

Structural aberrations in fluorosed human teeth: Biochemical and scanning electron microscopic studies

A. K. Susheela*[‡], Madhu Bhatnagar*,
N. Gnanasundram[†] and T. R. Saraswathy[†]

*Fluorosis Research and Rural Development Foundation, C-13 Qutab Institutional Area, New Delhi 110 016, India

[†]Madras Dental College, Chennai 600 018, India

The present investigation was carried out to provide biochemical and ultrastructural evidences on the aberrations that appear in teeth in human Dental Fluorosis (DF), a condition caused by excess intake of fluoride. Human fluorosed teeth were obtained from the OPD of Madras Dental College, Chennai. Normal tooth samples were also collected from patients who opted for denture. The samples were investigated for fluoride and calcium contents, besides the tooth surfaces were examined under scanning electron microscope to assess the morphological aberrations. An increase in fluoride content and decrease in calcium content in fluorosed human teeth were observed when compared to the control. The scanning electron micrographs of the enamel surface of fluorosed human teeth show pitted, uneven and rough surfaces. Cracks and fissures were also observed on the enamel surface of fluorosed teeth. The present study provides evidence to suggest that pitting, perforation and structural alterations in DF are the result of impaired enamel mineralization.

DENTAL fluorosis (DF) is a hypomineralization of tooth enamel caused by continuous ingestion of excessive amount of fluoride during tooth development. This results in a variety of pathological changes in the structure of the teeth^{1,2}. DF is characterized by opaque, lusterless

[‡]For correspondence. (e-mail: susheela@ndf.vsnl.net.in)

white patches in the enamel which may become striated, pitted and discoloured followed by the breakdown of mineralized layer of the enamel shortly after eruption^{3,4}. As revealed by studies in humans⁵⁻⁷ and several other mammalian species⁸⁻¹³, these alterations arise from fluoride effects on both secretory and maturation stages of amelogenesis. Studies on various animal models^{10,13} and in human¹⁴ support the view that the early maturation stage is the most critical developmental period for DF, but sufficiently high concentration of fluoride might effect the enamel at all stages of its formation^{9,15}.

In addition to diet, modern sources of ingestion of fluoride included a variety of dental products, some of which have been identified as risk factors for fluorosis^{16,17}. A highly significant association was found between the estimated fluoride ingestion from toothpaste and fluorosis¹⁸. In fluoridated toothpaste users, especially children in the age group 5–10 years and 10–14 years, even after rinsing their teeth satisfactorily with water fluoride level in circulation was enhanced within a few minutes¹⁹. It has been reported that DF and dental caries, diseases of different etiology, co-exist in the same individual at fluoride levels ranging from 0.5 to 5.0 mg/l and above²⁰. The calcium content is found depleted in fluorosed teeth and the tooth matrix

becomes demineralized^{20,21}. Studies conducted on fluorosed human tooth matrix molecules revealed that one of the sulphated isomers of glycosaminoglycans, i.e. dermatan sulphate accumulates as a result of fluoride ingestion and thus results in demineralization of tooth matrix. It is also a fact that the demineralized loci in teeth is unlikely to get remineralized due to the presence of dermatan sulphate in the matrix²². Embery *et al.*²³ have shown that in rat incisor teeth on exposure to fluoride, dermatan sulphate accumulated and provided evidence to suggest that fluoride ingestion in high amounts causes a major imbalance to the ground substance components of mineralized tissues. In the animal model it has also been reported that long-term fluoride administration leads to structural alteration on the enamel surface and adverse effects on biochemical constituents²⁴. Present studies have been designed to investigate the enamel surface of fluorosed teeth under scanning electron microscope (SEM) and to assess the fluoride and calcium contents of tooth samples.

The fluorosed and normal human teeth were obtained from the OPDs of Madras Dental College, Chennai. Teeth were collected in 70% alcohol. They were washed in 0.1 M phosphate buffer (pH 7.4), rinsed with deionized water and cut in two equal halves. One half was used for SEM studies and the other half for estimation of fluoride and calcium contents.

For SEM studies the teeth were washed in 25% hypochlorite solution to remove organic debris from the surface of the enamel and washed with deionized water, dehydrated in graded acetone. After critical point drying followed by sputter coating with gold, the enamel surface was examined under SEM (SEM model LEO 435 VP).

The half portions of the teeth were cut into small pieces and defatted for 3 days with a 12-hourly change of diethyl ether and acetone (1:1 v/v) and dried in acetone. Dried defatted pieces of teeth were weighed and ashed for 48 h at 550° C. The ash obtained was used for estimation of fluoride and calcium contents.

A known amount of ash was digested in 1 ml of 0.25 M HCl and neutralized by 0.125 M NaOH diluted by deionized water. The concentration of fluoride was determined using an Ion Meter (ION 85-ION Analyser, Radiometer Copenhagen)²⁵. The values are expressed as mg/kg ash wt.

A known amount of ash was digested in perchloric acid. After dilution with deionized water, the calcium content was estimated using an Atomic Absorption Spectrophotometer (model GBC 902). The values are expressed as mg/g ash wt.

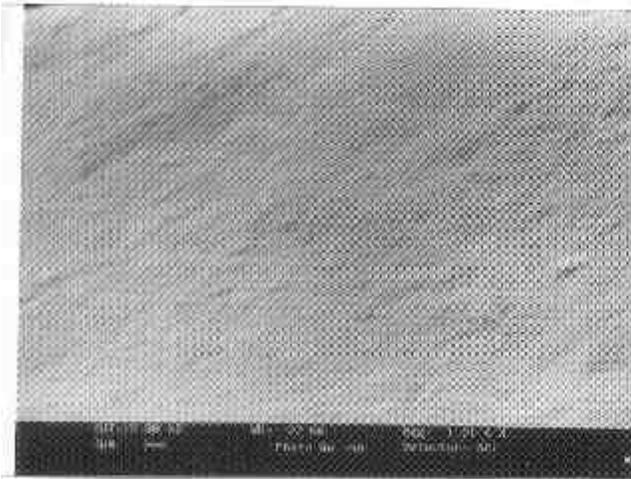


Figure 1. SEM of the enamel surface of normal human tooth showing smooth and even enamel surface ($\times 1,21$).

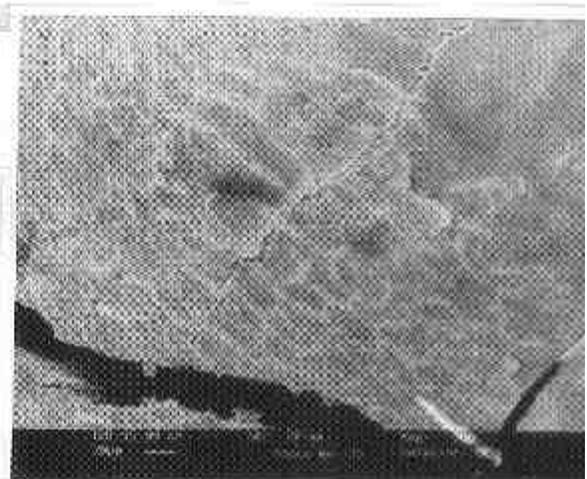


Figure 3. Enamel surface of fluorosed tooth showing many cracks (low magnification, $\times 286$).

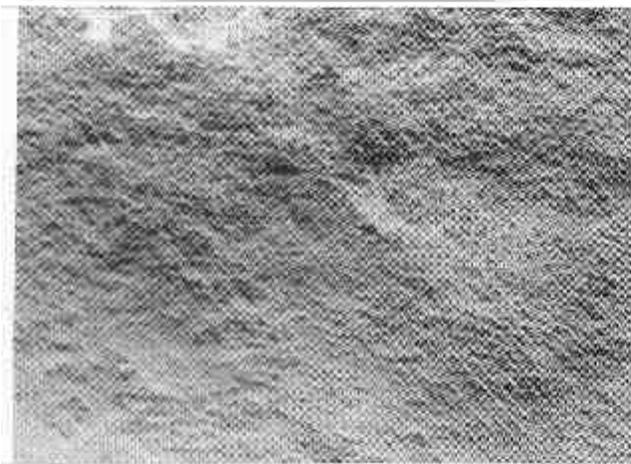


Figure 2. SEM of fluorosed human tooth showing an uneven, rough enamel surface ($\times 970$).

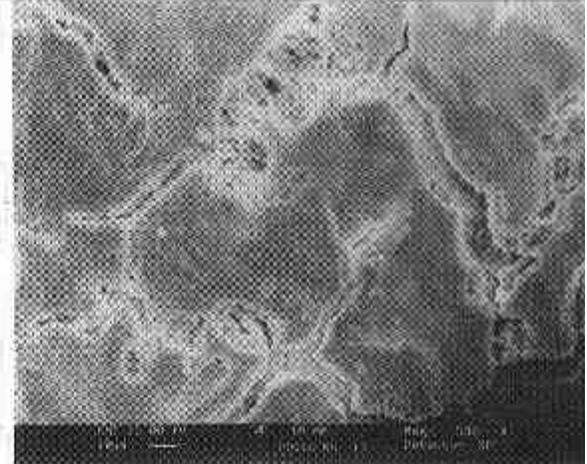


Figure 4. Enamel surface of fluorosed tooth showing deep cracks and fissures (high magnification, $\times 532$).

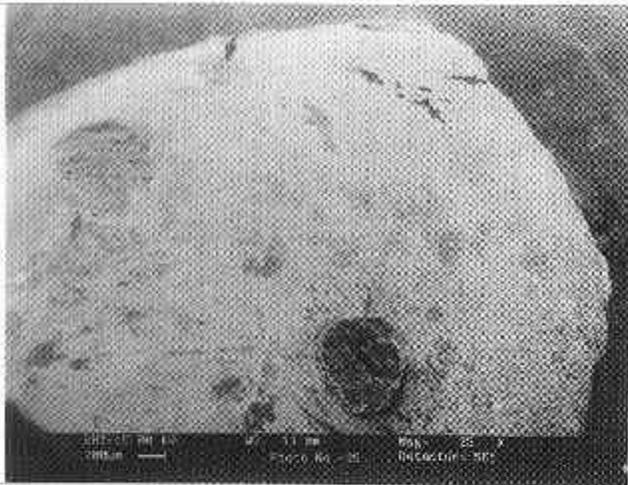


Figure 5. Pits on the enamel surface of fluorosed tooth ($\times 25$).

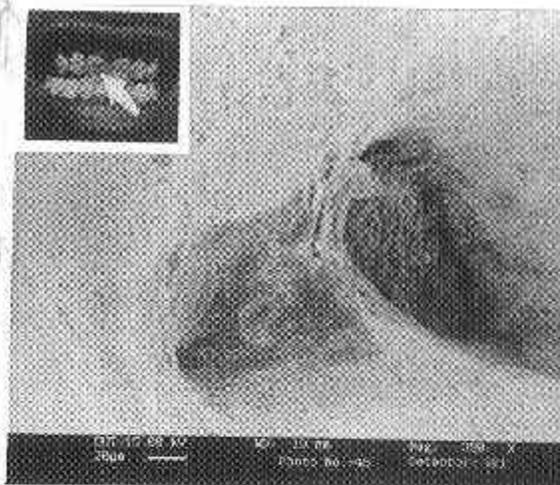


Figure 7. Pits appear as punched out areas on the enamel surface of fluorosed tooth ($\times 350$). Inset shows a set of fluorosed teeth; pits are seen in the discoloured area (arrow).

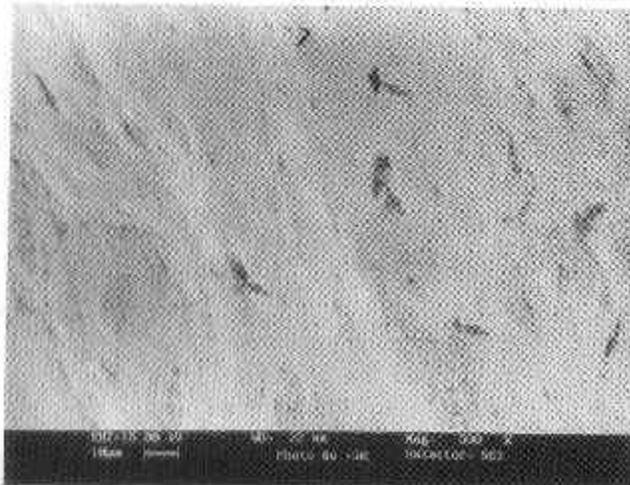


Figure 6. Pores of different dimensions seen on the enamel surface of fluorosed human tooth ($\times 598$).



Figure 8. Fluorosed tooth showing a pit on the enamel surface revealing structural damage ($\times 101$).

Table 1. Fluoride and calcium contents in normal and fluorosed human teeth

Tooth samples	Fluoride content in tooth (mg/kg ash wt.)		Calcium content in tooth (mg/g)	
	Mean	Mean	Mean	Mean
Control - 1	80	110	404	472
Control - 2	140		540	
Fluorosed - 1	675	671.6	283	275.3
Fluorosed - 2	840		272	
Fluorosed - 3	500		270	

The enamel surface of normal human teeth when viewed through SEM, revealed a smooth homogenous appearance and displayed a regular pattern of prisms (Figure 1). The enamel surface of fluorosed teeth appeared highly uneven and rough (Figure 2). Cracks and fissures

were also observed on it (Figures 3 and 4). The enamel surface showed pits of varying dimensions in the discoloured area of the teeth (Figures 5 and 6). The pits appeared to be lesions punched on the enamel surface, thus exposing the underlying porous enamel (Figures 7 and 8).

The fluoride content of fluorosed human teeth is found to increase from 110 to 671.6 ppm compared to normal. The calcium content was found to decrease from 472 to 275.3 mg/g ash wt compared to normal (Table 1). The appearance of enamel formation and the abnormalities in human teeth varies from small white spots to large hypoplastic areas^{26,27}. It is now well established that a linear relationship exists between fluoride dose and enamel fluorosis in human population²⁸. DF is a dose response condition, greater the intake of fluoride during the developmental period more severe the fluorosis will be²⁹⁻³¹.

In the present investigation, the SEM on human fluorosed teeth has shown pitted, uneven and rough enamel surface. Cracks and fissures are also seen on the enamel surface of fluorosed teeth which were not detected in normal teeth. The histopathology of DF has been investigated by polarized light microscopy^{5,32}, microradiographic appearances^{5,7,33}, and electron microscopic features^{7,33-35} by various authors. In most of the studies the affected tooth shows a pronounced accentuation of the perikymata, discrete pits and larger areas of hypoplasia of the enamel appeared to such an extent that the morphology of the tooth was lost^{1,5,6}. The hypomineralization and irregular prism pattern was observed on the enamel⁶. Fejerskov and Larsen²⁸ suggested that with increasing severity the sub-surface enamel along the tooth becomes increasingly porous and the lesion extends towards the inner enamel. It has also been reported that several years after eruption there is a trend towards an increasing severity of enamel surface destruction in children exhibiting pronounced degree of sub-surface enamel hypomineralization at the time of eruption³⁶. The microscopic changes in fluorosed human teeth are closely linked to the extent and degree of hypomineralization and to enamel thickness³².

An increase in fluoride content and a decrease in calcium content in fluorosed human teeth were observed in the present study compared to normal teeth. Similar observations have been reported earlier though not accompanied by ultra structural studies²⁰. The elevated fluoride content was found in fluorosed enamel in all stages of enamel development¹⁵. Due to long-term fluoride administration marked reduction in calcium and phosphorous content in mature enamel was reported by Shinoda⁸. Surface enamel that exhibits DF contains higher concentration of fluoride than unaffected enamel and the fluoride content generally increases with severity of the condition³⁷.

It is evident that long-term exposure to fluoride therefore interferes with the process of mineralization as Bhussry³⁸ had suggested that structural alterations of ameloblastic layer result in the retardation of enamel matrix formation and its mineralization. Calcium deficiency and generalized malnutrition disturb the physiological conditions that affect amelogenesis in human and can lead to variations in clinical appearance of DF at similar levels of fluoride intake³⁹. Considering the complexity of the biological mineralization process, the exact mechanism leading to DF is not fully understood³³. Nevertheless deviations in the natural stages of mineralization of human teeth enamel and the histopathology of fluorosed enamel have led to the hypothesis that DF is the result of impairment of enamel mineralization^{1,7}. Unlike normal teeth the fluorosed teeth readily get fractured which suggests gross structural alterations and decrease in mineral content in the teeth.

The present study investigating the human teeth surface under SEM and biochemical studies on

fluoride and calcium contents has provided evidence to suggest that pitting, perforation, and tooth getting fractured (chipped off) in DF are mainly due to accumulation of fluoride, depletion of calcium in the matrix and structural alterations on the enamel surface.

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