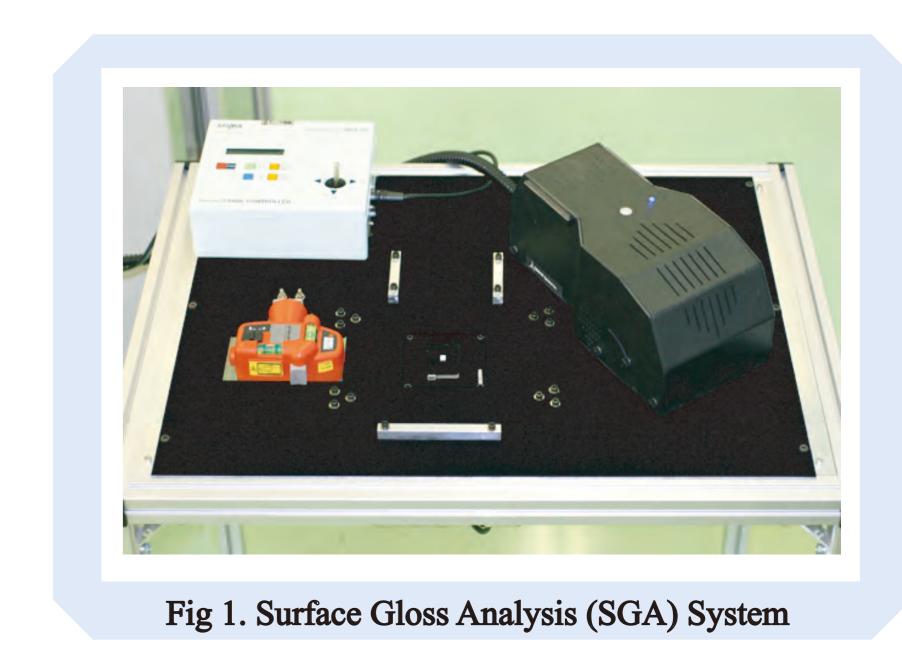




Fig 2. Brushing Machine

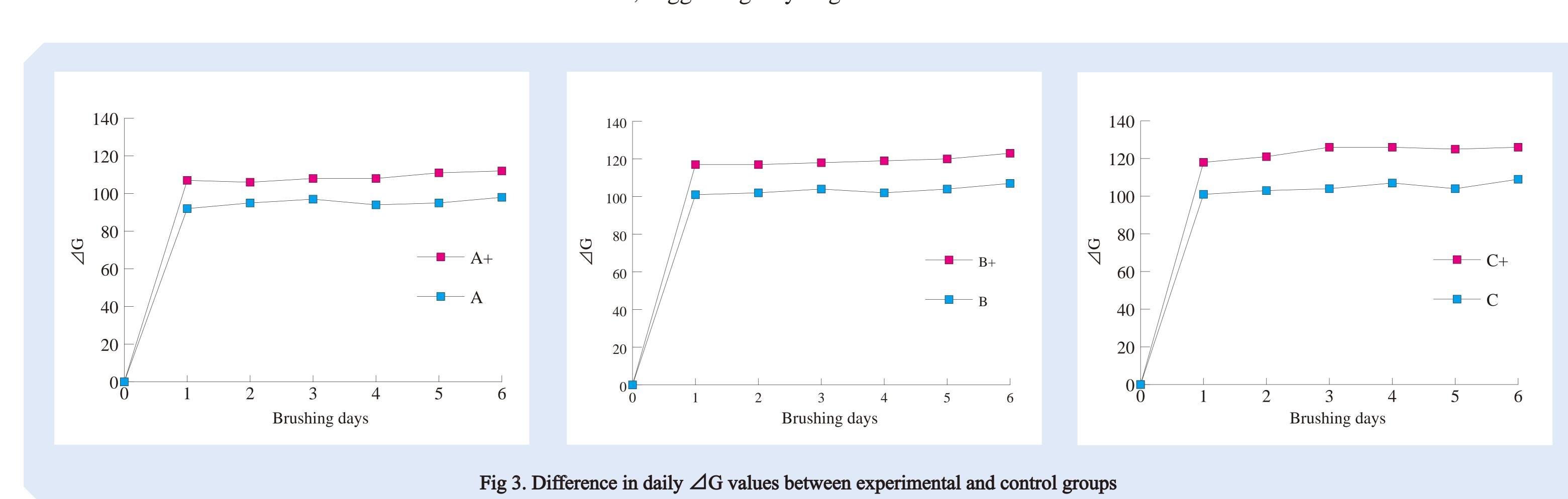
#### 4. Surface Analysis

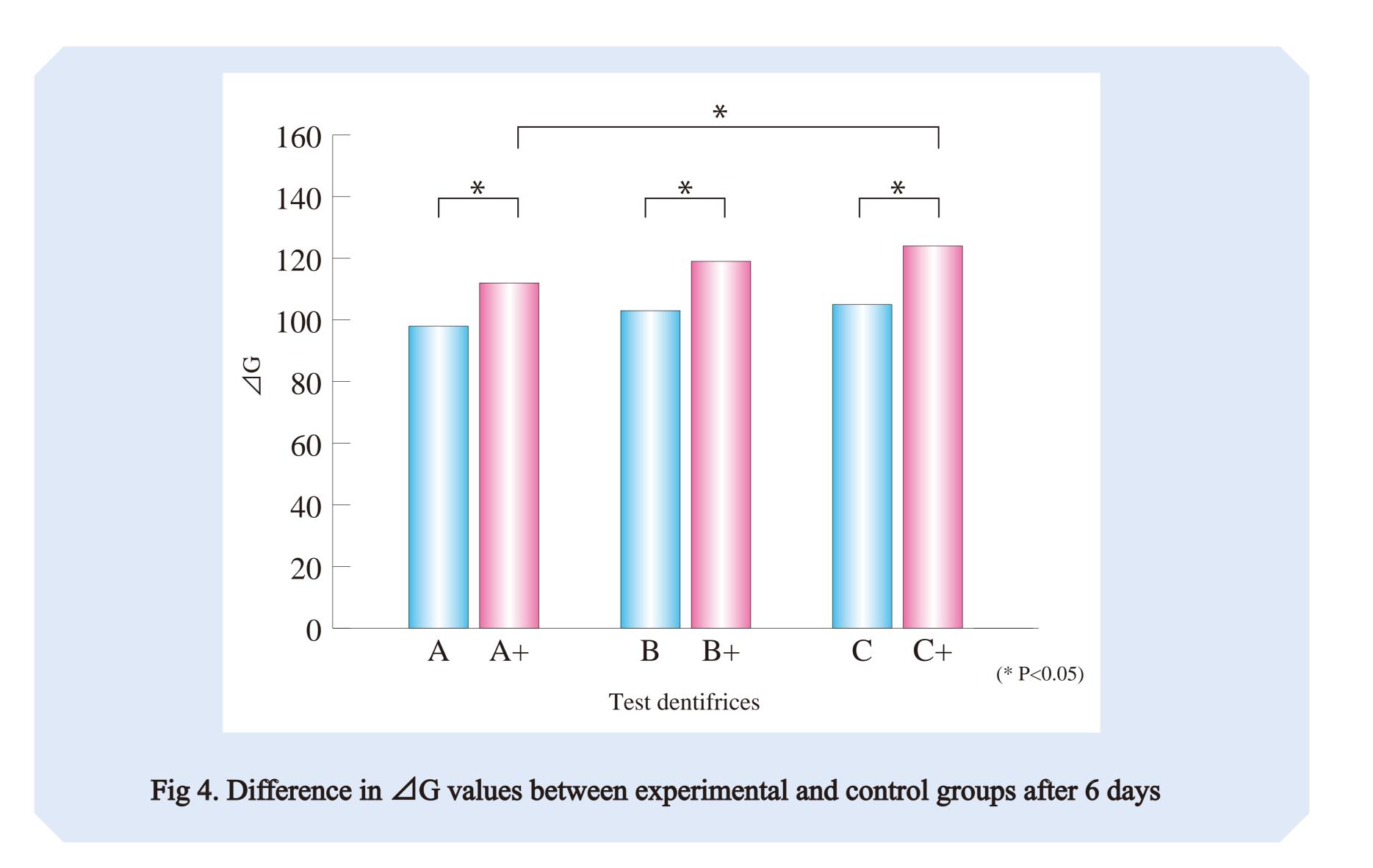
A test for the presence of HC in the enamel surface after brushing was also carried out. Teeth were analysed by time-of-flight secondary ion mass spectrometer (TOF-SIMS, ULVAC-PHI) at the Tokyo Metropolitan Industrial Technology Research Institute (Analytical conditions: Ga ion as the primary ion, output voltage 15kV, analysis area 100×100µm, detected positive secondary ion). Four samples were analysed: one tooth each brushed in group A dentifrice (paste A or paste A+) for 6 days, HC alone, dropped onto an aluminum plate, and a sample of sound tooth enamel. Before analysis, all samples were dried.

# Results

#### 1. Brushing Test and Gloss Measurement

- Gloss increased from the first day of brushing in both groups.
- Throughout the 6-day test,  $\triangle G$  for the experimental groups was greater than that of the control groups, and after 6 days,  $\triangle G$  for all experimental groups was significantly higher than for controls (P <0.05).
- The value of  $\triangle G$  increased with the concentration of nHAP, suggesting a synergistic effect of both HC and the nHAP concentration.

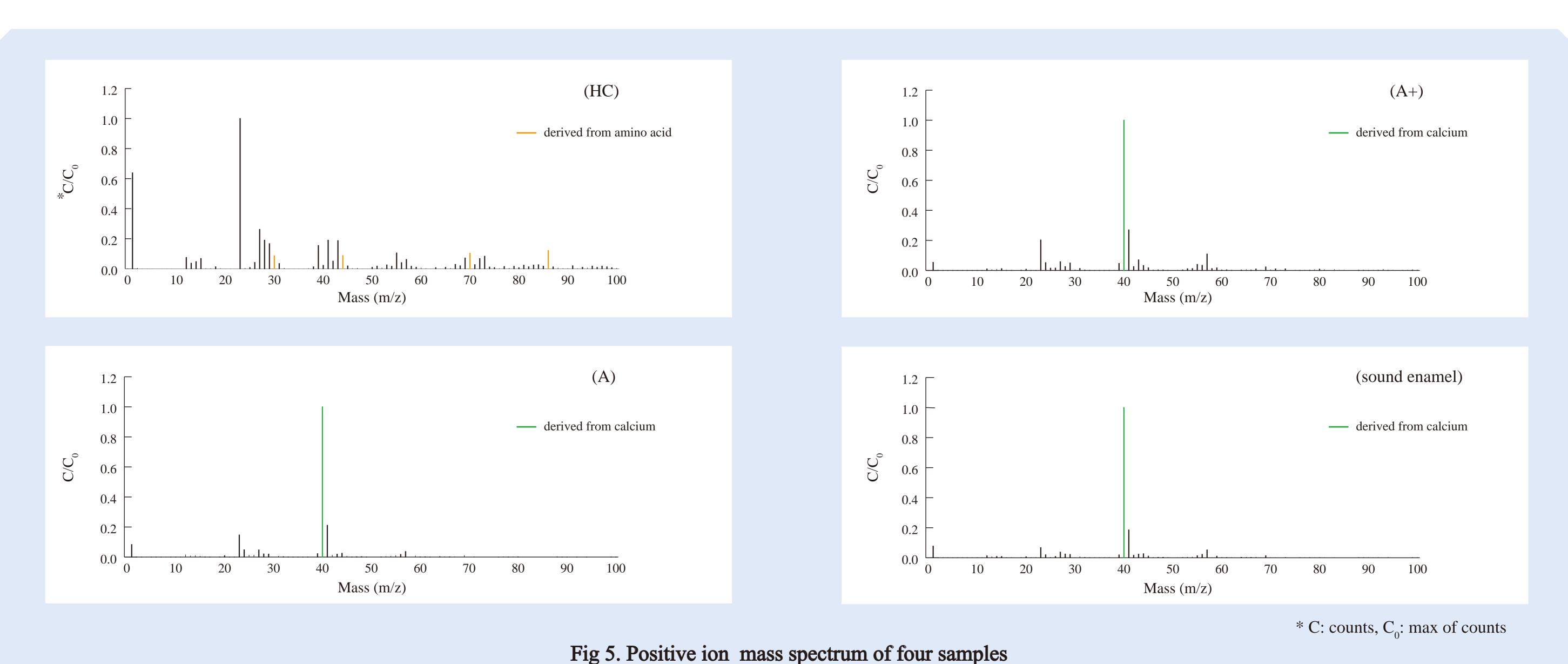




## 2. Surface Analysis

- In the resulting positive ion mass spectrum, some peaks derived from amino acid were detected in the HC sample, but only a main peak derived from
- calcium was detected in the A+, A and sound enamel samples.

   Results indicated that the enamel surface after brushing with HC toothpastes did not contain any HC.



## Conclusion

HC, in conjunction with nHAP, promotes increased enamel gloss. As no HC was detected in the enamel after surface analysis, we postulated that rather than acting directly on the enamel surface, HC may support the deposition of mineral on and into the enamel by nHAP.