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ORIGINAL RESEARCH

Nanostructure of Crystal Hydroxyapatite from Fluorosis: Affected Enamel

Abdillah Imron Nasution

Department of Oral Biology, Study of Dentistry Program, Faculty of Medicine, Aceh, Indonesia

Correspondence: Abdillah Imron Nasution, Department of Oral Biology, Study of Dentistry Program, Faculty of Medicine, Aceh Indonesia, e-mail: abdillah@karstaceh.com

ABSTRACT

Fluorosis is a condition due to ingestion of excessive amounts of fluor which can cause the change the teeth structure and strength. Currently, a little explanation was available to describe the surface and change of nanostructure crystal hydroxyapatite which contribute to influence the macrocharacteristic of fluorosis enamel.

Aims and objectives: To describe the change of surface structure, c-axis, a-axis and grain size of crystal hydroxyapatite on fluorosis enamel.

Materials and methods: This research was carried out the fluorosis and normal enamel specimen by using scanning electron microscopy/energy disperse X-ray (SEM/EDX) to determine fluor concentration and the surfaces structure of fluorosis enamel, and powder X-ray diffraction (XRD) to determine change of c- and a-axis of hydroxyapatite of fluorosis enamel.

Results: Fluor concentration were higher in fluorosis enamel and the surface increasingly roughness and porous. SEM/EDX also confirmed gaps areas between enamel rods and visible aprismatic zone in some regions. The axis on fluorosis enamel was a-axis = 9.3786 Å and c-axis = 6.8836 Å. The a-axis on normal enamel was = 9.4148 Å and c-axis = 6.8791 Å. Grain size of fluorosis enamel was 19.59 nm and normal enamel was 20.30 nm.

Conclusion: Fluor as most electronegative element changes the c-axis, a-axis, and grain size of crystal hydroxyapatite and generates the internal atomic bonding which influences the stability of enamel strength.

Keywords: Fluorosis enamel, Crystal hydroxyapatite, c-axis, a-axis, Grain size.

INTRODUCTION

Fluorosis enamel is conditions due to ingestion of excessive amounts of fluor which can cause the change in tooth structure and strength. Fluorosis has been spread in 25 states, with the estimate of affected population are 10 million.[1] Fluor is commonly found in volcanoes areas.[2] Most of volcanoes areas were found in Indonesia. 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 the characteristic of mottled enamel demonstrate porous enamel and surfaces are rough.[6]

Enamel formation is structured by enamel prism which is formed by crystal apatite.[7] Most apatite crystal founded is $Ca_{10}(PO_4)_6(OH)_2$ or known as hydroxyapatite (HA).[2] HA arranged the enamel prism whose length is 120 to 160 nm and width is 25 nm on the narrow side and 40 nm on the wide 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 Ca ion position on hexagonal corner to form calcium column. Ca ion position is perpendicular to c-axis. Ca ion position is also on cell central canal which formed Ca triangles. Space between Ca columns were placed by two ions PO_4 on the hexagonal side.[7,9,10] F ion position on c-axis higher than OH position. Calderyn[11] was reported that OH position on c-axis between 1/4 and 1/3, while ion F position is 1/4 above the Ca triangle.

The structure of enamel which formed by fluor apatite (FA) or $Ca_{10}(PO_4)_6F_2$ known have different structure than crystal HA. *In vitro* study reported high concentration of fluor in enamel showed the a-axis dimension of apatite crystal is 7.78 Å and c-axis is 6.03 Å.[8] Other research reported that retention of fluor in enamel decreasing of a-axis from 9.437 Å to 9.406 Å and increasing the length c-axis from 6.874 Å to 6.884 Å.[10]

Fluor is the highest electronegative and very reactive element.[10] Enamel structure which formed by fluor apatite demonstrate different structure than crystal HA. Brittleness of enamel on fluorosis enamel indicated the change of apatite crystal structure as enamel former. *In vitro* study reported high concentration of fluor in enamel, revealed the decreasing of grain size of apatite crystal.[12] Other research reported that retention of fluor in enamel decreasing lattice parameter of cell unit crystal of hydroxyapatite.[10]

There is no *in vivo* study in Indonesia focused on the change of nanostructure of apatite crystal in fluorosis enamel. According to this problem, the objective of this study is to describe the change of nanostructure of crystal HA in fluorosis enamel. Result of this research as initial explanation according to nanostructure of apatite crystal in scope of fluorosis enamel. Beside, this finding suggest as a basic contribution for the characteristic of enamel in nanotechnology research.

MATERIALS AND METHODS

Specimen Preparation

Specimens were divided into two groups: Normal and fluorosis enamel. Both groups soaked in alcohol 70% during 24 hours and wrapped in ringer-cotton for storage. Normal specimens were collected from a fresh tooth which extracted for orthodontic treatment at RSGM Jakarta Dentistry Faculty University of Indonesia. Fluorosis specimens were collected from various places in Jakarta. Fluorosis specimens collected were seven specimens and control was three specimen.

Data Acquisition and Processing

SEM analysis was carried out with a secondary electron emission for image acquisition of both surfaces of the specimens. Two separate magnifications were used: 20× and 500×. 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.[13]

The X-ray diffraction analysis was carried out with Philips Diffractometer PW 1710, X-ray tube was 1.5406 Å and filament tension is 30 kV, $2\theta = 5^{o}$-89^{o}, with the time of each phase is 1 second. XRD result was analyzed with the APD program and controlled by ICDD. This analysis is conducted for change of c- and a-axis parameter (Angstrom) of both specimens calculated with Bragg equation. The grain size (nm) of apatite crystal calculated with Scherer equation,[13] full width at half maximum (FWHM) was analyzed by UDF XRD data in BellaV2-1.

$$D = \frac{k \lambda \text{ Radian}}{\beta_1 \cos \theta}$$

Note: Scherer equation, with k = shape factor/constant 0.9, λ = X-ray wavelength, radian constant = 57.3, cos θ = cosinus 2θ, and β_1 = full width at half maximum (FWHM).

RESULT

General Aspect and Clinical Appearance

Enamel control shows the normal translucency of the glossy, creamy white enamel remains after wiping and drying of the surface. Control does not show the characteristic of fluorosis enamel. The enamel surface of normal revealed a smooth homogenous appearance and displayed a regular pattern. The characteristic clinical appearances of specimen 01 show translucency and thin white opaque lines are seen running across the tooth surface. The opaque white lines frequently merge to form small cloudy areas which scattered over the surface. Specimen showed a smooth brownish-dark discoloration and displayed an irregular pattern as showed a slight snow capping in the edge of cusp/incisal. Specimen 02 showed irregularly snowcap area. Cervical showed more homogenous area than cusp, opacities, and brownish appearance in the parts of mesioincisal. The small pits frequently occurred in opacities area and generate bands as showed on the surface. The characteristic clinical appearances of specimen 03 show the morphology was changed. The entire surface showed an obvious opacity (chalky white). Most of the outer enamel is lost and surrounding pits on the entire enamel showed an opaque surface.

Clinical appearance of specimen 04 showed the loss of almost entire surface which generate a change of tooth shape. Brownish discoloration and a slight snow capping of incisal edges showed in this specimen. Opaque white lines are frequently generating tooth into two areas. Characteristic clinical appearances of specimen 05 show loss of the part of occlusal, small white spot hypoplastic areas appearance on enamel formation which displayed a brownish snowcap on the surface. Clinical appearances of specimen 06 show the opaque lines which frequently generate small cloudy areas which scattered of the surface. Specimen also showed surrounding pits and major part of the outer enamel was change the anatomical shape of tooth. Enamel surface generally opacity and enamel was opaque. This condition revealed white to dark-gray striation in part of enamel. Specimen 07 shows loss of the part of the outer enamel results in a change of the anatomical shape of the surface, brownish and roughness area. General aspect and clinical appearance by SEM as demonstrated in Figure 1.

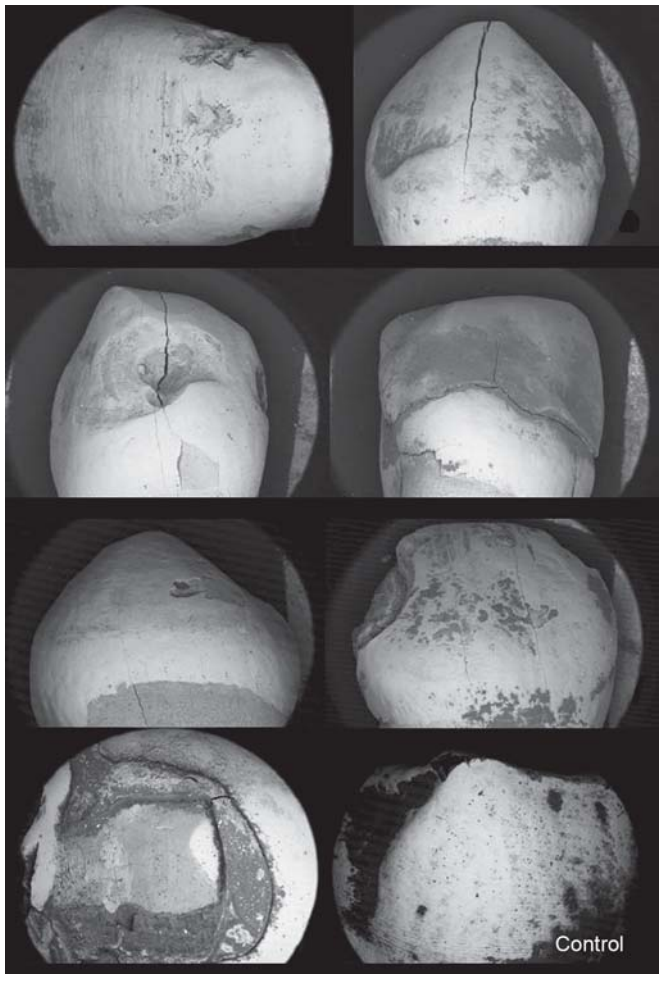


Fig. 1: SEM of all specimens (magnification 20×)

Surface Structure as Determinant by SEM/EDX

Crown surfaces in all fluorosis specimens demonstrated surface damage, the various defects ranging from scattered small pits to partial loss of the outermost enamel. Most of these areas had acquired dark-grey pigmentation. These conditions especially as showed on the specimen 3, 4 and 7. There is a loss of enamel on the mesioincisal area. Crack and fissure was also displayed on the surface particularly as showed on the specimen 3 and 4. These conditions made tooth become two irregular sections. 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 20 × by SEM.

Results of SEM/EDX show the highest to lowest fluor concentration are specimen 04, 03, 07, 06, 01, 02 and 05. Table 1 demonstrates fluor concentration from all specimens, fluorosis specimens showed high concentration rate than control.

Specimen 04 showed fluor concentration is 22.20%, SEM show loss of almost entire surface results in a change of tooth shape. Specimen 03 with the fluor concentration is 15.3%. SEM, show rough surface area and crack line rise from pit. Pits from occlusal are seen running across and reach the tooth cervical made the change of enamel shape. The fluor concentration on specimen 07 is 9.83%. SEM shows the rough surface and damage enamel. Fluor concentration on specimen 06 is 9.76%. SEM shows the rough surface, pits formation and damage enamel. The fluor concentration on specimen 01 is 7.35%. SEM show the rough surface, pits formation which spread away both in occlusal and cervical area. Specimen 02 showed fluor concentration 4.8%. Specimen 05 showed low fluor concentration level 4.04%. SEM showed entire of the enamel surface showed nonporous area. Small part of rough enamel as shown on the surface, demonstrate fluor concentration is higher than other areas in this specimen. Specimen control with the lowest fluor concentration value (0.23%) shows a solid substance, containing a lot of very small pore formation, indicate regular pattern. Sign (*) which found on fluor concentration mean fluor concentration on the surface of enamel control is zero.

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 (Fig. 2).

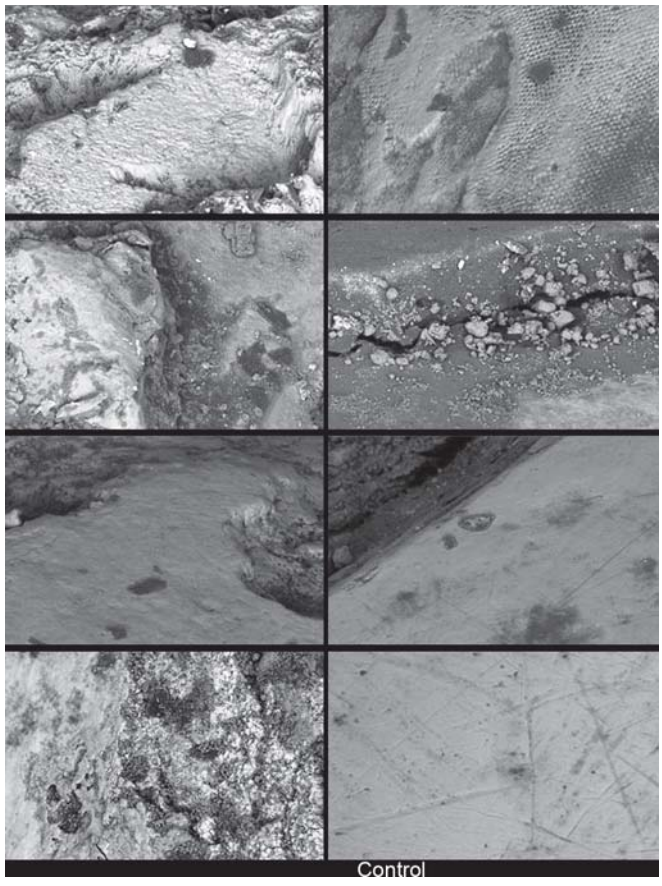


Fig. 2: Damage enamel surface (specimen 1 to 7) which visualized gap areas between enamel rods (magnification 500×)

The aim of 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 which conducted by examination of fluor concentration level on damage surface and the image was taken by magnification 500x. This analysis demonstrated fluor rate on damage enamel was higher than control/normal. The sequence of highest fluor concentrations in all specimens schematically shown on Table 2. SEM/EDX have confirmed gaps areas between enamel rods, and a surface aprismatic zone was visible in some regions.

XRD Analyze

XRD fluorosis enamel specimens, indicated of compound $Ca_5 (PO_4)_3F$ while graph XRD of control indicate $Ca_5(PO_4)_3$

Table 1: Fluor concentration on enamel surface

Specimens	*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

Table 2: Fluor concentration on damage enamel surface

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

*SEM/EDX analysis as indicated in Table 1, mean fluor concentration on enamel surface is zero.

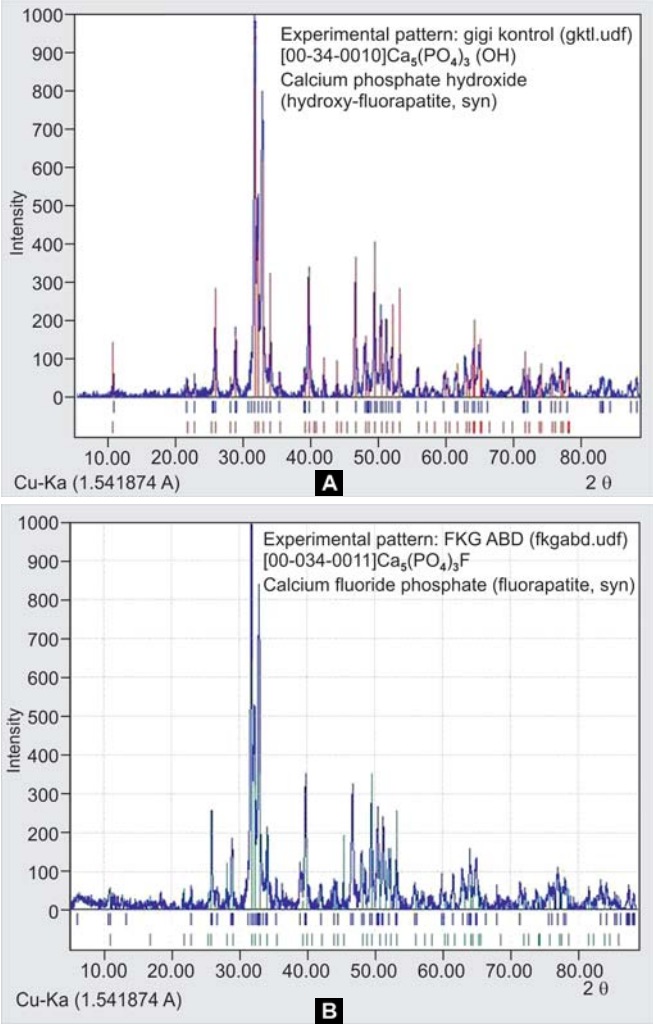
Table 3: Lattice parameter of enamel apatite

	a-axis (Å)	*c-axis* (Å)
Control (normal)	9.4148	6.8791
Fluorosis	9.3786	6.8836

Table 4: Grain size of crystal apatite

	Control (normal)	*Fluorosis enamel*
FWHM*	0.115	0.250
Grain size (nm)	20.30	19.59

*SEM/EDX analysis as indicated in Table 1, mean fluor concentration on enamel surface is zero



Figs 3A and B: XRD result

(OH) as shown on Figures 3A and B. Both pattern of graph fluorosis and control displayed a same pattern, especially at 2θ = 32. Analyses of UDF XRD data result by ICDD indicate the degradation of the intensity crystallinity on several curve on fluorosis specimen at angle 2θ = 31.89, 32.32, 33.04 and 34.17, while control shows the slime peaks at angle 2θ = 31.81, 32.29 and 32.98. Analyses of lattice parameter of a-axis and c-axis also indicate that the enamel fluorosis have the difference crystallinity from normal. Both analysis of lattice parameter on a-axis and c-axis as displayed on Table 3. From this result as shown on table that enamel control have better crystallinity than fluorosis enamel. Grain size result analysis of crystal apatite as displayed on Table 4. This table indicated decreasing of grain size of fluorosis enamel.

DISCUSSION

Fluorosis enamel is a condition due to ingestion of excessive amounts of fluor which can cause the change of teeth structure and strength.[1] SEM showed fluorosis enamel was porous, rough surface and damage enamel. Crack and fissure also displayed on the surface. The conditions made tooth become two irregular shapes. These phenomenons suggest fluor enhanced the porosity of enamel surface. Fejerskov[14] reported, SEM image of the surface of fluorosis enamel show pits and roughness area. According to Wright,[16] pits and roughness area occurs because of the increasing of concentration fluor level. Result of this study also show the fluor concentrations level on the surface which loss of the major part of the outer enamels were higher than control, that may be associated with between fluor concentration and enamel strength. Fluor concentration on the fluorosis enamel improved the brittleness of the enamel. This study result perfectly matched with previous research that reported surface of fluorosis enamel (mottled enamel) is more brittle than normal.[5,16]

Enamel formation is structured by enamel prism (enamel rod) which formed by crystal apatite.[7] Most apatite crystal founded is $Ca_{10}(PO_4)_6(OH)_2$ or known as hydroxyapatite (HA).[2] High concentration levels of fluor on fluorosis enamel suggest the forming of fluorapatite. This forming indicates the substitution OH^- by F^- on apatite crystal. This forming showed the ability of F^- to substitute OH^- in surface structure and lattice formation of crystal apatite.[10] High concentration level of fluor as showed on results indicate the important character of both interaction in the mechanism of detach and damage enamel on fluorosis enamel. Donadel[12] reported the enamel structure which formed by fluor apatite (FA) or $Ca_{10}(PO_4)_6F_2$ demonstrate different structure than crystal HA. Gaps areas between enamel rods and visible aprismatic zone in some regions as confirmed by SEM/EDX generate 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.[15] Gaps areas between enamel rods automatically generate the enamel into the irregular pattern and roughness surface of the enamel. This result matched with the clinical appearance and visualized by SEM that displayed a regular pattern and rough surface on fluorosis enamel.

XRD analysis displayed a similar graph of XRD pattern between HA and FA.[10] Furthermore, analysis data of UDF XRD showed changes of lattice arrangement of crystal apatite in a-axis and c-axis. The a-axis parameter of fluorosis enamel was smaller than control (9.3786 Å *vs* 9.4148 Å), but parameter c-axis showed an improving length which is 6.8791 Å in control and 6.8836 Å in fluorosis enamel. Fluor is very reactive element.

Pauling Scale for fluor is 4 that mean fluor has the highest electronegativity among the other elements.[10] This character will generate internal atomic bonding between enamel crystal units which structured enamel prism of apatite. This condition generates the excess of attractive force internal atomic bonding on enamel. The consequence suggested Ca atom will shift to the fluor atom.[16] Other research reported that decreasing of lattice parameter on a-axis of cell unit crystal of hydroxyapatite on fluorosis enamel will influence the stability of enamel strength.[18] It is established that fluor as most electronegative element generates the internal atomic bonding which influence the stability of enamel strength.

The change of c-axis occurs because the position of ion F is higher than c-axis position OH in apatite crystal. Ion F position on c-axis higher than ion OH position. Calderyn,[11] reported that OH position on c-axis between 1/4-1/3, while ion F position is 1/4 above the Ca triangle. Difference in position level of both ions in c-axis generates the elongation of c-axis of cell unit. This phenomenon indicated as attempting stability of apatite crystal to decreasing of interatomic bonding and minimalism the effect of microstrain in apatite crystal atoms.[10]

Analysis data XRD of control showed the slimmer peaks from fluorosis graph. The slimming peak of control indicates the alteration of grain size of crystal apatite. The grains size both of control and fluorosis crystal calculated by Scherrer equation. This equation calculated that grain size of normal crystal is 20.30 nm while the grain size of fluorosis enamel is 9.60 nm. Decreasing grain size of crystal occurred because the difference size of ion between ion F and ion OH. This study result perfectly matched with previous reported that application of fluor on synthetic apatite was decreasing of dimension of crystal.[17] Furthermore, as we explained above, the electronegativity of fluor will generate the attractive force us her the shifting atoms Ca which settled on canal position into fluor atom.[16] The shifting of Ca atoms to fluor atom which presented in the cell units of crystal apatite will generating the degradation of lattice dimension of crystal apatite which automatically will decreasing of grain size crystal apatite.[18]

CONCLUSION

High level fluor concentration on fluorosis enamel indicates the substitution of ion OH by ion F increasing the surface roughness of enamel surface. Gap areas between enamel rods and visible aprismatic zone in some regions generate the enamel into the irregular pattern and roughness surface of the enamel. Fluor, as most electronegative element, changes the lattice arrangement and grain size of crystal apatite. The changes of lattice arrangement and grain size of crystal apatite generates the excess of attractive force on internal atomic bonding of crystal cell units. The change of this internal atomic bonding will decrease of enamel strength on fluorosis enamel.

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