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A natural biomimetic porous medium mimicking hypomineralized enamel

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ABSTRACT

Objectives. In order to evaluate the clinical impact of low viscosity resin infiltration in hypomineralized enamel, it is necessary to obtain a biomimetic porous substrate capable of mimicking enamel. The specifications for the biomimetic porous medium are defined using the literature data on hypomineralized enamel. Based on these specifications, we propose to use deproteinized dentin, the latter being deproteinized by heat treatment.

Methods and Results. Thermogravimetry analysis (TGA), field emission scanning electron microscopy (FESEM) observations, mercury intrusion porosimetry (MIP) tests and nanoindentation are performed on the deproteinized dentin tissue. Heat treatment is shown to be an effective and reproducible method for removing organic fluids and protein residues in dentin. Deproteinizing dentin also enables forming nanovoids by eliminating its organic matrix. The interconnected open nanoporosities (porosities of less than 100 nm) created at 600 °C are distributed between 14 nm and 32 nm and the total porosity is 39% (including 36% due to nanoporosities). At 800 °C, they are distributed between 60 nm and 100 nm and total porosity is 37% (including 33% arising from the nanoporosities). The hydroxyapatite crystal structure is transformed less at 600 °C, so this temperature should be preferred.

Significance. Besides providing new understanding of the dentin tissue itself, this study led to characterizing a porous medium made of natural apatite, and proposing and validating its use as a porous medium mimicking hypomineralized enamel. The next logical step of this study is the characterization of resin infiltration in this medium and its mechanical reinforcement.

1. Introduction

Enamel is the most mineralized tissue of the organism with a mineral component evaluated at 96% by weight and 87% by volume. However, the mineral component may be affected by certain pathologies. In the case of hypomineralization, the rate of mineralization decreases without loss of volume. In this case the enamel is called hypomineralized enamel. In hypomineralized enamel, the mineral part made up of hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$) is smaller than in sound enamel, reaching only 50–80% by volume. At the macroscopic scale, hypomineralized enamel becomes opaque, leaving unsightly white spots. At the microscopic

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Table 1 – Specifications for the porous medium based on hypomineralized enamel characteristics.

Characteristics	Expected range
Porosity	20–40%
Pore size	20–200 nm
Young's modulus	7–21 GPa [7]
Other specifications	• Dimensions and homogeneity compatible with infiltration and mechanical tests. • Apatite shape close to the natural apatite shape found in enamel.

scale, it presents interconnected porosity (20–200 nm) due to ultrastructure alteration (enlargement of the inter-prismatic sheath [1,2] and localized acid dissolution of intra-prismatic crystals [3]). From the mechanical standpoint, hypomineralized enamel is weaker than sound enamel because mechanical properties like hardness and elastic modulus are strongly correlated with its degree of mineralization [4–6]. Its hardness and elastic modulus are around 0.5 ± 0.3 GPa and 14.5 ± 7.5 GPa [7], respectively, versus 3–6 GPa and 70–115 GPa for sound enamel [7–11].

The objective of the resin infiltration technique, a new treatment fully compatible with the concept of minimally invasive dentistry [12], is to mask the white lesions of hypomineralized enamel. This treatment is based on the use of a photopolymerizable low viscosity resin with a refractive index close to that of sound enamel [13,14]. Besides remaining a minimally invasive procedure [15–17], the treatment masks white opacity [18,19] and reinforces the mechanical characteristics [20] of hypomineralized enamel. The mineral network initially weakened is incorporated into a resin. It forms a semi-natural biocomposite with good mechanical properties, which is what makes it such an attractive biomimetic material. Regarding the new boom in therapeutic resin infiltration, an increasing number of infiltrants are expected on the market in the coming years, making it necessary to assess and compare them. Some of them have been studied separately from the lesion or by using a porous medium mimicking the lesion [21], that is to say the characteristics obtained (contact angle, surface tension, viscosity, hardening) do not take into account the medium invested. To bridge this gap, our aim is to define a reliable and reproducible model mimicking most of the characteristics of hypomineralized enamel (summarized in Table 1). This makes it necessary to perform infiltration and mechanical tests on pairs of resin/biomimetic porous medium.

The use of natural hypomineralized enamel seems ideal, but as hypomineralization is not an indicator for extraction, such samples are very difficult to gather in sufficient numbers for systematic testing. Therefore artificial hypomineralized enamel [22–24] or models made of synthetic hydroxyapatite can be considered. However, these candidates must be dismissed because either they cannot provide a specimen with a calibrated volume (necessary for infiltration and mechanical tests) or the crystal form is not sufficiently close to that of natural crystal. As surprising as it may seem, deproteineized dentin could be a very interesting alternative in response to our specifications. First, dentin is formed by the same odontogenesis mechanisms as enamel. Enamel and dentin have the same hydroxyapatite chemistry. Secondly, the mineral density of dentin (50% by volume) is certainly lower than in sound enamel (87% by volume), but is still very close to that of hypomineralized enamel (60–70% by volume). Thirdly, the mechanical properties of dentin [25,26] (hardness from 0.81 to 1.19 GPa and a Young's modulus from 18 to 25 GPa) are close to those of hypomineralized enamel. Fourthly, the use of dentin samples offers the advantage of allowing the preparation of calibrated samples compatible with laboratory tests. However dentin presents an abundant collagen matrix (mainly composed of type I collagen fibers) strongly linked to the crystal lattice. To obtain a porous mineral medium from the dentinal substrate, we propose to deproteinize the dentin to remove the collagen fibers and only keep the mineral matrix. Given the complexity of the organic matrix, deproteinization by heat treatment seems more relevant and efficient than selective chemical deproteinization.

The objective of this study is to evaluate the morphological and mechanical consequences of dentin deproteinization by heat treatment to validate the use of deproteinized dentin as a substrate mimicking hypomineralized enamel.

2. Materials and methods

2.1. *Sample preparation*

Twenty non-carious human molars, extracted according to the protocols approved by the review board of the Dental Faculty of Paris-Descartes University, were used in this study. They were stored directly in 1% chloramine-T solution. The teeth were prepared using a flat wet grinding machine (Planopol-3, STRUERS) to retain only the coronal dentin. Enamel, cementum and radicular dentin were removed. Three teeth were kept for the control group (D0). Seventeen specimens were cut vertically into two equal parts to form two equivalent groups (D600 and D800). All the specimens were also acid etched with 37% phosphoric acid for 15 s and then rinsed to remove the smear layer formed during the preparation procedures.

A conventional gradient ethanol dehydration process was applied to the D0 specimens reserved for SEM observation, and for the specimens of groups D600 and D800. In groups D600 and D800, the samples were deproteinized by heat treatment (see next section). After the preparation procedure, all the specimens were stored in a desiccation room.

The specimens reserved for nanoindentation (Fig. 1) were embedded before micron polishing. Specimens were rinsed in an ultrasound water bath (Transsonic 275, PROLABO) between each polishing step. Sound and hypomineralized enamel samples were also prepared in order to establish a comparison with the groups made from dentin. After embedding, the enamel samples and D0 group samples were stored in distilled water. Samples from groups D600 and D800 were again subjected to ethanol dehydration.

Sample preparation and the methodology are summarized in Fig. 1.

2.2. *Deproteinization by heat treatment (HT)*

In groups D600 and D800, the samples were deproteinized according to the thermodynamic principle of phase transition.