Remineralization of Dentin in vitro

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The remineralization of completely demineralized bovine dentin was studied in vitro by monitoring the velocities of fall of small particles through calcifying solutions. The density of a particle of dentin may be found from its velocity of fall through a fluid using Stokes' law.

The minimum concentration product of calcium and acid phosphate ions of the solution in which remineralization would take place was 3.6 (mM)$^2$ in the presence of 22 mM bicarbonate, pH 7.35, and ionic strength 0.1. This is just above the solubility product of brushite (CaHPO$_4$·2H$_2$O). Incubation of decalcified dentin in a phosphoprotein removed from dentin during demineralization, or in phosvitin, had no effect on remineralization.

The rates of remineralization and of the fraction remineralized were inversely proportional to particle size. This inverse correlation may be due to deposition of mineral in a surface layer of constant depth irrespective of particle size. The fraction of a particle remineralized was greatly increased by the use of highly supersaturated calcifying solutions or by the incorporation of fluoride into the solutions.

The empirical reaction order of remineralization for both calcium and phosphate ions was found to be unity, which is, within the error limits, equal to the order of growth of seed crystals of hydroxyapatite in calcifying solutions of the same composition.

Key words: Calcification — Dentin — Nucleation — Kinetics.

Introduction

Decalcified dentin, like bone and cartilage, can be remineralized in supersaturated solutions. An orderly deposition and growth of calcium phosphate salts within and around its fibers is often seen. Studies of the remineralization of dental hard tissues have been concerned for the most part with partially demineralized enamel and dentin, and with the repair of incipient caries in enamel or the repair of carious dentin (Koulourides, 1968). Posterior changes in the composition of enamel, especially in hypomineralized areas, have been taken as evidence of remineralization in vivo (Brudevold et al., 1960). Microradiographic studies have shown carious dentin which has regained high density, an observation that can be explained by remineralization (Amprino and Camanni, 1956).

In laboratory studies, dental tissues are exposed either to calcifying solution, supersaturated in calcium and phosphate salts, or to saliva. The extent of mineralization is determined by direct techniques in which the amount of mineral taken up by the tissue or depleted from the solution is measured, or by indirect techniques such as microradiography, polarizing microscopy, absorption of dyes, and measurement of surface hardness. Reviews on the remineralization of enamel

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and dentin have been given by Wei (1967), Koulourides (1968), Poole and Silverstone (1973), and Francis et al. (1973).

In the present study, we sought to apply a new technique (Chan and Eirich, 1973) for the study of the remineralization of dentin. This technique monitors the change in density of a particle of hard tissue by measuring its velocity of fall through a column of calcifying solution.

Materials and Methods

The sedimentation apparatus and its application have been described previously (Chan and Eirich, 1973). The apparatus consists of glass columns about 60 cm long, and a stand for mounting and rotating them in an air thermostat. Each column has a restricted channel near one end to center the falling particle within the column. The solution in the column is stirred at 1,000 rpm by a small stirring bar when readings are not being taken. The bar is held fixed to the side of the column by an external magnet during readings.

An experiment is initiated by filling the column with 220 ml of calcifying solution and by equilibrating to 37°C in the thermostat. The hard tissue sample is introduced into the column through a Teflon valve with a disposable pipette. The duration of fall of the particle between bench marks 30.5 cm apart is periodically measured with a stopwatch. Because calcification in solutions close to physiologic composition is a very slow process, readings were taken only once a day. An experiment usually lasted for 10 days, although in some instances, experiments were continued for 3 wk. This technique has the advantage that calcification can be followed for long periods of time without spontaneous precipitation or bacterial growth, since the columns are closed, air-free and air-tight and need not be opened for sampling.

Bovine dentin was used in the study. The lower jaws of calves were obtained from an abattoir on the day of slaughter. The teeth were removed, cleaned of soft tissue, and frozen until use. The roots were the source of dentin.

The dentin was cut into small cubes with a scalpel under a dissection microscope. The dimensions of the cubes were measured to the nearest 5 μm with a planimeter. The particles were generally about 0.03 cm in diameter because this proved to be the smallest size practical to cut and to observe. Small particles were desirable in order to shorten the duration of the experiments.

The calcifying solution, prepared immediately before each experiment from three stock solutions, was that described by Hirschman and Sobel (1965). The desired amount of calcium stock solution was added dropwise to the phosphorus stock solution in 100 ml of basal salt solution and 600 ml of water. Before the calcium was added, the pH of the solution was lowered by bubbling CO₂ through it. After the calcium was added and the solution adjusted to 1 l, the pH was raised by bubbling nitrogen through the solution. The final pH of the solutions was 7.35 ± 0.05. The amount of basal salt solution added was always 100 ml so that the calcifying solution contained 70 mM NaCl, 5 mM KCl, and 22 mM NaHCO₃; the ionic strength was 0.10. To prevent the loss of CO₂, the solution was immediately poured into the glass columns. At the end of the experiments the pH was remeasured and found to be unchanged.

Results

Nucleation. Dentin placed directly into calcifying solution in the columns would not calcify, even when incubated up to a month. Nucleation of fully demineralized dentin was obtained only by placing the dentin in calcifying solution for 1 h, after which time it was removed, rinsed with distilled water, and transferred to the column of calcifying solution. This technique is similar to that used by Bachra and Fischer (1968) to nucleate certain tissues. The minimum concentration product at which remineralization of freshly demineralized dentin occurred was $Ca \times P = 3.6 \text{ (mM)}^2$ in a solution with 22 mM bicarbonate, ionic strength $= 0.10$, pH 7.4 and 37°C.