ANALYSIS OF SYNCHROTRON X-RAY DIFFRACTION PATTERNS FROM FLUOROTIC ENAMEL SAMPLES

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ABSTRACT

With the introduction of fluoride as the main anticaries agent used in preventive dentistry, and perhaps an increase in fluoride in our food chain, dental fluorosis has become an increasing world-wide problem. Visible signs of fluorosis begin to become obvious on the enamel surface as opacities, implying some porosity in the tissue. The mechanisms that conduct the formation of fluorotic enamel are unknown, but should involve modifications in the basics physical-chemistry reactions of demineralisation and remineralisation of the enamel of the teeth, which is the same reaction of formation of the enamel’s hydroxyapatite (HAp) in the maturation phase. The increase of the amount of fluoride inside of the apatite will result in gradual increase of the lattice parameters. The hexagonal symmetry seems to work well with the powder diffraction data, and the crystal structure of HAp is usually described in space group P63/m. The aim of this work is to characterize the healthy and fluorotic enamel in human tooth using technique Synchrotron X-ray diffraction in order to determine the crystal structure and crystallinity of on fluorapatite (FAp) crystal present in fluoritic enamel. All the scattering profile measurements was carried out at the X-ray diffraction beamline (XRD1) at the National Synchrotron Light Laboratory - LNLS, Campinas, Brazil.
1. INTRODUCTION

Teeth, like other natural biomaterials, are essentially inorganic/organic composites with enviable strength and damage resistant properties. In human teeth, the enamel is the most mineralized tissue in the body and contains more than 95% of mineral at the end mineralization. The mineral content of the enamel is composed by hydroxyapatite crystals (HAp), Ca_{10}(PO_4)_{6}(OH)_2, resulting in a highly crystalline structure [1].

The chronic exposure to high levels of fluoride during tooth development induces a molecular or structural change in the enamel characterized microscopically as a subsuperficial hypomineralization of the enamel. Fluoride increases the rate of crystal precipitation, forming fluorapatite (FAp) instead of hydroxyapatite [2]. Some authors have pointed that this acceleration of enamel mineralization causes premature mineralization of the outer enamel, retaining the proteins into enamel and altering the enamel mineralization, resulting in the well mineralized superficial enamel and the hypomineralized subsurface lesion that, microscopically, resembles a caries white lesion [3].

Recent literatures have revealed significant increase of prevalence of dental fluorosis. The increase coincided with the widespread availability of discretionary fluorides such as fluoridated toothpaste, fluoride supplements and fluoride applications in the dental office. The main risk factors for fluorosis were water fluoridation, fluoride toothpaste use, fluoride supplement use and infant formula [4].

The diffraction of X-rays by matter results from the combination of two different phenomena: scattering by each individual atom and interference between the waves scattered by these atoms. Each atom emits a scattered wave that is coherent with the incident radiation, and these waves interfere with each other, so that we have a diffracted radiation whose intensity depends on the direction of observation. The diffraction pattern of a crystalline material records the X-ray intensity as a function of the scattering angle and it offers a clue to the crystal structure. Because the atoms form a regular array, the diffracted waves can reinforce at certain diffraction angles and cancel each other at all other angles. Thus, interference peaks are formed at these angles.

Any crystalline material immersed in a white X-ray beam diffracts along given directions, according to Bragg’s law:

\[ 2d_{hkl} \sin \theta_B = \lambda \]  

(1)

where \( d_{hkl} \) is the interplanar spacing of the \((hkl)\) crystallographic planes, \( \theta_B \) is the Bragg angle and \( \lambda \) is the wavelength of the incident beam.

The differential coherent cross section is given by

\[ \left( \frac{\partial \sigma}{\partial \Omega} \right)_{Coherent} = \frac{r_e^2}{2} (1 + \cos^2 \theta) F^2(q, Z) \]  

(2)
where $r_e$ is the classical electron radius, $\theta$ is the scattering angle, $F(q, Z)$ is the atomic form factor and $q$ is the momentum transfer argument given by $q = \sin(\theta/2)/\lambda$. The scattering angle $\theta$ between the transmitted primary beam and the scattered ray is twice the Bragg angle. $F(q, Z)$ is equal to the Fourier transform of the electron charge distribution, which is a property of the material [5].

Therefore, many aspects of the enamel crystals can be studied by X-rays diffraction (XRD), such as surface coating produced by fluoride agents, correlation of changes of pH with crystal size, precipitation of octacalcium phosphates, effects of calcium phosphate precipitation on acid resistance of enamel, effect of recombinant amelogenins on the microstructure of the mineral. It is important to determine whether enamel defects of mineralization are formed by normal hydroxyapatite crystals or altered crystals, since this will have implications for solubility and other properties of the enamel.

The aim of this work is to characterize the healthy and fluorotic enamel in human tooth to determine the crystal structure of on fluorapatite (FAp) crystal present in fluorotic enamel. The properties of synchrotron radiation are most useful for crystalline diffractometry. The X-ray diffraction station at National Synchrotron Light Laboratory (LNLS) was used for obtaining high-quality crystalline diffraction patterns.

2. MATERIALS AND METHODS

2.1. Teeth Extractions

Ten third molars teeth with fluorosis were collected at Venâncio Aires, Rio Grande do Sul, Brazil, in 2004. This collection was approved by the Ethics Committee for Human Research, the Faculty of Dentistry of Ribeirão Preto University of São Paulo. These teeth were stored at -20ºC until processing.

2.2. Preparation of Specimens

A piece of the teeth crown was previously fixed in buffered 4% paraformaldehyde for 48h, dehydrated in crescent degrees of alcohol, embedded in glycol methacrylate resin (Technovit® 7200 VLC, Kulzer, Wehrheim, Germany) and cut using a high precision diamond disk (150µm) in a sectioning machine (EXAKT, Germany). These sections were ground until the thickness of 80µm. Thickness was measured using a digital paquimeter (accuracy of ± 10µm).

2.3. X-ray Diffraction Measurement

X-ray diffraction experiments were performed on flat, human enamel surfaces with thickness about 100 µm in National Laboratory of Synchrotron Light (LNLS), in Campinas, São Paulo. The scattering measurements were carried out in θ-2θ reflection geometry using the x-ray diffraction beamline (XRD1). Then a double-crystal Si (111) pre-monochromator, upstream of the beamline was used to select a small band of energy ($\lambda\Delta\lambda/\lambda = 10^{-4}$) in 11 keV
(\(\lambda = 1.127 \, \text{Å}\)). Scattering signatures were obtained at intervals of 0.04° for angles of 17° to 35°.

3. RESULTS

The total intensity detected at a particular angle consists of the sample scatter and the background. The intensities measured in function of the scattering angle for each fluorotic enamel samples were compared with the intensities measured for the healthy enamel samples. The Figure 1 show a comparison between the patterns obtained for hydroxyapatite (HAp) and fluorapatite (FAp). Figures 2 to 4 show the patterns obtained for fluorotic and healthy teeth with 1/3 forms root, 2/3 formed root and fully formed root, respectively. Each figure shows a comparison between the patterns obtained for fluorotic enamel samples and healthy enamel samples.

![Figure 1. Comparison between hydroxyapatite and fluorapatite patterns.](image_url)
Figure 2. Comparison between fluorotic and healthy enamel. Teeth have 1/3 formed root.

Figure 3. Comparison between fluorotic and healthy enamel. Teeth have 2/3 formed root.
4. CONCLUSIONS

The Synchrotron X-ray diffraction patterns are unique and characteristics of each crystalline substance and can provide valuable information about the internal characteristics of a sample. Many workers can make use of the difference in the scattering properties of these samples to reach valuable qualitative and quantitative results.
From this preliminary result, it could be observed the agreement between the scattering patterns for fluorotic and health human enamel. The evident similarities of the diffraction patterns point out the analogy between the structures of the hydroxyapatite (HAp) and fluorapatite (FAp). It is expected because of the crystal structure of both hydroxyapatite (HAp) and fluorapatite (FAp) are usually described in space group P63/m, having lattice parameters very close and due to low levels of fluoride present in fluorotic enamel samples.

Further work will include quantitative phase analysis using powder human enamel samples. X-ray profile-fitting structure refinements will be made using the Rietveld method which have proved to be a powerful tool in detecting ionic substitution in the hydroxyapatite (HAp) structure.

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