Mimicking the Self-organized Microstructure of Tooth Enamel

Supporting Information

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Materials and Methods for CC Dissolution Kinetics of Tooth Enamel

Freshly extracted caries- and filling-free human permanent molars, stored for less than two weeks in 0.5 % Chloramine-T, were used. Each tooth was cut horizontally at the cemento-enamel junction to remove the root portion. The crown was cut vertically (parallel to the tooth axis) from the buccal and lingual sides to produce two enamel discs about 2 mm thick from each tooth.[1, 2] All cuts were made using a slow speed diamond saw (Buehler Isomet 1000 precision saw) with water irrigation. Samples were cleaned by ultrasonication in distilled water for 5 min before and after polishing and were serially polished using progressively finer silicon carbide papers (P800, P1000, P1200, P2400 and P4000) (Struers, Denemark). The use of human tissue specimens followed a protocol that was approved by our IRB/ethical committee and informed patient consent was obtained.[1, 2]

In the constant composition (CC) method, multiple titrant solutions containing HCl and NaCl were added simultaneously to the reaction solutions during the reactions. Titrants, were prepared according to eqns. (1) and (2), taking into account dilution effects.

$$T_{NaCl} = W_{Nacl} + 6C_{eff} \tag{1}$$

$$T_{HCl} = 14C_{eff} \tag{2}$$

In eqns. (1) and (2), *W* and *T* are the total concentrations in the reaction solutions and titrants, respectively; C_{eff} is the effective titrant concentration with respect to HAP (C_{eff}=1.00×10^{-4} mol L^{-1}).

CC curves, recorded as plots of added titrant volume, *V*, against time, *t*, were used to calculate the dissolution rates, *R*, from eqn. 3

$$R = (C_{eff} / A_T).(dV / dt) \tag{3}$$

The dissolution rate is expressed as equivalent moles of HAP dissolved per mm^2 of surface per min. A_T is the geometric surface area of enamel exposed to the reaction solutions.[1, 2]

The dissolution experiments, initiated by the introduction of known amounts of enamel sample, were conducted in magnetically stirred (450 rpm) double-walled Pyrex vessels thermostated at 37.0 ± 0.1 °C. Undersaturated solutions, prepared by mixing calcium chloride and potassium dihydrogen phosphate with sodium chloride to maintain the ionic strength at 0.15 mol L^{-1}, were adjusted to pH=4.50 by the dropwise addition of HCl (0.05 mol L^{-1}). The relative undersaturation with respect to HAP, σ, is defined by eqn.4,

$$\sigma = S - 1 = \left[\frac{IAP}{K_{sp}}\right]^{1/v} - 1 \qquad (4)$$

where S is the undersaturation ratio, v is the number of ions in a formula unit of the salt, and IAP and Ksp are the ionic activity and solubility products, respectively.

$$S = \left[\frac{(Ca^{2+})^{10}(PO_4^{3-})^6(OH^-)^2}{K_{sp}}\right]^{1/18} \qquad (5)$$

where (Ca^{2+}), (PO_4^{3-}), and (OH^-) are calculated by PHREEQE speciation program using the Davies extended form of the Debye-Hückel equation using mass balance expressions for total calcium and total phosphate with appropriate equilibrium constants by successive approximation for the ionic strength. K_{sp} is the solubility product at 37.0 °C (5.52×10^{-118}). A σ value of -0.902 was achieved using total molar concentrations: calcium, 1.00×10^{-3} mol L^{-1}, phosphate, 6.00×10^{-4} mol L^{-1}, and sodium chloride, 0.146 mol L^{-1} .[1, 2]

Titrant addition was triggered by a potentiometer (Orion 720A, U.K.) incorporating glass (Orion No. 91-01, U.K.) and reference Ag/AgCl (Orion 900100, U.K.) electrodes. During the dissolution, the electromotive force was constantly compared with a preset value and the difference, or error signal, activated motor-driven titrant burets to maintain a constant thermodynamic dissolution driving force. During the reactions, samples were periodically withdrawn, filtered (0.22 μm Millpore filters) and the solutions were analyzed for total calcium (atomic absorption) and phosphate (spectrophotometrically as the vanadomolybdate complex); concentrations remained constant to within 1%. [1, 2]

Crystallites for SEM and EDS investigations (Hitachi S-4000 FESEM, beam voltage at 20 kev) were collected from the bulk solution by filtration (Nucleopore N003 filter membranes) both during and at the end of the dissolution experiments and were dried at room temperature.

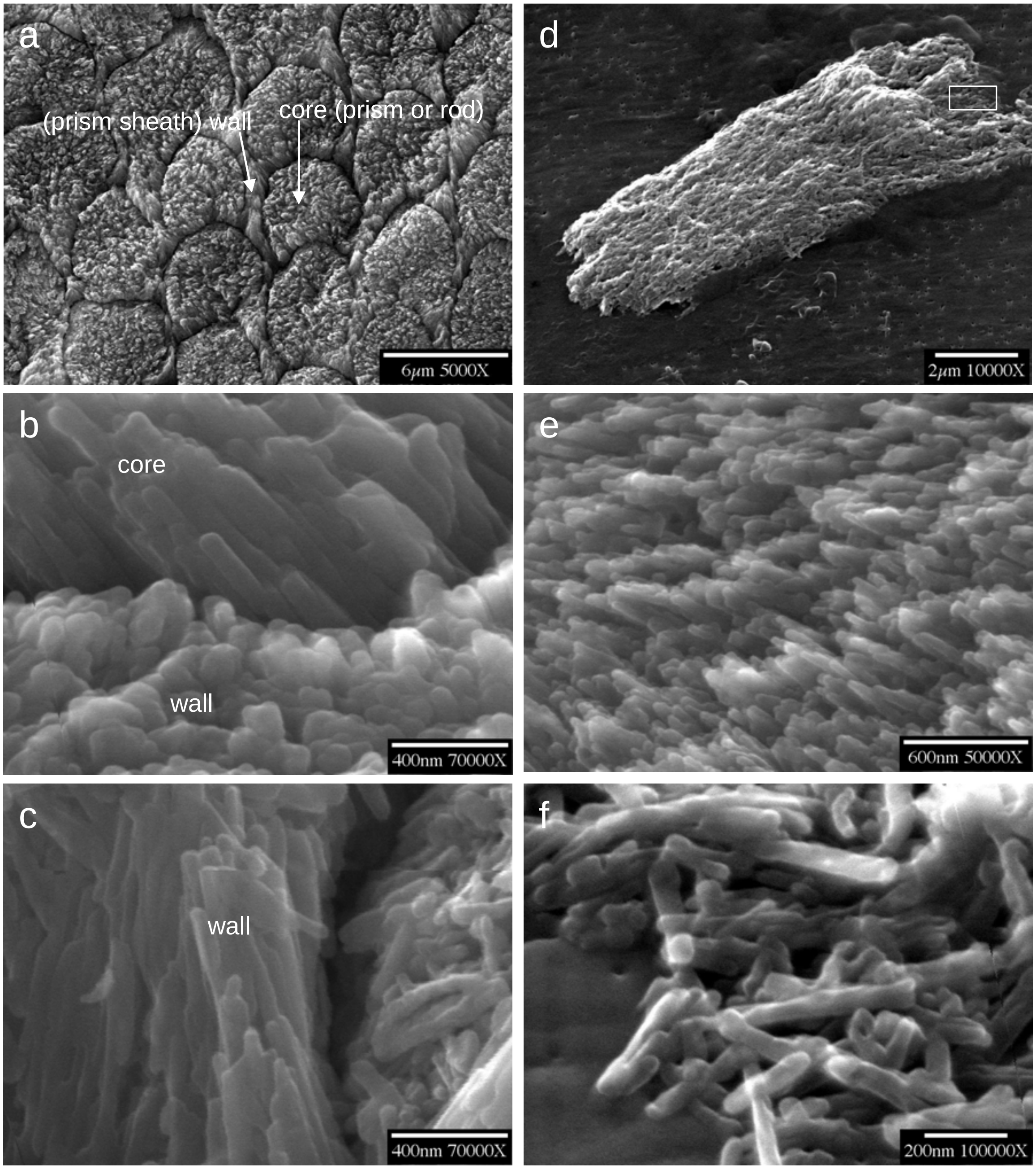

Figure S1. SEMs of enamel demineralization at different dissolution stages, showing organized structure after initial dissolution (a) and the nanorods at both cores and walls after 1 day dissolution (b, c). A detached small fragment (d) and the nanorod bundles (e) in an enlarged white rectangle in (d) are seen. The CC dissolution rate decreased virtually to zero after long reaction times, rod-like nanoparticles with the size about 200-300 nm long and 50-70 nm wide (f), collected from the bulk solution by filtration at the end of dissolution experiment, are kinetically resistant to further dissolution, even though the solution is undersaturated. All EDS results indicated that these rod-like crystallites were apatitic phases similar to those prior to enamel demineralization (not shown here, but see ref. 2).

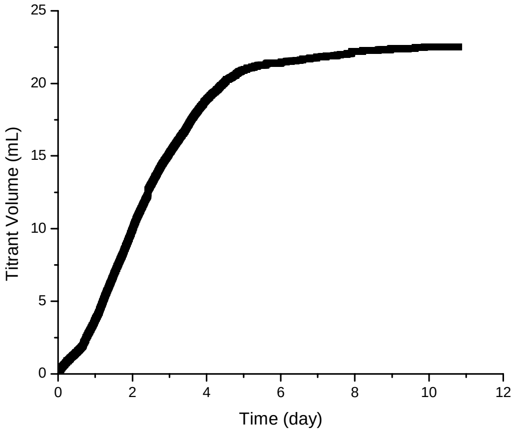

Figure S2. CC curve of tooth enamel dissolution (pH=4.5, *I*=0.15 mol L^{-1}, σ=-0.902, 37 ºC). The rate decreased virtually to zero at the end of dissolution reactions.

References

S1. L. J. Wang, R. K. Tang, T. Bonstein, P. Bush, G. H. Nancollas, *J. Dent. Res.* **2006**, *85*, 359-363.

S2. L. J. Wang, R. K. Tang, T. Bonstein, C. A. Orme, P. J. Bush, G. H. Nancollas, *J. Phys. Chem. B* **2005**, *109*, 999-1005.