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Surface Structure Study of Crystal Hydroxy-Apatite from Fluorosis Enamel

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ABSTRACT

Fluorosis is a condition due to ingestion of excessive amounts of fluor which can cause the change in tooth structure and strength. However, there is still lack of explanation on the surface structure of crystal hydroxyapatite that influences the microscopic characteristic of fluorosis enamel. **Objectives**: To investigate the surface structure of crystal hydroxy-apatite in fluorosis enamel. **Materials and Methods**: Determination of fluor concentration and the surface structure of normal and fluorosis enamel specimen were carried out by using Scanning Electron Microscopy/ Energy Disperse X-Ray (SEM/ EDX). **Results**: Fluor concentration of fluorosis enamel was significantly higher with increased surface roughness and porosity than normal enamel. SEM observation also showed gaps areas between enamel rods and visible aprismatic zone in some regions. **Conclusion**: High level of fluor concentration on fluorosis enamel indicated the substitution of OH^- by F^- increasing the surface roughness of enamel surface.

***Key words*:** Enamel, Fluorosis, Crystal hydroxyl-apatite, SEM/ EDX, Fluor

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INTRODUCTION

Fluorosis enamel is a condition due to ingestion of excessive amounts of fluor which can cause the change in tooth structure and strength. Fluorosis can be found in 25 states of USA, with the estimate of affected population was 10 million people[1]. Fluor is commonly found in volcanoes areas and most of volcanoes areas were found in Indonesia[2]. This possibility can make the fluorosis spread in some region in Indonesia.[3]

Enamel is the hardest substance in the body[4], but in several cases and research report, enamel surface and outer enamel of fluorosis enamel (mottled enamel) were detached and more brittle than normal enamel.[5] Recent data on electron microscopy studies reported that the characteristic of mottled enamel demonstrated porous enamel with rough surfaces. [6]

Enamel formation is structured by enamel prism which formed by crystal apatite.[7] Most apatite crystal found is $CA_{10}(PO_4)_6(OH)_2$ or known as hydroxyapatite (HA).[2] HA arranged the enamel prism with length of 120-160 nm and width of 25 nm on the narrow side and 40 nm on the width side. Each HA crystal is arranged by apatite cell unit in lattice arrangement of P and Ca, and also the lattice arrangement of O and H.[8] Every cell unit of apatite suggested ion Ca position on hexagonal corner to formed calcium column. Ca position is perpendicular to c-axis. Ca position is also on cell central canal which formed Ca triangles. The space between Ca columns were placed by two PO_4 ions on the hexagonal side.[7,9,10] F ion position on c-axis was higher than OH position. Calderyn reported that OH position on c-axis between ¼-⅓, while ion F position is ¼ above the Ca triangle[11].

Fluor is the highest electronegativity and very reactive element.[10] Enamel structure which was formed by fluor apatite (FA) or ($CA_{10}(PO_4)_6(F)_2$) demonstrated different structure than crystal HA. Brittleness condition in fluorosis enamel indicated the change of apatite crystal structure as enamel former. An in vitro study reported a high concentration of fluor in enamel and has revealed a decreasing grain size of apatite crystal.[12] Other research has reported a retention of Fluor in enamel decreasing lattice parameter of cell unit crystal of hydroxyapatite. [13]

OBJECTIVES

The aim of this study was to determine the surface structure crystal hydroxyapatite in fluorosis enamel. Result of this research as elementary data for the future research according to nanostructure of apatite crystal in scope of fluorosis enamel. Beside, this finding suggest as basic contribution disparity between of crystal structure in correlation with the characteristic of enamel in nanotechnology research.

MATERIALS AND METHODS

Specimen preparation

The specimens were divided into two groups: normal human enamel as control (n = 1) and fluorosis human enamel (n = 7). Both groups were soaked in alcohol 70% for 24 hours and wrapped in ringer-cotton for storage. Normal enamel specimens were collected from a fresh tooth which was extracted for orthodontic treatment at RSGM Jakarta- Faculty of Dentistry Universitas Indonesia.

Data acquisition and processing

The surfaces structure were visualized by SEM, which is capable of imaging features with a secondary electron emission for image acquisition of both surfaces of the specimens. We carried out two separate magnifications 20 X and 500 X to demonstrate the different restructuring (roughness) of normal and fluorosis enamels surface. EDX/ EDS were carried out to *determination* of fluor concentration of normal and fluorosis enamel. We carried out *EDX/ EDS studies* to establish whether the degree of roughening of the surfaces was dependent on the amount of fluoride on the enamel. EDX/ EDS analysis was carried out with backscattered emission, i-Probe 380-450 pA, accelerating voltage 12 kV. Wavelength is under 1000 mA. Result analysis is Fluor percentage rate (%) EDX/ EDS standard.[14]

RESULTS

General Aspect and Clinical Appearance

The human enamel used as control showed normal translucency with glossy creamy white enamel which remained after wiping and drying the enamel surface. The normal enamel surface revealed a smooth homogenous appearance and displayed a regular pattern. The clinical characteristic appearance of specimen 01 showed translucency and thin white opaque lines thet were seen running across the tooth surface. The opaque white lines frequently merged to form small cloudy areas which scattered over the surface. Specimen showed a smooth brownish-dark discoloration and displayed an irregular pattern as showed with slight snowcapping in the edge of the cusp/ incisal.

Specimen 02 showed irregularity snowcap areas. Cervical areas of the enamel showed more homogenous area than cusp with opacity and brownish appearance in the mesio-incical part. Small pits were frequently observed in opacity areas and generated bands as showed on the surface. The clinical characteristic appearance of fluorosis specimen 03 showed an alteration of the enamel morphology. The entire surface showed an obvious opacity (chalky white) with most of the outer enamel has disapeared and the surrounding pits on the entire enamel showed an opaque surface. Clinical appearance of specimen 04 showed the loss of almost of the entire surface which generated a

change in tooth shape. Brownish discoloration and a slight snowcapping of incical edges were also shown in these specimens. Opaque white lines were frequently generated into two areas of the tooth.

Clinical characteristic appearance of specimen 05 showed loss of the occlusal part with small white spot hypoplastic areas appearance on enamel formation and displayed a brownish snowcap on the surface. Clinical appearances of specimen 06, showed the opaque lines which generated small cloudy areas that scattered on the surface. The specimen also showed surrounding pits with major part of the outer enamel has changed the tooth anatomical shape. Enamel surface shown an opacity and opaque enamel. This condition revealed white to dark-gray striations in part of the enamel. Specimen 07 showed loss of the outer enamel which resulted a change in anatomical shape of the surface with brownish and rough area. General aspect and clinical appearance were carried out by SEM shown on Figure 1.

Surface Structure Determination by SEM/ EDX

Crown surfaces of all fluorosis specimens demonstrated surface damage, ranging from scattered small pits to partial loss of the outermost enamel. Most of these areas acquired dark-grey pigmentation. These conditions were especially shown on specimen 03, 04, and 07 which shown the enamel was loss on the mesio-incisal area. Crack and fissure were also displayed on the surface particularly in specimen 03 and 04. Crack and fissure conditions, made tooth become two irregular sections. In addition, some areas demonstrating no surface damage appeared cloudy to opaque and had entirely lost the normal translucency of intact enamel. The area beneath the thin surface layer generally was opaque when observed by magnification of 20X by SEM.

Results of SEM/ EDX showed the highest to lowest Fluor concentration on specimen 04, 03, 07, 06, 01, 02, and 05 respectively. Table 1 demonstrated fluor concentration from all specimens with fluorosis specimens showed higher concentration rate than control specimen. Sign (*) on SEM/ EDX analysis on Table 1 indicated that there was no fluor concentration on the enamel surface.

Table 1. Fluor concentration on enamel surface

Specimen	Fluor concentration on enamel surface (%)
Control	0.23*
01	7.35
02	4.80
03	15.31
04	22.20
05	4.04
06	9.76
07	9.83

Specimen 04 showed fluor concentration of 22.20% with loss of enamel surface which changes the tooth shape. In specimen 03, the fluor concentration was

Fig. 1. SEM of all specimens (Magnification 20X)

15.3% with rough surface area and crack line that rises from the pit. Pits from occlusal were seen running across to the cervical and changed the enamel shape. The fluor concentration on specimen 07 is 9.83%. SEM showed rough and damage enamel surface. Fluor concentration on specimen 06 is 9.76%. SEM showed rough surface with pits formation and damage enamel. The fluor concentration on specimen 01 is 7.35%. SEM showed rough surface and pits formation which spread away both in occlusal and cervical areas. Specimen 02 showed fluor concentration of 4.8 %. Specimen 05 showed low fluor concentration level of 4.04 % and SEM picture did not show porous area on the entire enamel surface. Small part of rough enamel as shown on the surface, demonstrated higher fluor concentration than other areas in this specimen. Specimen control with the lowest fluor concentration value (0.23%) showed a solid substance, with small pores, as regular pattern. Sign (*) which found on fluor concentration mean no Fluor concentration on the enamel surface.

Analysis is also carried out to evaluate Fluor concentration on the surface which loss of the major part of the enamel as demonstrate change of anatomical shape and surface. This analysis is to have the picture of surface and Fluor concentration on the surface which loss of the major part of the enamel. Table 2 demonstrates the Fluor concentration level on damage enamel and was taking by magnification 500 X. Results demonstrated Fluor rate on damage enamel was higher than control/ normal.

The sequent of highest fluor concentrations in all specimens schematically showed on Table 2. SEM/ EDX have confirmed gaps areas between enamel rods, and a surface aprismatic zone was visible in some regions.

Table 2. Fluor concentration on damage enamel surface

Specimens	Fluor concentration on damage enamel surface (%)
	I
Control	0.14*
01	6.13
02	5.28
03	28.35
04	26.44
05	4.01
06	6.71
07	7.35

DISCUSSION

Fluorosis enamel is a condition due to ingestion of excessive amounts of fluor which can cause the change of tooth structure and strength.[1] SEM showed fluorosis enamel that were porous, rough and damage enamel surface. Crack and fissure were also displayed on the surface. The conditions had shown a boundary that made tooth become two irregular shapes. These phenomenon suggested that fluor had enhanced the porosity of enamel surface. Fejerskov[15] reported, that SEM image of fluorosis enamel surface showed pits and roughness area.

According to Wright, [16] pits and roughness area occurred as a result of increased of fluor concentration level. Results of this study also showed that fluor concentrations level on the surface were higher than control with major loss of the outer part enamels, which may be associated with fluor concentration and enamel strength. Fluor concentration on the fluorosis enamel improved the brittleness of the enamel. This study result perfectly matched with the work of Koningswald and Sanders[5] that reported higher brittleness of fluorosis enamel surface (mottled enamel) than normal enamel.

Enamel formation is structured by enamel prism (enamel rod) which formed by crystal apatit that mostly composed of $CA_{10}(PO_4)_6(OH)_2$ or known as hydroxyapatite (HA).[2, 7] High concentration levels of fluor on fluorosis enamel formed Fluorapatit which indicated the substitution OH^- by F^- on surface structure and lattice formation of apatite crystal.[13.] High concentration level of Fluor indicated the important character of interaction and the mechanism of detach and damage enamel on fluorosis enamel. Donadel[8] reported that enamel structure formed by Fluor apatite (FA) or (CA_{10} $(PO_4)_6$ $(F)_2$) demonstrated different structure than crystal HA. Gap areas between enamel rods and visible aprismatic zone in some regions as confirmed by SEM/ EDX generated the decreasing the numbers of apatite crystals in the enamel rods of fluorosis enamel. As we know, enamel is also uniquely composed of extremely long and narrow crystals, packed into parallel arrays called enamel rod, which can form intricate interwoven pattern.[16] Gaps area between enamel rods will automatically generated irregular pattern and roughness on enamel surface that matched with the clinical appearance and visualization by SEM.

Pauling Scale for Fluor is 3.98 which mean that fluor is the highest electronegativity among other elements and is a very reactive.[10] This properties will generate internal atomic bonding between enamel crystal units which structured the enamel prism of apatite. According to Achille, charge of fluor atom in fluor apatite is -0.82 and OH is -0.78. This condition generates the excessive of force internal atomic bonding on atomic structure of enamel crystal. The consequence suggested, Ca atom which that position is closely with fluor atom, will shift to the fluor atom.[17] Other research reported that the decreasing of lattice parameter on a-axis of cell unit crystal of hydroxyapatite on fluorosis enamel will influence the stability of enamel strenght[18] generated by the internal atomic bonding of fluor as the most electronegative element.

Result of this research as elementary data for the future research according to nanostructure of apatite crystal in scope of fluorosis enamel. Beside, this finding suggest as basic contribution disparity between of crystal structure in correlation with the characteristic of enamel in nanotechnology research.

CONCLUSION

High level fluor concentration on fluorosis enamel indicated increasing surface roughness of enamel surface with gap areas between enamel rods and visible aprismatic zone in some regions as confirmed by SEM/ EDX. Fluor as most electronegative element generates the internal atomic bondings which influence the stability of enamel strength.

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