

Dental Caries Progression Along Enamel Lamellae – A Polarized Microscopic Study

Kumar Sourab

Lecturer, D. Y. Patil University, School of Dentistry,
Nerul, Navi Mumbai

Bhalinge Payoshnee

Lecturer, M. A. Rangoonwala College of Dental Sciences,
Pune

Tamgadge Avinash

Vice-Dean & Professor, Department of Oral Pathology,
Nerul, Navi Mumbai

Periera Treville

Professor & HOD, Department of Oral Pathology, Nerul,
Navi Mumbai

Tamgadge Sandhya

Professor, Department of Oral Pathology, Nerul, Navi
Mumbai

Jadhav Abhishek

Lecturer, D. Y. Patil University, School of Dentistry,
Department of Oral Pathology, Nerul, Navi Mumbai

Abstract: Lamellae or cracks are distributed throughout tooth enamel in both deciduous and permanent dentitions. While earlier authors postulated that lamellae may be pathways or entry for caries, no evidence was adduced and the theory appears to have been discounted. The present study seeks to show that at least in some cases, lamellae are permeable to dyes, may be associated with caries initiated in the dentin, supporting the hypothesis of Hardwick and Manly of lamellae penetration by *Streptococcus mutans* and lactobacilli. The enamel lamellae are shown to be a permeable pathway allowing caries-producing bacteria access to the dentinoenamel junction. Caries can thus be established within the tooth without visible evidence at the surface.

I. INTRODUCTION

Dental caries is all pervading in modern man living in highly industrialized societies. It is the major health problem affecting mankind, in that its manifestations persist throughout life despite treatment. There are practically no geographic areas in the world whose inhabitants do not exhibit some evidence of dental caries. [1] Dental caries is a unique multifactorial infectious disease. Our understanding of etiological factors; the progress of the disease, and the effectiveness of prophylactic procedures have led us to believe that we understand the disease.

The final result, “caries to be or not to be”, is a complex phenomenon involving a number of external factors. For example diet, microbial flora – colonizing the teeth, oral hygiene, age and internal defense factors such as general health, nutritional and hormonal status, tooth surface

morphology and saliva which is regarded to play a potent role in dental caries. [2]

The disease dental caries is a dynamic process taking place in the microbial deposits (dental plaque) which results in a disturbance of the equilibrium between tooth substance and the surrounding plaque fluid so that, over time, the result is a loss of mineral from the tooth surface. The disease caused by many factors and variety of different causative agents. Bacterial plaque is the only, immediate, direct factor causing caries. A number of different determinants control the level of bacterial activity (sugar) and resistance of the tooth to caries (saliva, fluorides).

Examining a thin ground tooth section with an ordinary light microscope reveals dental caries and other formations in enamel: bands of hunter-schreger, lines of retzius, enamel lamellae, enamel tufts and enamel spindles. [3] Similar studies have also been conducted to evaluate the penetration of

different adhesive resin systems into cavitated and non-cavitated artificial carious lesions.^[4]

In polarized light after microbial accumulation, there were clear alterations in the tissues and in the enamel surface, respectively. Visible signs of carious dissolution were found on the control surfaces of all the individuals and confirmed under polarized light by the increase of tissue porosity, showing classic subsurface lesions in different stages of progression.^[5] Lesion area measurement of enamel caries using polarized light microscopy is currently performed in a large number of studies, but measurements are based mainly on a mislead qualitative interpretation of enamel birefringence in a single immersion medium. The differences in their histopathological features derived from a qualitative versus a quantitative interpretation of enamel birefringence is found. Enamel birefringence leading to an underestimation of the depth of enamel caries mainly as the criterion of validating sound enamel.^[6]

The present study was designed to show the significance of the enamel lamellae as sites associated with caries, apparently initiated in the dentine, leading to the supposition that the lamellae, in this small incidence of cases, is the pathway for cariogenic bacteria.

II. AIMS AND OBJECTIVES

- ✓ To prove that enamel lamellae are permeable to dyes, and usually associated with caries initiated in the dentin.
- ✓ To evaluate whether or not presence of hidden caries.
- ✓ To study the path of progression of dental caries under Polarized Microscopy.

III. MATERIALS AND METHODOLOGY

STUDY SUBJECTS

30 Extracted tooth specimens of patients with caries affected individuals ranging from age 15-75years. (15 males and 15 females) and 20 Extracted tooth specimens of patients without caries which will act as control subjects from individuals ranging from age 15-75years. (10 males and 10 females)

Extracted tooth considered from the outpatient department of Oral Surgery, Dr. D.Y Patil University, School of Dentistry, Nerul, Navi Mumbai.

EXCLUSION CRITERIA

The following subjects were not taken as volunteers for the study, as these cases may alter the results of study by giving it a False positivity.

- ✓ Those patients who are under medication of any type.
- ✓ Those patients who have habits of any type.
- ✓ Pregnant females.
- ✓ Those patients with systemic diseases of any type.
- ✓ Children were excluded from the study.

STUDY DESIGN

In the study of dental caries correlation, many studies have proved a significance. Thus, dental caries pattern of progression and hidden caries were studied through the pathway of enamel lamellae.

IV. MATERIALS USED IN THE STUDY

- ✓ Mouth mirror
- ✓ WHO Probe⁽⁷⁾
- ✓ Surgical gloves and mouth masks
- ✓ 50 Extracted human teeth
- ✓ Quartz halogen lamp
- ✓ Varnish
- ✓ Orange-G stain
- ✓ Carborundum saw
- ✓ Electric lathe machine or grinding machine
- ✓ Arkansans Stone
- ✓ Methyl Alcohol
- ✓ Microscopic slide, DPX, Cover Slip
- ✓ Polarized Microscope

V. PROCEDURE FOR DENTAL CARIES PROGRESSION BY ENAMEL LAMELLAE

- ✓ Questionnaires with detailed case history and consent form taken.
- ✓ At all times extracted teeth were stored in water to prevent preparation shrinkage cracks from dehydration.
- ✓ Examination of extracted tooth with a quartz halogen lamp in subdued ambient light as exhibiting proximal enamel lamellae in posterior teeth. Labial or lingual lamellae were considered in anterior teeth.
- ✓ The teeth were sealed with varnish except in the vicinity of the lamellae.
- ✓ All teeth were photomicrographed under incident light and transillumination under stereomicroscope.
- ✓ The teeth were immersed in saturated Orange-G stain for 24 hours, then washed.
- ✓ The crowns were progressively sectioned using a carborundum saw and those showing stain penetration were examined and photographed.
- ✓ Serial sections of retained specimens performed.
- ✓ For examination under polarized light a selection of sections showing stain absorption were reduced to approximately 100µm thickness.
- ✓ These sections were dehydrated in ethyl alcohol and mounted in a microscopic slide. Thus examined under polarized light.
- ✓ The progression of dental caries lesions observed under the polarized microscope and results interpreted.

VI. PRINCIPLE

Enamel Lamellae Progression

The enamel lamellae have long been regarded as possible portals of entry. The peripheral portions of some lamellae under high magnification exhibit the presence of bacteria.

Thus we can prove that in a clinically caries-free tooth a lesion can be produced in the dentine by micro-organisms which invade and pass down the lamella.

The current study was designed to show the significance of the enamel lamellae as sites associated with caries, apparently initiated in the dentine, leading to the supposition that the lamella, in this small incidence of cases, is the pathway for cariogenic bacteria.

VII. RESULTS

TEST OF TRANS-ILLUMINATION

The first group of 50 teeth with proximal lamellae were examined but the lamellae were not detectable through trans-illumination. Since, trans-illumination being an excellent tool for detecting proximal lamellae, it did not disclose occlusal or proximal lamellae.

MICROSCOPIC EVALUATION UNDER POLARIZED LIGHT

NON-CARIOUS TEETH

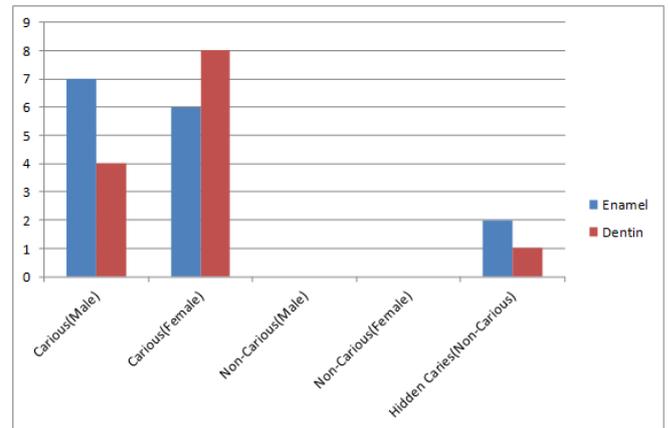
20 (40%) non-cariou teeth were involved in this research. Under polarized microscope, these tooth structures represented well-defined alternate dark and light zones with birefringence of portions of enamel and dentin. No demineralised areas were evident. 3(6%) of non-cariou teeth, showed no evidence of caries clinically, but on ground sections hidden/occult caries was found at the initiation of DEJ and appeared demineralised, darkly colored at DEJ.

CARIOUS TEETH

30(60%) of carious teeth were involved in this research. 21(42%) of carious teeth were associated with enamel lamellae. 12(23%) proved the initial caries progression through pit and fissure type caries evident clinically as white-spot lesions; 3(6%) proved with smooth surface caries and 6(12%) proved carious teeth including demineralised areas. Under polarized microscope, the reactions of tooth tissue varied from well-defined dark-zones to classic enamel-caries lesions. The characteristic which was observed clinically as a broad white-mark. These lesions presented a well-defined superficial layer, showing negative birefringence and the body of the lesion which represented the area of greatest tissue loss, presenting positive birefringence. The enamel lamellae presenting as thin fine line like projections which were associated with enamel caries and thus continuing along the carious lesions reaching upto DEJ. In case 22, which was confirmed by polarized microscope as single large carious lesion of enamel associated with two enamel lamellae at the peripheral ends. Highest number (21 carious teeth) proved its association of carious lesions with enamel lamellae. (As seen in Table 1; Bar Graph 1).

Non- Cariou (Male)	0	0
Non-Cariou(Female)	0	0
Hidden Caries(Non-cariou)	2	1

Table 1: Table showing progression of dental caries in enamel and dentin



Bar Graph 1

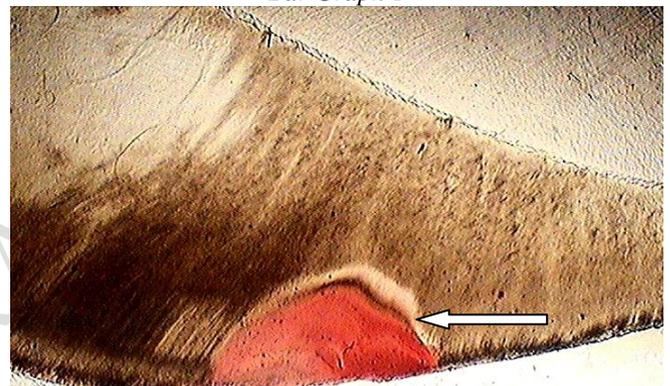


Figure 1: Caries associated with enamel lamellae (Ground section image through research microscope)

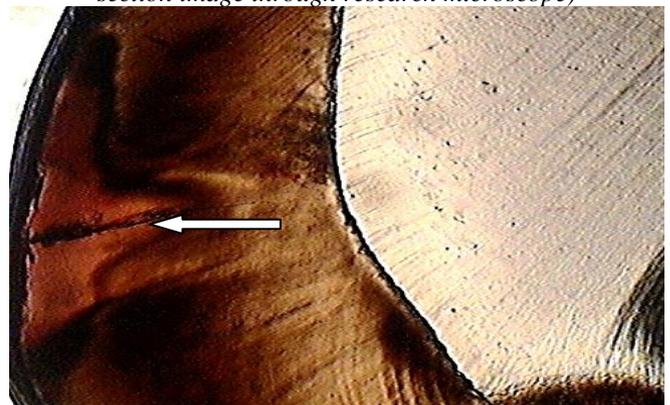


Figure 2: Enamel lamellae associated with dental caries under polarized microscope (40x view)

Status of teeth	Enamel	Dentin
Cariou(Male)	7	4
Cariou(Female)	6	8

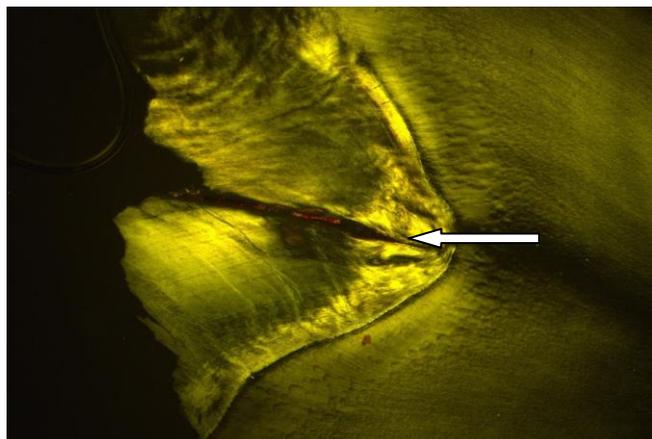


Figure 3: Enamel lamellae associated with dental caries under polarized microscope (40x view)

VIII. DISCUSSION

The study comprised 50 lamellae in this research, three cases lamellae entered dentine. Well-defined dark zones and pseudoisotropic areas were observed, which indicated a smaller amount of mineral loss. The superficial layer of the lesion was small and very often non-existent. As per our study also clinical and microscopic result proved a series of different rate of progression of dental caries. 20 (40%) non-carious teeth were involved in this research. Under polarized microscope, these tooth structures represented well-defined alternate dark and light zones with birefringence of portions of enamel and dentin. No demineralised areas were evident. 3(6%) of non-carious teeth, showed no evidence of caries clinically, but on ground sections hidden/occult caries was found at the initiation of DEJ and appeared demineralised, darkly colored at DEJ. 30(60%) of carious teeth were involved in this research. 21(42%) of carious teeth were associated with enamel lamellae. 12(23%) proved the initial caries progression through pit and fissure type caries evident clinically as white-spot lesions; 3(6%) proved with smooth surface caries and 6(12%) proved carious teeth including demineralised areas. Under polarized microscope, the reactions of tooth tissue varied from well-defined dark-zones to classic enamel-carries lesions. The characteristic which was observed clinically as a broad white-mark. These lesions presented a well-defined superficial layer, showing negative birefringence and the body of the lesion which represented the area of greatest tissue loss, presenting positive birefringence. The enamel lamellae presenting as thin fine line like projections which were associated with enamel caries and thus continuing along the carious lesions reaching upto DEJ. In case 22, which was confirmed by polarized microscope as single large carious lesion of enamel associated with two enamel lamellae at the peripheral ends. Highest number (21 carious teeth) proved its association of carious lesions with enamel lamellae. According to Weerheijm KL et al(1990) showed that atleast some lamellae are prone to bacterial invasion by caries –

causing bacteria. The mechanism of lamellae invasion on an intact surface followed by proliferation at the DEJ explains the clinical features of hidden caries.^[8,9] According to Holmen et al (1987) observed after 4 weeks ‘white spot’ lesions with subsurface demineralization in all individuals. After 1 week initial demineralization occurred as an outer translucent zone in polarized light. While in 2 to 3 week specimens showed varying degrees of subsurface demineralization. Quantitative assessments of tissue porosity disclosed in all stages of lesion development that the outer 3-9micrometer of the surface remained less porous than the immediately underlying tissue. Microscopic alterations showed well-defined dark zones and pseudoisotropic areas which indicated a smaller amount of mineral loss. The superficial layer of the lesion was small and very often non-existent.^[10]

REFERENCES

- [1] Soben Peter. Essentials of Preventive and Community dentistry (Text Book) Arya Medi Publishing house, New Delhi; 2004. [2nd edition]
- [2] Shafer, Hine, Levy. Text of Oral Pathology, Elsevier 2009[6th Edition]; 409-473
- [3] Rudy C. Melfi; Keith E. Alley. Permar's Oral Embryology and Microscopic Anatomy. A Textbook for students in oral pathology. Lippincott Williams and Wilkins 2000; X edition; 297 page.
- [4] El-Kalla I. H. ,Saudi HI, El-Agamy R. A. Effect of adhesive resin application on the progression of cavitated and non-cavitated incipient caries lesions. Am J Dent 2012 June; 25(3):176-80.
- [5] Susana Paim, Adriana Modesto, Jaime Aparecido Cury, Anders Thylstrup. Development and control of caries lesions on the occlusal surface using a new invivo caries model ; Pediatric Dentistry: Pesqui Odontol Bras 2003; 17(2): 189-195
- [6] De Medeiros RC, Soares JD, De Sousa FB. Natural enamel caries in polarized light microscopy: differences in histopathological features derived from a qualitative versus a quantitative approach to interpret enamel birefringence. J. Microscope. 2012 May;246(2):177-89
- [7] Kedjarune U, Migasen P, Changbumrung S, Pongpaew P, Jungtrongehitr R. Correlation of salivary flow rate. Caries Research. 1997; vol.2; 31:148-154.
- [8] WeerheijmKL, De Soet JJ, De Graaff J, Van Amerongen WE. Occlusal hidden caries: a bacteriological profile. J Dent Child 1990;57:428-32
- [9] TenCateJM. Oral histology. Development, structure and function. StLouis: Mosby, 1985:210.
- [10] Lisbelh Holmen, A. Thylstrup, B.Ogoard, F.Koagh. A polarized light microscopic study of progressive stages of enamel caries in vivo. Caries research, 1985, 19:348-354.