# Investigating the ultrastructure of enamel white spot lesions (WSL) using Optical Coherence Tomography at different length scales

**Submitted in partial fulfilment of the requirements for the**

**Degree of Clinical Doctorate in Paediatric Dentistry**

**University College London**

**Eastman Dental Institute**

**2014 – 2017**

**Shaima Mansour Sarkhouh**

**BDS, MFD (RCSI), MPaed Dent (RCSED)**

**Project supervisors:**

**Dr. Laurent Bozec** (Division of Biomaterial & Tissue engineering, Eastman Dental Institute- University Collage London)

**Dr. Susan Parekh** (Department of Paediatric Dentistry, Eastman Dental Institute- University Collage London)

**Dr. Maisoon Al-Jawad** (Institute of Dentistry, Queen Mary University of London)

**DECLARATION OF WORK**

I, Shaima Mansour Sarkhouh, declare that the work included in this thesis is original and my own work. Where data and information has been supported by others or performed in collaboration with other sources, I attest that this is acknowledged and indicated.

Name: Shaima Mansour Sarkhouh
Course: DDent (Paediatric Dentistry)
University Collage London- Eastman Dental Hospital
June 2017

## Abstract

White spot lesion (WSL) is the clinical presentation of early caries, which is a demineralisation that occurs at subsurface level, with a well-mineralised surface layer enclosing the lesion. Early diagnosis and treatment of WSL is crucial to prevent further destruction of tooth structure. The aim of this research is to investigate the potential of optical coherence tomography (OCT) to be used as an adjunct diagnostic clinical tool to evaluate the severity of such lesions. This research also compared the OCT outputs with traditional histology, X-ray Microtomography (XMT), Synchrotron X-ray Diffraction (SXRD) and Scanning Electron microscope (SEM).

All specimens were collected from patients undergoing dental treatment at Eastman Dental Hospital with informed consent following ethical approvall.

Initially, Artificial WSLs were induced on sound enamel surfaces using a buffered methylcellulose gel system at pH 4.6 for 7 and 14 days. Type-matched native WSL and healthy control teeth were selected based on ICDAS for comparison. Imaging of samples was obtained using OCT of whole teeth and by polarised microscopy, SXRD, XMT and SEM of polished 250 μm thick sections.

Polarised microscope, XMT and SEM confirmed the findings of the OCT results. Images showed that the more back scattered signals recorded, the deeper the destruction throughout enamel thickness. SXRD results showed changes in enamel texture, which was interpreted from measuring crystallite orientations and lattice parameter. SXRD result showed some correlation with OCT images, however more investigation is required to confirm the findings.

In conclusion, the variations observed in the back-scattered light in OCT experiment were because of mineral density variation within enamel structure, as well as the changes in prismatic structure and may be related to crystallite texture and orientation. OCT has shown to be a reliable non-destructive technique, that can investigate the internal structure, by measuring the back-scattered light from materials such as enamel and dentine. In healthy samples, OCT B-scans showed a homogenous pattern of scattering intensity throughout enamel structure, indicating healthy structure, while in both natural and induced white spot lesions, a non homogenous scattering intensity was observed, indicating changes in enamel structure.

**AKNOWLEDGEMENT**

I would like to express my gratitude to:

The programme director, **Dr. Paul Ashley**, we all appreciate and recognise his effort to make DDent programme a smooth sail for us.

My research supervisors, **Dr. Laurent Bozec**, **Dr. Susan Parekh** and **Dr. Maisoon Al-Jawad**, for their endless support and encouragement to complete this project.

My lovely **colleagues** in DDent programme for their support and always having each other's back during this fun-filled journey.

**Dr. Nicola Mordan, Dr.Graham Palmer, Dr. Khalifa Al-Azri** and **Mohammed Al-Moswi.** Thank you for your assistance.

**Kuwait Ministry of Health** for sponsoring my scholarship.

I would like to dedicate this thesis to the people in my life who have helped me achieving my goals:

My parents, **Mansour Sarkhouh** and **Eslah Baker**, the pillars upon which I stand tall. None of this would be possible without their endless care and support. Thank you for everything.

My sisters, **Sherifa Sarkhouh** and **Fatima Sarkhouh,** for believing in me and always lending an ear to all my dramatic meltdowns.

Lastly, I would like to thank my amazing friends **Noura, Asma, Fahad, Qamar, Abdul, Shaikha, Albatool, Ela, Nick, Lama, Soha, Dalal, Zoe, Rawan, Aspasia and Dimitra**. Thank you for always being there. This is the beginning of a lifelong friendship.

Table of Contents

DECLARATION OF WORK ........ 3

ABSTRACT ........ 4

AKNOWLEDGEMENT ........ 5

LIST OF FIGURES ........ 9

LIST OF TABLES ........ 12

LIST OF ABBREVIATIONS ........ 13

1 BACKGROUND ........ 15

1.1 BASIC TOOTH STRUCTURE ........ 16

1.1.1 Enamel formation ........ 17

1.1.2 Enamel ........ 17

1.1.3 Enamel biomineralisation ........ 17

1.1.4 Enamel microstructure ........ 19

1.1.5 Solubility of calcium hydroxyapatite ........ 21

1.1.6 Ion integration in enamel hydroxyapatite: ........ 21

1.2 CARIES ........ 22

1.2.1 Introduction ........ 22

1.2.2 Demineralization ........ 23

1.2.3 Pathway of caries attack and histological changes ........ 24

1.2.4 Microscopic features of caries enamel ........ 25

1.2.5 White spot lesions ........ 26

1.2.6 Artificial caries- like white spot lesions ........ 27

1.2.7 In vitro dissolution of hydroxyapatite ........ 28

1.2.8 A review of different demineralization protocols ........ 28

1.2.9 Summary ........ 29

1.3 CARIES DETECTION METHODS ........ 29

1.4 SUMMARY ........ 35

1.5 THE USE OF IMAGING TECHNIQUES TO STUDY ENAMEL STRUCTURE ........ 35

1.5.1 Optical coherence tomography (OCT) ........ 35

1.5.2 Synchrotron X-ray diffraction (SXRD) ........ 40

1.5.3 X-ray Microtomography (XMT) ........ 47

1.5.4 Scanning electron microscopy (SEM) ....... 50

2 AIM AND OBJECTIVES ....... 52

2.1 AIM ....... 52

2.2 OBJECTIVES ....... 52

3 TISSUES COLLECTION ....... 52

3.1 STUDY REGISTRATION AND ETHICAL APPROVAL ....... 52

3.2 PATIENT IDENTIFICATION AND RECRUITMENTS ....... 52

3.3 SAMPLES SIZE AND SELECTION ....... 53

3.4 SAMPLES STORAGE AND DISINFECTION ....... 53

3.5 SAMPLES PREPARATION ....... 53

3.5.1 Induction of caries like lesion ....... 54

4 THE ASSESSMENT OF THE SAMPLES USING ICDAS, POLARISED MICROSCOPE AND OCT ....... 56

4.1 RATIONALE OF THIS CHAPTER ....... 56

4.2 MATERIALS AND METHODS ....... 56

4.2.1 Material and methods for ICDAS ....... 56

4.2.2 Materials and methods for OCT ....... 56

4.2.3 Materials and methods for Polarised microscope ....... 58

4.3 RESULTS ....... 59

4.3.1 Results of ICDAS ....... 59

4.3.2 Results of Optical Coherence Tomography (OCT) ....... 60

4.3.3 Results of Polarised Microscope ....... 70

4.4 DISCUSSION ....... 72

5 ASSESSMENT OF ENAMEL STRUCTURE USING SXRD ....... 74

5.1 DISCLAIMER ....... 74

5.2 RATIONALE OF THIS CHAPTER ....... 74

5.3 MATERIALS AND METHODS ....... 74

5.4 OBTAINING DIFFRACTION PATTERN IN ENAMEL, DENTINE AND AIR ....... 76

5.5 RESULTS ....... 79

DISCUSSION ....... 86

6 ASSESSMENT OF ENAMEL STRUCTURE BY OTHER IMAGING TECHNIQUES SUCH AS XMT AND SEM ....... 88

6.1 DISCLAIMER ....... 88

6.2 RATIONALE OF THIS CHAPTER ....... 88

6.3 MATERIALS AND METHODS ....... 88

6.3.1 Material and methods of XMT experiment ....................................88
6.3.2 Materials and method of SEM experiment ....................................89
6.4 RESULTS ....................................................................................................... 91
6.4.1 Results of XMT experiment ....................................................................91
6.4.2 Results of SEM experiment ....................................................................93
6.5 DISCUSSION .................................................................................................. 97
7 SYNOPSIS OF ALL IMAGING MODALITIES ................................................ 99
8 LIMITATIONS AND FUTURE WORK........................................................... 103
9 CLINICAL RELEVANCE ............................................................................. 103
10 CONCLUSION .......................................................................................... 104
11 REFERENCES........................................................................................... 105
12 APPENDIX 1 ............................................................................................. 115
13 APPENDIX 2 ............................................................................................. 117
14 APPENDIX 3 ............................................................................................. 119
15 APPENDIX 5 ............................................................................................. 122

## LIST OF FIGURES

**Figure 1-1:** Basic tooth structure ........................................................................ 16
**Figure 1-2:** A) Crystallite structure. B) Arrangement of ions in Hydroxyapatite crystal (Siddiqui, 2014) ........................................................................................... 18
**Figure 1-3:** A) Single prism structure, B) Geometry of enamel prism (An et al., 2012) 20
**Figure 1-4:** map that shows cusp region with high texture and crystal alignment (Al-Jawad el al., 2007) ............................................................................................ 21
**Figure 1-5:** A simplistic view of caries development (Selwitz et al., 2007) ................. 24
**Figure 1-6:** Diagram represents caries zones. 1. Translucent zone, 2. Dark zone, 3. Body zone, 4. Surface zone. ............................................................................. 25
**Figure 4-1:** Image shows the different parts of OCT instrument ; 1) 10 Santec light source, 2) OCT scanning probe, 3) Adjustable platform ............................................. 57
**Figure 4-2:** A) Sample after sectioning and polishing. B) Sectioning instrument . ...... 58
**Figure 4-3:** a clinical photograph shows the scanning frame of the OCT on the tooth surface. ................................................................................................................ 60
**Figure 4-4:** OCT Image of the distal surface of the control sample. ........................... 61
**Figure 4-5:** OCT image of the distal surface of control sample set two. ...................... 62
**Figure 4-6:** OCT scan of the distal surface of the maxillary first permanant molar ..... 63
**Figure 4-7:** OCT scan of the distal surface of Mandibular first molar. ......................... 64
**Figure 4-8:** OCT scan shows the distal side of Maxillary first molar (induced caries like lesion sample (7 days). ........................................................................................ 66
**Figure 4-9:** OCT scan shows the distal surface of induced caries lesion sample (14 days). ................................................................................................................. 66
**Figure 4-10:** OCT b- scans of set one the corresponded a- scans of the area of interest. A) OCT b- scan of the control sample (area of interest highlighted by yellow lines. B) Scattering intensity profile (a-scan) of the area of interest of the control sample. C) b- scan of natural white spot lesion sample. D) a- scan of natural white spot lesion. E) b-scan of the induce caries like lesion sample. F) a- scan of the induced white lesion sample. ........................................................................................... 68
**Figure 4-11:** OCT b- scans and a- scans of set two. A) b- scan of the control sample (yellow lines indicated area of interest). B) a- scan of control sample. C)b- scan of natural white spot lesion sample. D) a- scan of natural white spot lesion sample. E) b-scan of induced caries like lesion sample. F) a- scan of induced white spot lesion sample. ............................................................................................................... 69
**Figure 4-12:** Polarised Microscope images show: A) control sample B) induced caries like lesion sample C) natural white spot lesion sample. .............................................. 70

**Figure 4-13:** Polarised microscope images show: A) Control Sample B) Induced caries like lesion sample C) Natural white spot lesion sample. ............................................ 72

**Figure 5-1:** A) Image represents SXRD setup (Al-Jawad et al., 2007) B) Image shows the experiment set up at Diamond beamline; 1. 2D x-ray detector; 2. the adjustable platform; 3. Mounted samples on a hard cardboard; 4. X-ray beam tube. .................. 75

**Figure 5-2:** Image shows a sample with the position of the selected tracks on enamel structure moving from DEJ towards enamel surface. ................................................. 76

**Figure 5-3:** Typical diffraction patterns obtained from this type of experiment. a) Identifying the preferred orientation of the crystallites from 002 reflection. b) Diffraction pattern of the dentine appears as thick 002 reflection with no reflection variations. c) Diffraction pattern of the enamel showing the variations in diffraction pattern. d) Image shows the change in crystal orientation in a different diffraction pattern within the enamel. Reproduced with permission from(Al-Jawad et al., 2007). ............................ 77

**Figure 5-4:** A) Image showing the fitted two peaks that correlates to the two arches in Debye ring. Gaussian curve was fitted to measure the width of the peaks. FWHM is indicated by a green indicator representing the width of the peak. B) Intensity against azimuthal angle plot in dentine. ........................................................................ 78

**Figure 5-5:** Image shows four peaks, which represent two populations in the enamel structure. First population is indicated by green arrows, second population indicted by the blue arrows. ......................................................................................... 79

**Figure 5-6:** The resultant FWHM values of control sample in set one. A) The position of the tracks in relation to dentine horn represented by green line. B) Graphs show the trends in FWHM as a function of distance (From enamel surface towards DEJ). ........ 80

**Figure 5-7:** The resultant FWHM values of control sample in set two. A) The position of the tracks in relation to dentine horn represented by green line. B) Graphs show the trends in FWHM as a function of distance (From enamel surface towards DEJ). ........ 81

**Figure 5-8:** Images of the three selected enamel tracks of the samples in set one; A) Control sample. B) Natural caries lesion sample C) Induced caries like lesion sample (7 days)........................................................................................................... 82

**Figure 5-9:** Graphs represent FWHM values; A) Graphs of FWHM of the first track of the control, natural caries, and induced caries like lesion samples. B) Graphs of FWHM of the second tracks of the control, natural and induced caries like lesion samples. C) Graphs of FWHM of the third tracks of the control, natural and induced caries like lesion samples. ......................................................................................... 83

**Figure 5-10:** Images of the three selected tracks on the samples of set two (14 days demineralisation) indicated by green line; A) Control sample. B) Natural caries lesion sample. C) Induced caries like lesion sample. .......................................................... 85

**Figure 5-11:** Graphs represent FWHM values against the distance from enamel surface towards DEJ; A) Graphs of FWHM values of the first tracks of the control, natural and induced caries like lesion samples. B) Second tracks of control and natural caries and induced caries like lesion samples. C) Third tracks of the samples. .......... 86

**Figure 6-1:** Image to demonstrate X-ray Microtomography (XMT) set-up................... 89

**Figure 6-2:** Image shows the six samples coated with gold- palladium layer and mounted on samples holder to be tested using SEM.................................................. 90

**Figure 6-3:** FEG-SEM instrument. ................................................................................ 90

**Figure 6-4:** High contrast XMT images show the distribution of mineral concentration of A) Control sample (C161SS), B) Natural caries sample (MIH83), Induced caries like lesion sample (C166SS). ........................................................................................ 91

**Figure 6-5:** Images show the distribution of mineral concentration of Second set of samples; A) Control sample (C162SS), B) Natural caries sample (60CONTROLAS), C) Induced caries like lesion sample for 14 days (C167SS). ........................................... 92

**Figure 6-6:** SEM images showing the prismatic structure of the control samples; A) Control sample of set one (C161SS) and B) Control sample of set 2 (C162SS) at a scale of 100 μm with 200x magnification...................................................................... 93

**Figure 6-7:** Scanning electron microscope image of a carious lesion. ........................ 94

**Figure 6-8:** Images show SEM results of samples in set one; A) The natural caries lesion sample (MIH83) at 100 μm scale. B) Natural caries sample at 20 μm scale. C) Induced caries like lesion sample for 7 days (C166SS) at 100 μm scale. D) induced caries like lesion sample at 20 μm. .................................................................... 95

**Figure 6-9:** Images show SEM results of the samples in set two; A) Natural caries sample (ICDAS 2) at 100 μm scale. B) Natural caries sample (ICDAS 2) at 20 μm scale. C) Induced caries like lesion sample (14 days) at 100 μm scale. C) Induced caries like lesion sample at 20 μm scale. ...................................................................... 96

**LIST OF TABLES**

**Table 1-1:** Table demonstrates ICDAS scoring system. .............................................. **34**
**Table 4-1:** Table shows the demographic and ICDAS scores of the samples. ............ **59**
**Table 7-1:** A summary of all imaging modalities ........................................................... **100**

**LIST OF ABBREVIATIONS**

| | |
|---|---|
| AU | Arbitrary unit |
| Ca | Calcium |
| CCD | Charged Coupled Device |
| CEJ | Cemento- Enamel Junction |
| DD | Diagnodent |
| DEJ | Dentine Enamel Junction |
| DIFOTI | Digital Imaging Fiber Optic Transillumination |
| ECM | Electric Conductance Monitor |
| ECJ | Enamel cementum junction |
| FD-OCT | Fourier Domain- Optical Coherence Tomography |
| FOTI | Fiber Optic Transillumination |
| ICDAS | International Caries Detecting and Assessment System |
| IEE | Inner Enamel Epithelium |
| LCI | Low coherence interferometry |
| LED | Light emitted diode |
| MD | Mineral density |
| MIH | Molar Incisor Hypomineralisation |
| OCP | Octacalcium Phosphate |
| OCT | Optical Coherence Tomography |
| OEE | Outer Enamel Epithelial |
| PD | Photo detector |
| PS- OCT | Polarisation sensitive Optical coherence tomography |
| QLF | Quantitative light Fluorescence |

| | |
|---|---|
| SEM | Scanning Electron Microscope |
| SLD | Super luminescent light diode |
| SXRD | Synchrotron X-ray Diffraction |
| TD-LCI | Time Domain- Low Coherence interferometry |
| WHO | World Health Organization |
| WSL | White spot lesion |
| XMT | X- ray Microtomography |

# 1 BACKGROUND

Dental enamel is considered a unique and strong biological tissue with a well-mineralised ultrastructure. It consists of about 96% of carbonated hydroxyl apatite by weight (Kay et al., 1964). Enamel is the outer most layer that covers the dentine and protects the dental pulp. Caries is known as a disease of calcified dental tissues as a result of changes in oral environment and chemical imbalance in the oral cavity. Dental caries is considered the most common human disease, affecting about 60-90% of school children worldwide and also prevalent in the adult population with nearly 100% of adults have dental caries (Petersen et al., 2005). Thorough examination and diagnosis of early enamel decay is crucial to prevent further enamel destruction and an established caries lesion, which will affect the integrity and the strength of the enamel structure. White spot lesions in dental enamel are the clinical presentation of early dental caries and is defined as “demineralization at the subsurface layer that is enclosed with a layer of well mineralized enamel” (Darling et al., 2006)

Dentists and patients are faced with caries and its subsequent problems on a daily basis. Patient related problems include pain that may cause disturbance in eating and sleeping, aesthetics, discomfort and the expense of managing caries, costing 5-10% of health care expenditure worldwide (Petersen et al., 2005). Clinician related problems include pain management and restoring the aesthetics and function of decayed teeth.

Many systems are used to detect dental caries. Diagnostic systems used depend on the position of the caries (proximal surfaces, occlusal surfaces), and include visual and radiographic assessment. The International Caries Detection and Assessment System (ICDAS) is a system for detection and visual assessment of dental caries. It was developed for epidemiological studies and it is considered a gold standard system in the detection of dental caries (Ismail et al., 2007).

The aim of this study is to investigate the ultrastructure of enamel white spot lesions using different imaging systems and to test the potential of Optical Coherence Tomography (OCT) to be used as a clinical tool for identify early caries lesions, and to see if it can go beyond ICDAS, the gold standard system in detecting caries. OCT is a non-ionizing imaging system, which has been used in many studies to assess enamel ultrastructure, and it is discussed in further details in section 1.5.1.

In this study, induction of artificial early enamel caries on healthy enamel was produced in vitro to understand the changes that occur in enamel structure in the very early periods of acid attack. Methylcellulose gel buffered with lactic acid was used in a demineralisation protocol for 7 and 14 days. Different tests were done to evaluate the changes in enamel ultrastructure in natural early (incipient) enamel caries and induced early enamel caries-like lesion compared to sound control enamel.

In order to understand the ultrastructure of early enamel decay it is important to us to know basic tooth structure.

### 1.1 Basic tooth structure

Teeth are important in mastication and preparation of food for digestion throughout life. Therefore teeth withstand huge loads, strains and stresses during mastication process yet still keep structural integrity and strength.

Teeth consist of four major layers (Figure 1-1), the outer most layer which is enamel, laying over dentine, cementum and pulp. Enamel is the hardest part, translucent layer that covers the dentine. The dentine is less mineralized layer that support enamel integrity and protect the pulp together with the enamel. Enamel and dentine interface line is called enamel dentine junction (DEJ) Pulp contains nerves and blood supply to the tooth. Cementum is a layer that covers the dentine in the root part. And the interface between the crown and root is called Enamel- cementum Junction (ECJ).

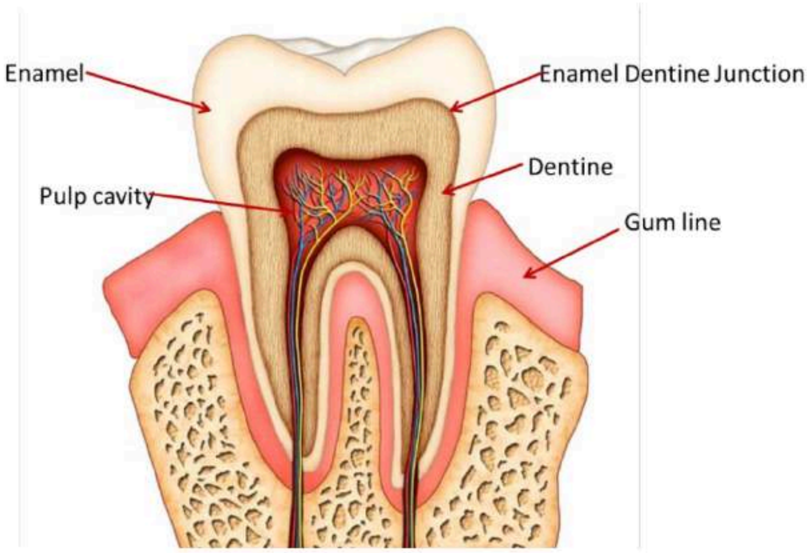



**Figure 1-1: Basic tooth structure**

**(Image taken from http://beta.classmint.com )**

### 1.1.1 Enamel formation

Enamel formation is also known as amelogenesis. Amelogenesis is divided into three stages; formation stage, where the enamel matrix starts to form. The next stage is when the enamel matrix begins to mineralise. The final stage is maturation, where enamel crystals enlarge and mature. Any disturbance at any stage will affect enamel structure and crystallography (Siddiqui, 2014) .

### 1.1.2 Enamel

Enamel is the hardest substance in human body as it contains more well oriented crystallites than any other mineralized tissue in the body. It is thin and translucent layer that covers and protect the dentine. Enamel consists of organic and inorganic materials. The inorganic material is composed of a highly organised crystalline calcium phosphate known as hydroxyapatite ($Ca_{10}$ $(PO_4)_6$ $(OH)_2$ (Kay et al., 1964, Young, 1974), which occupies over 95% of the enamel volume. Enamel varies in thickness over the surface of tooth, the thickest part is at the cusp with 2.5 mm thickness (Nanci, 2012) and thinnest part at the cementum and cemento-enamel junction which is usually an edge to edge relationship between the enamel and the cementum (Schroeder and Scherle, 1988). Enamel does not contain collagen as it present in other hard tissues such as bone and dentine, but it composed of unique proteins such as amelogenins and enamelins. Although the role of these proteins is not fully understood, it is believed that they play a major aspect in enamel formation (Margolis et al., 2006).

### 1.1.3 Enamel biomineralisation

In order to understand enamel demineralisation process, it is vital to understand enamel biomineralisation. Enamel crystallites originate by ions crystallising from super-saturated solutions. The initiation of crystal formation, the position and crystal shape and orientation are all influenced by genetic control. The main mineral component of the mature enamel crystallite is non-stoichiometric carbonated calcium hydroxyapatite (Simmer and Fincham, 1995). The first enamel crystallite to appear is in the form of octacalcium phosphate. The octacalcium phosphate (OCP) acts as a precursor and as a template for hydroxyapatite precipitation. Octacalcium phosphate is less stable than hydroxyapatite and it can hydrolyses to give hydroxyapatite. Octacalcium phosphate is less liable to crystal growth inhibitors and has flatter plates that are similar to first enamel crystals. Octacalcium phosphate is kinetically favored thus; it forms easier and faster than hydroxyapatite. Hydroxyapatite has hexagonal crystals that are more stable than OCP.

Therefore, there are three stages for enamel crystal growth. The first stage is the incipient seed formation, which involves grouping and crystallization of ions into a crystal. In the second stage, two-dimension growth of the seed, octacalcium crystal grows in length and width but not in thickness. The third stage involves hydrolysis of one unit cell thickness of OCP into two units' cells of hydroxyapatite. The hydrolysis reaction is as following (Simmer and Fincham, 1995):

$$Ca_8H_2\,(PO_4)_6.5H_2O + 2Ca^{2+} \leftrightarrow Ca_{10}\,(PO_4)_6\,(OH)_2 + 4H^+ + 3H_2O$$

Enamel crystallites originate at the DEJ as thin ribbons, 10–15 nanometres in width and 1–2 nanometres in thickness (Kerebel et al., 1979). Each crystallite is thick enough to adapt a one-unit cell of octacalcium phosphate or two unit cells of hydroxyapatite. Hydroxyapatite crystallite is considered to have a hexagonal system, which composed of a series of hexagonal plates that are stacked on top of each other (Figure 1-2A). Each plate has a hydroxyl ion at the centre that is surrounded by two triangles of ions, one composed of calcium ions and the other triangle is composed of phosphate ions (Figure 1-2B). All enclosed within a hexagon that is composed of calcium I (Kay et al., 1964)

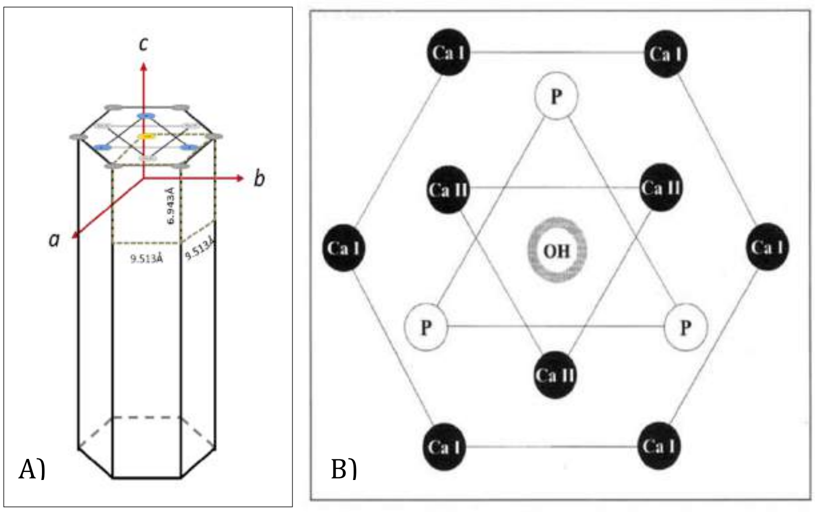


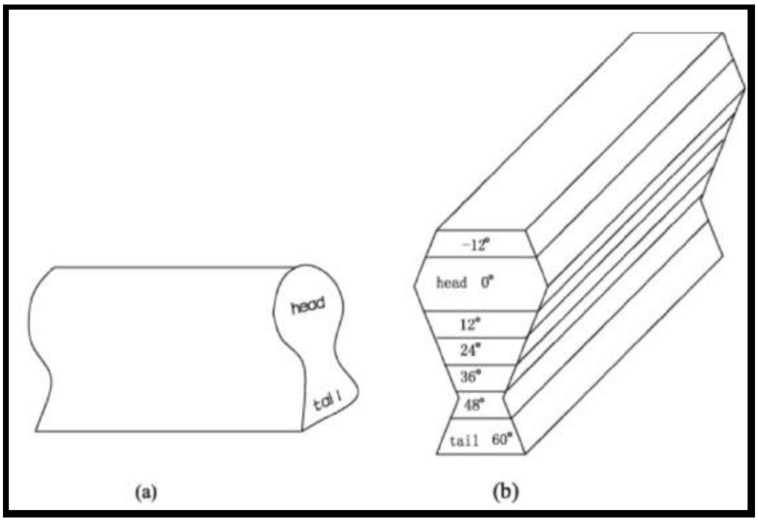

**Figure 1-2: A) Crystallite structure. B) Arrangement of ions in Hydroxyapatite crystal (Siddiqui, 2014)**

Growth of enamel crystallites is a result of deposition of ions onto the crystal surfaces a, b and c (1-2A). These surfaces can be identified as the crystallographic axes of the hydroxyapatite crystals structure. Rapid elongation of the crystallite occurs when the ions deposited on the c-axis direction. Followed by Deposition of ions on B-axis leading to growth of the crystallite in width then thickness (a axis) (Simmer and Fincham, 1995). Enamel crystallites have a pyramidal shape with the apex tip toward the ameloblasts and base towards the dentine-enamel junction. Crystallites increase in thickness until they contact adjacent crystallites. The morphology of crystallites changes from pyramidal to rod shape, with the same cross section at both base and tip. The carbonated hydroxyapatite crystal has a width of 50nm, 25nm thickness and is 1mm long and runs from the DEJ to the tooth surface (Johansen, 1965).

### 1.1.4 Enamel microstructure

Mature dental enamel is composed of basic structure called prisms. Each prism contains about 1000 hydroxyapatite that runs from the DEJ to the surface. In cross section, enamel prisms resemble a keyhole structure with the head towards the tooth surface and the tail positioned cervically and this permits a tight packing of enamel prisms (Figure 1-3). The size of Enamel prism is about 3-6 μm in diameter and 9 μm in height (Nanci, 2012). Many studies that used electron microscope and x-ray diffraction confirmed that hydroxyapatite crystallites are arranged with their long axis almost parallel to the long axis of enamel prism, however, the arrangement of hydroxyapatite crystallites gradually change in the tail region of the keyhole like structure of the prism. The borders between enamel prisms are called interprismatic regions. These regions are composed of crystallites that are arranged in a different direction than those found within the enamel prisms. Each prism is enclosed by prism sheath with a thickness of 800 nm. These prism sheaths have a high protein content and low mineral crystallites that exhibit different orientations compared to enamel prisms (Poole and Brooks, 1961).

Crystallite orientation within the prism affects the mechanical behaviour of enamel. When enamel undergoes compressive load, plastic deformation progresses throughout enamel prisms. Studies have shown that the stiffness for uniform arrangement and non-uniform arrangement of hydroxyapatite is the same, but more energy dissipation was observed in non-uniform arrangement of hydroxyapatite crystals within prisms, resulting in more fracture resistance (An et al., 2012). Outer enamel has an uniformed crystallite orientation whereas inner enamel exhibits non uniformed crystallite orientation, therefore inner enamel exhibits a higher fracture resistance than outer enamel (An et al., 2012). The highly organized crystals correspond to the regions where great forced is expected as biting surfaces (Al-Jawad et al., 2007).




**<u>Figure 1-3 A) Single prism structure, B) Geometry of enamel prism (An et al., 2012)</u>**

The variation in enamel prisms organisation and crystallite orientation within the prisms creates a heterogeneous structure, which is the most durable and hardest tissue in the human body. At the cusp of the tooth, the average direction of the hydroxyapatite crystallites is almost parallel to the direction of the prisms so the arrangement of the crystallites is perpendicular to the biting forces (Simmons et al., 2011). Yet, at the side of the crown, the crystallites direction is arranged away from the long axis of the prism (Meckel et al., 1965, Poole and Brooks, 1961). Highest order and arrangement of Hydroxyapatite crystallite occurs at the occlusal surface then the arrangement decreases moving towards DEJ (Low, 2004). The regions, which have high crystallite arrangement, can withstand biting forces that is expected on the occlusal surfaces, as shown in Figure 1- 4 (Al-Jawad et al., 2007). Lynch (2013), demonstrated that primary teeth enamel has less crystallites when compared with permanent teeth enamel, and the prisms are smaller in dimension and composed of slightly larger crystallite, however, crystallites arrangement in both dentition is similar (Lynch, 2013).

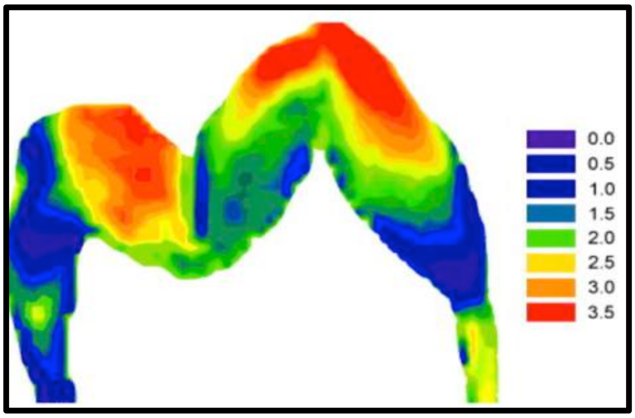



**Figure 1-4: map that shows cusp region with high texture and crystal alignment (Al-Jawad el al., 2007)**

### 1.1.5 Solubility of calcium hydroxyapatite

Hydroxyapatite behaviour is affected by the concentration of calcium ions and the pH of the oral cavity. Saliva is considered a rich source of enamel minerals in the form of calcium and phosphate ions. Calcium ion concentration in saliva is about 1 mmol $L^{-1}$, which is considered, supersaturated with respect to enamel hydroxyapatite. Salivary pH is normally around 6.9 – 7.4. If the oral cavity pH drops below the critical limit of 5.5, hydroxyapatite will start to dissolve and if the calcium concentration falls, demineralisation will occur. Demineralisation is also called dissolution of dental hard tissues. So high pH and saturation of calcium ions promotes hydroxyapatite precipitation and remineralisation, whilst low pH and calcium ion concentrations will trigger hydroxyapatite disolution and demineralisation (Al-Jawad and Anderson, 2014).

Remineralisation of dental enamel can be achieved in early stages by up taking of calcium, phosphate and fluoride ions. Fluoride works as a catalyst for calcium and phosphate ions dissolution into enamel, which will remineralise crystallite structure in the lesion. It was reported that high pH with high concentration of calcium will support remineralisation of enamel structure (Al-Jawad and Anderson, 2014).

### 1.1.6 Ion integration in enamel hydroxyapatite:

Each atomic site of hydroxyapatite crystals is subjected to substitutions that in turn will alter the properties of the enamel crystals. Incorporation of ions into enamel apatite can occur during development or after tooth eruption. Carbonate can replace either hydroxyl or phosphate ions, and because of the poor fit of carbonate into the lattice, a less stable and more acid soluble apatite is generated. Carbonate concentration increases from the enamel surface (2%) towards the dentine which is 4-6% (Robinson et al., 2000). This means enamel acid solubility increases toward the DEJ. The incorporation of fluoride ions occurs by either filling or displacing the hydroxyl ion in the c-axis. Fluoride ions have high charge density and symmetry, giving a closer fit for fluoride ion within the Calcium ion triangles, and this will result in lowering the lattice energy and stabilize the crystal structure. Fluoridated crystals have less acid solubility and are more difficult to dissolve. The behaviour of flouroapatite crystals is crucial in the role of fluoride in preventing or controlling dental caries. Magnesium has a very limited possibility to replace calcium (Featherstone et al., 1983, Terpstra and Driessens, 1986). Magnesium has the same effect as carbonate on hydroxyapatite lattice, as it increases acid solubility of enamel crystals.

## 1.2 Caries

### 1.2.1 Introduction

Dental caries is considered one of the most common oral diseases, affecting 60 – 90% of school children worldwide (Petersen, 2003). Dental caries is a complex lesion which is initiated by bacteria within the biofilm or dental plaque (Manji and Fejerskov, 1990, Kidd and Fejerskov, 2004). The biofilm is defined as “a thin deposit covering any solid surface which has adequate amount of moist and nutrients” (Kidd and Fejerskov, 2004). The dental hard tissues such as enamel, dentine and cementum are oral solid surfaces that are covered by pellicle to which the microbial cells attach. The pellicle is a film that is formed from proteins, which develops in seconds after teeth brushing and cleaning. Over the pellicle, the biofilm that consists of large amount bacteria develops then colonises by primary and secondary organisms, to create a matrix within which cells grow (Selwitz et al., 2007).

The biofilm is always metabolically active leading to fluctuations in the pH. A fall in pH will cause mineral loss from the tooth structure while rising in pH will lead to mineral gain. There are specific locations on the tooth where biofilms tend to form and mature, particularly in the occlusal surfaces, the proximal surfaces below the contact point and along the gingival margin where the biofilm is protected from mechanical wear by the tongue, cheeks and tooth brushing. Caries lesions tend to appear in areas where the biofilm stagnates and remains undisturbed for prolonged periods of time (Selwitz et al., 2007).

If the biofilm is disturbed or removed partially, mineral loss may be interrupted and stopped. That is to say that the caries process can be arrested at any stage of caries formation. There are a number of factors that also contribute in the caries process and influence pH fluctuation, including: diet, fluoride ion concentration, and salivary secretion rates (Fejerskov, 1997).

### 1.2.2 Demineralization

The chemical changes that occur in enamel structure during the process of demineralization and caries destruction are complex, due to hydroxyapatite and the way it behaves during dissolution. As mentioned previously, demineralisation is also called dissolution of dental hard tissue. Dissolution starts when endogenous bacteria especially mutans streptococci (Streptococcus mutans and Streptococcus sobrinus) and Lactobacillus in the biofilm, ferment carbohydrates producing weak organic acid (Lactic acid), as a by-product of metabolism. Lactic acid will cause a drop in pH resulting in dissolution of enamel structure (West and Joiner, 2014).

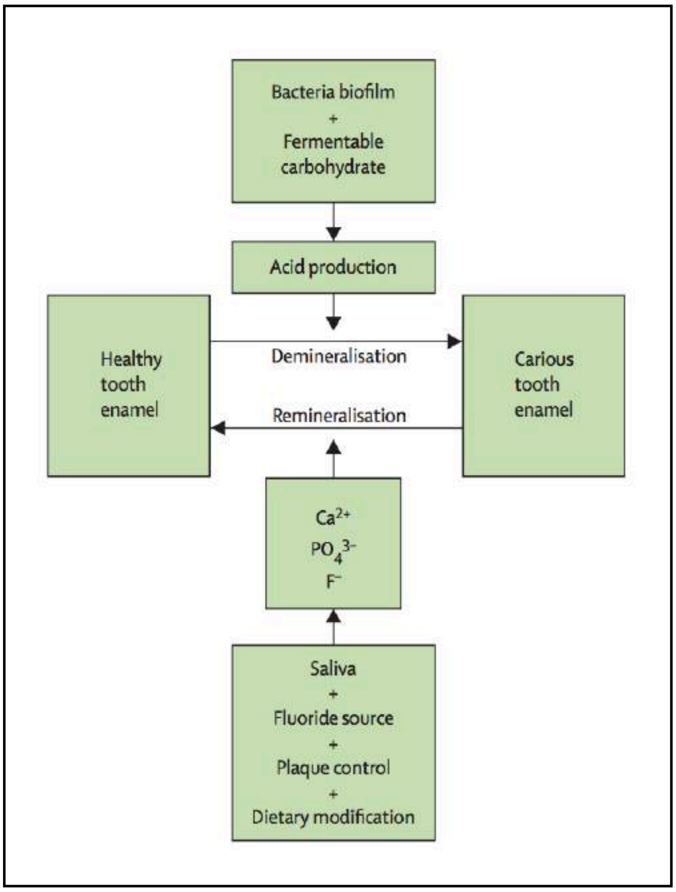



**Figure 1-5: A simplistic view of caries development (Selwitz et al., 2007)**

### 1.2.3 Pathway of caries attack and histological changes

Histological examination of caries progression throughout the enamel suggested that the most soluble and most accessible material is removed from the periphery of the prisms (Darling et al., 1961), and this indicates the lower crystal packing at the periphery of the prism, making it easier for acids and protons to diffuse into the tissue and removing mineral ions out (Robinson et al., 2000).

Histologically, enamel caries is described as having four zones (Figure 1-6) that is recognized by polarized light microscopy according to optical birefringence (Darling et al., 1961). These zones are demonstrated as they appear from the enamel surface towards DEJ. First, the surface zone, it is an intact layer with a thickness of 35-130 μm (Cochrane et al., 2012). it is believed that the surface zone remains intact because of the presence and the deposition of ions such as $Ca^{2+}$ and $PO_4^{3-}$ from the saliva. Second zone, the body of the lesion, this zone exhibits a considerable mineral loss which is about 24% and it displays changes in crystallites morphology (Nanci, 2003).

Third, the dark zone, displays enlargement of pores and exhibits 6% mineral loss (Silverstone, 1967b). The forth and last zone is the translucent zone, this zone is deepest and considered to be the advancing front of caries lesion.it characterise by proteins loss followed by removal of inorganic substances. About 1-2 % of the mineral loss has been demonstrated to be derived from interprismatic and periphery of the prism region due to ease of ion and acid flux in these areas (Darling et al., 1961, Arends and Ten Cate, 1981). Voids and pores present along the border of the prisms which makes the crystals in the outer layer dissolve as a result of the easiness of the penetration of hydrogen ion during caries process (Johnson, 1967, Boyde, 1989).

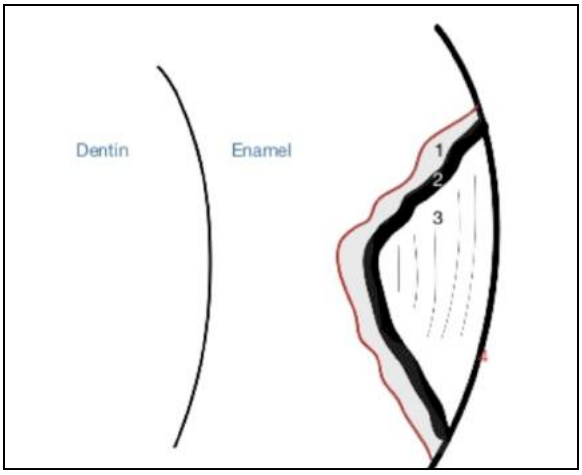



**Figure 1-6: Diagram represents caries zones. 1. Translucent zone, 2. Dark zone, 3. Body zone, 4. Surface zone.**

**Image taken from https://www.slideshare.net/nurved/dental-caries-bacterial-tooth-loss**

### 1.2.4 Microscopic features of caries enamel

Under light microscope examination, sound dental enamel appears as interchanging dark and light bands which known as Hunter-Schreger bands. These bands represent prisms arrangement in transverse section (Lynch et al., 2010, Lynch et al., 2011, Yilmaz et al., 2015) A review examining hydroxyapatite crystals demineralization in cross sectional views with electron microscopy (high resolution), and identified a central dark line, surrounded by white spots within the crystallite. Closer examination revealed that these spots are defects in the hydroxyapatite crystals (Yanagisawa and Miake, 2003).

A study examining 10 human premolars with early caries lesions using Scanning Electron Microscopy (SEM), demonstrated a honeycomb like enamel surface. Micro cavities and holes identified and viewed under higher magnification showed the cavities were wedge shaped holes (Worawongvasu, 2015). The key characteristic of this study is that surface layer can be re mineralised by coating it with amorphous crystalline layer (Worawongvasu, 2015).

### 1.2.5 White spot lesions

Clinically, white opaque areas of subsurface demineralization that appear on the enamel surface are called white spot lesions. This is related to the refractive indices of the enamel (1.62), water (1.33) and air (1.00). In white spot lesions, enamel porosity increases and then it imbibed with a medium of watery consistency (saliva) resulting in a refractive index of 1.33 (Kidd and Fejerskov, 2004). The variation in refractive indices of both matters will affect the scattered light therefore the lesion appears opaque. When drying the enamel using air that has a refractive index of 1.0, the lesion becomes distinguished. This is because of the difference between refractive index of the air and the enamel is greater than the difference between water and enamel which subsequently will make the lesion more recognizable (Kidd & Fejerskov 2004).

The porosity of the white spot lesion can help in determining the penetration of the lesion into the enamel surface. If the lesion is visible on a dried tooth surface, it is most likely to be in the outer enamel, while lesions that appear visible on wet enamel surfaces suggest penetration has progressed through the enamel and possibly reached the dentine (Kidd & Fejerskov 2004). Porosity is considered the foundation of many caries detection techniques, for example, radiography and light induced fluorescence (Kidd and Fejerskov, 2004).

Researchers have proven that caries lesions develop as a progression of caries white spot caries lesions if left untreated with non-invasive interventions. Carious lesions can be classified to two types: active lesions and inactive lesion. Inactive lesions have greater mineral content than active lesions, which make active lesions easier to re-mineralize when the appropriate treatment is provided. Clinically, it was found that there is no significant difference between the active and non-active lesions. However, the active lesion exhibits increased porosity when compared with non active lesion (Cochrane et al., 2012). If the biofilm is disturbed, mineral loss will be stopped or may be reversed to mineral gain (Kidd and Fejerskov, 2004).

Another type of white spot lesion results from orthodontic therapy, especially fixed appliances that compromise providing conventional tooth brushing and proper oral hygiene leading to creation of dental caries on tooth surfaces that exhibit low caries prevalence (Øgaard et al., 1988). Many studies confirmed that cariogenic activity increases underneath and around orthodontic band and brackets therefore, increased caries incidence on labial as well as lingual surfaces throughout orthodontic treatment (Øgaard et al., 1988, Ingervall, 1962, Zachrisson and Zachrisson, 1971, Gorelick et al., 1982, Årtun and Brobakken, 1986). A study conducted by Gorelick el at. Stated that no difference was recorded between using band in orthodontic fixed appliances or bonded brackets in term of carious white spot lesion formation (Gorelick et al., 1982). Also they found that WSL formation was the highest in maxillary incisors whereas the lowest incidence was in maxillary posterior teeth. No WSL formed on mandibular canines on lingual surfaces after prolonged use of lingual bonded retainers. The author justified these findings by the presence of salivary flow at these particular surfaces. No fluoride regime was used in the study as preventive measures. Also they found that maxillary lateral incisors showed higher incidence of WSL when compared to maxillary central incisors, they suggested that the reason behind this is the distance between orthodontic brackets and labio-gingival distance, they stated that when there is an adequate distance between the gingiva and the bracket, effective oral hygiene can be delivered. The authors' concluded that preventive fluoride programme is needed to limit WSL formation during orthodontic treatment (Gorelick et al., 1982).

### 1.2.6 Artificial caries- like white spot lesions

To understand the caries process and the changes that occur in the enamel ultrastructure during caries formation, artificial caries-like lesions were formed in vitro. Several studies and experiments were preformed to mimic the caries process in laboratory settings, but it is vital for us to understand that caries develops by a complicated multifactorial process, which makes it hard to reproduce in laboratory settings. However, studies that induced caries like lesion in vitro has a great impact in identifying the disease and in understanding the pathophysiology of caries process. The more knowledge we gain from understanding the caries process, the more effective treatment modalities can be developed to prevent the disease. In vitro, induction of artificial caries-like lesions can be performed in several methods by using strong acids and gel buffers (Robinson et al., 1995).

### 1.2.7 In vitro dissolution of hydroxyapatite

Hydroxyapatite crystals are composed of 40% Calcium, 57% Phosphate, 2% hydroxyl ions. When oral pH drops, trivalent phosphate converts to divalent phosphate, which leads to alterations in properties, this changes from a stable and water insoluble mineral into monetite or brushite that is less stable (Robinson et al., 1995). The trivalent phosphate ions act as holders for calcium ions and when it converts into divalent phosphate the bond between calcium and phosphate is weakened releasing calcium ions therefore mineral dissolution occurs. At low pH, $PO_4$ converts to be $HPO_4$, which will buffer the acidic solution by engaging hydrogen ions creating $H_2PO_4$. When pH reaches the critical level (5 to 5.5), phosphate becomes incapable to buffer with Hydrogen ions. Eventually, apatite crystal will bind with protons increasing its porosity (Robinson et al., 1995, Ehrlich et al., 2009).

### 1.2.8 A review of different demineralization protocols

Since 1950, numerous studies have attempted to induce artificial caries like lesions in vitro, using two basic systems; (i) nutrient systems and (ii) micro-bacteriological cultures, which uses only chemical techniques (Ingram and Silverstone, 1981). Even though organic acid solutions are an acceptable method to be used as a destructive agent to produce artificial caries in vitro, when examined histologically, the structural changes that occur in enamel are different from the changes seen in natural enamel caries (Silverstone, 1966). For example, the intact surface zone and the dark zone are not produced when diluted lactic acid is used alone. So, although the presence of acid solution is important to initiate caries process, the presence of a substance that acts as plaque has an impact on modifying the acid attack, to give us the characteristic changes that is seen in natural early caries lesion (Silverstone, 1965, Silverstone, 1966, Silverstone, 1967a).

By using viscous gels that are buffered with organic acids, simulation of caries lesion in vitro became possible (Von Bartheld, 1961, Silverstone, 1967a). Using gelatinous gels, such as cellulose derivatives of 10-20 % concentration with one of the organic acids to induce artificial caries, the created lesion appeared indistinguishable when examined under polarized microscopy from enamel affected naturally by caries lesion (Silverstone, 1967a). A pH of below 4.1 and more than 5.3 for the organic acids, means that subsurface caries lesion cannot be formed (Robinson et al., 1995). In this project, the induction of an artificial caries like lesion followed a protocol was based on previous studies (Milly et al., 2015). The protocol chosen was a cellulose derivatives gel, methylcellulose, to mimic the plaque layer, with a concentration 8% methylcellulose and then buffered with a layer of 0.1 mol/L Lactic acid. The choice of lactic acid was made with regards to natural caries formation process, as this specific acid is produced as a result of fermentation of carbohydrate by bacteria in biofilm (plaque). The pH was adjusted to 4.6 to promote subsurface lesion into the enamel structure. In this protocol, an average depth of 70 to 100 μm subsurface lesion was created in the enamel by keeping samples undisturbed for 14 days (Milly et al., 2015)

### 1.2.9 Summary

Enamel is well-mineralized tissue with a complex microstructure. Many studies have identified the enamel ultrastructure and investigated enamel changes in carious lesions, using techniques such as light microscopy, scanning electron microscopy, optical coherence tomography and X ray imaging.

In this project, OCT will be used to examine early carious white spot lesions. Then we will compare the outcome with well-established imaging techniques to benchmark OCT and to develop this technique as a clinical diagnostic tool for caries detection.

Over the years, many techniques have been developed to assess and detect caries lesions. Early diagnosis of caries is crucial to prevent rapid progression of caries in dental tissues, to prevent pain and infection. Any diagnostic methods need to have certain characteristics to be considered as a useful tool, such as validity. To consider a system as a valid diagnostic tool, it should have high sensitivity and specificity. Sensitivity is the proportion of true disease identified correctly by the diagnostic system, and specificity is the proportion of non-caries teeth identified correctly. (Attrill and Ashley, 2001). Also the diagnostic tool should be reliable and reproducible, safe and non invasive. There are several methods of detecting caries, and these will be considered in greater detail in the next section.

## 1.3 Caries detection methods

1. **Visual and tactile**

   In this method, occlusal caries is detected by drying the teeth and examined under dental light using a sharp explorer. This method is no longer used because of the potential risk of creating irreversible traumatic damage in the dental enamel

(Ekstrand et al., 1987). The use of a sharp explorer to identify a “sticky” fissure has no role in detecting caries, but it means that the explorer tip size fits well with pits and fissure size (Deery, 2013). Also this method can jeopardize the remineralisation process and it can cause inoculation of fissures with cariogenic bacteria. It was found that this technique gives a little information to help in caries diagnosis (Ekstrand et al., 1997). For proximal caries, orthodontics separators can be used to detect carious lesions with direct vision. However, the patient might feel pain and discomfort from the elastic separators which reaches the peak one day after placing the separator then decrease and pain disappear within one week (Giannopoulou et al., 2006)

A systematic review on visual and visual/tactile technique found that the majority of diagnostic systems are indefinite and incapable of measuring the different stages of the carious lesion (Ismail et al., 2007).

**2. Radiographic examination**

Radiographs are an ionised technique widely used to detect dental caries whether the lesion is cavitated or non-cavitated, and if the lesion is active or arrested. Bitewings radiographs are used to detect occlusal and interproximal caries. A clinical study comparing visual diagnosis vs. bitewings for dental caries showed that 22.9- 32.9% of proximal caries was detected visually while 93.1- 97.1% was detected by radiographs. For occlusal caries, between 75.9-83.9% was identified by visual examination whereas between 33.1- 42.6% was detected by bitewings radiographs (Hopcraft and Morgan, 2005).

**3. Dyes for caries detection**

This method employs non- specific protein that stains infected or less mineralised dentine therefore easy identification and removal of carious dentine, and were first introduced in 1972 (McComb, 2000). It was found that these dyes stain sound dentine at the DEJ and that not all-stainable dentine was infected. The use of dyes in caries detection lacks specificity (Yip et al., 1994). Also it was found that these dyes stain collagen in less mineralised organic matrix instead of the infected dentine therefore, there is increased potential for excessive removal of sound dental tissue which may cause unnecessary mechanical pulp involvement (McComb, 2000). Caries detector dyes such as procion dyes can cause irreversible staining of dental tissues, which is unacceptable clinically. This caries detection technique lacks scientific evidence that support the used of this method (McComb, 2000).

**4. Quantitative light induced fluorescence (QLF)**

For several years, the changes in mineral content of dental hard tissues has been known to cause alteration in visual and optical properties (Stookey, 2004). In 1982, Swedish researchers showed the ability of auto fluorescence lasers to assess mineral loss of dental hard tissues. Also they reported that when the proper filters are used with fluorescence laser instead of white light, an improved contrast can be gained between sound and infected enamel (Sundstrom et al., 1985). This method (QLF) employs violet blue light of a wavelength of 290- 450 nm. The incident light will be absorbed by the tooth and transmitted as fluorescence. A charged coupled device (CCD) micro-camera detects and captures the fluorescent image and data is analysed by computer software.

The limitations of this method are that the result can be affected by several factors such as the presence of staining, plaque and stains. Also the degree of tooth dehydration can affect can affect the outcome. This method cannot detect lesions at mesio-buccal or disto-buccal surfaces as QLF light has to be perpendicular on the tooth surface (Heinrich-Weltzien et al., 2003). A clinical study to compare visual examination and QLF in detection of non-cavitated carious lesions concluded that small and non cavitated occlusal caries are more likely to be detected using QLF however, it is impractical and time consuming (Kühnisch et al., 2007). A systematic review showed that the accuracy of QLF has insufficient scientific evidence (Twetman et al., 2013).

**4. Fiber optic transillumination (FOTI)**

White light with high intensity is used in this method on a clean and dry tooth. The light is directed to the tooth and then the scattered light is collected from the other side on a mirror system to be processed in a digital electronic instrument. Dark shadows appear if caries is present. The main advantage of this technique is that it can be used to detect all tooth surfaces including the interproximal area. There are several limitations of FOTI; one of the limitations is that it cannot measure the depth of the lesion. Also the data produced cannot be recorded as an image which makes the system more subjective to the examiner visual evaluation of the appearance of the scattered light (Pretty, 2006).

### 5. Digital imaging fiber optic trans-illumination (DIFOTI)

This imaging tool is the digital version of FOTI. It employs a light with high intensity with 2 heads camera, one to examine occlusal surfaces and the second to inspect smooth surfaces. The images produced can be saved and archived on computer for retrieval at the follow up visits. The limitation of this system is that the images cannot be quantified and the analysis of the image is also subjective depending on the examiner's visual evaluation of the scattering appearance (Pretty, 2006).

### 6. Diagnodent (DD)

This method is manufactured by Kavo™, and detects caries by employing fluorescence to the tooth of interest. The system uses a red light of 655 nm wavelength, with 2 intra-oral tips, one for pits and fissures and one for smooth surfaces. This system does not provide images of the examined tooth but it gives numerical reading that display on 2 LED screens. One shows the peak value while the second shows the current value. Measurement of bacterial activity and metabolised by-products induce fluorescence detected by DD. The depth of the lesion depends on the reading, so the higher the reading, the deeper the carious lesion (Pretty, 2006). A study compared the outcome of occlusal caries detection in 58 primary teeth by two examiners using visual examination and radiographs examination and DD. The outcomes were not statistically significant and using visual and radiographic methods produced a similar result (Attrill and Ashley, 2001). The sensitivity scores provided by DD were 0.77- 0.80 and the specificity scores were 0.82- 0.85 (Attrill and Ashley, 2001).

### 7. Electric conductance monitor (ECM)

This method uses a fixed frequency with single alternating current to calculate the bulk resistance within dental hard tissues. To examine a particular surface, the device probe is applied on site of interest and held in place for 5 seconds for measurement cycle. The tip of the ECM probe will produce compressed air directed to the surface to be examined and measure the drying profile. The mechanism of this device is to measure the porosity, which is generally known to be associated with dental caries. Subsequently, the data collected can give a valuable information about the lesion features and characteristics (Pretty, 2006). The limitations of this system are that the results can be influenced by many factors such as, tissue thickness, moisture, tooth temperature and immature or hyperplastic tooth (Pretty, 2006). Pretty showed that ECM had sensitivity values of 74.8 (± 11.9) in site specific and 63 (±2.8) surface specific, whereas the specificity values at site specific 87.6 (±10) and 79.5 (± 9.2) at surface specific evaluation (Pretty, 2006)

**8. International Caries Detection & Assessment System (ICDAS)**

This system developed in 2002 by a group of restorative dentists, researchers and epidemiologist during the International Consensus Workshop on Caries Clinical Trials (Pitts and Stamm, 2004), which demonstrated the necessity of non- cavitated lesions detection. Also they concluded that clinical studies that only measure cavitated lesions, as outcome measures are considered out of date. The workshop also referred to many inconsistent and unreliable caries detection systems (Ismail et al., 2007). ICDAS is based on the work of previous studies which assessed the reproducibility of occlusal demineralisation depth assessment in three methods and its accuracy (Ekstrand et al., 1997). ICDAS is considered a standardized caries detection system based on scientific evidence, to deliver valuable information about caries diagnosis and prognosis therefore, aiding in clinical management of dental decay. This system was constructed to identify the carious process in six stages, starting from early changes in dental enamel to the most extensive cavity caused by demineralisation. ICDAS detect and assess caries according to whether the lesion is cavitated or non- cavitated, tooth topography that includes smooth surfaces, pits and fissures, the anatomy of the tooth, and sealant restoration status (Ismail et al., 2007). The system covered examination protocol such as examining a clean and dry surface of interest (Ismail et al., 2007).

ICDAS classifies caries status into 6 codes from zero to six (table 1-1). Code 0, the tooth should be sound with no signs of carious demineralisation after prolonged time of drying by air, recommended to be 5 seconds. Tooth surface that exhibits dental defects such as amelogenesis imperfect, fluorosis, stains (extrinsic and intrinsic), tooth wear such as erosion, attrition and abrasion should be considered as sound surface. Tooth surface with pits and fissure stains that is associated with non-carious habits such as frequent coffee or tea drinkers should also be considered as sound surfaces (Ismail et al., 2007). Code 1, this code is given to the tooth surface when no signs of carious demineralisation or colour change is observed when the surface is dry. But after prolonged drying time of about 5 seconds, opacity or change in colour either to white or light brown discoloration is evident on tooth surface. Code 2, when the tooth surface is wet and carious opacity with change in discoloration (white spot lesion) is visible without drying. Or brown discoloration of pits and fissures that appears wider than natural features of stained pits and fissures. Code 3, without drying, carious opacity or change in discoloration to brown colour with wider feature of fissure or fossa is evident when compared to clinical appearance of sound pits and fissures or fossa. Once prolonged air drying is applied, enamel structure loss can be viewed within or at the margins of pits or fissures. A localized breakdown of enamel structure is evident without visible dentine in the carious cavity. To confirm the visual findings, ball ended probe can be passed gently on fissures and pits to confirm the initial assessment. Code 4, the lesion shows an intact enamel surface or localized enamel cavity or breakdown but with a shadow of underlying discoloured dentine. The colour of the intrinsic shadow may appear brown, blue and grey discolouration that is visible through the intact enamel surface. This shadow can be detected without drying when the tooth is wet. The examiner should evaluate the origin of the shadow whether the carious lesion started from the tooth surface that being examined or it started from an adjacent surface with no evidence of caries on the surface of interest. If confirmed that the caries originate from adjacent tooth surface but the shadow reflects on the surface being tested then it should be scored with code 0 as sound surface. Code 5, this code is given to the lesion when a distinct cavity on dental enamel is detected with visible dentine showing underneath enamel cavitation. When the surface is examined wet, the dark dentine will show through dental enamel. But when air is applied for 5 second, a frank cavity will be visually detected with evidence of carious enamel demineralisation, which may be present as opaque or dark brown on cavity walls especially in the pits and fissures. To confirm surface evaluation, the examiner can use ball end explorer and slide it gently along the cavity. If the ball entered the cavity reaching the dentine then the surface should be recorded as code 5. The last code is 6, deep with extensive loss of dental structure with dentine evidently visible at the wall and base of the cavity. The cavity may extend to involve half of tooth surface with a increased likelihood to reach the dental pulp (Ismail et al., 2007).

| ICDAS Lay Terms | Sound | Early Stage Decay | | Established Decay | | Severe Decay | |
|---|---|---|---|---|---|---|---|
| ICDAS Dental Terms | Sound | First visual change in enamel | Distinct visual change in enamel | Localised enamel breakdown | Underlying dentine shadow | Distinct cavity with visible dentine | Extensive cavity within visible dentine |
| ICDAS Detection | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| ICDAS Activity | ICDAS Activity +/- | | | | | | |

**Table 1-1: Table demonstrates ICDAS scoring system. Image taken from https://www.icdas.org**

## 1.4 Summary

ICDAS is considered the gold standard for detection caries including the white spot lesion. However, it cannot assess the depth and the progression of the lesion into enamel structure. There is a need for new systems that can help the clinicians to determine the advancement of caries lesion so better treatment modality can be provided.

In the following section, I will discuss in details about OCT and the potential of developing this technique as a clinical diagnostic tool.

## 1.5 The use of imaging techniques to study enamel structure

### 1.5.1 Optical coherence tomography (OCT)

**Principles of OCT**

Optical coherence tomography (OCT) is defined as a non-destructive imaging system that employ near infrared light to create an in-depth images of internal biological structures such as ocular, skin, intravascular, oral soft and hard tissue (Jones et al., 2006b). OCT has the same concept of ultrasound but light is used instead. For both technique the back scattered signals is measured (Jones et al., 2006). OCT is able to measure reflected or back scattered light from surface tissue which measures signal intensity and tissue birefringence (Jones et al.,2006). It creates a two dimensional cross sectional images (XZ) that called b-scans Figure 1-7A. By superimposing b-scans in the Y direction, a three dimensional image can be produced. Over the last twenty years, Optical Coherence Tomography was used in medical and dental fields.

OCT calculates the backscattered signal from scattering surface and produces an a-scan, which represents a signal intensity of the imaged tissue Figure 1-7B. (Fried et al., 2002). OCT use low coherent interferometry (LCI) visible and near infrared wavelength (Fercher, 2010). Two imaging resolutions can be created by OCT, depth and lateral resolution image. Another type of OCT called polarised sensitive OCT employs polarised light instead. Consequently, the backscattered polarised light creates a colour coded scale image (Manesh et al., 2009, Hee et al., 1992).

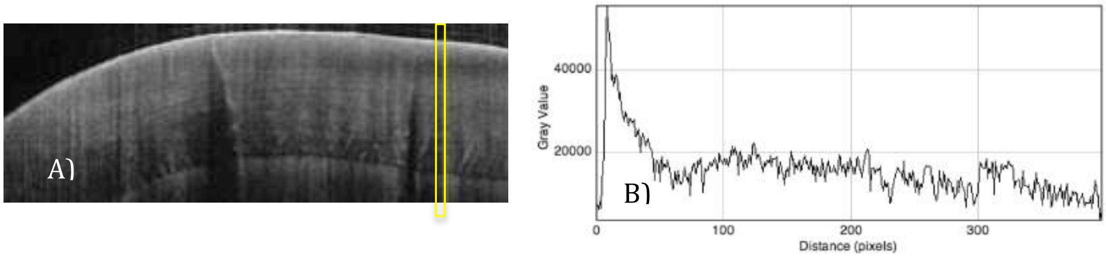



**Figure 1-7: A) b-scan of OCT instrument . B) a-scan**

Frecher, 2010 described the two different schemes of OCT, which is based on low coherence interferometry, the Time-Domain LCI (TD-LCI) and Fourier-domain LCI (FD-LCI). The former technique, TD- LCI, it records structure depth of a specimen by using a series of partial time coherence interferograms. These interferograms are created by light reflecting specimen sites and a reference beam reflected on a moving mirror. The second scheme, FD-LCI, can be based either on tuneable laser which known as frequency tuning FD-LCI or fFD-LCI, or based on a spectrometer named Spectral FD-LCI or sFD-LCI (Fercher, 2010). It was cited that both schemes have similar result in terms of resolution. Low coherence a-scans and b-scan images can be created by both techniques. However, TD-OCT has less sensitivity when compared to FD-OCT (Leitgeb et al., 2003, Choma et al., 2003).

Initially, multi-mode diode lasers were the source of light for OCT. These types of light sources give confusing results caused by their periodic coherence functions. Then, super luminescent light diodes (SLD) were used as light source to produce low coherence interferometry for OCT (Huang et al., 1991, Fercher, 2010). These light sources give high depth resolution due to their ability to produce broader spectral emission by monotonic coherence functions. Later, femtosecond Titanium-sapphire lasers were used offering extra-high resolution. Sub- micrometre resolution was achieved using photonic crystal as OCT light source (Huang et al., 1991, Hartl et al., 2001, Povazay et al., 2002, Bourquin et al., 2003).

In the past, FD- OCT showed imperfect tunability due to the use of wavelength tuneable light source. It offered either a slow tuning but high depth resolution or low depth resolution with fast tuning. Currently, tuneable lasers use cavity tuning 'swept laser sources' instead of external- cavity tuneable laser diodes which allow performing the spectral filtering inside the laser cavity (Fercher, 2010). However, SLDs remain the dominant light source.

OCT technique has several acquisition systems. The standard OCT systems, FD-OCT and TD-OCT, produce images with depth oriented cross-sections in planes normal to the frontal plane. These depths oriented cross sections called b-scans. Where as the en face OCT systems, produce frontal sections of the sample of interest creating an images called c-scans, this technique perform two dimensional transverse scan in high speed with only slow move in axial direction of the coherence access (Fercher, 2010). A different acquisition system used is Linear OCT, which considered an alternative to TD-OCT yet it show less sensitivity and resolution (Koch et al., 2004, Fercher, 2010). Other acquisition systems are developed such as depth of field OCT, High depth-resolution OCT, High speed and volumetric OCT, High lateral resolution and full field OCT systems. Each acquisition is used according to its specific application.

OCT has many optical signal property detection systems. Polarisation sensitive OCT system (PS-OCT) is one of these systems mentioned previously in this section. This system is founded by Hee el al (Hee et al., 1992). In this system, illumination of the sample by circularly polarized light and then use two channels of polarization sensitive detectors (Fercher, 2010). Many optical signal detection systems were developed such as Differential phase contrast OCT, system sensitivity, spectrometry and refractometry.

Dental hard tissues such as Enamel and dentine are considered to be a scattering material. To develop a useful imaging optical tool it is vital to us to understand how visible and near infrared light transmit through enamel and dentine. During enamel demineralisation, partial dissolution of enamel forms micro pores in its structure that behave as scattering centers for visible and near infrared light (Darling et al., 2006). The magnitude of scattered light in enamel is likely to reduce by $1/\lambda^3$, $\lambda$ is the incident light wavelength (Darling et al., 2006). This is due to the dimension of light scatterers (crystals) in dental enamel. As a result, near infrared region ranging from 780 to 1550 nm present the ideal optical imaging techniques because of the weak absorption and scattering in dentine and enamel. Longer infrared wavelengths will increase the absorption of the light by the water in the material therefore less penetration of the light through the tissue (Darling et al., 2006, Fried et al., 2002).

The principle of OCT instrument is demonstrated in Figure 1-8. Swept source (SS) produces a low coherent light, which inters the Beam Splitter then splits into two beams. One beam targets the reference mirror and the second beam targets the sample. Both beams will be back reflected from the sample and the reference mirror and then united through the fiber coupler. After that the back reflected light would be identified by photo detector (PD), which called Michelson interferometer. The interference signal can be detected if the length of the back reflected light is in the range of coherence length of the actual source (Otis et al., 2000). The depth of the light penetration into tooth structure can be calculated because the position of reference mirror is known (depth resolution). These depth signals are known as a- scans, which are processed in digital signal processing system where OCT image is produced. B-scan is formed of constructing several a scans (Fercher et al., 2003).

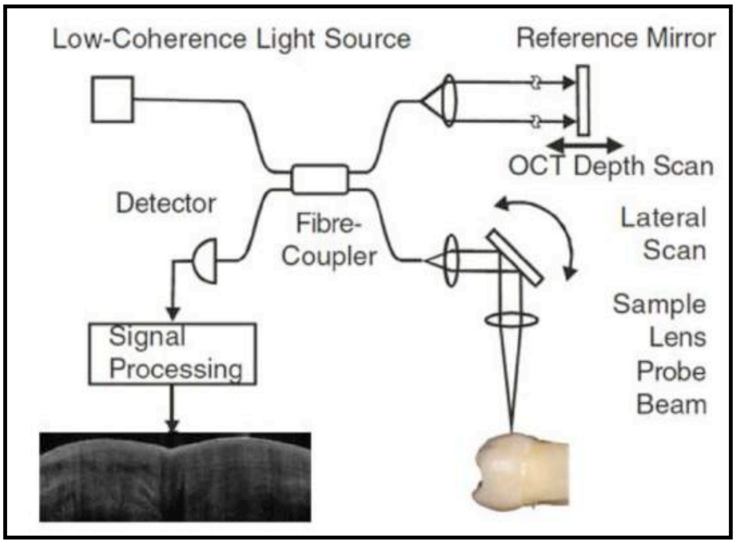



**Figure 1-8: Diagram demonstrating the principles of OCT (Fercher et al., 2003). Image modified by Khalifa Al-Azri.**

**Dental hard tissue refractive index:**

Refraction happens when the light propagates from one medium to any other medium. Refractive index is the amount of alteration of light velocity that occurs when light travels from air to any different material. If the refractive index is large, the velocity of light would be slow in the medium (material). Many studies confirmed that air refractive index is 1. OCT measures light refraction that occurs when light passes from air into dental structure. Refractive index considered one of the major optical measurements of biological structures (Meng et al., 2009). Every biological structure has a refractive index that controls light propagation within the structure that shows the scattering properties of the material (Hariri et al., 2012b). Knuttel et al stated that the material scattering properties depend on the result of the difference of refractive index in between the materials (Knu et al., 2004). It was cited in many studies that carious white spot lesion could alter the refractive index of sound enamel which in terms aids in diagnosing carious WSL (Besic and Wiemann, 1972).

OCT has been tested in many studies to evaluate the methods used in measuring tooth refractive index such as focus tracing method (Song et al., 2000, Haruna et al., 1998). One of the disadvantages of this method is the difficulty to perform, as multiple focus points should be adjusted with specific calibration of lens. A different method can be performed; it is called opal path length matching (OPL) (Meng et al., 2009, Hariri et al., 2012a). there are several advantages of this method such as easy to perform, fast with high accurateness (Meng et al., 2009). They cited enamel refractive 1.631 ± 0.007, dentine refractive index 1.540 ± 0.013 and cementum 1.582 ± 0.01 which corresponds to the findings in other studies (Kienle et al., 2006, Ohmi et al., 2000, Hsieh et al., 2011).

**Implementation of OCT**

The first application of OCT technique was in medicine in ophthalmology (Swanson et al., 1993, Fercher, 2010). In 1991, Fercher et al obtained a two dimensional image of human eye fundus using OCT(Fercher et al., 1991). Frecher et al described advantages of OCT in 2010. They stated that OCT is a non- destructive and contact free technique. Nevertheless, the main disadvantage of this technique is that light penetration into the sample of interest is limited (Fercher, 2010). OCT was also applied in dermatology for examining and diagnosing dermatological diseases such as bullous and inflammatory conditions (Welzel, 2001). In gastroenterology, endoscopic OCT is used to examine gastrointestinal tract disorders.

In dental studies, OCT was extensively used to investigate enamel, dental caries and artificially induced carious lesions and remineralisation. Jones et al examined artificial occlusal caries using PS-OCT by measuring the magnitude of backscattered light in the enamel (Jones et al., 2006a). They cited that by evaluating the backscattered changes and observing near infrared light depolarisation artificial occulsal caries can be measured and this technique is promising in caries detection and diagnosis (Jones et al., 2006a). Fried et al studied root, occlusal, interproximal caries and recurrent caries under composite fillings. They concluded that PS-OCT is capable to image such lesions (Fried et al., 2002). Another study conducted by Azevedo et al investigated the ability of OCT to measure demineralisation amount in artificially induced dentine caries. They stated that OCT is an invaluable tool to examine carious lesions depth (Azevedo et al., 2011). A study conducted by Feldchtein et al to examine in vivo oral soft and hard tissues and dental restorations such as composite, amalgam and compomer

(Feldchtein et al., 1998). They showed defects that occurs underneath dental restorations that can be detected using OCT. Maia et al., investigated the potential of OCT in detecting caries in deciduous teeth. They employed two different techniques of OCT. the first one was TD- OCT with wave length of 1280 nm and sFD- OCT with wave length of 840 nm. They found that OCT is capable of detecting caries and monitoring lesion progression (Maia et al., 2010). Another study was conducted to examine root caries using PS- OCT and compared the outcome with TMR. They demonstrated the potential of OCT in replacing conventional caries detection systems such as radiographs and avoid X-ray ionizing hazards directed to patients (Amaechi et al., 2004).

OCT has been used to study early carious white spot lesion. However, it is vital to benchmark OCT findings with well-established imaging methods as Polarised microscopy, Synchrotron X-ray diffraction and scanning electron microscopy. These techniques will be explained in detail in the next sections.

### 1.5.2 Synchrotron X-ray diffraction (SXRD)

**SXRD principle**

In 1895, a physicist called Wilhelm Röntgen discovered rays able to pass through human body. And due to their unknown nature, he called them X-rays. X-rays have been used in a wide range of medicine, scientific, as well as food and cosmetic industry. X-ray techniques can reveal internal features of the material such as chemical and physical properties as well as it structural composition. X-rays are high frequency with short wavelength photons, which situated between gamma rays and ultraviolet light on electromagnetic spectrum as shown in Fig 1-9. X rays have a wavelength range from 0.1 and 100 Ångstrom (Å) and energy ranging from 100 eV-100 KeV. The X-rays used in diffraction studies have wavelength between 0.5 to 2.5 Å as it depends on bonds distances of a crystal and its dimension (Cullity, 1978). When the X-ray is directed to an object it interact with its atoms leading to either absorption or scattering of photons energy which will cause attenuation of x-ray energy. The degree of attenuation depends on material density, atomic number of the material and x-ray energy.

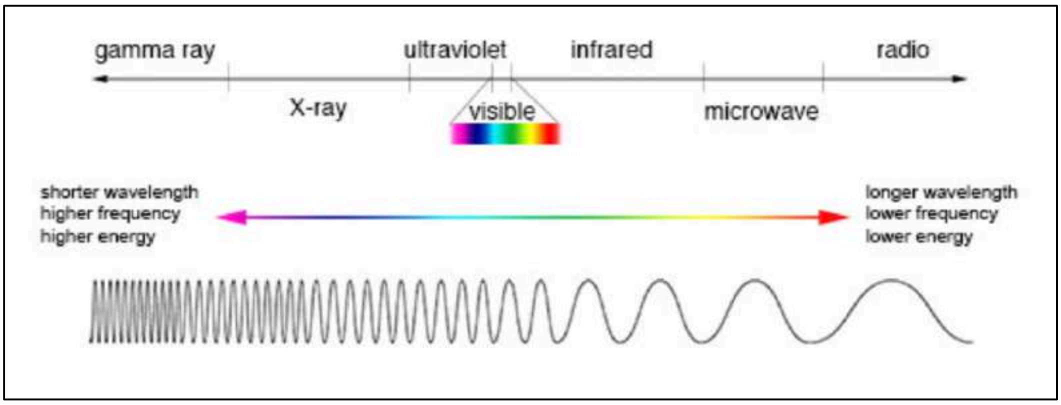



**Figure 1-9: electromagnetic spectrum**

X-rays are produced by electron transition interactions when accelerated, highly charged electrons hit a metal target. There are 2 sources of X-ray, monochromatic which produce single wavelength and polychromatic that produces multiple wavelengths. X-ray can be generated by either X-ray tube (conventional method) or by synchrotron facilities, which has higher flux and larger energy range than the conventional method.

Synchrotron facilities are used for a wide range of research such as food industry, pharmacology, material science, chemistry and physics and there are about 70 synchrotron light facilities around the world. These facilities provide a flux and energy range unattainable by laboratory (conventional) sources. Two of the most powerful synchrotron X-ray sources are the Diamond Light source (DLS) in the United Kingdom and the European Synchrotron Radiation Facility (ESRF) in France.

Synchrotron radiation was first discovered in 1947. Synchrotron is a system where electrons are accelerated to a very high speed, almost near to the light speed, and then deflected by magnetic field. When electrons decelerate by passing through a magnetic field, they lose this energy in the form of photons (light), which is the synchrotron light. Bundles of electrons are created in the electron gun and directed to a long linear accelerator (16 m long) then they enter the booster ring to get accelerated to almost near the speed of light. Electrons are then inserted into storage ring where they are guided to follow a circular path by the strong magnetic field (Figure 1-10).

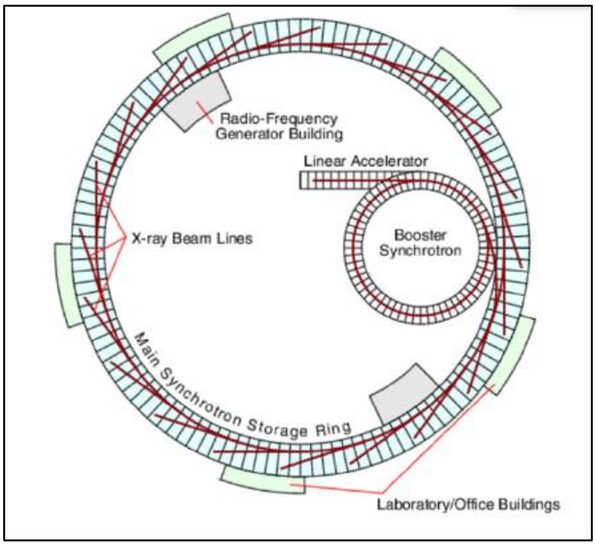



**Figure 1-10: Diagram represents synchrotron radiation system.**

**(Image taken from http://pd.chem.ucl.ac.uk/pdnn/inst2/work.htm)**

The radiation is emitted when moving electrons at relativistic velocities forced to deviate from straight line motion by the use of bending magnetic (BM) and insertion device (ID) which are placed in the straight areas of the orbit. The purpose of the magnets is to increase radiation intensity and to regulate X-ray properties to the beamline requirements. Each beamline has specific properties that depend on the requirements of particular experiment.

Synchrotron X-ray radiation produce a very intense, well collimated beam with continuous photon flux which permit deeper penetration of the radiation into the matter and the short wavelength allows us to study the fine structure of the materials. The beam properties such as the size and optics can be adjusted for specific experiment, which allow rapid collection of data with high quality sets. For these advantages, synchrotron facilities are on high and continuous demand by researchers. Only successful research proposals are awarded beam time, which undergo thorough and competitive peer review process.

X-ray diffraction is a technique that use x-rays with short wavelength to investigate the properties of a material in an atomic length scale. Laue first discovered this technique in 1912. He concluded that crystals with uniformly spaced atoms could behave as scattering matters for X-ray. When an incoming X-ray has a wavelength similar to the distance between the atoms in the crystals, it should then diffract. Analysis of X-ray diffraction patterns reveals the chemistry and crystallography of a material, the crystal size, and the orientation of crystallites (texture).

**Bragg's Law**

In 1915, Lawrence Bragg and his father William Bragg won The Nobel Prize for utilizing X-rays to analyse crystal structure. The principle of diffraction is mainly demonstrated in Bragg's law (figure 1-11). Crystal materials are well ordered atoms, which arrange in three-dimensional planes. Atoms in crystalline materials are distanced 'd' from each other and arranged in organised planes, as a result, it exhibit periodicity. Diffraction is defined as X-ray scattering from crystal planes. When the magnitude of X-ray wavelength is of the same distance between atoms a constructive interference will occur.

Bragg's law means that when parallel rays of incident beam are scattered at angle ø the subsequent reflections produce coherent interference of the diffracted beam Bragg's law is a simple mathematical model which express the essential condition for X-ray diffraction. Bragg's law is $\lambda = 2\, d \sin ø$

Where;

n is an integer

$\lambda$ is the X ray wavelength.

d is the distance between lattice planes

ø is the angle between incident/diffracted ray and the lattice plane.

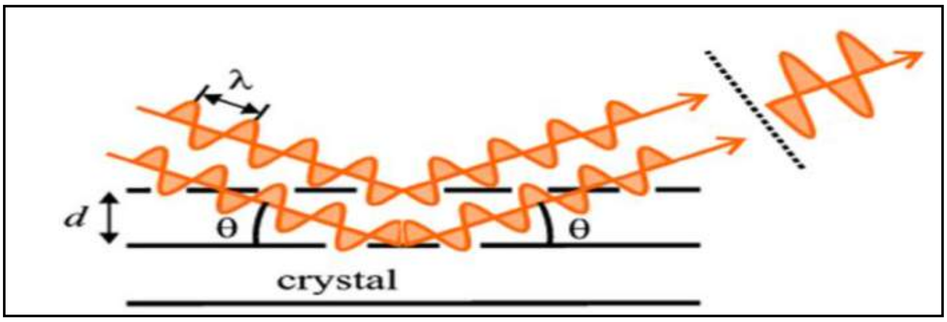



**Figure 1-11: Simplified diagram demonstrates Bragg's law. (Image taken from http://pd.chem.ucl.ac.uk/pdnn/powintro/braggs.htm)**

In powder diffraction, a polycrystalline is used instead of a single crystallite and the scattering of the diffracted beam of the polycrystalline material will produce continuous cones of intensity, which is known as Debye Scherer cones. Diffracted beam direction can help in identifying the shape and size unknown crystal.

**The analysis of diffraction data**

SXRD diffraction patterns provide quantitative information of the tested material at crystallite level. These information can be extracted by fitting the peaks that shown on the produced diffraction pattern. Figure 1-12 demonstrates examples of different two-dimensional (2D) diffraction patterns comparing the difference between a single crystal patterns, a powder (or polycrystalline) diffraction pattern, and that of a polycrystalline material with some organised arrangement of crystallites (texture). Figure 1-12A shows a typical 2D diffracted pattern of a single crystal. If this pattern is integrated azimuthally around 360$^{o}$ the plot in Figure 1-12a will be produced, i.e, the produced profile of intensity is two lines vertically positioned which represent the Debye ring of the diffracted pattern. In contrast, Figure 1-12B shows a 2D powder diffraction pattern, which appears as a continuous solid Debye ring due to the random orientation of crystallites with no particular pattern of arrangement. When this pattern is plotted against azimuthal angle, a horizontal line is produced (Figure 1-12b). When a polycrystalline specimen with well organised crystallites is examined, the diffraction pattern appears to fall somewhere between these two patterns, i.e. has two intensity “arcs” in the 2D pattern (Fig 1-12C). This is due to the well-arranged and oriented crystals. And when plotted against azimuthal angle, the intensity profile produced show two peaks, which correspond to the two peaks in Debye ring of the diffracted pattern (Fig 1-12c). This phenomenon in known as texture.

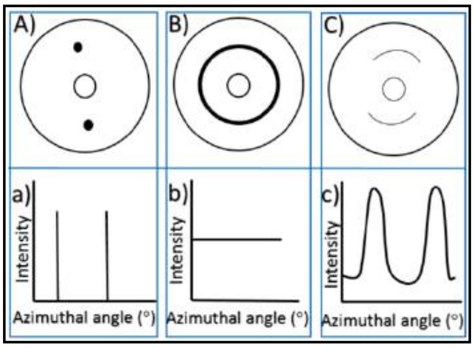



**Figure 1-12: A) Diffraction pattern of a single crystal. B) Diffraction pattern of powder or polycrystalline sample. C) Diffraction pattern of enamel – a polycrystalline sample with organised crystallites.**

The texture direction and magnitude of the crystallites can be calculated by measuring the width of the intensity peaks that shown in the diffraction image by plotting the intensity against azimuthal angle as demonstrated in Figure 1-13. This can be achieved by fitting Gaussian curve of the peaks and then obtain the Full Width Half Maximum (FWHM) for each peak as indicated in Figure 1-13.

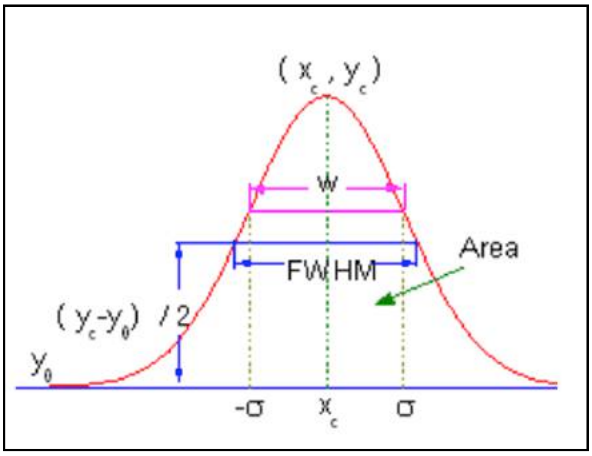



**Figure 1-13: Demonstration of FWHM of the peak**

**(Image taken from OriginLab®)**

**Examples of SXRD to study the texture in dental enamel**

Enamel microstructure has been investigated using many techniques such as AFM and SEM (Marshall et al., 2001, Habelitz et al., 2001). The dental enamel qualitative features were studied by these techniques. However, employing these techniques does not provide information regarding the subsurface structure of dental hard tissues. Although they gave prisms arrangement in the enamel, quantification and spatial distribution of crystallite organisation could not be provided using these techniques (Al-Jawad et al., 2007, Simmons et al., 2011). 2D- SXRD can be used to quantify the spatial distribution of physical features using an X-ray beam in transmission, and moving the sample relative to the beam in order to “map” 2D regions of interest.

Low et al conducted a study in 2004 to investigate the alignment of enamel crystals from EDJ toward enamel surface. He found that crystallite near EDJ were less organized when compared to crystallites at the surface of the enamel (Low, 2004). Using SXRD with a micro-focused beam spot allows the ability to detect abnormalities in the specimen in micron scale (Simmons et al., 2011). Also in SXRD technique, intact specimen can be used which provides a quality information about enamel crystallites and its spatial dimension (Al-Jawad et al., 2007, Simmons et al., 2011). For example, to understand the phenotype properties that found in abnormal dental enamel that found in type II and type IVA Mucopolysaccharidosis, it is important to compare the information of sound enamel and abnormal enamel (Al-Jawad et al., 2012). A study to evaluate HA crystallites size of dental hard tissues like enamel and dentine in patients with Mucopolysaccharidosis condition type I (that is associated with a hereditary condition called Hurler’s syndrome) found that there is no difference in hydroxyapatite crystal structure of the enamel of patients with this condition and control enamel (Guven et al., 2008). However, another study was conducted to study the orientation of crystallites between normal and affected teeth of patients diagnosed with type II and Type IVA Mucopolysaccharidosis. In contrast to the earlier study, by using 2D XRD they were able to observe a clear difference in affected enamel when compared to normal teeth (Al-Jawad et al., 2012). Siddiqui et al studied the remineralisation process of enamel crystallographic texture using SXRD. They found that there is a possibility to remineralise the affected demineralized enamel if a proper protocol is followed (Siddiqui et al., 2014)

### 1.5.3 X-ray Microtomography (XMT)

**Principles of XMT**

In 1982, X- ray microtomography was first identified and described by Elliot (Elliott and Dover, 1982). The system is also named as micro computerised tomography (micro-CT). The concept of XMT is similar to the medical computerised tomography but with a reduced size version and higher spatial and contrast resolutions. The technique of XMT is capable of providing three-dimensional scans of an object of variable sizes from millimetres to few centimetres at 5 μm resolution. It is able to test the density distribution and internal structure properties of the specimen. Huang et al cited that XMT is a non invasive technique used to produce a high resolution data (Huang et al., 2010, Huang et al., 2007). It is vital to state that although the non destructive nature of XMT, it utilises an extremely high radiation dosage to image a sample and it consumes a very long time to provide the data consequently, this imaging technique is limited to laboratory studies and it is not applicable for clinical use (Davis et al., 2013).

XMT can determine X-ray absorption distribution in a solid material without destruction of the object. When the X-ray beam is projected on the sample, the absorbed x-ray photons by the material is measured by a detector, mostly a film or a device called CCD device which is charge coupled based detector (Salvo et al., 2003). The produced image is a 2D image then to construct a 3D image, multiple radiographs should be performed as the sample rotate around 360 $^{o}$. Then the radiographs are processed by software to create a 3D image.

There are several XMT systems, synchrotron based type, commercial or in- house developed desktop systems. Each system can produce different type of x-rays. Monochromic X-rays are produced by synchrotron based system while polychromatic x-rays are produced by commercial and in-house desktop systems (Huang et al., 2007, Kinney and Nichols, 1992, Cooper et al., 2004, Prymak et al., 2005).

Wong et al, 2004, stated that mineral concentration distributions in dental hard tissues could be determined by X-ray microtomography with resolution range between 5 – 30 μm. They also cited that for XMT, the thickness of the specimen depends only on X-ray beam size, which make the specimen size constant with no irregularities as specimens produced by physical cutting instrument s. Much thinner specimen slices with minimum size can be also produced as it depends only on x-ray beam size as mentioned (Wong et al., 2004).

XMT has an immovable X- ray source and detector whereas the sample is attached on a turning stage that rotate with vertical line. When the sample rotates around $360^o$, X-ray incident beam attenuation is recorded producing grey-scale image. Beside the reconstruction of 3D image that can be provided, mineral density quantification is also achievable (Davis et al., 2013).

XMT scanner was developed in a 4 generations. The first generation has pencil beam which is a narrow beam generated by source collimator, a second collimator is used to eliminate scattered radiation. The accuracy of the obtained image is the priority of this system instead of the speed in obtaining it (Davis et al., 2010). In the following scanner generation, 2D detector is used in place of the point detector, which was used in the first system. The third generation, the structural quantification was main concern rather than the precision of linear attenuation coefficient as in the first generation. The third generation use area detectors to increase the amount of detected photons. Davis et al., 2010 stated that the polychromatic X-ray source creates a range of photon energy, which can cause artefacts such as beam hardening and ring artefact. In the fourth scanner system, a fixed ring detector utilised to avoid ring artefacts. MuCAT scanner is an example of $4^{th}$ generation. It has the same principal of the third microtomography scanner generation but with addition of the time delay integration charge coupled device TDI CCD readout. TDI CCD is a method helps in eliminating ring artefacts (Davis et al., 2010, Davis and Elliott, 1997). High quality image can be produced by this system.

X-ray source spot size is small in X-ray microtomography, which produce low flux when compared to medical scanner that has a full size x-ray spot (Davis et al., 2013). There is a link between X-ray exposure and image contrast, when the exposure is lessened, more noise will be produced giving a low contrast image or low signal-to-noise ration (SNR). To overcome this problem and enhance the SNR, the time of x-ray exposure can be modified to a longer period. However, errors can appear on the image by increasing exposure time such as ring artefact. These artefacts can be reduced by moving the specimen or the detector in a random way between the projections (Davis et al., 2013). Davis et al, 2013, stated that High SNR images could be produced by developing XMT scanner with the technique of time-delay integration readout. This technique helps in obtaining high spectrum of X-ray projections. Further improvements in data processing and correction of beam hardening have provided a high SNR images with high accuracy of mineral density quantification of hard tissues (Davis et al., 2013). Recently, many dental researchers became familiar with XMT technique especially the commercial scanners which are not adjusted to create the optimal results for mineral concentration teeth. However, improvement in the commercial XMT scanners is noted recently inn terms of the accuracy as well as the quality (Davis et al., 2013).

**Implementations of XMT**

XMT has been used extensively in many researches to determine the distribution of mineral concentration in bone and dental hard tissues in vitro. Wong et al studied the variation in mineral concentration of the enamel in 11 deciduous molars from different surfaces related to enamel dentine junction. They reported an increase in mineral concentration distribution from EDJ moving towards enamel surface. The conclusion of this study was that the baseline distribution of mineral concentration should be recorded to assess caries progression in caries researches (Wong et al., 2004). Another study conducted by Huang et al, 2010, to study mineral density (MD) in seven natural white spot lesions using XMT with Nano indentation. They concluded that MD is lesser in the body of the lesion and increases towards the surface of WSL however, not as high as sound enamel mineral density level (Huang et al., 2010). Farah et al conducted a study using ten sound teeth matched with ten MIH teeth to investigate mineral density for whole enamel thickness in sound and hypomineralised teeth using commercial XMT. They concluded that mineral density in MIH enamel was 19% less than enamel in sound teeth. Also they found that there is a strong link between the colour of enamel defect and mineral density in MIH samples (Farah et al., 2010). Fearne et al studied the mineral content of teeth with enamel defect such as hypomineralised enamel in permanent molars. They cited that about 20% decrease of mineral concentration of enamel was reported in teeth that are affected by hypomineralisation when compared to the non affected enamel. They also examined a full thickness of enamel and reported that hypomineralised teeth showed an opposite ingredient of mineral concentration to that of non affected enamel, which indicates disruption in the maturation stage during tooth formation (Fearne et al., 2004).

### 1.5.4 Scanning electron microscopy (SEM)

**The principles of SEM**

Scanning Electron Microscopy (SEM) is a technique that is used to provide a high-resolution view of morphological features and structural characteristics of a sample using focused beam of electrons. It is widely used in many researches to examine various materials as it provides important information on the ultrastructure properties and elemental composition of the specimen. SEM technique employs electron beam to provide images of specimens with high magnification that allow better understanding of material properties.

A beam of electrons is generated from electron gun to scan the sample surface of interest. Electrons become focused by passing through lenses and electromagnetic fields, which will eventually be delivered by a fine probe to examine the area of interest. As soon as the focused beam hits the sample surface, Auger and backscattered electrons will be generated and then detected by a detector to form an image. The samples must be coated with a layer of electronically conductive material allowing the interaction between the sample and electrons to occur. Two types of electron will be produced; Secondary electrons, which initially show the sample morphology as well as topography with contrast of sample composition. Incident electron hits the sample, secondary electrons will escape their orbit which will result in having images with high resolution (Vernon-Parry, 2000). The other type of electrons is the backscattered, which produce greater contrast because of the higher atomic mass elements. Therefore this type of electrons considered to be the most valuable in contrast illustration of specimen composition as well as crystallographic properties. Backscattered electrons are the incident electrons that reach closely to the atom's nucleus resulting in a large angle scattering then re-emerge again from the specimen surface. This type of electrons has higher energies when compared to the secondary electron (Vernon-Parry, 2000).

**Implementation of SEM**

SEM has been benefited widely to study enamel ultrastructure (Jälevik et al., 2005, Boyde, 1975, Azinović et al., 2003). Worawongvasu in 2015 used SEM to examine incipient lesions in 10 human premolars. They found that at low magnification enamel surface layer of some samples has small depressions, which is a result of dissolution of enamel prism ends. Also an alternating smooth and rough areas with some focal holes giving the incipient lesion a very characteristic feature as shown in Figure 1-14a.

At higher magnifications, the small depressions had the appearance of wedge shape as in figure 1-14b. In another set of samples, at low magnification, the enamel appeared like a fish scales due to the dissolution of adjacent prism ends (Figure 1-14c). These samples when examined at high magnification showed the dissolution of enamel prism core and sheath is more abundance than the inter-prismatic area. The crystals in the affected area has enlarged inter-crystalline space while in the unaffected area a well packed HA crystals were identified. Some samples showed an amorphous layer covering a rough pitted enamel, which indicate remineralisation. The amorphous layer seemed to be a result of coalescence of HA crystals. In the remineralised surfaces, a more regular and homogenous appearance when compared to demineralized areas. This finding implies that the natural early caries lesion has alternating periods of demineralization and remineralisation.

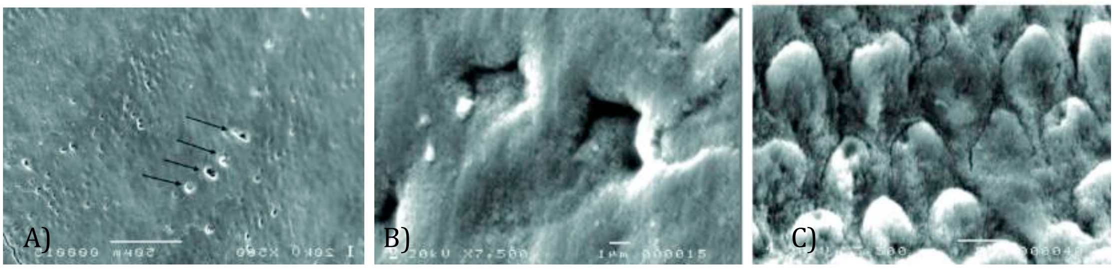



**Figure 1-14: A) Focal holes indicated by arrows. B) Wedge shape appearance of enamel. C) Fish scales enamel surface (Worawongvasu, 2015)**

## 2 Aim and objectives

### 2.1 Aim

To test the potential of Optical Coherence Tomography (OCT) in detecting early carious white spot lesion and correlate the findings with well established imaging techniques.

### 2.2 Objectives

1. To produce artificial caries-like lesions on healthy sound enamel in vitro to understand the changes in enamel dissolution at early stages and compare it with natural early caries lesions and healthy enamel.
2. To study the effect of demineralization on enamel structure at microscopic and crystallographic levels.
3. To compare the results of OCT with various analytical techniques to understand at which level OCT respond to changes in enamel structure. XMT investigates the mineral density, SXRD studies the crystallographic structure and SEM evaluates the prismatic structure of the enamel.

## 3 Tissues collection

### 3.1 Study registration and ethical approval

Ethical approval for this research is obtained from The National Health Services Research Ethics Committee on 11$^{TH}$ of August 2011, R & D (Reference number 11/LO/0777).

### 3.2 Patient identification and recruitments

Eligible patients who attended the department of Paediatric Dentistry at the Eastman Dental Hospital (EDH) were invited to participate in this research. The patients were identified from patient's clinical notes and the general anaesthesia theatre list. Inclusion criteria included patients who required the extraction of first permanent molars (FPM) as part of their treatment plan.

Exclusion criteria included FPMs with extensive caries or teeth that showed signs of enamel structure defects and patients who did not speak and understand English sufficiently to consent for participation in the study. Full explanation of the project was given to the parents and patients verbally along with information leaflets (Appendix 1 and Appendix 2). All patients/ parents gave informed consent to collect and use extracted teeth in the project (Appendix 3, 4 and 5). Each participant signed three copies of the consent form. One was filed in patient notes, a second one was given to the participant, and the last one was kept with project's supervisor.

### 3.3 Samples size and selection

A total of nine permanent first molar teeth were collected from patients after obtaining consent. Only six teeth were selected according to the inclusion and exclusion criteria. All the samples were assessed by two clinicians and the samples were categorised into three groups; 2 control teeth, 2 teeth with natural white spot lesions and 2 sound teeth, which were used to create artificial white spot lesions. Each group had samples with lesions on the same tooth surface.

### 3.4 Samples storage and disinfection

All samples were collected and stored according to The Human Tissues Act 2004 (Price, 2005). All collected samples were given an anonymised ID number to comply with data protection Act. Samples were collected in a plastic pot filled with normal saline until taken to the Eastman Dental Institute laboratory. Samples were washed under running water then cleaned, debrided with a surgical scalpel, and disinfected by storing it in 70% Ethanol for 48 hours. Then afterwards transferred to 0.1% Thymol and stored at 4°C in a fridge in biomaterial laboratory at EDH as per department policy. The use of 0.1% Thymol as a storage medium was according to the department policy. Further discussion about the effect of Thymol on the samples will be explained later on in the discussion chapter.

### 3.5 Samples preparation

The six permanent first molar teeth were then divided into 2 groups, composed of 3 samples that were matched in terms of tooth type and tooth surface. Set 1, consisted of 3 samples of maxillary first permanent molars. One control sample, one natural WSL sample (ICDAS: score 1) and the last sample was to induce caries like lesion (7 days) on sound enamel. The tooth surface that was tested was the distal side for all samples. Set 2, consisted of 3 samples of mandibular first permanent molars. One control sample, one natural white spot lesion sample (ICDAS: score 2) and one sample to induce white spot lesion (14 days) on sound enamel. The tooth surface that was tested was the distal side. Selection criteria of selected samples were based on ICDAS scoring. Increasing the number of samples was planned but it was a limitation that will be discussed in further detail in the discussion section.

### 3.5.1 Induction of caries like lesion

Samples were removed from 0.1% Thymol solution and washed under running water the patted to dry with paper tissue. The protocol used to induced caries like lesion was 8 % methylcellulose gel buffered with Lactic acid (Milly et al., 2015).  A window of 4 mm X 4 mm was created on each sample by polishing all tooth surfaces by a transparent nail varnish (Rimmel™, London) leaving the window free of the transparent nail varnish but covering the rest of the sample. Following that, each sample was mounted on a putty rubber base impression material that was place at the base of a container (Figure 3- 1A). A layer of 8% methylcellulose was placed on the top of the tooth then buffered with 0.1 mol/L lactic acid with 4.6 pH, infiltration paper separating the methylcellulose gel and acid, as shown in figure (3- 1B). Samples were placed in a locked laboratory refrigerator at 37º.  One sample was kept in the demineralization gel for 7 days, in attempt to match natural white spot caries lesion of score 1 in ICDAS in the same samples set. The second sample was kept for 14 days in attempt to match the natural white spot lesion of score 2 ICDAS. On the last day of the demineralization period, samples were washed with distilled water and placed in 0.1% Thymol until imaged by different imaging techniques.

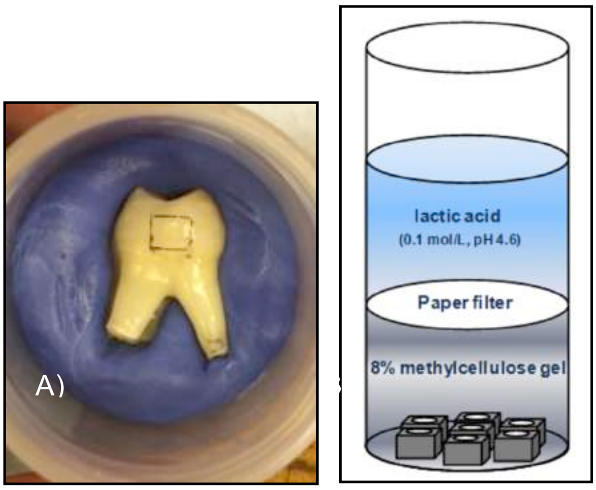



**Figure 3-1: A) Image showing sample preparation for WSL induction.  B) Image showing demineralisation protocol used to create caries like lesion**

After inducing caries like lesion, all the samples were dried using paper tissues, then placed on a black colored background to increase contrast enhancement between the sample and the background. Each tooth was labelled with tooth number, surface and sample ID. Photographs of all the samples were obtained using Canon™ Camera that can provide high resolution, model number DS6041 with ring flash, model number MR-14EX. Figure 3- 2 and Figure 3- 3 show the clinical pictures of set one and set two respectively.


**Figure 3-2 Shows the three samples of maxillary first molars in set one; A) Induced caries like lesion for 7 days sample. B) Natural WSL with ICDAS score 1 sample. C) Control sample.**

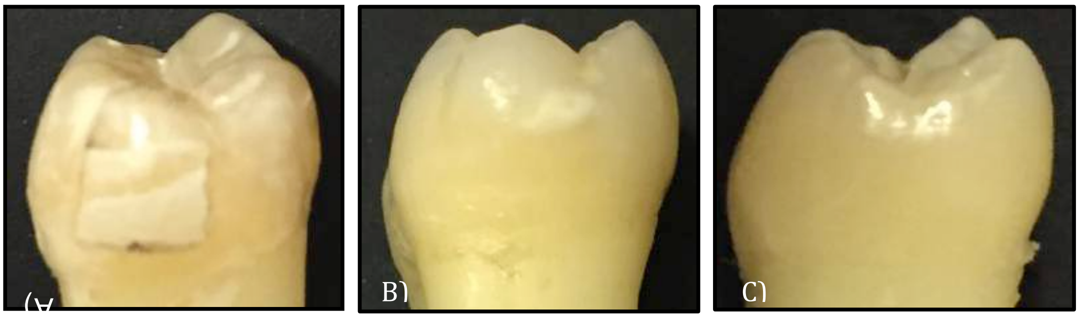



**Figure 3-3 Shows the distal of three samples of mandibular first molars in set two; A) induced cares like lesion for 14 days. B) Natural WSL with ICDAS score 2 sample. C) Control sample.**

# 4 The assessment of the samples using ICDAS, Polarised Microscope and OCT

In this chapter, the assessment of all samples using the visual caries diagnostic tool (ICDAS) scoring system, polarised microscope and OCT will be discussed.

## 4.1 Rationale of this chapter

To examine the samples using the gold standard caries diagnosis technique (ICDAS), then investigate the histology of each sample to detect the depth of caries lesion into enamel structure using polarised microscope. OCT was used before sectioning the samples to examine and compare the results of each technique and to confirm that OCT can detect the early changes in enamel structure.

## 4.2 Materials and methods

### 4.2.1 Material and methods for ICDAS

The International Caries Detection and Assessment System (ICDAS) was used to characterise the carious lesion stage of the selected samples. Two clinicians completed an e-learning training course (https://www.icdas.org/icdas-e-learning-course) to use the ICDAS scoring system. This e-learning course was a 90 minute programme that has been developed to support training in ICDAS. It demonstrated ICDAS examination protocol and provided a full explanation of the scoring system. At the end of the course, the clinician was assessed by a test on a range of clinical photographs of different stages of dental caries. The course was completed by the clinicians successfully. The samples were removed from 0.1% Thymol solution and washed under running water. Next, each sample was tested under dental chair light before and after prolonged drying for 5 seconds using the three in one air- water syringe. Scores were recorded and the samples were placed back in the container until the next experiment. The results will be discussed in details the result section.

### 4.2.2 Materials and methods for OCT

Optical Coherence Tomography (OCT) was then used to image the samples before sectioning, the instrument used was the VivoSight$^{TM}$ OCT scanner which is manufactured by Michelson Diagnostics, United Kingdom, and has a multi-beam Swept-source frequency Domain scanner. The light used in OCT instrument is near infrared with 1305 nm wavelength, which is considered a class one laser. In tissues, It has less than 7.5 µm of optical resolution laterally and 5 µm optical resolution axially.

The depth of focus is 1 mm and can scan an area of up to 6 mm (width) X 6 mm (length) with depth of about 1.2 mm to 2.0 mm of the scanned tissue. It can scan 35 frames in one second with 10 kHz scan rate.

The OCT instrument consists of light source, Santec HSL-2000-12-MDL, the processing system- Dell Precision T3600, image display, and mounted OCT scanning probe (Figure 4-1). The scanning probe was mounted on adjustable arm, which gives more stability for the probe. An adjustable platform was used for sample mounting to allow modification in the distance between the sample and OCT probe to enabling us obtaining the best image quality.


**<u>Figure 4-1: Image shows the different parts of OCT instrument ; 1) 10 Santec light source, 2) OCT scanning probe, 3) Adjustable platform</u>**

The samples were removed from the refrigerator the day before examination to test them at room temperature. Samples were removed from the storage solution (0.1% Thymol) and excess Thymol was wiped off using paper tissues. The sample was mounted on a glass slab using soft modelling material (plasticine) to stabilize the sample in a perpendicular position to the OCT scanning probe, and then positioned on adjustable platform below the OCT scanning laser. The same OCT setting was used to scan all the samples (60 frames) and the size of the scanned area (6 mm X 6 mm). The scan began cervically from the cemento-enamel junction (CEJ) to the cusps of the tooth coronally. Multiple frames were taken for each sample using a multi-one pre-setting; which takes 60 frames with 100 μm between each frame. OCT images (b-scans) were analysed using software called ImageJ™, which is a processing programme developed by the National Institutes of Health in Maryland (USA).

For OCT data analysis, there were two approaches to analyse the data. The first approach was qualitative analysis of OCT images by investigating the changes in enamel structure in caries and control samples. The second approach was measuring the back scattered signals intensity when the laser travels into the depth of enamel structure. Both approaches were performed to analyse the obtained OCT images of all samples.

### 4.2.3 Materials and methods for Polarised microscope

Preparation of the samples was done prior to histology investigation using Polarised Microscopy. The samples were removed from storage solution, rinsed by distilled water then mounted on impression compound material. Each sample was fixed vertically on an adjustable handle in the cutting instrument (accuton-5, Denmark) (Figure 4-2A), and sectioned longitudinally in a Mesio- Distal direction by fine blade of 300 μm under water irrigation coolant. Each sectioned sample was about 350- 400 μm then the samples were hand polished using polishing sand papers to reach a thickness of 250 – 300 μm (Figure 4-2B). Extreme care was given during handling and polishing the delicate samples to avoid losing any enamel structure. The preparation of the samples was carries out in hard tissue Laboratory at Dental Physical Science Department in Queen Mary University London. Samples were placed in Thymol solution until ready to be tested.

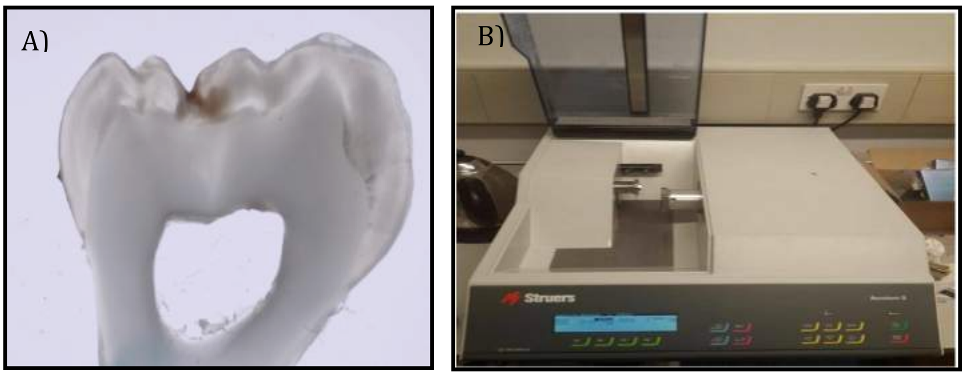



**Figure 4-2: A) Sample after sectioning and polishing. B) Sectioning instrument .**

All samples were imaged by polarised microscope (LEIT2 DMRD) to examine them qualitatively. Samples were removed and washed under running water then placed in petri dish and immersed with distilled water. Low magnification of 5x was used to examine the samples with a polarisation filter. When polarized light hits the sample, the image produced depends on the order of crystallites. Well ordered crystallites reflected the light creating bright colour while less ordered and destructed crystals produced a darker colour. After the experiment, the samples were placed back in the storage solution

### 4.3 Results

#### 4.3.1 Results of ICDAS

A total of 6 samples were examined by ICDAS, Polarised microscope and OCT imaging. The recorded scores were within score 0 to 2. Score 0 means that after proper drying of tooth surface there is no evidence of visual changes happened to the surface and it is completely sound. Score 1 means that after proper drying of tooth surface; a white spot lesion can be detected visually. Score 2 means that without proper drying of the sample surface, the white spot lesion can be seen even though the tooth is still in a wet medium. The main difference in the characteristic features between score 1 and 2 relied on whether the lesion was visible before drying or not visible. The results of ICDAS scores of all samples are presented in table 4-1.

| Tooth Notation | Patient age (Years) | Patient gender | Ethnicity | Sample ID | ICDAS score |
|---|---|---|---|---|---|
| UR6 | 9 | Female | Asian | C161SS | 0 |
| UR6 | 15 | Female | White | C166SS | 1 |
| UR6 | 10 | Female | Asian | MIH 83 | 1 |
| LL6 | 9 | Female | Asian | C162SS | 0 |
| LL6 | 15 | Female | White | C167SS | 2 |
| LL6 | 6 | Male | White | 60ControlAS | 2 |

**Table 4-1: Table shows the demographic and ICDAS scores of the samples.**

The ICDAS scores for the two samples (C166SS and C167SS) that were used to induce caries white spot lesion were score 0. The enamel surface for both samples was sound with no visible changes. After applying the demineralisation protocol, ICDAS scores were recorded again as it shown in the table (4-1).

### 4.3.2 Results of Optical Coherence Tomography (OCT)

The six teeth were imaged by OCT, the scanning area was 6 mm X 6 mm as shown in Figure 4-3 with depth of 2 mm. As mentioned previously, the setting for OCT instrument was the same for all examined samples. The acquisition used was pre-set multi-one system for all samples, 60 frames were provided with 100 µm distance between each. the obtained OCT images were transported and saved as TIFF files.

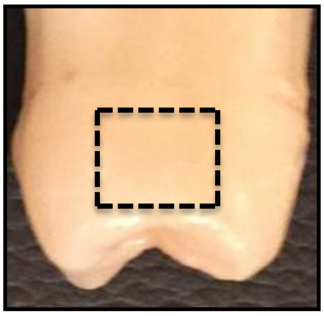


**Figure 4-3: a clinical photograph shows the scanning frame of the OCT on the tooth surface.**

**Results of control samples**

The clinical photograph along with OCT image of the interproximal area (distal surface) of the control sample (C161SS) are shown in Figure 4- 4. From the clinical photograph, it is clear that the enamel surface is sound and free from enamel defects with no caries lesions. A solid black line indicates the position of the area of interest across the enamel surface. Control sample of set one is demonstrated in Figure (4- 4).

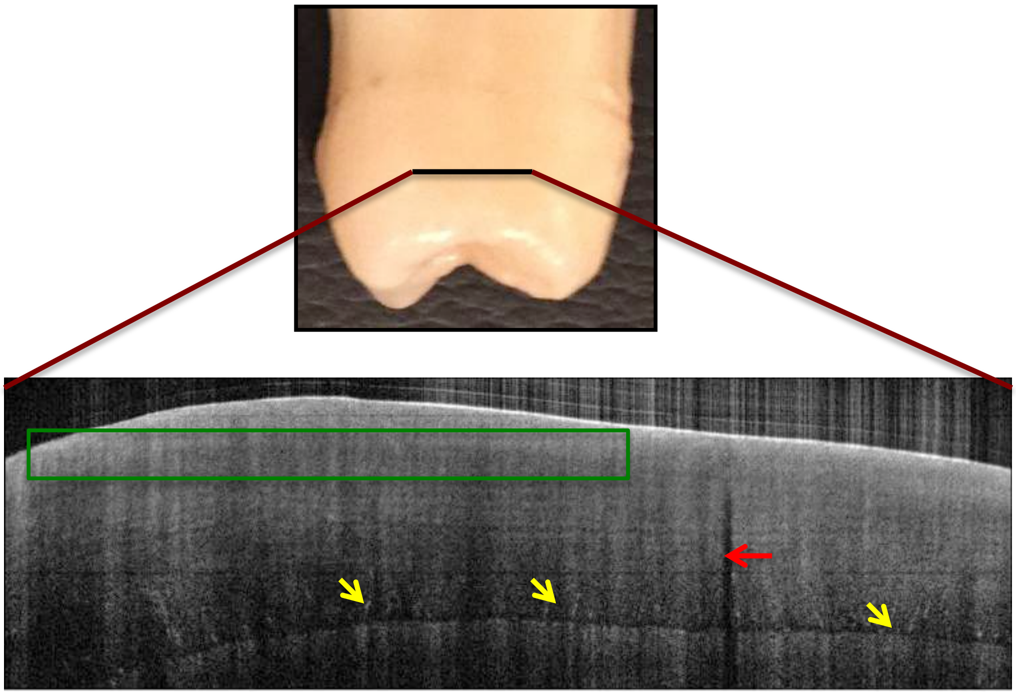


**Figure 4-4: OCT Image of the distal surface of the control sample.**

In the obtained OCT image, basic layers of the crown are demonstrated. Enamel, dentine with EDJ between the two layers. The enamel structure in b-scan exhibits a uniform scattering. There is a crack extending from dentine into enamel structure indicated by red arrow in Figure 4- 4. Also on DEJ, there are small opaque feathers like structures, which are, resemble enamel spindles (yellow arrows). Area of less intense zone can be observed in enamel structure (Green box), this is due to the increased scattering on enamel surface that caused shadowing underneath these areas. The dimensions of the image are 1500 X 460 pixels corresponding to 6000 μm X 1840 μm.

OCT image and clinical photograph of the control sample (C161SS) in set two is demonstrated in Figure 4-5. As mentioned previously, the OCT image shows three layers of tooth structure; the enamel, dentine and EDJ. In this sample, two cracks can be identified (indicated by red arrows). A homogenous and uniform scattering in enamel structure can be recognised from OCT scan. At enamel surface, there is an increase in brightness when compared to the rest of b-scan (indicated by the green arrows). This is because of the greater scattering signals from this region. Opaque feather like structure is also present in this image, which represents enamel spindles as, mentioned previously (Indicated by yellow arrows). It was believed that enamel spindles are formed during tooth formation as a result of the extension of the odontoblasts beyond the dentine-enamel junction. The dimensions of this image are 1500 X 460 pixels that correspond to 6000 μm X 1840 μm.

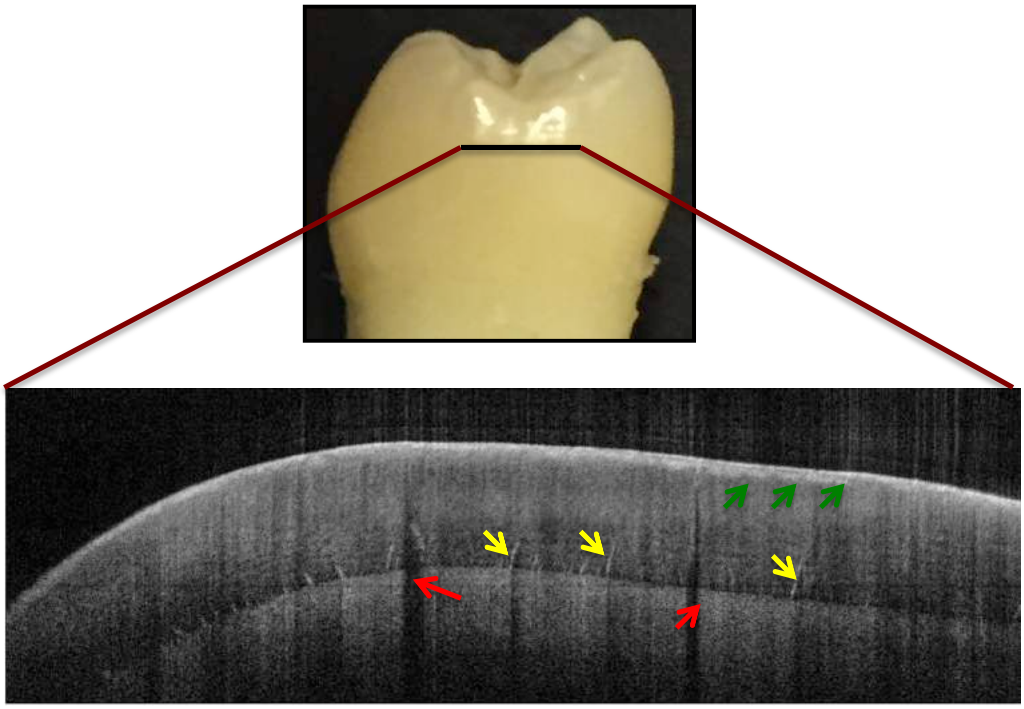


**Figure 4-5: OCT image of the distal surface of control sample set two.**

**Red arrows indicate the cracks, green arrows indicate the increase scattering at enamel surface and yellow arrows indicate enamel spindles.**

The figure below shows a histological picture of enamel spindles located at the dento-enamel junction, which is comparable to the image obtained in the OCT b-scans. (Figure 4-6).

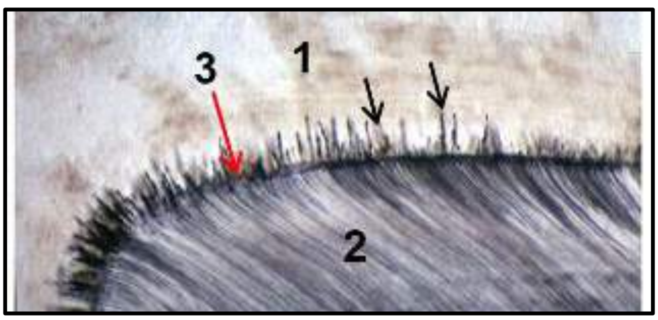



**Figure 4-6: Image shows the enamel spindles that is located at DEJ (black arrows). 1. Enamel, 2. Dentine, 3. DEJ.**

Image taken from:
**http://course.jnu.edu.cn/yxy/eruption/zuzhitupu/Cards/tth/09_bb.html**

**Results of natural white spot lesions samples**

The OCT scan of natural caries white spot lesion sample (C166SS) of set one is demonstrated in Figure 4- 7. In this OCT scan, unlike the previous scans of the control sample, only the enamel is shown and the dentine and DEJ are not visible. This is due the thickness of the enamel at this region. The position of the scan taken was located on the maximum interproximal curvature at the contact point of the distal surface of FPM as shown from the clinical photograph in Figure 4- 7. No subsurface cracks can be identified from the OCT scan. There is increase brightness at the surface of the enamel due to the increase of scattering intensity of the incident light (indicated by green arrows). Underneath the surface of increased brightness, the region appears darker just below the enamel surface. On the palatal side of the OCT scan of the sample, it can be seen that there is a darker region which suggests a reduction in scattering intensity at the subsurface region under an area of increased intensity (indicated by white circle). Unlike the buccal side which exhibits a uniform enamel structure Figure 4- 7.

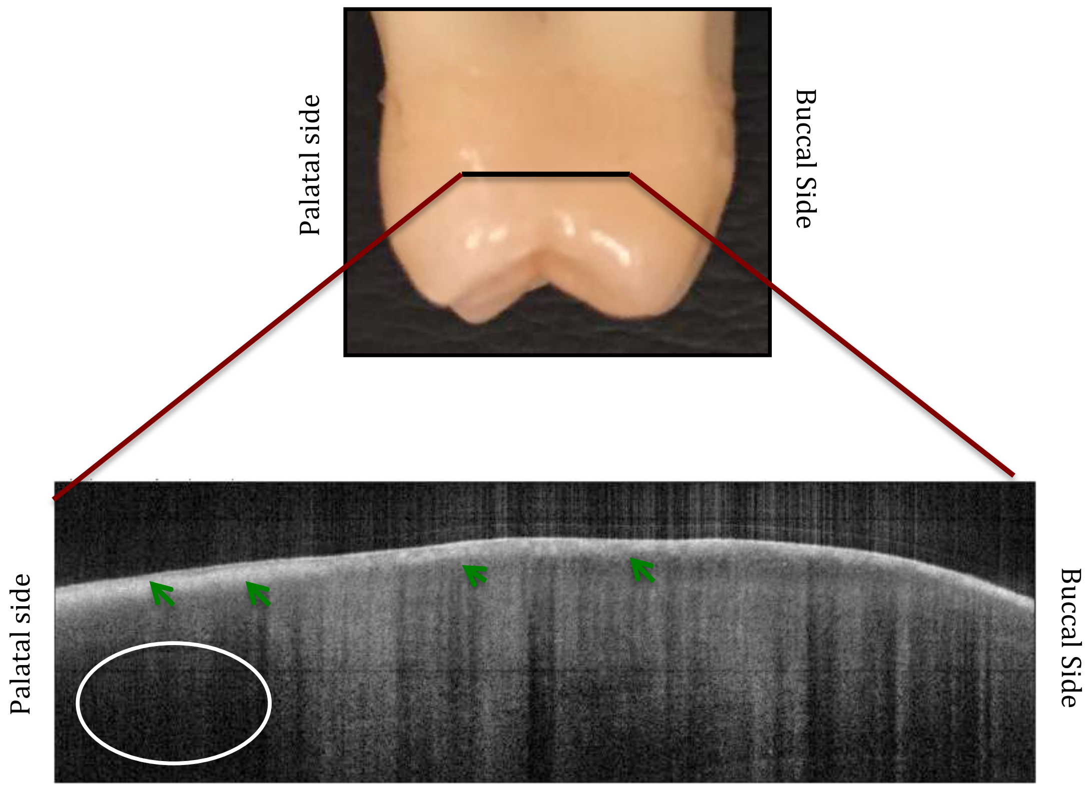



**Figure 4-7: OCT scan of the distal surface of the maxillary first permanant molar**

The OCT scan of the natural white spot lesion in set two (C167SS) is demonstrated in Figure 4- 8. The clinical picture of the sample shows clearly the white spot lesion on the distal side of the tooth with the black solid line that indicate the position of the scan. In OCT scan obtained, only the enamel is visible. As mentioned previously, it is due to the increased in the enamel thickness at the region where the OCT scan was taken. The enamel structure looks non-homogenous with variations in scattering pattern (indicated by white circle). The white spot lesion in the clinical photograph looks non cavitated. However, from OCT scan, there is a change in enamel structure, which is correlated to the white spot lesion on the clinical photograph. There is a subsurface crack can be identified in the buccal side of the scan (indicated by red arrow). As it shown from the OCT scan, the brightest colour due to the highest scattering pattern is present at the surface of the enamel (indicated by green arrows).

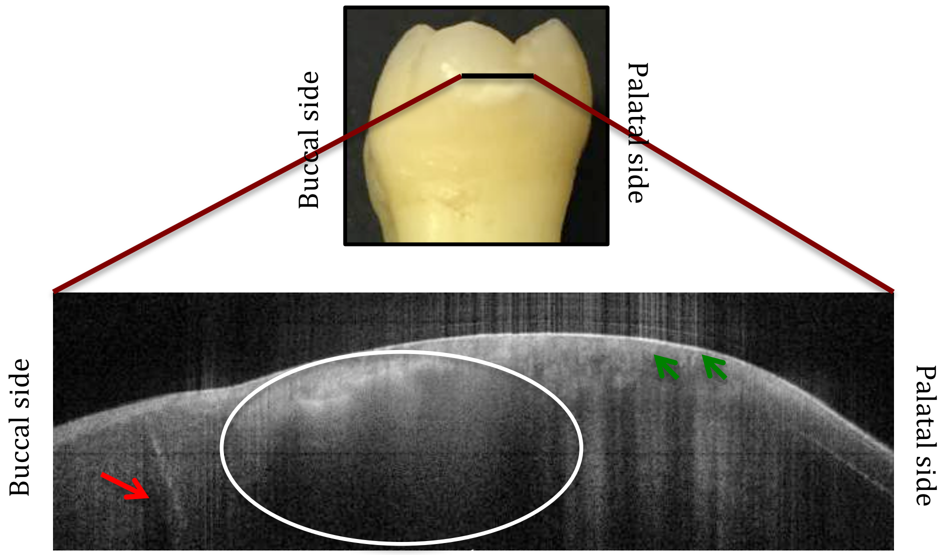



**Figure 4-8: OCT scan of the distal surface of Mandibular first molar.**

**Results of Induced caries like lesion samples**

The sample of induced caries like lesion that was exposed to lactic acid for 7 days (C166SS) in set one is demonstrated in figure 4- 9. It shows the 4 mm X 4 mm demineralised area that corresponded to enamel surface that was free from the transparent nail varnish. The OCT image obtained only the enamel structure as the area of interest lied on the maximum convexity of the interproximal side as shown in the clinical photograph. At the surface of the enamel, on the palatal side, there is minimal increase in brightness, which corresponds to the demineralised area (indicated by green arrows). Otherwise, Enamel structure exhibits a homogenous scattering pattern.

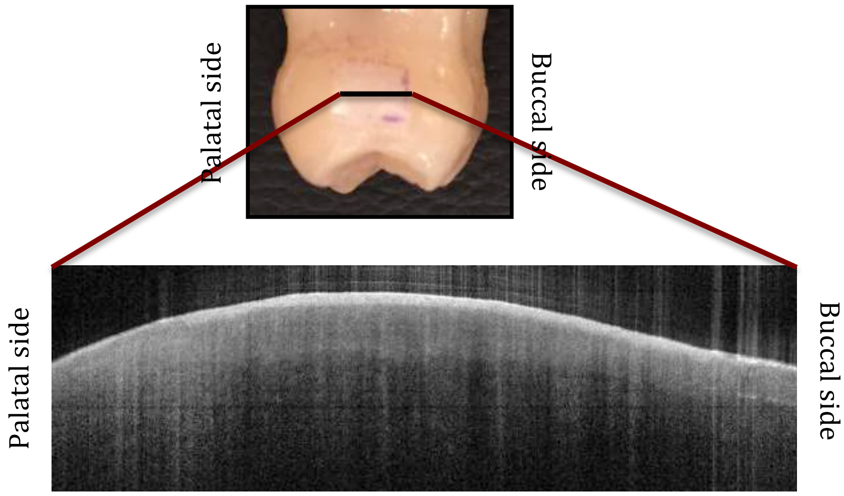



**Figure 4-9: OCT scan shows the distal side of Maxillary first molar (induced caries like lesion sample (7 days).**

The clinical photograph of the induced caries like lesion sample for 14 days (C167SS) is shown in Figure 4- 10. The 4 mm X 4 mm area of demineralisation appears on the distal surface of the tooth. From the OCT scan, only the enamel structure can be seen as the area of interest lies just below the disto-buccal cusp of the sample where the enamel thickness if the enamel is high compared to other parts of the tooth. The transparent nail varnish can be identified (indicated by red arrow). An area of increased brightness can be seen at the surface of enamel that corresponded to varnish free area (Green arrows) however, the region that is below the nail vanished appeared homogenous with the rest on enamel structure (yellow arrows).

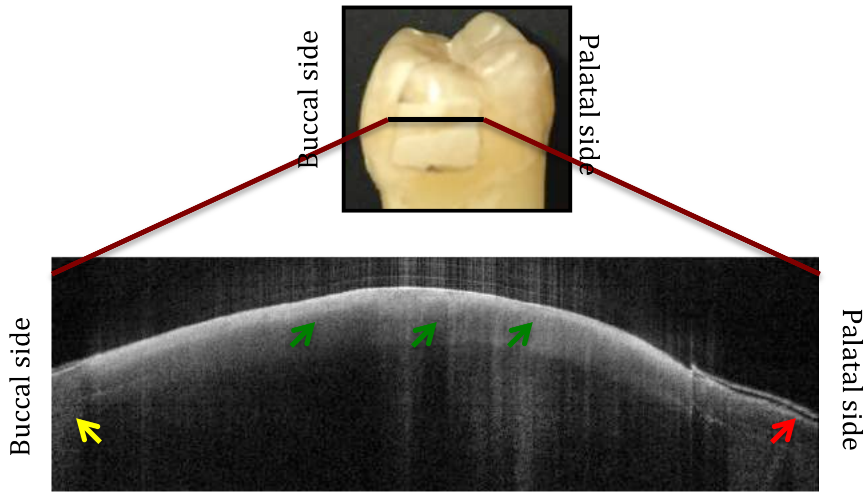



**Figure 4-10: OCT scan shows the distal surface of induced caries lesion sample (14 days).**

The increase in brightness that corresponds to the varnish free area is due to the increase of scattering intensity of the laser photons. Underneath this area, there is a darker region suggesting changes in the enamel structure

Scattering intensity profiles were then measured on b- scans of the samples as a function of depth. The width of selected area on the b- scans was ten pixels, which corresponds to 40.5 µm for all samples.

The OCT b- scans were transferred to Image J software to obtain the signal intensity profiles which is called a- scans. From Image j software, the information about OCT scan width was provided. The width was 6000 µm, corresponding to 1.500 pixels, this means that one pixel is equal to 4 µm. Consequently, the selected rectangular area of interest has a width of 40 µm. Next, the data from the scattering intensity profiles were extracted and plotted as a function of depth using Originlab pro 2015.

All the samples were investigated using this approach. All the selected regions on the b-scan were taken in areas with no subsurface cracks whenever possible as it might affect the scattering pattern of enamel structure.  An attempt was made selected regions in the b- scans were taken in an area all samples the a- scan demonstrated a sharp peak of signal intensity (spike- like) at the surface of the enamel as soon as the laser hit the surface. As the laser travelled through the depth of enamel, any changes in enamel structure will result in changes in scattering pattern. These changes can be detected and measure from the a- scan.

<u>**OCT signal intensity profiles for set one (7 days lactic acid demineralisation)**</u>

A signal intensity profile of all samples in set one is shown in figure 4- 11. For the control sample in the OCT b- scan, it is shown that the scattering is uniform through enamel structure with few cracks. The area of interest is highlighted with yellow lines (Figure 4- 11A). The a-scan shows the signal intensity, which at the beginning of the profile appeared non-scattering as the laser, travelled in the air (Figure 4- 11B). However, a sharp peak of scattering was produced as soon as the laser hit the sample at air-enamel interface (indicated by red arrow). The reason behind the sharp peak is that the photons travelled from a medium (Air) that has a lower refractive index (1) Compared to the enamel (1.63). Following the sharp peak, the profile showed a rapid decrease in the intensity as the photons pass into the depth of the enamel structure.

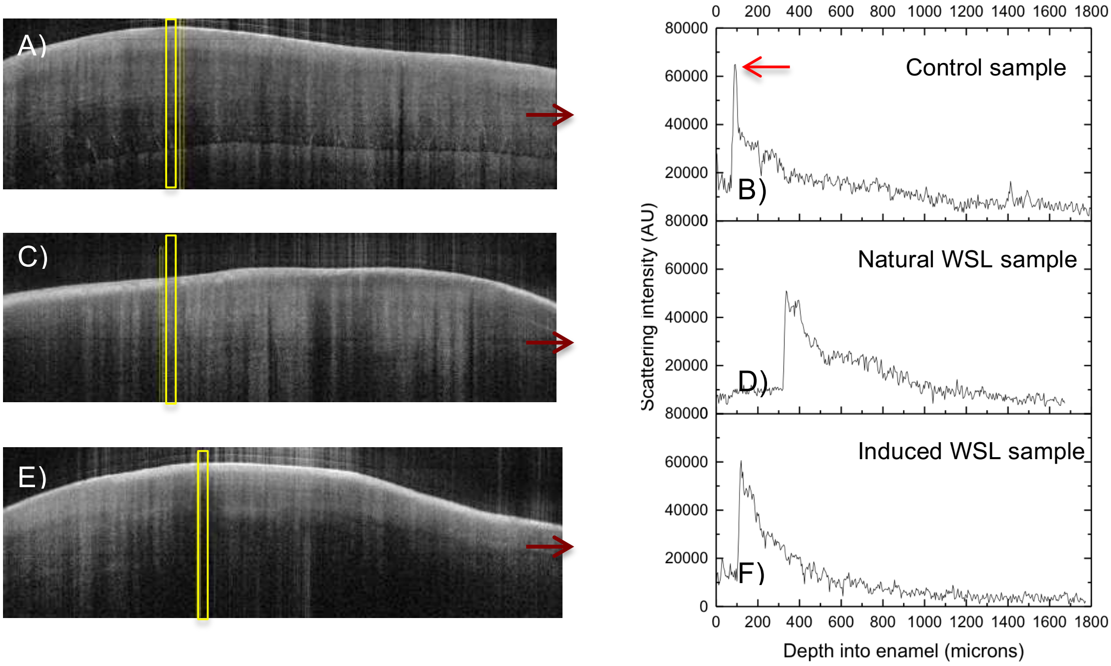



**Figure 4-11: OCT b- scans of set one the corresponded a- scans of the area of interest. A) OCT b- scan of the control sample (area of interest highlighted by yellow lines. B) Scattering intensity profile (a-scan) of the area of interest of the control sample. C) b- scan of natural white spot lesion sample. D) a- scan of natural white spot lesion. E) b-scan of the induce caries like lesion sample. F) a-scan of the induced white lesion sample.**

For the natural white spot lesion, the area of interest is highlighted by yellow vertical line on the b-scan of the OCT (Figure 4- 11C). Enamel structure appears homogenous but there is an increase in brightness at the surface of the enamel. Cracks can be identified as well. In the a-scan, an area of no-scattering intensity can be seen at the beginning of the profile (Figure 4- 11D). A high peak is developed at interface between the air and the enamel, which persisted for about 100 μm then the scattering intensity decayed when the photon travelled through the depth of the enamel.

For the induced caries like lesion sample, the b-scan appeared uniform in the scattering intensity except for the surface of the enamel, which correspond, to the area of induced demineralisation (Figure 4- 11E). From the scattering intensity profile, which was extracted from the highlighted yellow line on the b-scan, an area of no scattering was produced followed by a strong peak at the surface of the enamel. The peak persisted for about 50 μm then the scattering decreased by depth (Figure 4- 11F).

**OCT scattering intensity profiles of set two (14 days lactic acid demineralisation)**

The OCT images with scattering intensity profiles of set number 2 are shown in figure 4-9. Control sample shows homogenous scattering pattern when compared to the b-scan of natural white lesion sample (Figure 4- 12A and C). In the a-scan of the control sample, a sharp peak is produced at the surface followed by rapid decay of scattering intensity (Figure 4-12B). In natural white spot lesion, the sharp peak is produced at the surface of the enamel followed by slight decrease in scattering intensity that persists for about 500µm into enamel depth then fade away (Figure 4-12D). The persistence in scattering intensity suggests changes in enamel structure that led to changes in scattering pattern. In the induced caries like lesion sample (14 days), the demineralised area can be clearly identified (Figure 4-12E). At the start and end of the b-scan, the transparent nail varnish is shown as a layer on the top of enamel surface. A part of the b-scan (yellow line) was analysed and the resultant a-scan shows a strong back-scattered intensity at the surface that continues at a high point for about 200µm before in rapidly decays (Figure 4-12F).

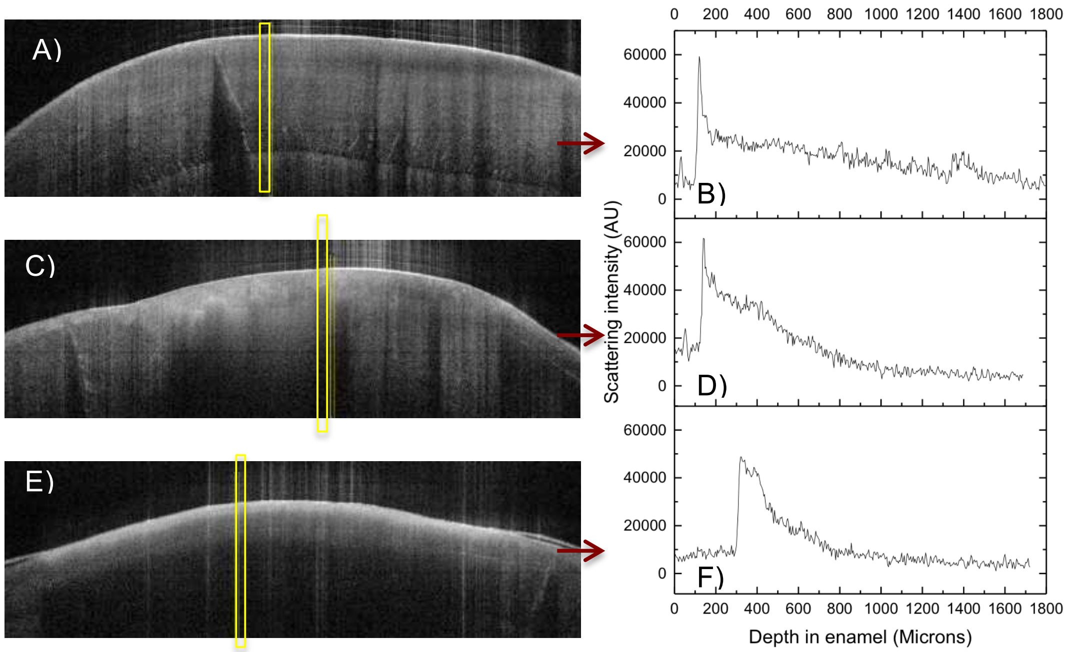



**Figure 4-12: OCT b- scans and a- scans of set two. A) b- scan of the control sample (yellow lines indicated area of interest). B) a- scan of control sample. C)b- scan of natural white spot lesion sample. D) a- scan of natural white spot lesion sample. E) b- scan of induced caries like lesion sample. F) a- scan of induced white spot lesion sample.**

### 4.3.3 Results of Polarised Microscope

In sample set 1, from the image obtained for control sample, tooth structure can be identified such as the enamel, dentine, and Dentine- enamel junction (DEJ). There is a variation in enamel thickness with the thickest part at the cusp area and the thinnest part at Cemento- enamel junction. Loss of tooth structure can be identified at the occlusal region with changes in enamel structure in enamel structure adjacent to the lesion. Above the dentine horn, there is a variation in the contrast in enamel structure as a lesion can be recognised just above dentine horn. At the DEJ, an interchanging dark and bright colored bands can be noted which is known as Hunter- Schreger bands. A crack can be seen at cervical third of the sample extending from enamel surface to the dentine (Figure 4- 13A). In the induced caries like lesion sample (Figure 4- 13C), changes at almost the full thickness of the enamel structure can be seen. There is an irregularity at the outer layer of enamel surface, while in the control and natural white spot lesion samples, a smooth enamel surface is noted. In the image obtained for natural white spot lesion sample (4- 13B), there is a change in the contrast at the enamel structure near enamel surface the advances minimally through enamel structure (Indicated by black arrows). Enamel crack and changes in contrast at fissure area can be identified (Figure 4- 13B).

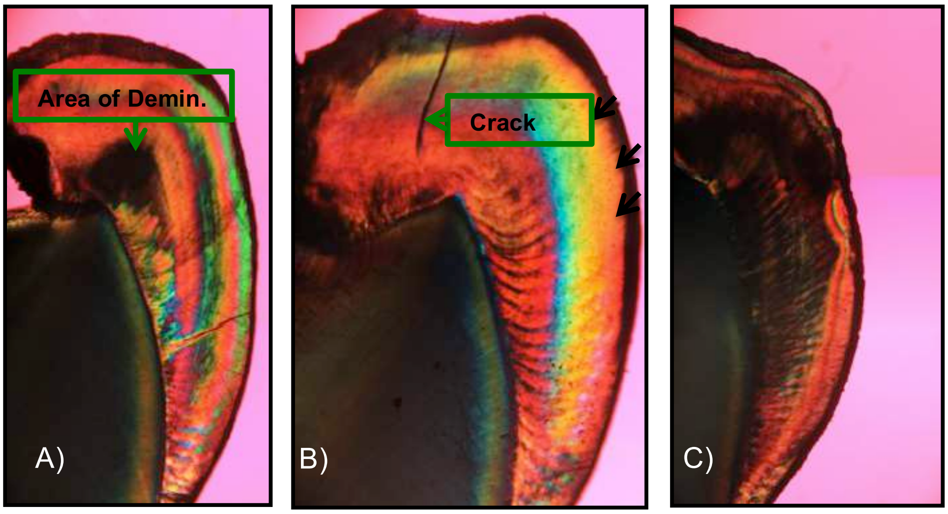



**Figure 4-13: Polarised Microscope images show: A) control sample B) induced caries like lesion sample C) natural white spot lesion sample.**

In sample set 2, the resultant image of control tooth shows clearly tooth structure layers; the enamel, dentine with enamel- dentine junction (DEJ) separating them. From the image obtained, it can be seen that there is a variation in enamel thickness; the thickest part is at cusp region while the thinnest part is at cemento-enamel junction (CEJ). Interchanging dark and bright bands that travel from DEJ towards enamel surface can be identified, Hunter- Schreger bands. Hunter- Schreger bands are considered the key characteristic of sound enamel. A crack can be recognised at the cusp region from enamel surface propagating though the two-third of enamel structure towards the dentine. Enamel structure at the fissure region appeared dark in colour when compared with the rest of enamel structure. As the dentine is less mineralised structure, it appeared dark in colour when it compared to the enamel (Figure 4-14A).

For the induced WSL sample (14 days), enamel, dentine and DEJ can be clearly identified. Hunter- Schreger bands also present from dentine towards enamel surface. The outer enamel layer appears darker at the surface, which advances minimally into the enamel structure. This is related to the window that was created on the sample to expose the surface to demineralisation protocol. However; it is considered minimal when compared with natural white spot lesion sample (4- 14B and 4- 14C).

The image obtained for the natural white spot lesion reviled the size and the depth of the caries lesion through enamel structure. The lesion located just below the level of dentine horn and it appeared as a triangle with it base at enamel surface and the apex towards DEJ. Hunter- Schreger bands were present as well within enamel structure (Figure 4-14C).

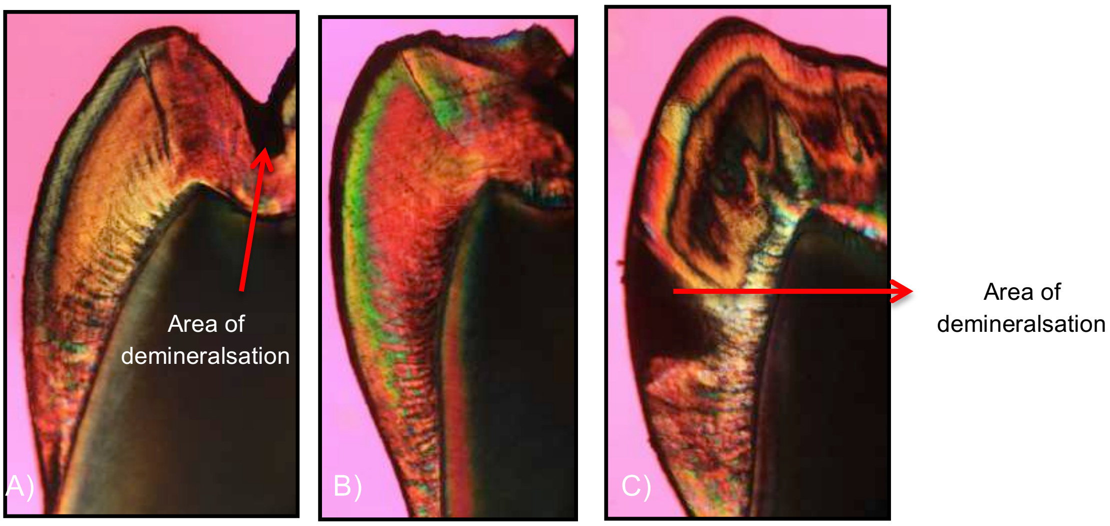



**Figure 4-14: Polarised microscope images show: A) Control Sample B) Induced caries like lesion sample C) Natural white spot lesion sample.**

## 4.4 Discussion

Dental caries is a complex disease, and a number of caries detectig tools have been produced to help in identifying and detecting the presence of carious lesions. ICDAS is considered to be the gold standard in caries lesions assessment and detection. The scores correspond to six stages of carious lesions, starting from early changes on enamel surface that can be visible clinically, to established and extensive caries. ICDAS is a simple, practical and evidence based system that can be used in clinical setting, public health programmes and dental research. However, this system is unable to detect the depth of early white spot lesion before it become established caries. The other method to assess the depth of the lesion is the use of radiographs. However, in clinical situation, radiographs is not indicated to detect caries white spot lesions. Also, radiographs uses ionising radiation that has a potiential to cause health risks.

In this project, all the samples were examine by ICDAS. Each sample was given a score according to ICDAS critieria. In the early white spot lesion sample, the lesion appeared localised with minimal changes in enamel structure without drying the sample therefore, a score 2 was given. However, polarised microscope image of the sample revealed deep caries progression towards the dentine-enamel junction which could not be anticipated without sectioning the sample and testing it under polarised microscope. OCT b-scans and a-scans have demonstrated the changes in enamel structure that correlate to the lesion in polarised microscope. In the induced caries-like lesion samples (7 days and 14 days), mild changes were detected in the polarised microscope which was detected by both OCT b-scan and the scattering intensity profile. Longer periods to produce caries like lesion should be considerd as no subsurface lesion was demonstrated in polarised microscope and mild changes were shown by OCT images.

When the gold standard clinical diagnostic tool (ICDAS) was compared with OCT results, OCT provided more description of lesion depth either by b-scans (qualitatively) or a- scans (scattering intensity profiles). A further details about choosing ICDAS as the gold standard will be discussed later on in chapter 6.5.

From other studies, the OCT findings in this project showed to be in consistent with the literature findings. A study to compare between conventional OCT and Polarisation sensitive OCT in investigating tooth structure as well as caries lesions, concluded that both OCT techniques can provide adequate information on caries depth and location, as well as investigation of tooth sturcture (Baumgartner et al., 2000).

It was noticed that there were variations in the interaction patten between laser light photons and the enamel when different samples were tested. Therefore, it is important to understand the reason behind the different behaviour of incident light with the enamel structure. An attempt to understand whether the photons interacts with enamel structure at prismatic level, crystallographic level or mineral density level is important to identify the reason behind the various behaviour of the laser in different circumstances.

In this project, enamel ultrastructure in sound, natural caries white spot lesion and induced caries like lesion were investigated in terms of crystallographic structure using SXRD, mineral dentisty (XMT) and prismatic structure (SEM). A correlation between OCT results and these multi-scale techniques will be discussed in the following chapters.

# 5 Assessment of enamel Structure using SXRD

## 5.1 Disclaimer

Data extraction and analysis of SXRD experiment was carried out in collaboration with Mr Mohammed Al- Mosawi, at the Institute of Dentistry, QMUL.

## 5.2 Rationale of this chapter

Synchrotron x-ray Diffraction (SXRD) is an advanced imaging technique that can identify crystallite orientation in enamel structure The purpose of using SXRD in this project was the evaluate the enamel structure at crystallographic level to enable us to understand at which level of enamel structure is the OCT laser scattering contrast generated. By comparing OCT and SXRD results, we might have a better interpretation of the reasons behind the changes in intensity in OCT results analysis.

## 5.3 Materials and methods

SXRD experiments were conducted at The Diamond light source, Oxford, United Kingdom. The same six samples from the previous experiment were used here; set one consisted of one control, one natural white spot lesion (ICDAS 1) and one induced caries-like lesion (7 days), and second set consisted of one control, one natural white spot lesion (ICDAS 2) and one induced caries-like lesion (14 days). Each set had samples that matched in term of tooth type, surface and tracks location. the reason behind this is that crystallite orientation varies according to tooth type and according to location within the same tooth (Al-Jawad et al., 2007, Simmons et al., 2011).

Beamline B16 was used in this experiment with a wavelength of 0.757 Angstroms and 16.4 keV energy. The size of the beam spot was 20 μm X 26 μm. All the samples were mounted on a hard card and then placed on adjustable platform that moved in 2 directions (X and Y direction) perpendicular to the beam direction.  SXRD experiment general setup is demonstrated in figure (5- 1A and 5- 1B).

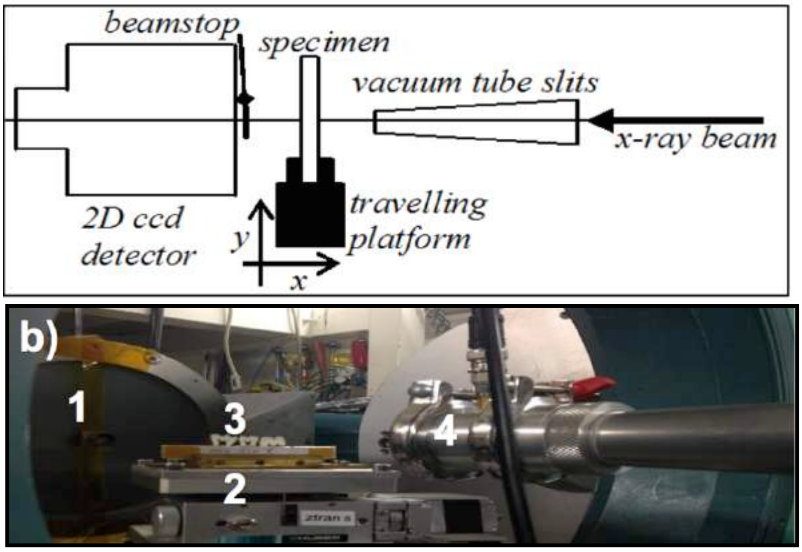



**Figure 5-1: A) Image represents SXRD setup (Al-Jawad et al., 2007) B) Image shows the experiment set up at Diamond beamline; 1. 2D x-ray detector; 2. the adjustable platform; 3. Mounted samples on a hard cardboard; 4. X-ray beam tube.**

The 2D x-ray detector was placed behind the mounted samples so, when the x-ray beam hit the sample, the photons will scatter from the sample and project on the 2D detector creating a diffraction pattern that provides information about the crystallographic structure. The 2D detector is a X-ray Image Star 9000 with 3056 X 3056 pixels and the beam located in its center. We selected several horizontal tracks from DEJ towards the enamel surface within a region of interest in each specimen. Diffraction patterns were collected for selected tracks on the sample (Figure 5-2) and the position of each track in sample was measured from a reference point, which was the tip of the dentine horn of the tooth. The position of the tracks of all samples in the two sets were correlated according the dentine horn reference point therefore, the same enamel region was evaluated in each sample set.

**Figure 5-2: Image shows a sample with the position of the selected tracks on enamel structure moving from DEJ towards enamel surface.**

**Analysing the Diffraction Patterns**

The 2D diffraction patterns demonstrate the existence of crystallographic orientation and texture in the (002) reflection. Yet, to quantify crystallographic texture in the selected areas of interest, further processing was needed. The obtained diffraction patterns were analysed and processed using software called FIT2D (Hammersley, 1997). This software was used to integrate azimuthally the two dimensional diffracted image around the (002) reflection. This produced a one dimensional plot of intensity (Arbitrary unit, AU) against azimuthal angle (degrees) for the (002) reflection. Initially, Originlab Pro 2015 software was used to fit the produced 1D plots to Gaussian curves, to calculate the full width half maximum (FWHM), which represents the width of the produced peaks. Due to the large amounts of data obtained from the SXRD experiments, an automated in-house written programme in MatLab R2015b software was used to batch-analyse the data.

Once the values of FWHM were extracted, they were plotted using Originlab Pro software. These values were plotted against the distance from enamel surface towards DEJ, and only data from enamel FWHM was collected for this study. Higher FWHM values indicate less texture enamel whereas lower values indicate more textured enamel.

## 5.4 Obtaining Diffraction pattern in enamel, dentine and air

The obtained diffraction patterns in this experiment were divided into two diffraction patterns types; enamel and dentine. Figure 5- 3 shows the characteristic features of diffraction pattern obtained from enamel and dentine. Variations observed in the intensity of Bragg reflections in Debye ring are considered a feature of crystallites orientation and texture. So in enamel structure, more variations in the patterns obtained were identified, indicating increased crystals orientation and increased texture.

In the diffraction patterns of the dentine, a wide peak of 002 reflection patterns was observed with fewer variations in the intensity azimuthally indicating reduced dentine texture and crystallites orientation. The preferred crystallites orientation can be determined from the pictorial view of the Debye ring as demonstrated in figure 5- 3. An example of the obtained plot of the intensity of Bragg's reflection against the azimuthal angle is demonstrated in figure 5- 3.

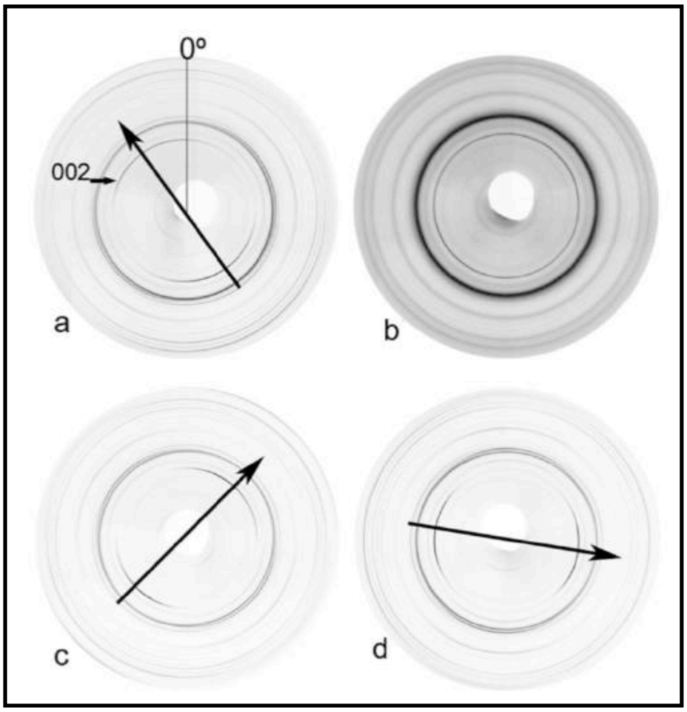
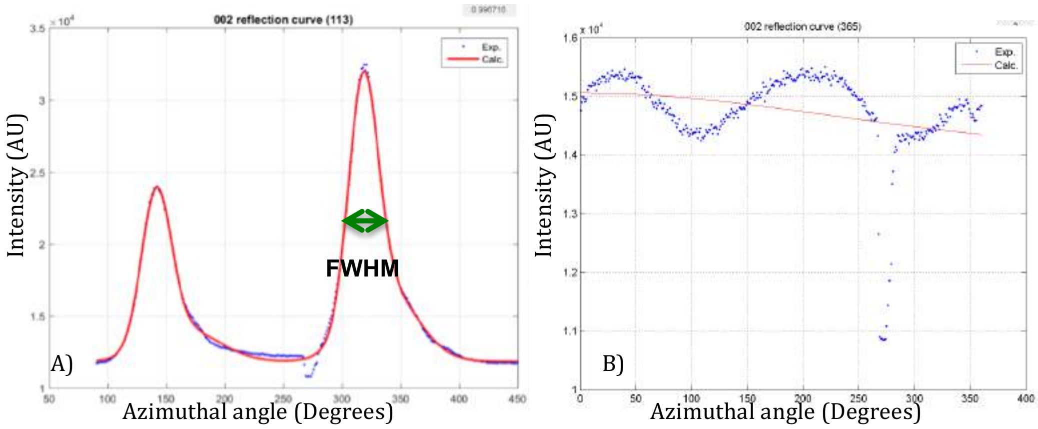



**Figure 5-3: Typical diffraction patterns obtained from this type of experiment. a) Identifying the preferred orientation of the crystallites from 002 reflection. b) Diffraction pattern of the dentine appears as thick 002 reflection with no reflection variations. c) Diffraction pattern of the enamel showing the variations in diffraction pattern. d) Image shows the change in crystal orientation in a different diffraction pattern within the enamel. Reproduced with permission from(Al-Jawad et al., 2007).**




**<u>Figure 5-4: A) Image showing the fitted two peaks that correlates to the two arches in Debye ring. Gaussian curve was fitted to measure the width of the peaks. FWHM is indicated by a green indicator representing the width of the peak. B) Intensity against azimuthal angle plot in dentine.</u>**

Figure 5-4A shows the sharp and intense peaks, which in turn will produce a low FWHM values, this is a typical feature in highly organised crystallites indicating high textured structure. While in diffraction pattern that have broad and less intense peaks will give high FWHM values indicating less or lack of crystallites organisation and less textured structure (Figure 5- 4B).

The resultant plots of enamel diffraction patterns often consist of two peaks that represents the “head” and “tail” of a single population of crystallites oriented in the same direction within the enamel structure. However, in the samples tested in this project, four peaks in 002 reflection of the diffracted pattern were recognised meaning that the enamel structure in the selected regions consists of two populations of crystals with two distinct orientation directions (see Figure (5- 5)). The average FWHM of the first population (Peak 1 and Peak 3) was calculated and the average FWHM of the second population was calculated (Peak 2 and Peak 4) (Figure 5- 5) then the values plotted as a function of distance (μm) from the enamel surface (towards the EDJ) (Figure 5-6).

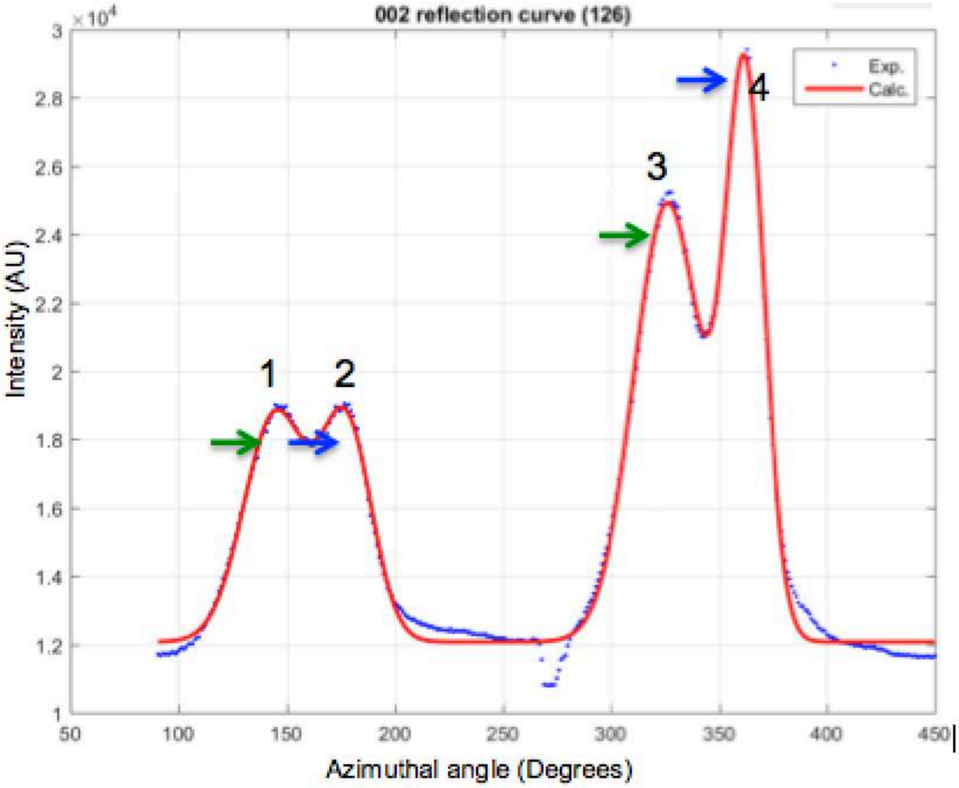



**Figure 5-5: Image shows four peaks, which represent two populations in the enamel structure. First population is indicated by green arrows, second population indicted by the blue arrows.**

## 5.5 Results

### Control samples

The results of six samples were plotted. In each sample three tracks were selected according to the distance to the dentine horn and plotted using Orginlab Pro. Figure 5-6A shows the selected tracks of the control sample in set one, and figure 5-6B illustrates FWHM values against the distance (μm) moving from enamel surface towards DEJ. The first population is represented by the black line while the second population is represented by the red line.

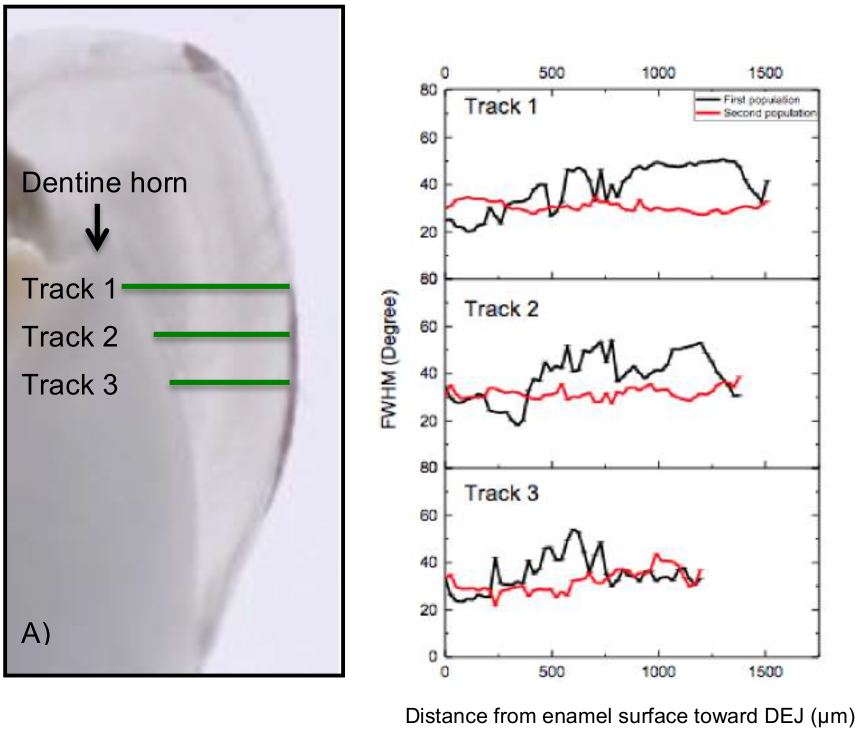


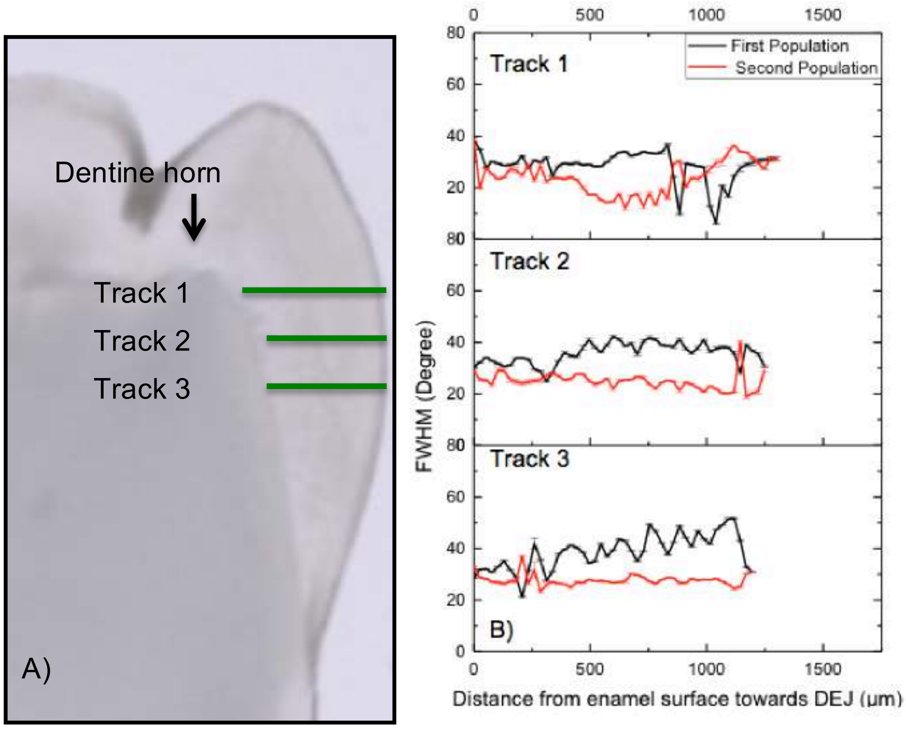

**Figure 5-6: The resultant FWHM values of control sample in set one. A) The position of the tracks in relation to dentine horn represented by green line. B) Graphs show the trends in FWHM as a function of distance (From enamel surface towards DEJ).**

The graph demonstrates the changes in FWHM values, which represents the crystallographic texture of the enamel. In track one of the control sample, the track length within enamel structure was 1600 µm. From the graph of track one, the first 200 µm exhibits low FWHM values, followed by a gradual increase moving further into enamel structure towards the DEJ. While in the second population, FWHM values persisted within the values of 20 to 40$^{o}$ FWHM throughout the track indicating that the second population of crystallites are more highly textured deeper into the bulk of the enamel.

In track 2, the full length of the selected region was 1400 µm, which was shorter than track 1 due to the reduced enamel thickness in this region. Low FWHM values can be seen at the first 300 µm from the enamel surface of the first population, the values then increased gradually towards the DEJ. For the second cycle, the trend of FWHM values remained at the same level throughout the enamel thickness (30- 40 degrees).

In track 3, at the enamel surface, low FWHM values (20 degrees) increased to 40 degrees at 250μm depth into enamel structure towards DEJ. For the second population, the trends of FWHM were similar to the previous tracks suggesting highly textured crystallites at this region.

The results for the control sample in set two are demonstrated in figure 5- 7. The position of the tracks of interest in relation to the dentine horn is indicated by a green line (Figure 5- 7A). Full width half maximum values of the selected tracks were plotted against the distance from enamel surface towards DEJ as demonstrated in Figure 5- 7B.




**<u>Figure 5-7: The resultant FWHM values of control sample in set two. A) The position of the tracks in relation to dentine horn represented by green line. B) Graphs show the trends in FWHM as a function of distance (From enamel surface towards DEJ).</u>**

The first track length of the control sample was about 1300 µm. FWHM values in the first population of track 1 were within 20° to 40° from the enamel surface to around 800 µm into enamel structure. Then FWHM values dropped towards DEJ. In the second population of the first track, FWHM values were within 20°-30° then slightly increased towards DEJ (Figure 5- 7B).

Track 2 of the control tooth, showed values within the same range in FWHM in both the first and second populations, which were positioned mainly between 20° to 40° region of width indicating a highly textured enamel.

Track 3 showed a similar changes of FWHM values at 250 µm from the enamel surface then the values increased gradually towards DEJ for the first population while the second population remained flat until the end of the track.

**SXRD results of natural caries and induced caries like lesion**

**Results of set one (7 days demineralisation)**

The three matched enamel tracks for control, natural caries and induced caries-like lesion (7 days) samples in set one are demonstrated in figure 5-8.

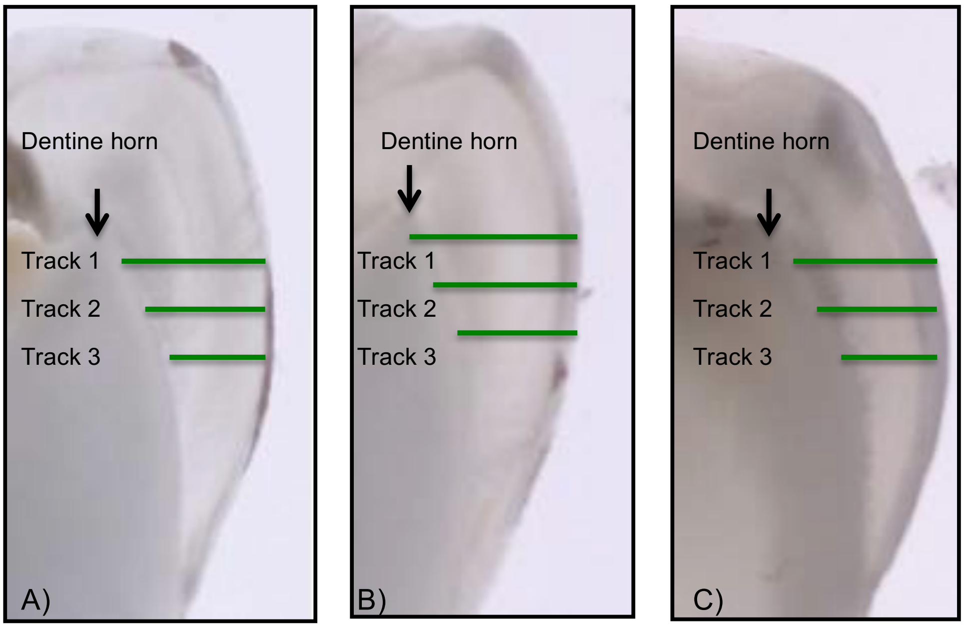



**Figure 5-8: Images of the three selected enamel tracks of the samples in set one; A) Control sample. B) Natural caries lesion sample C) Induced caries like lesion sample (7 days).**

As mentioned previously, the position of enamel tracks was measured from the dentine horn, which is indicated by black arrows on each sample. Then the tracks position were matched for the control, natural and induced caries like lesion sample (Green lines). The values of FWHM of each track are shown in Figure 5-9.

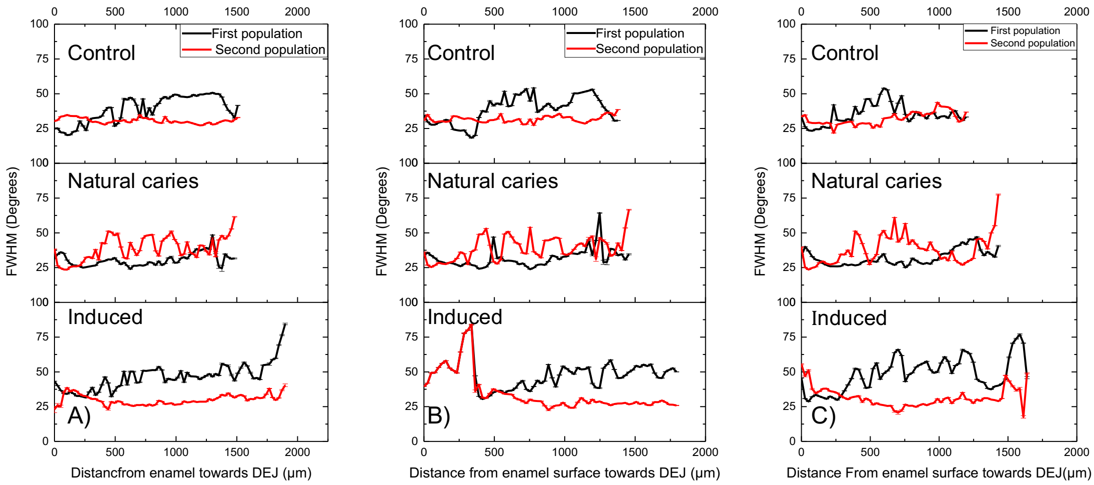



**Figure 5-9: Graphs represent FWHM values; A) Graphs of FWHM of the first track of the control, natural caries, and induced caries like lesion samples. B) Graphs of FWHM of the second tracks of the control, natural and induced caries like lesion samples. C) Graphs of FWHM of the third tracks of the control, natural and induced caries like lesion samples.**

In the first track of the control sample (Figure 5-9A), two populations were identified. FWHM values of the first population started at 25° of width at enamel surface then the values increased to 50° to a distance of 1300 μm into the enamel structure indicating less ordered and less textured crystallites at this region then it descended to 30° at DEJ. The values of FWHM of the second population were almost at the same range indicating only a small variation in the crystallites orientation and enamel texture. In track 1 of the natural caries sample, both populations started at 30° at enamel surface. There were minimal changes in FWHM values throughout the first population track with a slight increase towards DEJ. But in the second population of the same track, FWHM values inclined with a degree of fluctuation at the region between 250 μm and 1500 μm, indicating changes in the preferred orientation of enamel crystals at this region (figure 5-9A). In track 1 of the induced caries like lesion, first population showed a gradual increase towards DEJ suggesting less textured enamel at DEJ region when compared to enamel surface. The second population FWHM values were at the same range along the track.

For the second track of the control sample (figure 5-9B). FWHM values of the first population was at 25° at enamel surface then dropped to 15° at about 400 µm followed by an increase to 45°-50° at a distance of 500 µm from enamel surface before it declined to 40° towards DEJ. The second population of the second track of the control sample was almost the same as the second population of the first track of the same sample. In the natural caries sample, the first and second population showed similar features as track one of the same sample. In the induced caries like lesion sample, FWHM started at 40° as one population that increased to a width of 80° at about 400 µm of enamel surface followed by rapid decline to 25° at 500 µm. At a distance of 500 µm, two populations were identified, first population increased gradually towards DEJ while second population remained at the same region (25°) to the end of the track (figure 5-9B).

For the third track of the control sample, increased FWHM values in the first population at the region between 250 µm to 750 µm indicating change in preferred crystal orientation at this region of enamel structure. The second population showed similar features as the first and second track of the same sample (figure 5-9C). For the natural caries sample, first population FWHM reached the highest approximately 65° at 700 µm from enamel surface then declined to 25° followed by rapid increase toward DEJ. The second population showed low FWHM values along the track with slight increase at Dentine- enamel junction (figure 5-9C). First population of the induced caries like lesion sample showed higher FWHM values when compared to first population of the natural caries sample as it reached to 75° towards DEJ. However, the second population, FWHM started at 50° from enamel surface then decreased to 25° region that persisted then increased to 50° again at DEJ (figure 5- 9C).

**Results of set two (14 days demineralisation)**

Samples of set two (14 days demineralisation) with the position of the selected tracks are demonstrated in Figure 5-10. Tracks were selected in the same way as set one according to the position of the dentine horn. The three tracks were matched in all samples. The graphs corresponding to the matched enamel tracks in control, natural caries lesion and induced caries like lesion samples are demonstrated in figure 5- 10. Track one of each sample is shown in Figure 5-11A. FWHM values for all the samples started at the region of 20° - 40° at enamel surface except for the second population of the natural caries lesion sample which started slightly higher at 45°. In the first population of the induced sample, fluctuations in FWHM values can be seen from 250 µm to 1000 µm. On the other hand, in the first population of the natural caries lesion show a steep increase in FWHM values from 20° to 45° at a distance of 750 µm toward Dentine- enamel junction. And in the second population, an increase of FWHM values at about 200 µm from enamel surface. FWHM values in second population of control and induced caries like lesion sample were low then increase gradually moving to DEJ while for natural caries lesion the values decreased at a distance of 1200 µm from 30° to 15° before raising toward DEJ.

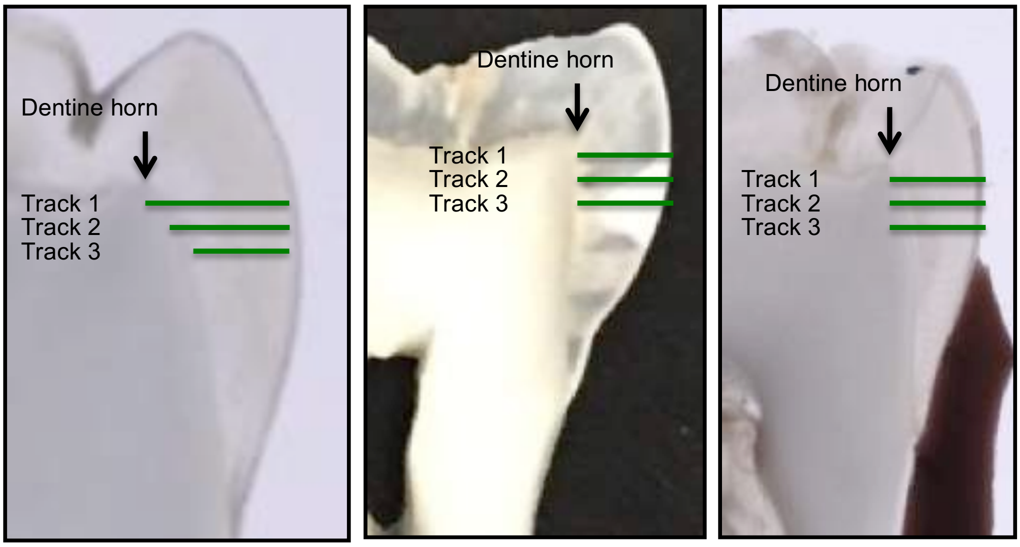



**Figure 5-10: Images of the three selected tracks on the samples of set two (14 days demineralisation) indicated by green line; A) Control sample. B) Natural caries lesion sample. C) Induced caries like lesion sample.**

The second tracks of set two samples (Figure 5-11). In the control sample, the first population had FWHM started at 25° at enamel surface then slightly increased to 40° followed by a decreased to a width of 20° towards DEJ while in the second population the values of FWHM stayed low below 25° until a distance of 1250 µm into enamel structure then a sharp peak was produced with it highest value 40° at DEJ. The values of FWHM in the natural caries sample were low at the surface of the enamel then increased from the distance of 500 µm to 1100 µm then decreased slightly at EDJ and the second population showed a gradual increase in FWHM values from the beginning of the track at the surface to DEJ indicating less textured enamel toward Dentine-enamel junction. The FWHM values in the induced caries like lesion sample in the first population was low at Enamel surface then decreased to 20° at 500 µm distance which persisted to a 1000 µm increased towards DEJ. And for the second population, the values of FWHM increased gradually from the beginning of the tract until 1500 µm to the width of 60° then decreased towards DEJ.

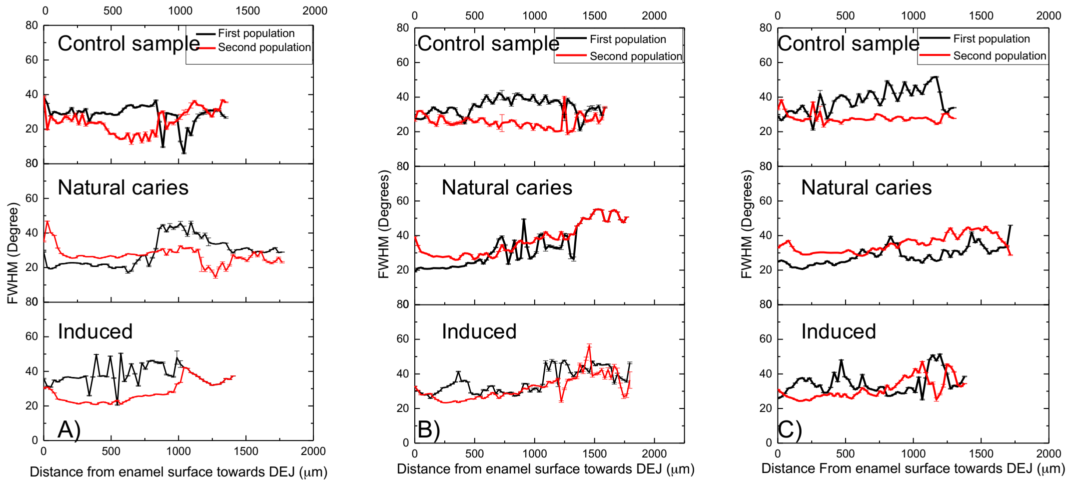



**Figure 5-11: Graphs represent FWHM values against the distance from enamel surface towards DEJ; A) Graphs of FWHM values of the first tracks of the control, natural and induced caries like lesion samples. B) Second tracks of control and natural caries and induced caries like lesion samples. C) Third tracks of the samples.**

Lastly, the FWHM values of the third track of the natural caries sample were the lowest at the surface similar to the value of the control sample, which was 25° followed by a decrease to 25° then an increase towards DEJ. For the second population the values were almost flat with a slight increase at DEJ. The third track of induced caries lesion sample showed a similar range of values as the previous track of the sample however, the highest value was 50° at 1250 µm from the enamel surface. For the second population of the track, FWHM values were at the same range as the second population of the second track of the same sample (figure 5- 11C).

## Discussion

Synchrotron X-ray Diffraction (SXRD) was used to investigate the enamel structure at the crystallite level. The orientation of the crystals was examined to understand the reason behind the different back-scattering responses of the OCT laser. SXRD gives us an information about enamel texture by measuring the intensity of the diffracted light around the Debye ring. As mentioned previously, high FWHM values indicated a less oriented and less textured enamel while low FWHM indicated well oriented crystallites and high textured enamel. In this experiment, each sample was matched with a control and compared in terms of tooth type, surface and track position in relation to the dentine horn as crystallite orientation can be affected by tooth surface, position and tooth type. Two populations were identified in the enamel structure of these samples. It was found that population two had more intensity in the patterns than population one. In the control samples of both sets, the second population, which had more pattern intensity, showed that FWHM values were low at the enamel surface and then increased towards the DEJ meaning that the enamel texture is high at the surface and decreased moving towards DEJ. This finding is consistence with other studies on enamel crystallites (Al-Jawad et al., 2007).

Tooth samples that were demineralised for 7 days showed no changes in enamel texture at the enamel surface, and a slight decrease of texture between 250 μm to 450 μm, which might be due to 7 days exposure to the Lactic acid. Tooth sample with 14 days demineralisation showed a change in texture between 250 μm to 1000 μm that showed increase in FWHM (less texture) indicating a subsurface lesion. More samples with longer period of lactic acid exposure are needed to confirm this finding.

The values of FWHM in the naturel caries lesion showed more severe change in enamel texture of natural caries sample in set two (ICDAS 2) than the natural caries sample in set one (ICDAS 1) -as might be expected - as FWHM values between 750 μm to 1300 μm of enamel structure were high indicating less enamel texture of this region.

The results of SXRD of the control samples showed no changes in enamel texture, which coincides with the homogenous, back scattered light of OCT however, the sharp peak with highest back scattered light at the surface was not correlated to the reduced FWHM values at enamel surface of the samples. There was an increase in enamel texture at the surface of the enamel in natural caries sample (ICDAS 2) in the second set, which was correlated to the increased scattering at OCT images.

The results obtained from the tracks of the samples using SXRD were massive; therefore in the future we might consider taking the average of both populations to facilitate results analysis.

# 6 Assessment of enamel structure by other imaging techniques such as XMT and SEM

## 6.1 Disclaimer

All XMT data presented in this project were obtained in collaboration with Dr Graham Davis at the Institute of Dentistry at Queen Mary University of London (QMUL).

## 6.2 Rationale of this chapter

In this section, the aim was to elucidate the effect of mineral density using XMT and the enamel structure at prism level using SEM to understand the reason behind OCT laser reaction within the enamel structure.

## 6.3 Materials and methods

### 6.3.1 Material and methods of XMT experiment

**MuCat 2 system**

The MuCat 2™ scanner was used in this experiment. This system is a fourth generation scanner, acquired from the first generation system (Elliott and Dover, 1982) in the Department of Dental Physical Science at Queen Mary's University to be used for dental research (Davis et al., 2013). This system employs 225 kV micro-focus X-ray generator with a focal spot of 5 µm in size (Tring, Hertfordshire). It has a demountable source that allows the target and the filaments of the X-ray to be changed to produce a higher power than a fixed source. Both the camera and the sample are placed on a mechanical stage from Physique Instrumente (Palmbach, Germany). The CCD camera with a slow cooled scan, 800S series from (Spectral Instruments Inc, USA) was used with 16 megapixel Fairchild CCD485 sensor connected by a fiber- optic faceplate to a columnar caesium iodide scintillator of 100 µm supplied from Applied Scintillation technologies (Essex, UK).

TDI, Time delay integration, was used in MuCat2™. The samples were placed in the path of the beam therefore; the projection will be detected when the camera move across the beam and the CCD scanner read out simultaneously around 360 degrees.

A 3D image can be constructed from a sequence of 2D images stacked on each other. The CCD camera was mounted at 90 degrees to aid in overcome errors such as ring artefact. A simple representation of the set-up is demonstrated in figure 6-1.

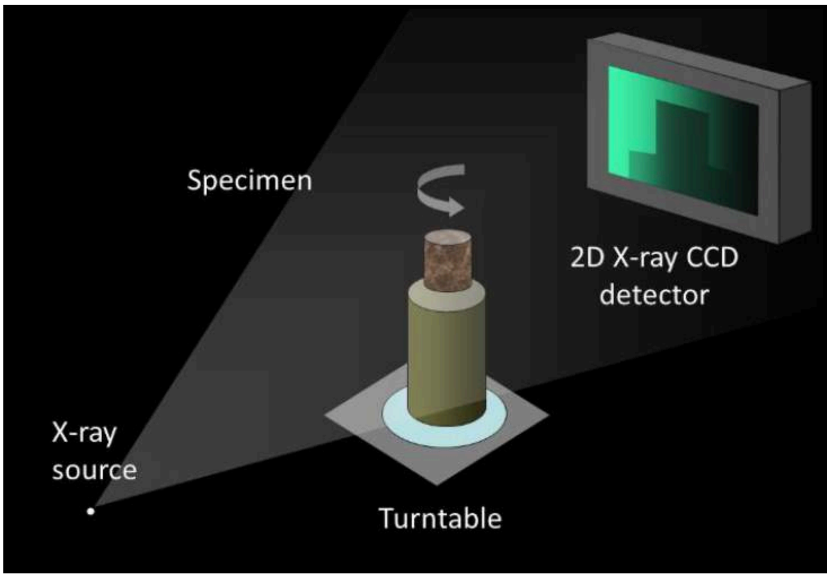



**Figure 6-1: Image to demonstrate X-ray Microtomography (XMT) set-up.**

The 3D images obtained were transferred to Tomview™ software (version 1.1 provided by Graham Davis at QMUL). This software was produced specifically to view and direct MuCat 2™ three dimensional images.

### 6.3.2 Materials and method of SEM experiment

SEM is an advanced imaging technique that is used to provide a high resolution view of the sample's morphological features as well as structural changes that occur at its surface. SEM instrument was manufactured by Philips XL30 (FEI company, the Netherlands). It employs Field Emission gun (FEG-SEM) to enhance the resolution. This method is widely used in many research fields to investigate the materials and obtain a significant information on samples microstructure. SEM electron beam of 5 KeV was generated from SEM electron gun and directed to the samples resulting a discharge of secondary electrons from the surface of interest. The chemical composition and the shape of the samples influence the intensity if the secondary electrons which will eventually be collected on SEM detector.

**Sample preparation For SEM imaging**

A total of six samples were etched using 6% citric acid for 60 seconds in an attempt to remove the smear layer, which was created as a result of sample polishing. Then the samples were washed with distilled water and left to dry for 24 hours. Following this, the samples were placed on an aluminium holder and fixed by adhesive discs. A coat of gold- palladium (Au-Pd) was painted on the surface of the samples (Figure 6-2). Extra-care was taken when treating the samples with Au-Pd to avoid losing any part of the areas of interest in the samples. The reason behind coating the samples is to improve the images resolution by increasing the conductivity between the samples and electron beam.

**Figure 6-2: Image shows the six samples coated with gold- palladium layer and mounted on samples holder to be tested using SEM**

The samples were placed in the samples stud inside a high vacuum chamber of SEM instrument to be analysed (Figure 6- 3).

**Figure 6-3: FEG-SEM instrument.**

## 6.4 Results

### 6.4.1 Results of XMT experiment

High contrast images were extracted from the Tomview™ software. The difference in mineral density (MD) distribution within enamel structure was observed in the grey-scale. A bright coloured region indicated a high mineral density while darker colour scale indicated a low mineral concentration in enamel structure of the selected region. The images of set one; control (C161SS), Natural caries (MIH83) and induced caries like lesion sample (C166SS) are demonstrated in figure 6-4.

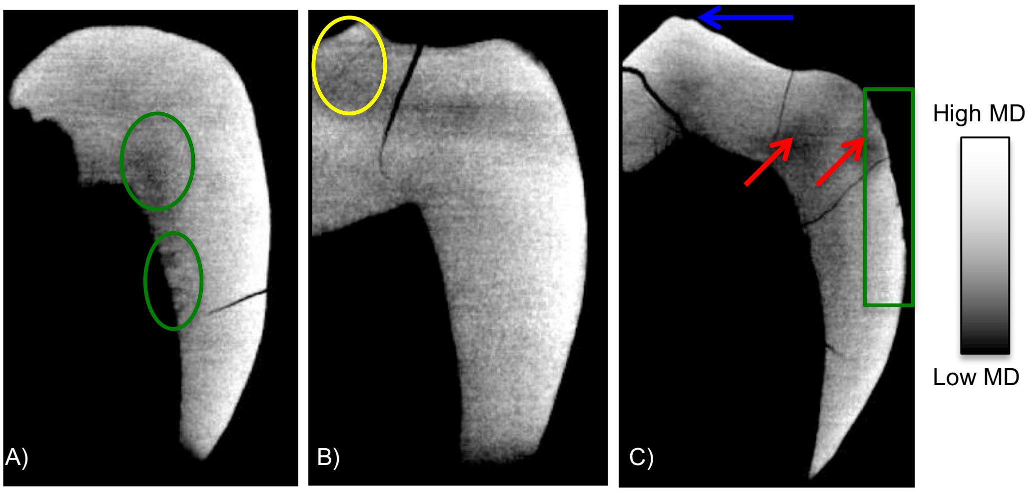



**Figure 6-4: High contrast XMT images show the distribution of mineral concentration of A) Control sample (C161SS), B) Natural caries sample (MIH83), Induced caries like lesion sample (C166SS).**

In the control sample (figure 6-4A), the enamel appeared cracked at the cervical one-third part of enamel surface. The mineral concentration of this sample decreased from the enamel surface towards DEJ. The enamel region near the DEJ just above dentine horn showed decreased mineral concentration compared to other parts of the enamel (green circles). For the natural caries sample (ICDAS 1), the enamel surface appeared intact except at the occlusal surface as vertical cracks were seen extending from the surface towards DEJ (figure 6-4B). The mineral concentration of this sample decreased from the enamel surface to the DEJ as in the previous healthy enamel, however a degree of mineral loss was evident at the occlusal surface, indicated by the yellow circle in figure 6-4B. The enamel of induced caries like lesion sample (figure 6-4C) showed a few cracks over the enamel structure extending all the way to the DEJ. The highest mineral concentration in this sample was at the tip of the cusp (indicated by blue arrows). Darker areas of low mineral concentration were indicated by red arrows. The interproximal enamel surface showed some roughness and irregularities (green box).

Figure 6- 5 shows XMT results of the second set; control sample C162SS, natural caries sample with ICDAS score 2 (60CONTROLAS) and induced caries like lesion sample (C167SS).

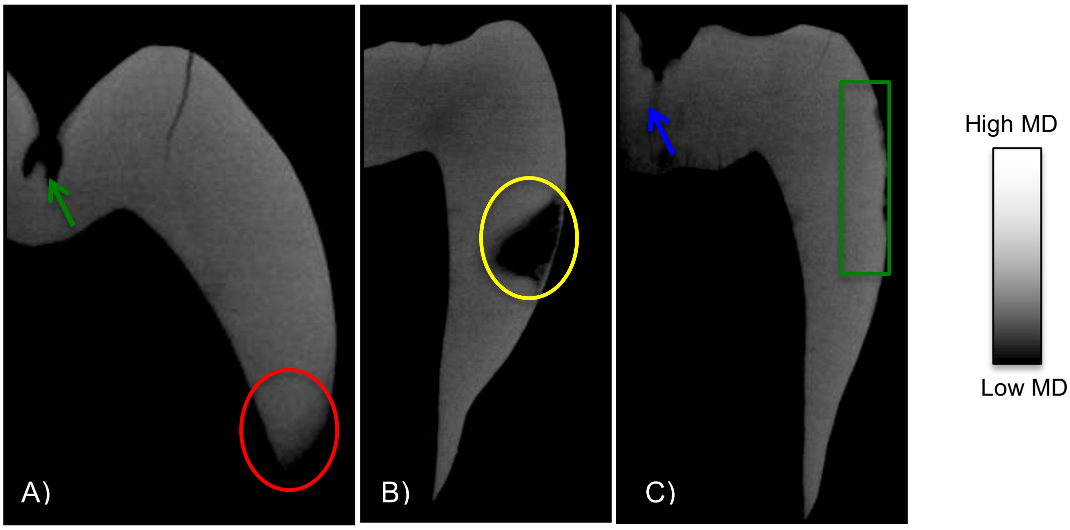



**Figure 6-5: Images show the distribution of mineral concentration of Second set of samples; A) Control sample (C162SS), B) Natural caries sample (60CONTROLAS), C) Induced caries like lesion sample for 14 days (C167SS).**

The control sample showed an intact smooth interproximal enamel surface, however a crack at the cusp region was evident and a dark area at the fissure region was identified, indicating low mineral concentration represented by the green arrow (figure 6- 5A). Mineral concentration throughout the enamel thickness in the healthy sample showed a similar finding as the previous control sample in set one. The dark area at the cervical part of the tooth was not due to mineral loss, but because the sample was not placed flat enough during scanning (red circle). The natural caries sample (ICDAS 2) showed an area of low mineral concentration (yellow circle) traveling from enamel surface towards DEJ (figure 6- 5B). The induced caries like lesion sample (figure 6- 5C) showed a decrease of mineral concentration at the interproximal surface corresponding to the area that was exposed to the lactic acid for 14 days (green box). Also a dark area was identified at fissure region indicating decreased mineral content (Blue arrow).

### 6.4.2 Results of SEM experiment

The result of Scanning Electron Microscope of the control samples of set one (C161SS) and set two (C162SS) are shown in figure 6-6. The images were taken at a scale of 100 µm. Enamel prisms of control sample (C161SS) had a regular arrangement with no signs of prisms dissolution (figure 6-6A). In the control sample (C162SS), horizontal lines can be identified, which is a result of sample polishing however, no signs of prisms destruction was observed (figure 6-6B).

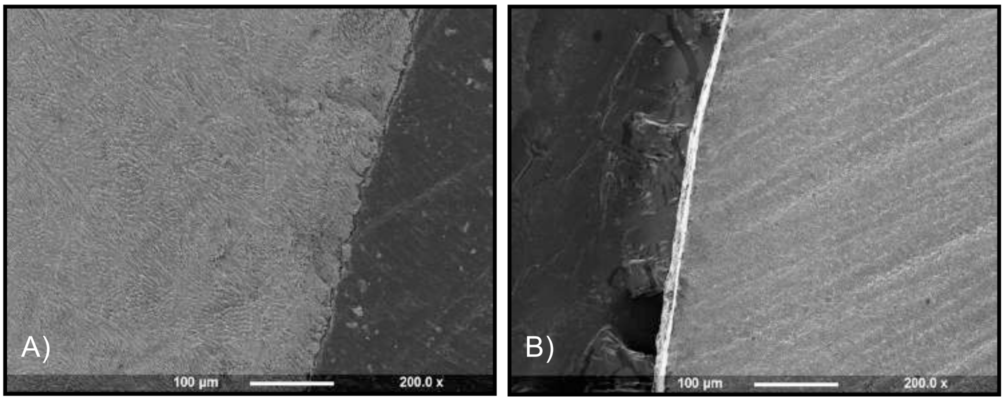



**Figure 6-6: SEM images showing the prismatic structure of the control samples; A) Control sample of set one (C161SS) and B) Control sample of set 2 (C162SS) at a scale of 100 µm with 200x magnification.**

Another region of the control sample (C161SS) that was affected with caries was tested (figure 6-7). The image was taken at 100 µm scale and clearly showed the changes to enamel prisms when they were affected by caries. Prisms were regularly arranged, tightly packed and the boundaries of enamel prisms were identifiable but at the carious part of the enamel, the prisms appeared destructed with loss of enamel prisms boundaries at the carious part as indicated by yellow circle in figure 6-7. An amorphous appearance was identified at the carious lesion as a result of prisms dissolution and the lack of prismatic structure (red arrows).

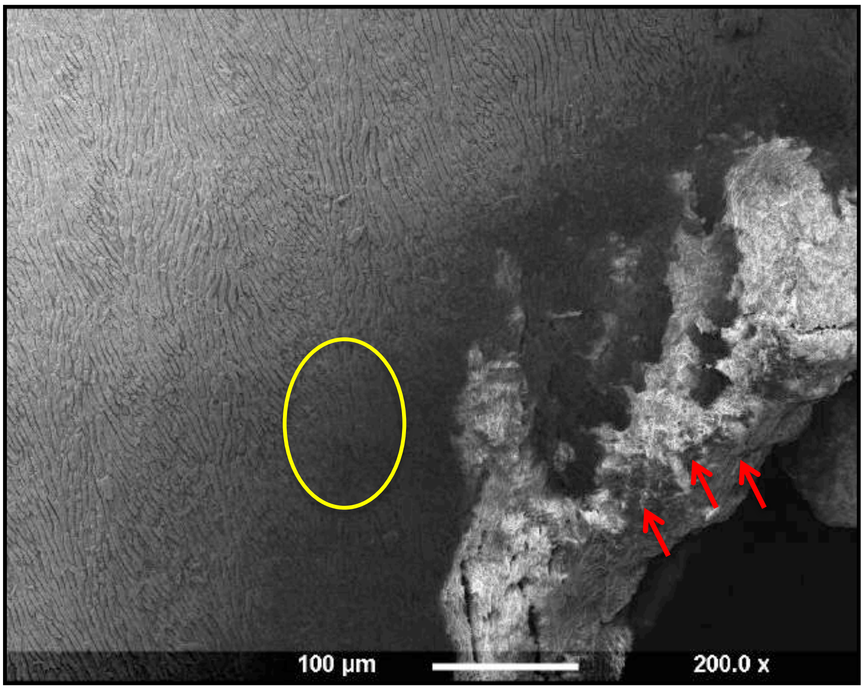



**Figure 6-7: Scanning electron microscope image of a carious lesion.**

The results of natural caries (MIH83) and induced caries like lesion for 7 days (C166SS) samples are shown in figure 6- 8. Both samples were images at 100 µm scale with 200.0x magnification (figure 6- 8A) and 20 µm scale with 20x magnification (figure 6-8B). The image at 100 µm scale showed a dark area at enamel surface indicating a dissolution of enamel prisms. When the sample was investigated with a higher magnification 1000.0x, dissolution of enamel prisms can be identified however the structure of the prisms showed a regular intact prisms moving towards DEJ (figure 6-8B).

The enamel of the induced caries like lesion sample appeared to be coated with a white amorphous material at the enamel surface. Several oblique lines could be identified as a result of sample polishing (green arrows). At 20 µm scale, a difference in enamel prisms orientation can be identified. The amount of prisms dissolution was less when compared with natural caries lesion (blue arrow).

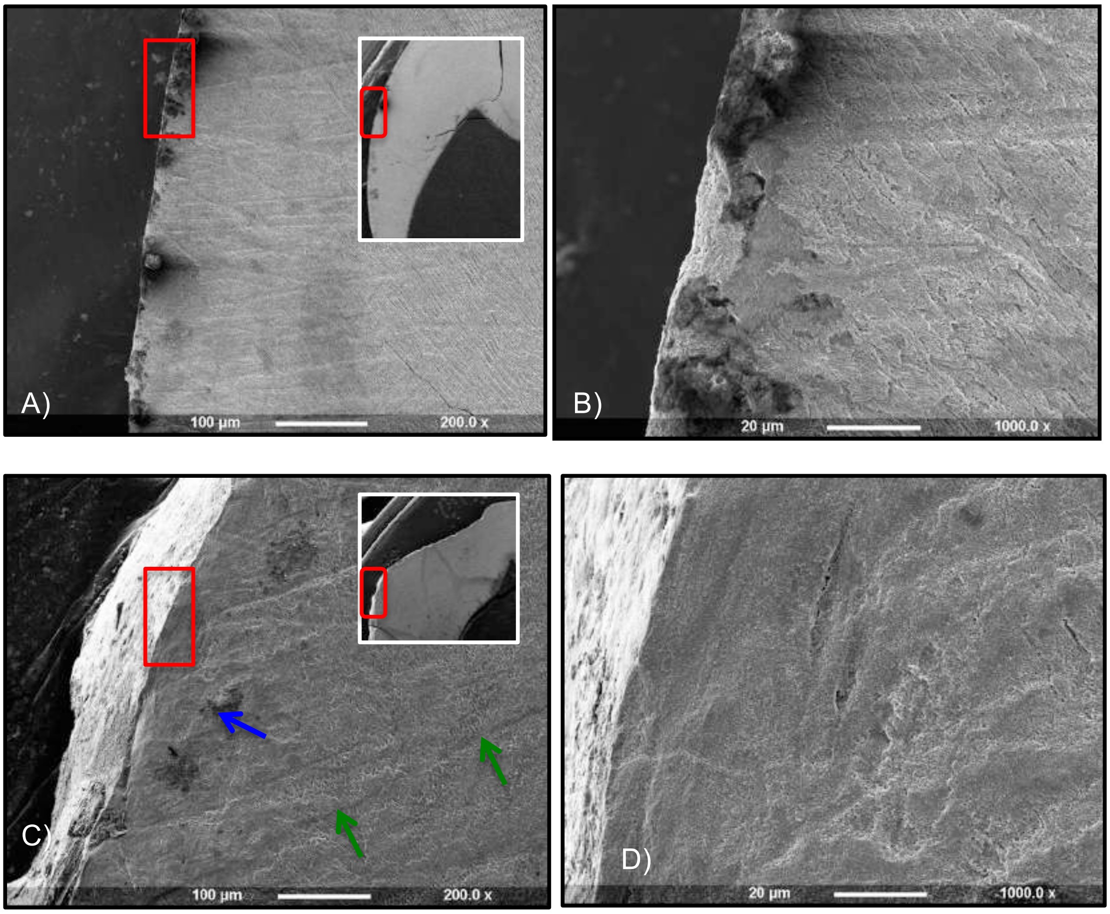



**Figure 6-8: Images show SEM results of samples in set one; A) The natural caries lesion sample (MIH83) at 100 µm scale. B) Natural caries sample at 20 µm scale. C) Induced caries like lesion sample for 7 days (C166SS) at 100 µm scale. D) induced caries like lesion sample at 20 µm.**

Figure 6-9A and B, are natural caries lesion (ICDAS 2) images at 200.0x and 1000.0x magnification respectively, showing a white layer on the enamel surface that appears to be an amorphous material. The prismatic structure appeared destructed with some openings or pores present just underneath this layer indicated by yellow arrows. The prisms had more regular appearance traveling towards DEJ (Figure 6-9A). Enamel microstructure at a higher magnification at 20 µm scale showed dissolution of the prisms, loss of prisms boundaries and structure at enamel surface (figure 6-9A-B).

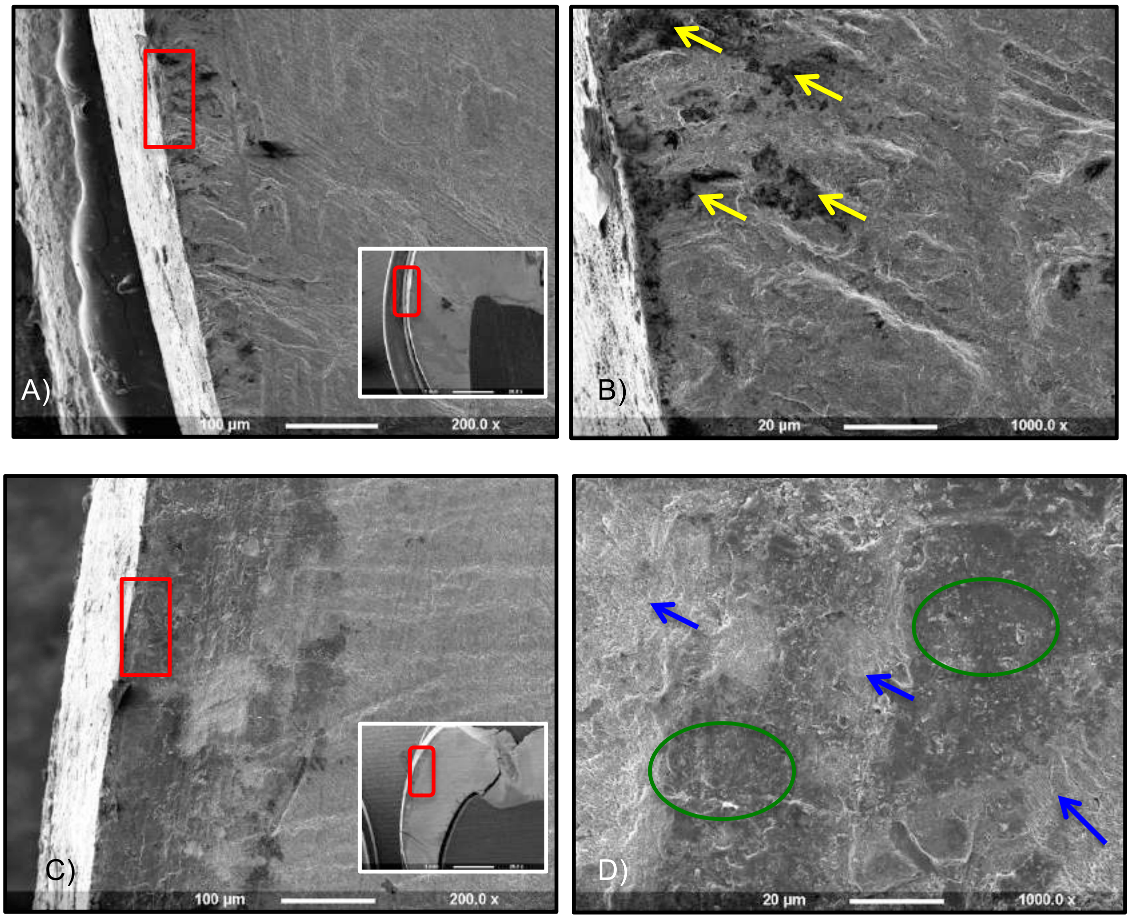



**Figure 6-9: Images show SEM results of the samples in set two; A) Natural caries sample (ICDAS 2) at 100 µm scale. B) Natural caries sample (ICDAS 2) at 20 µm scale. C) Induced caries like lesion sample (14 days) at 100 µm scale. C) Induced caries like lesion sample at 20 µm scale.**

The image of the enamel on the induced caries lesion sample which was exposed to lactic acid for 14 days (C167SS) is shown in figure 6-9C and D. The white amorphous layer was also present at the enamel surface. At a magnification of 200.0x of 100 µm scale, a darker area underneath this layer was identified indicating dissolution of enamel prisms. The same sample when imaged at a higher magnification of 1000.0x at 20 µm scale, the prisms boundaries could not be identified with uneven enamel structure (green circle). The amount of enamel prisms disolution was less severe when compared to the natural caries sample. In figure 6-9D, enamel structure showed areas with normal enamel prisms (blue arrows) in between areas that appeared to have prisms disolution.

## 6.5 Discussion

### Demineralisation protocol

Several protocols have been developed in attempt to produce caries like lesion in vitro. The use of organic acids is generally accepted as a demineralisation agent to induced caries in dental enamel, however enamel that was exposed to such acids alone showed changes which differ histologically from the structural changes observed in natural caries lesion (Ingram and Silverstone, 1981). It is known that the initiation of caries must be associated with the presence of acids but dental caries is not a simple process of demineralisation due to acids dissolution. The presence of plaque is important in order to modify the acid attack and create the characteristic features of natural caries lesion. Using a technique that employs viscous gel to mimic plaque then acidified with organic acid is important to simulate caries in vitro (Ingram and Silverstone, 1981). The protocol used in this project to induce caries like lesion was 8% methylcellulose to mimic dental plaque buffered with the acid that is produced by fermentation of the carbohydrates.

### Samples Storage protocol

All the samples were stored in 0.1% Thymol as per the Department policy. However, the use of thymol as a storage medium can influence the optical properties of the samples. Shi et al conducted a study to test the influence of the storage medium on the reading of DIAGNOdnet. They found that the teeth that were stored in thymol showed a decrease in the fluorescence when compared to teeth stored in formalin (Shi et al., 2001). Francescut et al., conducted a study to evaluate the influence of commonly used storage medium, on the infrared laser fluorescence response. They concluded that teeth stored in formaline, chloramine and thymol showed a statistically significant reduction in light fluorescence intensity over 2 years (Francescut et al., 2006).

### ICDAS

In this project, ICDAS was used as the gold standard for caries detection. This system has only two codes to characterise carious white spot lesions; code 1 and code 2. The reason behind choosing this system was because it's considered a standardised caries detection clinical tool based on evidence. However, this technique cannot assess the extent of the lesion into enamel structure and it is not 100% accurate in detecting early carious lesions. In the future, considering another technique such as polarised microscope as the gold standard is required.

### X-ray Microtomography (XMT)

XMT technique provided an understanding of the enamel structure in healthy enamel as well as enamel that was affected by early carious lesion. It gave a qualitative evaluation of mineral density of enamel in both control and caries affected samples, which was represented by a grey-scale image of the samples sections.

In this study, healthy enamel samples showed a slightly higher mineral density at the surface of the enamel, which decreased gradually as you moved to the DEJ. Many authors reported this trend of mineral density gradient. Wong et.al reported the primary teeth showed an increase of mineral density from DEJ to enamel surface (Wong et al., 2004). Also Fearne et.al reported that the mineral density increased from the DEJ to the surface of the enamel (Fearne et al., 2004). However, another study found that mineral density is highest at the cusp region and decreased towards the cervical area (Farah et al., 2010).

A decrease in mineral density was found in the region that was affect by caries when compared to other healthy enamel parts of the same sample of natural caries sample (60CONTROLAS). This sample was scored 2 in the ICDAS scoring system.

In the induced caries like lesion samples, the sample that was exposed to lactic acid for 7 days showed an irregular enamel surface, which indicates a decrease in mineral density at the surface of the enamel. Also an area of decreased mineral density was observed throughout the bulk of the enamel. Irregularities at enamel surface were also observed on the surface of the enamel surface of the induced caries like lesion sample (C167SS), which corresponded to the window that was created to expose the surface to the lactic acid.

### Scanning Electron Microscope (SEM)

The SEM technique gave an insight into the prismatic structure of the area of interest. Healthy enamel samples showed a packed enamel prisms with distinctive prisms boundaries. A key feature identified in many samples was the presence of an amorphous layer on the enamel surface, which could be an indication of remineralisation. Within this layer some pores can be identified. These pores can act as channels that enable the diffusion through this layer aiding in surface layer regeneration. Many authors reported similar findings (Palamara et al., 1986, Worawongvasu, 2015).

In the natural caries samples, (MIH83 and 60CONTROLAS), The prismatic structure showed an intermittent regular and irregular packing of enamel prisms with pores or concavities that were scattered in the outer enamel structure. These concavities represent the destruction/ dissolution of enamel prisms within this region. Prisms dissolution were severe and more deeper in natural caries sample with ICDAS score 2 (60CONTROLAS) than natural caries sample with ICDAS score 1 (MIH83).

In the 7 days and 14 days demineralisation samples (C166SS and C167SS respectively), The area of destructed enamel prisms were present corresponding to the area that was exposed to lactic acid 7 and 14 days. The 14 days demineralisation sample, loss of prisms boundaries can be observed with alternating areas of regularly packed prisms and irregularly packed prisms. However, natural caries samples showed an advanced disolution of enamel prisms when compared to the induced caries like lesions samples.

In XMT and SEM experiments, enamel cracks and desiccation were evidenced on the images obtained (Figure 6-4, 6-5, 6-8 and 6-9). This is due to the process of the sample preparation for SEM imaging and the tendency of enamel delamination during XMT and SEM imaging.

# 7 Synopsis of all imaging modalities

The aim of this project was to investigate the potential of developing the OCT as a clinical diagnostic tool for incipient caries. An investigation of the enamel structure using well-established techniques such as polarised microscope, SXRD, XMT and SEM. The reason behind employing these imaging techniques was to gain a better understanding of OCT laser response to healthy and affected enamel microstructure.

## OCT and X-ray Microtomography (XMT)

When we compared OCT results with XMT in chapter 6, we found that the healthy enamel samples had an even distribution of mineral density through the enamel structure between the DEJ and enamel surface but mineral density decreased at the outer enamel surface in the samples that were affected with natural incipient caries. This finding corresponded to OCT results of healthy and affected samples. In b-scans of the healthy samples, the enamel appeared homogenous with only increased back-scattered light intensity at the meeting surface between the air and enamel surface. The a-scans of the same samples showed a sharp peak at air/enamel interface then light intensity decreased gradually towards DEJ. Enamel surface that was affected with incipient caries exhibited a low mineral density when compared with other enamel regions in the same sample. These regions were identified in the b-scans of OCT as well as the a-scans as a persistence of high intensity of the back-scattered light in the affected regions.

By comparing OCT results with XMT outcomes, am important correlation had observed between both techniques. But, XMT technique can not be used to detect early carious lesions clinically because it is associated with high radiation exposure as well as long time required to obtain a scan.

**OCT and Synchrotron X-ray Diffraction (SXRD)**

Synchrotron X-ray diffraction technique is a well established and advanced method used to evaluate the enamel at crystallites level. Enamel crystal orientation and texture were examined to check if the results observed when the samples were scanned by OCT was due to enamel crystallites orientation and organisation.

In this project, it was essential to select and match the control with natural caries and induced caries like lesion samples in terms of tooth type, tooth surface and location. This is because the effect of these factors on crystallites orientation as it was found that there is a difference identified in crystal orientation from one tooth to another as well as one part to another within the same tooth (Al-Jawad et al., 2007). It was possible in this project to match two sets of samples that were matched with regards to tooth natation, surface and part within the sample. The analysis of the obtained data from SXRD experiment showed the lesion did not affect that crystallites orientation and texture as mentioned previously in chapter 5.

By comparing OCT and SXRD results, it was found that there is some correlation between OCT response and crystallites texture in the healthy enamel and that affected by caries. The OCT result of the natural caries sample with ICDAS score 2 (60CONTROLAS) showed an increase in the back-scattered light to a distance of about 500 μm from enamel surface, corresponding to a lower texture in the enamel from enamel surface to around 200 μm from SXRD data. Texture in the second population of the control sample (C162SS) showed an almost flat trend throughout the track from enamel surface towards DEJ, which coincides with OCT result of the sample except for the high peak of back-scattered light at air/enamel interface. Further investigation is needed to confirm these correlations.

**OCT and Scanning Electron Microscope (SEM) and Polarised Microscope**

Scanning Electron Microscope technique investigates enamel structure at prismatic level. The comparison done between SEM and OCT showed that there is a correlation between prisms disolution and back-scattered light intensity of OCT laser. For example, in the control samples, OCT b-scans images showed a homogenous grey-scale scattering of OCT laser, which correlated to the well packed prisms of enamel structure at the same region. SXRD results could correlates to SEM as FWHM values observed in natural caries sample (60CONTROLAS) were high at the outer enamel surface indicating less organised and textured enamel crystals and at prisms level, the prisms were dissolved with no distinctive prism boundaries.

The images obtained from the polarised microscope identified the presence and the depth of the caries lesion into enamel structure, which was corresponded with OCT b-scans and a-scans.

A table that summaries the findings and correlation between all the imaging techniques is shown in Table 7-1.

## Table 7-1: A summary of the imaging modalities.

| Type of lesion | OCT | SXRD | XMT | SEM |
|---|---|---|---|---|
| Healthy samples | • Enamel and dentine can be identified.<br>• Homogenous pattern of scattering intensity throughout enamel structure | • FWHM values trend was almost flat with little increase towards DEJ indicating a gradual decrease in texture and orientation towards DEJ. | • Mineral density distribution was uniform throughout enamel structure with gradual decrease towards DEJ. | • Regular enamel prisms with no signs of dissolution |
| Natural caries samples | • Only enamel can be identified.<br>• An increase in scattering intensity to a distance of 100 µm from enamel surface in sample (MIH83) and about 500 µm from enamel surface of sample (60CONTROLAS). | • A decrease in enamel texture and orientation in the region of 250 to 1500 µm (MIH83).<br>• Decrease in texture and orientation to a distance of 200 µm from enamel surface. | • The sample (60CONTROLAS) with ICDAS score 2 showed low mineral density at enamel surface extending towards DEJ. | • Enamel dissolution at the surface of the enamel at 100 µm scale.<br>• Amorphous layer can be identified at enamel surface of the sample (60CONTROLAS). |
| Induced caries like lesion samples | - Only enamel can be identified.<br>- Slight increase in scattering intensity at enamel surface about 50 µm in the 7 days demineralisation sample, and an increased scattering intensity of about 200 µm from enamel surface in 14 days demineralisation sample. | • Decrease in enamel texture in the distance between 250 to 450 µm in the 7 days demineralisation sample and between 250 to 1000 µm in 14 days demineralisation. | • Roughness at the surface of the sample indicating low mineral density at the area exposed to lactic acid. | • Amorphous layer covering the enamel in both samples.<br>• Minimal enamel prism dissolution at enamel surface. |

## 8 Limitations and future work

The sample size was one of the limitations in this project. This is because of the restricted criteria of having an incipient caries lesion that matched with control sample of the same tooth type and surface. Also incipient caries lesion is not considered an indication for teeth extraction, which means that the collection of samples was also limited.

Another limitation was the setup of SXRD experiment. This experiment was highly competitive, as the proposal had to be peer-reviewed to be accepted. Also it has time restriction and it was difficult to repeat so it had to be well planned prior to the experiment date to gain the maximum utilisation of experiment time.

In the future, the samples should be exposed to a longer period to the demineralisation protocol, 8% methylcellulose gel buffered with lactic acid of 0.1 mol/L to create a subsurface lesion in the enamel structure. Also the oral cavity should be simulated by using a phantom head model with a pink modelling wax rims at the maxilla and mandibular region to maximise the representation of the clinical setting. OCT probe used in this project was not made to be used for intra-oral examination, so developing a smaller movable OCT probe is important in order to be used in the clinical set-up. Furthermore, increasing the number of samples is required to confirm the observed findings and to confirm that OCT can diagnose and monitor the changes in mineral density that occurs in carious lesions.

## 9 Clinical relevance

Dental caries is considered one of the most common oral diseases, affecting 60 – 90% of school children worldwide (Petersen, 2003). Early detection and treatment of incipient caries is crucial to prevent pain and further enamel destruction. To date, there is no one universal diagnostic tool that can be used to detect carious lesions at the very early stages. In this project, the aim was to evaluate Optical Coherence tomography and to understand its light response to enamel changes so a better clinical diagnostic tool can be developed to identify early changes in enamel structure when it is affected by caries. The findings in this project showed that OCT is able to detect and assess the depth of WSL. OCT is considered not ionizing technique so teeth will be examined without exposing the patient to radiation hazard. OCT is a simple technique that does not consume a long time to investigate enamel structure. Employing OCT in clinical setting will aid in early caries diagnosis, which consequently will help in early intervening and reversing demineralisation process. Also, it can be used evaluate the tooth after removing the carious lesion ensuring that all the lesion has been removed before restoring the tooth with the restorative material to maximise its durability. Also, OCT might have a great relevance in monitoring the remineralisation and demineralisation process in vivo.

# 10 Conclusion

Evaluation of the potential of Optical Coherence Tomography (OCT) in detecting early carious white spot lesion was the main aim of this project. The production of artificial caries-like lesion on healthy enamel was performed to confirm that OCT is capable to detect the early changes that occur in the enamel structure at the early carious process. The demineralisation protocol used was able to produce a caries-like lesion, however longer period is required to create more advanced subsurface lesion. It was found that carious lesion had an effect on enamel mineral density and enamel prisms. Dental caries could have an effect on crystallite texture and orientation, however further investigation is required.

The variations observed in the back-scattered light in OCT experiment were because of mineral density variation within enamel structure as well as the changes in prismatic structure and may be related to crystallite texture and orientation. Further investigation of incipient caries lesion is required to confirm this finding.

# 11 References


AL-JAWAD, M., ADDISON, O., KHAN, M. A., JAMES, A. & HENDRIKSZ, C. J. 2012. Disruption of enamel crystal formation quantified by synchrotron microdiffraction. *Journal of dentistry,* 40**,** 1074-1080.

AL-JAWAD, M. & ANDERSON, P. 2014. Biomineralization and Biodemineralization of Enamel. *Handbook of Oral Biomaterials***,** 57.

AL-JAWAD, M., STEUWER, A., KILCOYNE, S. H., SHORE, R. C., CYWINSKI, R. & WOOD, D. J. 2007. 2D mapping of texture and lattice parameters of dental enamel. *Biomaterials,* 28**,** 2908-2914.

AMAECHI, B. T., PODOLEANU, A. G., KOMAROV, G., HIGHAM, S. M. & JACKSON, D. A. 2004. Quantification of root caries using optical coherence tomography and microradiography: a correlational study. *Oral health & preventive dentistry,* 2.

AN, B., WANG, R. & ZHANG, D. 2012. Role of crystal arrangement on the mechanical performance of enamel. *Acta biomaterialia,* 8**,** 3784-3793.

ARENDS, J. & TEN CATE, J. 1981. Tooth enamel remineralization. *Journal of Crystal Growth,* 53**,** 135-147.

ATTRILL, D. & ASHLEY, P. 2001. Diagnostics: Occlusal caries detection in primary teeth: a comparison of DIAGNOdent with conventional methods. *British dental journal,* 190**,** 440-443.

AZEVEDO, C. S. D., TRUNG, L. C. E., SIMIONATO, M. R. L., FREITAS, A. Z. D. & MATOS, A. B. 2011. Evaluation of caries-affected dentin with optical coherence tomography. *Brazilian oral research,* 25**,** 407-413.

AZINOVIĆ, Z., KEROS, J., BUKOVIĆ, D. & AZINOVIĆ, A. 2003. SEM analysis of tooth enamel. *Collegium antropologicum,* 27**,** 381-386.

ÅRTUN, J. & BROBAKKEN, B. O. 1986. Prevalence of carious white spots after orthodontic treatment with multibonded appliances. *The European Journal of Orthodontics,* 8**,** 229-234.

BAUMGARTNER, A., DICHTL, S., HITZENBERGER, C., SATTMANN, H., ROBL, B., MORITZ, A., FERCHER, A. & SPERR, W. 2000. Polarization–sensitive optical coherence tomography of dental structures. *Caries research,* 34**,** 59-69.

BESIC, F. C. & WIEMANN, M. 1972. Dispersion staining, dispersion, and refractive indices in early enamel caries. *Journal of dental research,* 51**,** 973-985.

BETA.CLASSMINT.COM. 2017. *Classmint.com - Awesome Study Notes*. [online] Available at: http://beta.classmint.com [Accessed 17 Jul. 2017].

BOURQUIN, S., AGUIRRE, A., HARTL, I., HSIUNG, P., KO, T., FUJIMOTO, J., BIRKS, T., WADSWORTH, W., BÜNTING, U. & KOPF, D. 2003. Ultrahigh resolution real time OCT imaging using a compact femtosecond Nd: Glass laser and nonlinear fiber. *Optics express,* 11**,** 3290-3297.

BOYDE, A. 1975. Scanning electron microscopy of enamel surfaces. *British medical bulletin,* 31**,** 120-124.

BOYDE, A., 1989. Enamel. In *Teeth* (pp. 309-473). Springer Berlin Heidelberg.

CHOMA, M. A., SARUNIC, M. V., YANG, C. & IZATT, J. A. 2003. Sensitivity advantage of swept source and Fourier domain optical coherence tomography. *Optics express,* 11**,** 2183-2189.

COCHRANE, N., ANDERSON, P., DAVIS, G., ADAMS, G., STACEY, M. & REYNOLDS, E. 2012. An X-ray microtomographic study of natural white-spot enamel lesions. *Journal of dental research,* 91**,** 185-191.

COOPER, D., MATYAS, J., KATZENBERG, M. & HALLGRIMSSON, B. 2004. Comparison of microcomputed tomographic and microradiographic measurements of cortical bone porosity. *Calcified tissue international,* 74**,** 437-447.

CULLITY, B. 1978. Element of X-ray diffraction, Addition–Wesley. *Reading, MA***,** 100.

DARLING, A., MORTIMER, K., POOLE, D. & OLLIS, W. 1961. Molecular sieve behaviour of normal and carious human dental enamel. *Archives of oral biology,* 5**,** 251-273.

DARLING, C. L., HUYNH, G. D. & FRIED, D. 2006. Light scattering properties of natural and artificially demineralized dental enamel at 1310nm. *Journal of biomedical optics,* 11**,** 034023-034023-11.

DAVIS, G. & ELLIOTT, J. 1997. X-ray microtomography scanner using time-delay integration for elimination of ring artefacts in the reconstructed image. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment,* 394**,** 157-162.

DAVIS, G., EVERSHED, A., ELLIOTT, J. & MILLS, D. Quantitative X-ray microtomography with a conventional source. SPIE Optical Engineering+ Applications, 2010. International Society for Optics and Photonics, 78040I-78040I-8.

DAVIS, G. R., EVERSHED, A. N. & MILLS, D. 2013. Quantitative high contrast X-ray microtomography for dental research. *Journal of dentistry,* 41**,** 475-482.

DEERY, C. 2013. Caries detection and diagnosis, sealants and management of the possibly carious fissure. *British dental journal,* 214**,** 551-557.

EHRLICH, H., KOUTSOUKOS, P. G., DEMADIS, K. D. & POKROVSKY, O. S. 2009. Principles of demineralization: Modern strategies for the isolation of organic frameworks: Part II. Decalcification. *Micron,* 40**,** 169-193.

EKSTRAND, K., QVIST, V. & THYLSTRUP, A. 1987. Light microscope study of the effect of probing in occlusal surfaces. *Caries research,* 21**,** 368-374.

EKSTRAND, K., RICKETTS, D. & KIDD, E. 1997. Reproducibility and accuracy of three methods for assessment of demineralization depth on the occlusal surface: an in vitro examination. *Caries research,* 31**,** 224-231.

ELLIOTT, J. & DOVER, S. 1982. X-ray microtomography. *Journal of Microscopy,* 126**,** 211-213.

FARAH, R., DRUMMOND, B., SWAIN, M. & WILLIAMS, S. 2010. Linking the clinical presentation of molar-incisor hypomineralisation to its mineral density. *International Journal of Paediatric Dentistry,* 20**,** 353-360.

FEARNE, J., ANDERSON, P. & DAVIS, G. 2004. 3D X-ray microscopic study of the extent of variations in enamel density in first permanent molars with idiopathic enamel hypomineralisation. *British dental journal,* 196**,** 634-638.

FEATHERSTONE, J., MAYER, I., DRIESSENS, F., VERBEECK, R. & HEIJLIGERS, H. 1983. Synthetic apatites containing Na, Mg, and CO3 and their comparison with tooth enamel mineral. *Calcified Tissue International,* 35**,** 169-171.

FEJERSKOV, O. 1997. Concepts of dental caries and their consequences for understanding the disease. *Community dentistry and oral epidemiology,* 25**,** 5-12.

FELDCHTEIN, F., GELIKONOV, V., IKSANOV, R., GELIKONOV, G., KURANOV, R., SERGEEV, A., GLADKOVA, N., OURUTINA, M., REITZE, D. & WARREN, J. 1998. In vivo OCT imaging of hard and soft tissue of the oral cavity. *Optics Express,* 3**,** 239-250.

FERCHER, A., HITZENBERGER, C. & JUCHEM, M. 1991. Measurement of intraocular optical distances using partially coherent laser light. *Journal of Modern Optics,* 38**,** 1327-1333.

FERCHER, A. F. 2010. Optical coherence tomography–development, principles, applications. *Zeitschrift für Medizinische Physik,* 20**,** 251-276.

FERCHER, A. F., DREXLER, W., HITZENBERGER, C. K. & LASSER, T. 2003. Optical coherence tomography-principles and applications. *Reports on progress in physics,* 66**,** 239.

FRANCESCUT, P., ZIMMERLI, B. & LUSSI, A. 2006. Influence of different storage methods on laser fluorescence values: a two-year study. *Caries research,* 40**,** 181-185.

FRIED, D., XIE, J., SHAFI, S., FEATHERSTONE, J. D., BREUNIG, T. M. & LE, C. 2002. Imaging caries lesions and lesion progression with polarization sensitive optical coherence tomography. *Journal of biomedical optics,* 7**,** 618-627.

GIANNOPOULOU, C., DUDIC, A. & KILIARIDIS, S. 2006. Pain discomfort and crevicular fluid changes induced by orthodontic elastic separators in children. *The Journal of Pain,* 7**,** 367-376.

GORELICK, L., GEIGER, A. M. & GWINNETT, A. J. 1982. Incidence of white spot formation after bonding and banding. *American journal of orthodontics,* 81**,** 93-98.

GUVEN, G., CEHRELI, Z. C., ALTUN, C., ŞENÇIMEN, M., IDE, S., BAYARI, S. H. & KARAÇAY, Ş. 2008. Mucopolysaccharidosis type I (Hurler syndrome): oral and radiographic findings and ultrastructural/chemical features of enamel and dentin. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology,* 105**,** 72-78.

HABELITZ, S., MARSHALL, S. J., MARSHALL, G. W. & BALOOCH, M. 2001. The functional width of the dentino-enamel junction determined by AFM-based nanoscratching. *Journal of structural biology,* 135**,** 294-301.

HARIRI, I., SADR, A., NAKASHIMA, S., SHIMADA, Y., TAGAMI, J. & SUMI, Y. 2012a. Estimation of the enamel and dentin mineral content from the refractive index. *Caries research,* 47**,** 18-26.

HARIRI, I., SADR, A., SHIMADA, Y., TAGAMI, J. & SUMI, Y. 2012b. Effects of structural orientation of enamel and dentine on light attenuation and local refractive index: an optical coherence tomography study. *Journal of dentistry,* 40**,** 387-396.

HARTL, I., LI, X., CHUDOBA, C., GHANTA, R., KO, T., FUJIMOTO, J., RANKA, J. & WINDELER, R. 2001. Ultrahigh-resolution optical coherence tomography using continuum generation in an air–silica microstructure optical fiber. *Optics letters,* 26**,** 608-610.

HARUNA, M., OHMI, M., MITSUYAMA, T., TAJIRI, H., MARUYAMA, H. & HASHIMOTO, M. 1998. Simultaneous measurement of the phase and group indices and the thickness of transparent plates by low-coherence interferometry. *Optics letters,* 23**,** 966-968.

HEE, M. R., SWANSON, E. A., FUJIMOTO, J. G. & HUANG, D. 1992. Polarization-sensitive low-coherence reflectometer for birefringence characterization and ranging. *JOSA B,* 9**,** 903-908.

HEINRICH-WELTZIEN, R., KÜHNISCH, J., VAN DER VEEN, M., DE JOSSELIN DE JONG, E. & STÖBER, L. 2003. Quantitative light-induced fluorescence (QLF)--a potential method for the dental practitioner. *Quintessence international,* 34.

HOPCRAFT, M. S. & MORGAN, M. V. 2005. Comparison of radiographic and clinical diagnosis of approximal and occlusal dental caries in a young adult population. *Community dentistry and oral epidemiology,* 33**,** 212-218.

HSIEH, Y.-S., HO, Y.-C., LEE, S.-Y., LU, C.-W., JIANG, C.-P., CHUANG, C.-C., WANG, C.-Y. & SUN, C.-W. 2011. Subgingival calculus imaging based on swept-source optical coherence tomography. *Journal of biomedical optics,* 16**,** 071409-071409-8.

HUANG, D., SWANSON, E. A., LIN, C. P., SCHUMAN, J. S., STINSON, W. G., CHANG, W., HEE, M. R., FLOTTE, T., GREGORY, K. & PULIAFITO, C. A. 1991. Optical coherence tomography. *Science (New York, NY),* 254**,** 1178.

HUANG, T. T., HE, L.-H., DARENDELILER, M. A. & SWAIN, M. V. 2010. Correlation of mineral density and elastic modulus of natural enamel white spot lesions using X-ray microtomography and nanoindentation. *Acta biomaterialia,* 6, 4553-4559.

HUANG, T. T., JONES, A. S., HE, L. H., DARENDELILER, M. A. & SWAIN, M. V. 2007. Characterisation of enamel white spot lesions using X-ray micro-tomography. *Journal of dentistry,* 35, 737-743.

INGERVALL, B. 1962. The influence of orthodontic appliances on caries frequency. *Odontol Revy,* 13, 175-190.

INGRAM, G. & SILVERSTONE, L. 1981. A chemical and histological study of artificial caries in human dental enamel in vitro. *Caries research,* 15, 393-398.

ISMAIL, A., SOHN, W., TELLEZ, M., AMAYA, A., SEN, A., HASSON, H. & PITTS, N. 2007. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. *Community dentistry and oral epidemiology,* 35, 170-178.

JOHANSEN, E. 1965. Comparison of the ultrastructure and chemical composition of sound and carious enamel from human permanent teeth. *Tooth enamel.* Wright Bristol.

JOHNSON, N. 1967. Some aspects of the ultrastructure of early human enamel caries seen with the electron microscope. *Archives of oral biology,* 12, 1505IN231519IN251521-1518IN241520IN26.

JONES, R., DARLING, C., FEATHERSTONE, J. & FRIED, D. 2006a. Imaging artificial caries on the occlusal surfaces with polarization-sensitive optical coherence tomography. *Caries research,* 40, 81-89.

JONES, R. S., DARLING, C. L., FEATHERSTONE, J. D. & FRIED, D. 2006b. Remineralization of in vitro dental caries assessed with polarization-sensitive optical coherence tomography. *Journal of biomedical optics,* 11, 014016-014016-9.

JÄLEVIK, B., DIETZ, W. & NORÉN, J. 2005. Scanning electron micrograph analysis of hypomineralized enamel in permanent first molars. *International Journal of Paediatric Dentistry,* 15, 233-240.

KAY, M. I., YOUNG, R. & POSNER, A. 1964. Crystal structure of hydroxyapatite. *Nature,* 204, 1050.

KEREBEL, B., DACULSI, G. & KEREBEL, L. 1979. Ultrastructural studies of enamel crystallites. *Journal of dental research,* 58, 844-851.

KIDD, E. & FEJERSKOV, O. 2004. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. *Journal of dental research,* 83, C35-C38.

KIENLE, A., MICHELS, R. & HIBST, R. 2006. Magnification—a new look at a long-known optical property of dentin. *Journal of dental research,* 85, 955-959.

KINNEY, J. H. & NICHOLS, M. C. 1992. X-ray tomographic microscopy (XTM) using synchrotron radiation. *Annual review of materials science,* 22, 121-152.

KNU, A., BONEV, S. & KNAAK, W. 2004. New method for evaluation of in vivo scattering and refractive index properties obtained with optical coherence tomography. *Journal of biomedical optics,* 9**,** 265-273.

KOCH, P., HÜTTMANN, G., SCHLEIERMACHER, H., EICHHOLZ, J. & KOCH, E. 2004. Linear optical coherence tomography system with a downconverted fringe pattern. *Optics letters,* 29**,** 1644-1646.

KÜHNISCH, J., IFLAND, S., TRANÆUS, S., HICKEL, R., STÖSSER, L. & HEINRICH-WELTZIEN, R. 2007. In vivo detection of non-cavitated caries lesions on occlusal surfaces by visual inspection and quantitative light-induced fluorescence. *Acta Odontologica Scandinavica,* 65**,** 183-188.

LEITGEB, R., HITZENBERGER, C. & FERCHER, A. F. 2003. Performance of fourier domain vs. time domain optical coherence tomography. *Optics express,* 11**,** 889-894.

LOW, I. M. 2004. Depth-Profiling of Crystal Structure, Texture, and Microhardness in a Functionally Graded Tooth Enamel. *Journal of the American Ceramic Society,* 87**,** 2125-2131.

LYNCH, C. D., O'SULLIVAN, V., DOCKERY, P., MCGILLYCUDDY, C. T., REES, J. S. & SLOAN, A. J. 2011. Hunter–Schreger band patterns and their implications for clinical dentistry. *Journal of Oral Rehabilitation,* 38**,** 359-365.

LYNCH, C. D., O'SULLIVAN, V. R., DOCKERY, P., MCGILLYCUDDY, C. T. & SLOAN, A. J. 2010. Hunter-Schreger Band patterns in human tooth enamel. *Journal of Anatomy,* 217**,** 106-115.

LYNCH, R. J. 2013. The primary and mixed dentition, post-eruptive enamel maturation and dental caries: a review. *International dental journal,* 63**,** 3-13.

MAIA, A., FONSECA, D. D., KYOTOKU, B. B. & GOMES, A. S. 2010. Characterization of enamel in primary teeth by optical coherence tomography for assessment of dental caries. *International Journal of Paediatric Dentistry,* 20**,** 158-164.

MANESH, S. K., DARLING, C. L. & FRIED, D. 2009. Polarization-sensitive optical coherence tomography for the nondestructive assessment of the remineralization of dentin. *Journal of biomedical optics,* 14**,** 044002-044002-6.

MANJI, F. & FEJERSKOV, O. 1990. Dental caries in developing countries in relation to the appropriate use of fluoride. *Journal of dental research,* 69**,** 733-741.

MARGOLIS, H., BENIASH, E. & FOWLER, C. 2006. Role of macromolecular assembly of enamel matrix proteins in enamel formation. *Journal of dental research,* 85**,** 775-793.

MARSHALL, G., BALOOCH, M., GALLAGHER, R., GANSKY, S. & MARSHALL, S. 2001. Mechanical properties of the dentinoenamel junction: AFM studies of nanohardness, elastic modulus, and fracture. *Journal of biomedical materials research,* 54**,** 87-95.

MCCOMB 2000. Caries-detector dyes-how accurate and useful are they? *J Can Dent Assoc,* 66**,** 195-8.

MECKEL, A., GRIEBSTEIN, W. & NEAL, R. 1965. Structure of mature human dental enamel as observed by electron microscopy. *Archives of oral biology,* 10, 775IN3783-782IN8.

MENG, Z., YAO, X. S., YAO, H., LIANG, Y., LIU, T., LI, Y., WANG, G. & LAN, S. 2009. Measurement of the refractive index of human teeth by optical coherence tomography. *Journal of biomedical optics,* 14, 034010-034010-4.

MILLY, H., FESTY, F., ANDIAPPAN, M., WATSON, T. F., THOMPSON, I. & BANERJEE, A. 2015. Surface pre-conditioning with bioactive glass air-abrasion can enhance enamel white spot lesion remineralization. *Dental Materials,* 31, 522-533.

NANCI, A. 2003. Ten Cate's oral histology: development, structure, and function. Mosby. *Inc.: New York*, 256-257.

NANCI, A. 2012. *Ten Cate's Oral Histology Development, Structure, and Function, 8/e*, Elsevier India.

OHMI, M., OHNISHI, Y., YODEN, K. & HARUNA, M. 2000. In vitro simultaneous measurement of refractive index and thickness of biological tissue by the low coherence interferometry. *IEEE transactions on biomedical engineering,* 47, 1266-1270.

OTIS, L. L., EVERETT, M. J., SATHYAM, U. S. & COLSTON, B. W. 2000. Optical coherence tomography: A new imaging: Technology for dentistry. *The Journal of the American Dental Association,* 131, 511-514.

ØGAARD, B., RØLLA, G. & ARENDS, J. 1988. Orthodontic appliances and enamel demineralization: Part 1. Lesion development. *American Journal of Orthodontics and Dentofacial Orthopedics,* 94, 68-73.

PALAMARA, J., PHAKEY, P., RACHINGER, W. & ORAMS, H. 1986. Ultrastructure of the intact surface zone of white spot and brown spot carious lesions in human enamel. *Journal of Oral Pathology & Medicine,* 15, 28-35.

PETERSEN, P. E. 2003. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century–the approach of the WHO Global Oral Health Programme. *Community Dentistry and oral epidemiology,* 31, 3-24.

PETERSEN, P. E., BOURGEOIS, D., OGAWA, H., ESTUPINAN-DAY, S. & NDIAYE, C. 2005. The global burden of oral diseases and risks to oral health. *Bulletin of the World Health Organization,* 83, 661-669.

PITTS, N. & STAMM, J. W. 2004. International Consensus Workshop on Caries Clinical Trials (ICW-CCT)—final consensus statements: agreeing where the evidence leads. *Journal of Dental Research,* 83, C125-C128.

POOLE, D. & BROOKS, A. 1961. The arrangement of crystallites in enamel prisms. *Archives of oral biology,* 5, 14IN5-26.

POVAZAY, B., BIZHEVA, K., UNTERHUBER, A., HERMANN, B., SATTMANN, H., FERCHER, A. F., DREXLER, W., APOLONSKI, A., WADSWORTH, W. &

KNIGHT, J. 2002. Submicrometer axial resolution optical coherence tomography. *Optics letters,* 27**,** 1800-1802.

PRETTY, I. A. 2006. Caries detection and diagnosis: novel technologies. *Journal of dentistry,* 34**,** 727-739.

PRICE, D. 2005. The human tissue act 2004. *The Modern Law Review,* 68**,** 798-821.

PRYMAK, O., TIEMANN, H., SÖTJE, I., MARXEN, J. C., KLOCKE, A., KAHL-NIEKE, B., BECKMANN, F., DONATH, T. & EPPLE, M. 2005. Application of synchrotron-radiation-based computer microtomography (SRµCT) to selected biominerals: embryonic snails, statoliths of medusae, and human teeth. *JBIC Journal of Biological Inorganic Chemistry,* 10**,** 688-695.

ROBINSON, C., KIRKHAM, J. & SHORE, R. 1995. *Dental enamel: formation to destruction*, CRC.

ROBINSON, C., SHORE, R., BROOKES, S., STRAFFORD, S., WOOD, S. & KIRKHAM, J. 2000. The chemistry of enamel caries. *Critical Reviews in Oral Biology & Medicine,* 11**,** 481-495.

SALVO, L., CLOETENS, P., MAIRE, E., ZABLER, S., BLANDIN, J., BUFFIÈRE, J.-Y., LUDWIG, W., BOLLER, E., BELLET, D. & JOSSEROND, C. 2003. X-ray micro-tomography an attractive characterisation technique in materials science. *Nuclear instruments and methods in physics research section B: Beam interactions with materials and atoms,* 200**,** 273-286.

SCHROEDER, H. E. & SCHERLE, W. F. 1988. Cemento-enamel junction–revisited. *Journal of periodontal research,* 23**,** 53-59.

SELWITZ, R. H., ISMAIL, A. I. & PITTS, N. B. 2007. Dental caries. *The Lancet,* 369**,** 51-59.

SHI, X.-Q., TRANAEUS, S. & ANGMAR-MÅNSSON, B. 2001. Validation of DIAGNOdent for quantification of smooth-surface caries: an in vitro study. *Acta Odontologica Scandinavica,* 59**,** 74-78.

SIDDIQUI, S. 2014. *Crystallographic and Microstructural Studies of Dental Enamel using Synchrotron X-ray Diffraction and Complementary Techniques.* Queen Mary University of London.

SIDDIQUI, S., ANDERSON, P. & AL-JAWAD, M. 2014. Recovery of crystallographic texture in remineralized dental enamel. *PloS one,* 9**,** e108879.

SILVERSTONE, L. Translucent zone of enamel lesions produced in vitro. journal of dental research, 1965. sage publications inc 2455 teller rd, thousand oaks, ca 91320 usa, 1174-+.

SILVERSTONE, L. 1967a. Observations on the dark zone in early enamel caries and artificial caries-like lesions. *Caries research,* 1**,** 261-274.

SILVERSTONE, L. M. 1966. The primary translucent zone of enamel caries and of artificial caries-like lesions. *British dental journal,* 120**,** 461-471.

SILVERSTONE, L. M. 1967b. Observations on the dark zone in early enamel caries and artificial caries-like lesions. *Caries research,* 1, 261-274.

SIMMER, J. P. & FINCHAM, A. G. 1995. Molecular mechanisms of dental enamel formation. *Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists,* 6, 84.

SIMMONS, L. M., AL-JAWAD, M., KILCOYNE, S. H. & WOOD, D. J. 2011. Distribution of enamel crystallite orientation through an entire tooth crown studied using synchrotron X-ray diffraction. *European journal of oral sciences,* 119, 19-24.

SONG, G., WANG, X., REN, H., ZHANG, W., ZHANG, L. & FANG, Z. 2000. Simultaneous measurements of the thickness and refractive index of microstructures in obscure specimen by optical coherence tomography. *Optik,* 111, 541-543.

STOOKEY, G. 2004. Optical methods—quantitative light fluorescence. *Journal of Dental Research,* 83, C84-C88.

SUNDSTROM, F., FREDRIKSSON, K., MONTAN, S., HAFTSTROM-BJORKMAN, U. & STROM, J. 1985. Laser-induced Fluorescence From Sound and Carious Tooth Substance-Spectroscopic Studies. *Swedish Dental Journal,* 9, 71-80.

SWANSON, E. A., IZATT, J., LIN, C., FUJIMOTO, J., SCHUMAN, J., HEE, M., HUANG, D. & PULIAFITO, C. 1993. In vivo retinal imaging by optical coherence tomography. *Optics letters,* 18, 1864-1866.

TERPSTRA, R. & DRIESSENS, F. 1986. Magnesium in tooth enamel and synthetic apatites. *Calcified tissue international,* 39, 348-354.

TWETMAN, S., AXELSSON, S., DAHLÉN, G., ESPELID, I., MEJÀRE, I., NORLUND, A. & TRANÆUS, S. 2013. Adjunct methods for caries detection: a systematic review of literature. *Acta Odontologica Scandinavica,* 71, 388-397.

VERNON-PARRY, K. 2000. Scanning electron microscopy: an introduction. *III-Vs Review,* 13, 40-44.

VON BARTHELD, F. 1961. Membrane phenomena in carious dissolution of the teeth. *Archives of Oral Biology,* 4, 120-121.

WELZEL, J. 2001. Optical coherence tomography in dermatology: a review. *Skin Research and Technology,* 7, 1-9.

WEST, N. X. & JOINER, A. 2014. Enamel mineral loss. *Journal of Dentistry,* 42, S2-S11.

WILSON, R., ELLIOTT, J. & DOWKER, S. 1999. Rietveld refinement of the crystallographic structure of human dental enamel apatites. *American mineralogist,* 84, 1406-1414.

WILSON, R. M., ELLIOTT, J. C., DOWKER, S. E. & SMITH, R. I. 2004. Rietveld structure refinement of precipitated carbonate apatite using neutron diffraction data. *Biomaterials,* 25, 2205-2213.

WONG, F., ANDERSON, P., FAN, H. & DAVIS, G. 2004. X-ray microtomographic study of mineral concentration distribution in deciduous enamel. *Archives of Oral Biology,* 49, 937-944.

WORAWONGVASU, R. 2015. A Scanning Electron Microscopic Study of Enamel Surfaces of Incipient Caries. *Ultrastructural pathology,* 39, 408-412.

YANAGISAWA, T. & MIAKE, Y. 2003. High-resolution electron microscopy of enamel-crystal demineralization and remineralization in carious lesions. *Journal of electron microscopy,* 52, 605-613.

YILMAZ, E. D., SCHNEIDER, G. A. & SWAIN, M. V. 2015. Influence of structural hierarchy on the fracture behaviour of tooth enamel. *Phil. Trans. R. Soc. A,* 373, 20140130.

YIP, H., STEVENSON, A. & BEELEY, J. 1994. The specificity of caries detector dyes in cavity preparation. *British dental journal,* 176, 417-421.

YOUNG, R. 1974. Implications of atomic substitutions and other structural details in apatites. *Journal of dental research,* 53, 193-203.

YOUNG, R. & MACKIE, P. 1980. Crystallography of human tooth enamel: initial structure refinement. *Materials Research Bulletin,* 15, 17-29.

ZACHRISSON, B. U. & ZACHRISSON, S. 1971. Caries incidence and orthodontic treatment with fixed appliances. *European Journal of Oral Sciences,* 79, 183-192.

# 12 APPENDIX 1

## Parent Information Leaflet

**A Study of the genetics and the physical properties of dental anomalies**

**Research team:**

**Dr Susan Parekh,**

**Dr Agnes Bloch Zupan,**

**Dr Peter Brett,**

**Dr Laurent Bozec**

**Mashael Abdullatif**

**Nurjehan Ibrahim**

**Nabilah Harith**

**Amanda O'Donnell**


**Contact details:**

Dr Susan Parekh

Tel: 020 3456 1067

Fax: 020 3456 2329

Unit of Paediatric Dentistry

The Eastman Dental Hospital and Institute

256 Gray's Inn Road

London WC1X 8LD

s.parekh@eastman.ucl.ac.uk

Website: www.uclh.nhs.uk


UCL Hospitals cannot accept responsibility for information provided by external organisations

**UCL HOSPITALS**

If you need a large print, audio or translated copy of this document, please contact us on 020 3456 1067. We will try our best to meet your needs.

If you wish to discuss this study with a member of the research team or an independent expert who is not part of the research team, please ask Dr Susan Parekh

Thank you for taking the time to read this leaflet.



University College London Hospitals NHS Foundation Trust

A study of the genetics and the physical properties of dental anomalies


Publication date: 07/07/11
Date last reviewed
Version number 2


1

**Invitation**

Your child is being invited to take part in a research study. Before you make a decision, it is important that you know why the research is being done and what it would involve from your child. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if anything is not clear at any time before or after participating. If you need more information we are willing to spend more time to satisfy you before taking any decision.

**What is the purpose of the study?**

To gather more information about dental anomalies, such as enamel defects (Amelogenesis Imperfecta), and dentine defects (Dentinogenesis Imperfecta). We want to use this information to improve our knowledge of genetics and how they affect the properties of the teeth.

**Why has my child been chosen?**

We are asking all patients who have been diagnosed with dental anomalies and members of their families with the same or other dental conditions to participate in the study.

**Does my child have to take part?**

No. It is up to you to decide. If you do decide to participate we will ask you to sign a consent form. If you, or your child, change your mind, you are free to withdraw at any time, without giving a reason. The standard of care your child receives will not be affected in any way.

**What will happen to me if my child takes part?**

We will ask you and your child some questions about your child's teeth, take photographs, and a saliva sample. The saliva sample will be used to link your child's DNA with the physical properties of their teeth. We will also measure the colour of the front teeth using a machine called the spectroshade™ micro, which rests gently on the teeth and uses a light to record the shade of the tooth (see information sheet provided). If any teeth need to be extracted as part of your child's treatment, these will be collected for laboratory testing of the teeth. Your child will not need to do anything else. If any member of your family has similar teeth, we will invite them to take part as well, as this will help to detect the common dental genes in families. If you do not want other members of your family to participate, you can refuse and your child's treatment will not be affected in any way.

**What are the possible disadvantages or risks of taking part?**

There are no risks anticipated. None of your answers will affect your treatment in any way.

**What are the possible benefits?**

We cannot promise the study will help you, but the information we get might help treat young people with dental anomalies in the future.

**What will happen with the results?**

Any samples that we collect will be stored using a study ID number, so that they cannot be directly linked to your child. We hope to publish the results of the study on completion.

**Will taking part in the study remain confidential?**

Yes. We will keep your information in confidence. This means we will only tell those who have a need or right to know. The safety and security of the data will be the responsibility of the principal investigator (Dr Susan Parekh). The information will also be stored in a database developed by Strasbourg University (phenodent database), who we work closely with. All information will be anonymised before putting on the phenodent database.

**What happens if something goes wrong?**

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against UCLH NHS Trust, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

**Who has reviewed the study?**

All research in the NHS is looked at by independent group, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Joint Research Ethics Committee. Thank you for reading this, please ask any questions if you need to.

2

# 13 APPENDIX 2

**Research team:**
**Dr Susan Parekh,**
**Dr Agnes Bloch Zupan,**
**Dr Peter Brett,**
**Dr Laurent Bozec**
**Mashael Abdullatif**
**Nurjehan Ibrahim**
**Nabilah Harith**
**Amanda O'Donnell**

**Contact details:**
Dr Susan Parekh
Tel: 020 3456 1067
Fax: 020 3456 2329
Unit of Paediatric Dentistry
The Eastman Dental Hospital and Institute
256 Gray's Inn Road
London WC1X 8LD
s.parekh@eastman.ucl.ac.uk
Website: www.uclh.nhs.uk

UCL Hospitals cannot accept responsibility for information provided by external organisations.

UCL HOSPITALS

If you need a large print, audio or translated copy of this document, please contact us on 0207 915 1022. We will try our best to meet your needs.

If you wish to discuss this study with a member of the research team or an independent expert who is not part of the research team, please ask Dr Susan Parekh

Thank you for taking the time to read this leaflet.


University College London Hospitals NHS
A study of the genetics and the physical properties of dental anomalies

Publication date: 08/08/11
Date last reviewed
Version number 3

## Patient's Information Leaflet

**A Study of the genetics and the physical properties of dental anomalies**

**Invitation**

You are being invited to take part in a research study. Before you make a decision, it is important that you know why the research is being done and what it would involve from you. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if anything is not clear at any time before or after participating. If you need more information we are willing to spend more time to satisfy you before taking any decision.

**What is the purpose of the study?**

To obtain and gather more information about dental anomalies, such as Enamel defects (Amelogenesis Imperfecta AI), and dentine defects (Dentinogenesis Imperfecta DI). We want to use this information to improve our knowledge of genetics and the properties of the teeth, to provide better support and long term care.

**Why has I have been chosen?**

We are asking all patients who have been diagnosed with dental anomalies and members of their families with the same or other dental conditions to participate in the study.

**Do I have to take part?**

No. It is up to you to decide. If you do decide to participate we will ask you to sign a consent form. If you change your mind, you are free to withdraw at any time, without giving a reason. The standard of care you will receive will not be affected in any way.

**What will happen to me if I take part?**

We will ask you some questions about your teeth and your medical history, and examine your teeth, take photographs, and a saliva sample. The saliva sample will be used to link your DNA with the physical properties of your teeth. If you require any teeth to be extracted as part of your treatment, these will be collected for laboratory testing of the teeth. You will not need to do anything else. If any member of your family has similar teeth, we will invite them to take part as well, as this will help to detect the common dental genes in families. If you do not want other members of your family to participate, you can refuse and your treatment will not be affected in any way.

**What are the possible disadvantages or risks of taking part?**

There are no risks anticipated. None of your answers will affect your treatment in any way.

**What are the possible benefits?**

The information from this study will hopefully be used to help us expand our knowledge about the genetics of dental anomalies, and relate this to the appearance of the teeth, identify affected families and provide better support and treatment.

**What will happen with the results?**

Any samples that we collect will be stored using a study ID number, so that they cannot be directly linked to you. We hope to publish the results of the study on completion. All confidential information will be coded and you will not be identifiable in any way.

**Will my taking part in the study remain confidential?**

Yes. All information that is collected about you during the research will remain strictly confidential and will be seen only by the investigators named on this sheet. The safety and security of the data will be the responsibility of the principal investigator (Miss Susan Parekh). This information will be recorded in such a way that it is completely anonymous and you cannot be individually identified in anyway.

The information will also be stored in a database developed by Strasbourg University (phenodent database), who we work closely with. All information will be anonymised before putting on the phenodent database.

**Who has reviewed the study?**

All research in the NHS is looked at by independent group, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Joint Research Ethics Committee. If you would like to see a summary of the findings from the study when it is completed, please tell Miss Parekh or any of the other dentists you see.

## 14 APPENDIX 3

University College London Hospitals NHS
NHS Foundation Trust

**The Eastman Dental Hospital**
256 Gray's Inn road
London
WC1X 8LD

Telephone: 020 3456 - 7899
Direct Line: 020-3456 - 1067
Fax: 020-3456-2329
Web-site: www.uclh.nhs.uk

Version 1
Study Number:.....
Patient Identification Number for this trial: .........

**PARENT CONSENT FORM**

Title of Project:

**A Study of the genetics and the physical properties of dental anomalies.**

Name of Researchers: Dr Susan Parekh, Dr Agnes Bloch-Zupan, Dr Peter Brett, Dr Laurent Bozec, Miss Amanda O'Donnell, Moshael Abdullatif, Nurjehan Mohamed Ibrahim and Nabilah Narith.

Please initial box

**1**. I confirm that I have read and understood the information sheet dated 21/12/10 (version 1) for the study. I have been allowed some time to think about this, ask questions, and have had these answered in a way that I understand. ☐

**2**. I understand that my child's is voluntary and that I am free to withdraw at any time, without giving any reason , without their medical care or legal rights being affected. ☐

**3**. I understand that sections of any medical notes may be looked at by the researchers and responsible individuals from regulatory authorities where it is relevant to my child taking part in research. I give permission for these individuals to have access to my child's records. ☐

**4**. I give permission to the investigators to pass clinical data collected from my child's examination to my General Practitioner or General Dental Practitioner. ☐

**5**. I understand that the samples taken from my child may be stored and used for the purpose of further research at a later date. I understand that these results will also remain anonymous. ☐

**6**. I understand that (this project or future research) will include genetic research aimed at understanding the genetic influences on dental defects in children. ☐

**7**. I agree for my child to take part in the above study. ☐

| Name of Patient | Date | Signature of parent |
|---|---|---|
| Name of Person taking consent | Date | Signature |

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes.

UCL HOSPITALS

UCL Hospitals is an NHS Foundation Trust comprising: The Eastman Dental Hospital, The Heart Hospital, Hospital for Tropical Diseases, National Hospital for Neurology and Neurosurgery, The Royal London Homoeopathic Hospital and University College Hospital (incorporating the former Middlesex and Elizabeth Garrett Anderson Hospitals).

S Parekh Version 1

For further information about this study please contact Dr Susan Parekh

Phone : 020 3456 1067 email: s.parekh@eastman.ucl.ac.uk

UCLH welcomes feedback from their patients who have been involved in research. In the first instance, you should inform the Principal Investigator. If you are not satisfied with the response of the research team then you should address your complaints to the UCLH complaints manager at UCLH postal address or through our website http://www.uclh.nhs.uk/Contact+us/. To help us identify the research study you have been involved in, please mention the title and the name of the research doctor or principal investigator. You can find this information on the Patient Information Sheet.

APPENDIX 4

University College London Hospitals NHS
NHS Foundation Trust

**The Eastman Dental Hospital**
256 Gray's Inn road
London
WC1X 8LD

Telephone: 020 3456 7899
Direct Line: 020-3456-1067
Fax: 020-3456-2329
Web-site: www.uclh.nhs.uk

Version 1
Study Number:.....
Patient Identification Number for this trial: ..........

**PATIENT CONSENT FORM**

Title of Project

**A Study of the genetics and the physical properties of dental anomalies.**

Name of Researchers: Dr Susan Parekh, Dr Agnes Bloch-Zupan, Dr Peter Brett, Dr Laurent Bozec, Miss Amanda O'Donnell, Mashael Abdullatif, Nurjehan Mohamed Ibrahim and Nabilah Narith

Please initial box

**1.** I confirm that I have read and understood the information sheet dated 21/12/10 (version 1) for the study. I have been allowed some time to think about this, ask questions, and have had these answered in a way that I understand. ☐

**2.** I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason , without my medical care or legal rights being affected. ☐

**3.** I understand that sections of any medical notes may be looked at by the researchers and responsible individuals from regulatory authorities where it is relevant to my part in the research. I give permission for these individuals to have access to my records. ☐

**4.** I give permission to the investigators to pass clinical data collected from my examination to my General Practitioner or General Dental Practitioner. ☐

**5.** I understand that the samples taken from me may be stored and used for the purpose of further research at a later date. I understand that these results will also remain anonymous. ☐

**6.** I understand that (this project or future research) will include genetic research aimed at understanding the genetic influences on dental defects in children. ☐

**7.** I agree for to take part in the above study. ☐

| | | |
|---|---|---|
| Name of Patient | Date | Signature of patient |
| Name of Person taking consent | Date | Signature |

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

UCL HOSPITALS

UCL Hospitals is an NHS Foundation Trust comprising: The Eastman Dental Hospital, The Heart Hospital, Hospital for Tropical Diseases, National Hospital for Neurology and Neurosurgery, The Royal London Homoeopathic Hospital and University College Hospital (incorporating the former Middlesex and Elizabeth Garrett Anderson Hospitals).

S Parekh Version 1

## 15 APPENDIX 5

**Consent form for the Phenodent database**

You/ your child have been asked to participate in the database project entitled "Diagnosing Dental Defects database D [4] / Phenodent.

The establishment of this registry has received the favorable opinion of CCTIRS 11.09.2008, and authorization of the CNIL on 18/05/2009 (Registration No. 908416).

I can at any time obtain additional information from Miss Susan Parekh (primary investigator) or Prof. Agnes Bloch-Zupan, Project Manager, the Reference Centre of dental manifestations of rare diseases. Department of Oral Health Care, University Hospital Strasbourg, Hôpital Civil, 1 place Hospital, F-67000 Strasbourg Cedex France or email: agnes.bloch@chru-strasbourg.fr

I authorize the registration of anonymous data and pictures in the database yes ☐ no ☐
and my ethnic background (via the collection of country and city of birth) yes ☐ no ☐
This information may also be used for teaching purposes

For data files, I authorize the possible dissemination of all images, yes ☐ no ☐
or only intraoral pictures yes ☐ no ☐

YOUR AGREEMENT TO PARTICIPATE IN THIS REGISTRY
My signature certifies that I clearly understood the information regarding my participation in this registry

| | | |
|---|---|---|
| Name of Patient | Date | Signature |
| Name of Parent | Date | Signature |
| Name of Person taking consent | Date | Signature |

This document is to be performed in two original copies:
A copy kept by the person giving consent (or by the holders of parental authority if minor)
The other copy to be kept by the primary investigator, Miss Susan Parekh

**Retrospective Audit of record keeping of trauma patients attending the Paediatric Department**

**Submitted in partial fulfilment of the requirements for the Degree of Clinical Doctorate in Paediatric Dentistry**
**University College London**
**Eastman Dental Institute**
**2014 – 2017**

**Shaima Mansour Sarkhouh**
**BDS, MFD (RCSI), MPaed Dent (RCSED)**

## Table of Contents

1 Background.......... 7

2 Dental trauma types and the prognostic factors.......... 8

2.1 Enamel infarction and fractures.......... 8

2.2 Enamel dentine fractures.......... 8

2.3 Enamel dentine pulp fracture.......... 9

2.4 Crown root fractures.......... 10

2.5 Root fracture.......... 10

2.6 Alveolar bone fracture.......... 11

2.7 Concussion.......... 11

2.8 Subluxation.......... 11

2.9 Extrusion.......... 12

2.10 Lateral luxation.......... 12

2.11 Intrusion.......... 13

2.12 Avulsion.......... 14

3 Current guidelines for record keeping.......... 14

4 Record keeping for patient with dental trauma.......... 15

4.1 General considerations.......... 16

4.1.1 Relevant information.......... 16

4.1.2 Dental visits elsewhere.......... 16

4.2 Examining dental trauma.......... 17

4.2.1 Extra-oral examination.......... 17

4.2.2 Intra-oral examination.......... 17

4.2.3 Special tests.......... 18

4.2.4 Diagnosis.......... 19

4.2.5 Treatment.......... 19

4.2.6 Avulsed teeth.......... 19

4.2.7 Children.......... 20

5 Summary.......... 21

6 Why this audit.......... 21

7 Aim.......... 21

8 Standards.......... 21

9 Methods ... 21

10 Data collection sheet ... 22

11 Results of first cycle ... 23

12 Clinical staff interviewing and questionnaire ... 24

13 Results of first and second cycles ... 26

14 Discussion ... 28

15 References ... 30

## List of figures


Figure 1: Interviewing analysis .................... 24
Figure 2: Interviewing analysis .................... 25
Figure 3: The revised trauma proforma .................... 25
Figure 4: Summary of the first and second cycles. .................... 26
Figure 6: Summary of results of the first and second cycles (Clinical examination) .................... 27

**List of tables**

Table 1: Results of the first and second cycles (Other information)....................27

**List of abbreviations**

GDC General Dental Council

FGDP Faculty of General Dental Practitioner

# 1 Background

Oral traumas occur frequently and comprise 5% of all body injuries. In preschool children, the figure of oral traumas is 18% of all injuries (Flores et al., 2007). The most common dental injuries are crown fractures and luxations (Flores et al., 2007). A proper diagnosis and appropriate treatment plan is important to achieve a good prognosis of traumatised teeth and oral cavity. All the clinicians have to examine the patients thoroughly and ask the right questions to reach an accurate diagnosis and provide a clear treatment plan. Also, clinicians should keep and maintain patient's records in order to ensure that other clinicians could seamlessly continue with the patients care.

Thorough documentation of dental trauma information at the first and the following visits is crucial for proper patient management. A study investigating the importance of a standardised trauma form in dental trauma found that when a standardised questionnaire was not used an average of only 53.3% of the information was recorded (Andreasen and Andreasen, 1985). Also they stated that Intraoral photographic registration of the traumatised dentition is of value in follow-up treatment and is a helpful part of the patient record in the event of insurance claims or legal action (Andreasen and Andreasen, 1985). Textbooks on dento-alveolar trauma described the importance of a thorough and systematic approach in taking trauma history and clinical examination (Andreasen et al., 2013, Andreasen et al., 2011, Curzon, 1999, Roberts and Longhurst, 1996). Some dental organisations such as American Academy of Paediatric dentistry has recommended and published a structured form for taking dento-alveolar history, which was described originally by Andreasen and Andreasen (Andreasen and Andreasen, 2013).

Five factors were considered to be important to document at the time of dental trauma (Day and Duggal, 2006). First, the history of the accident should be recorded which must includes what happened, where and when. By recording when the trauma happened and the time of receiving the treatment, the effect of the delay in receiving the proper treatment can be examined and the prognosis of the affected dentition could be determined (Andreasen et al., 2002). Second, the current medical history should be updated at the time of presentation. Third, loss of consciousness; this should be recorded with positive response or negative even if the patient has not lost consciousness. Fourth, a thorough documentation of extra and intra oral examination findings. Fifth, if all teeth and fragments were accounted for if the dental trauma was resulted in avulsion or tooth fracture (Day and Duggal, 2006).

As mentioned previously, the different types of dental trauma have a different effect on the traumatised teeth and can influence the prognosis of pulpal, periodontal ligament healing and the survival of the affected teeth. In the following section, different dental trauma types and their effect on the prognosis will be discussed in detail.

## 2 Dental trauma types and the prognostic factors

### 2.1 Enamel infarction and fractures

Enamel infraction is an incomplete fracture in the enamel structure (crack). It does not progress and usually arrested when it reach to the dentio-enamel junction (Bechtle et.al., 2010). Enamel infraction creates pathways for the bacteria to invade the root canal system (Love, 1996). Pulp necrosis was reported to reach 3.5% of teeth that enamel infraction was the only trauma injury that affected the tooth (Güngör, 2013). However, the figure increased to 34.5% when its associated with periodontal injury (Güngör, 2013).

### 2.2 Enamel dentine fractures

Several factors can have a significant effect on the prognosis of the traumatised tooth which include:

- **Periodontal injury involvement.** It has been shown that there is a significant relation between the degree of periodontal ligament injury and the risk of pulpal necrosis. It was stated that if there was no periodontal injury involved in the trauma, the chance of pulp necrosis was 3%. While with concussion and subluxation, the chances of pulp necrosis increased to 6% and 30% respectively (Robertson et al., 2000, Ravn, 1981).
- **The time between the trauma and treatment.** A study revealed that if treatment was delayed for more that 3 days, the chances for pulp necrosis increased from 7 % to 24 %, however, this study did not mention if there was periodontal ligament injury involved (Oulis and Berdouses, 1996).
- **Apical maturity.** It has been shown that there is no effect of apical maturity on pulpal survival unless periodontal injury was involved. A study had shown

28 % of teeth, which suffered enamel dentine fracture and periodontal injury, lead to pulp necrosis, 81 % of those teeth has closed apex.

- **The extent of enamel dentine fracture.** It has been shown that enamel dentine fracture that involved gingival margin had poorer prognosis for pulp necrosis when compared with enamel dentine fractures that do not involve gingival margins (Ravn, 1981). This may be related to the type of material used and the difficulties that associated with the complexity with the isolation.
- **Sensibility test response at the time of trauma.** It has been shown that of the 97% of teeth with enamel dentine fractures which responded positively to sensibility test at the time of presentation, only 4% subsequently became non- vital. Of teeth that did not respond to sensibility test at the initial presentation, 28% subsequently became non- vital (Ravn, 1981). However, the response of sensibility tests at the first visit is not always accurate as it can be affected by the operator skills and the patient behaviour. Andreasen and Andreasen stated that sensibility test requires a patient with co-operative and relaxed behaviour in order to prevent false responses (Andreasen and Andreasen, 1994). Usually, children who have suffered a dental trauma present with limited co-operation. In addition their teeth are often covered with blood and can be tender to examine properly without local anaesthetic. This will limit the use of sensibility testing.

### 2.3 Enamel dentine pulp fracture

- **Periodontal injury involvement.** The association of periodontal injuries can raise the risk of pulp necrosis from 0% to 14% (Robertson et al., 2000).
- **Apical maturity.** A study found that apical maturity has no effect on pulpal healing (Robertson et al., 2000). The treatment provided was a mixture between pulpotomy and pulp capping, no more details were given. High success rate of pulpotomies was reported which was 96% regardless of the status of apical maturity (Cvek, 1978). for pulp capping procedures, Apical maturity reduced pulp survival, however, it was not at a significant level (Ravn, 1982). A retrospective study was inconclusive whether tooth maturity had an effect on the success rate of the treatment provided because pulpotomy was performed only on teeth that were considered immature and pulp capping was provided only for teeth that considered mature with closed apex (Fuks et al., 1982).

### 2.4 Crown root fractures

- **Periodontal injury involvement.** As mentioned previously, periodontal injuries would reduce the chances of pulp survival.
- **Apical maturity.** There was no studies that I could identify that relate apical maturity with pulp survival. However, It is assumed that apical maturity will definitely influence the treatment choice and a greater chance of pulp survival for immature teeth because of their robust blood supply (Day and Duggal, 2006).
- **Extent of fracture.** It is important to determine if the fracture extends below or above the alveolar bone. The treatment options will be determined based on the extent of the sub-gingival fracture, plup involvement, root length and morphology and the aesthetic result required (Olsburgh et al., 2002).

### 2.5 Root fracture

- **Concomitant Crown fracture.** A study has shown that the concomitant crown fracture had a significant effect on the prognosis of pulp survival in teeth that had root fracture (Welbury at al., 2002). However, this was not proved in another study (Andreasen et al., 2004a).
- **Periodontal injury involvement.** Studies have shown that the type of root fracture healing is significantly affected if periodontal injury was involved with the coronal part (Andreasen et al., 2004b, Cvek et al., 2001, Welbury et al., 2002). Periodontal injury involvement was also found to have a negative association with pulp survival of the coronal part of root fracture (Andreasen et al., 2004b).
- **The degree of displacement of the coronal part of root fracture.** The degree of the coronal part displacement gives an idea about the extent of periodontal ligament involvement. A study has been shown that the degree of the displacement affect significantly on pulp survival and the type of healing of the fractured root parts (Andreasen et al., 2004b).
- **Occlusal interference.** This can be assessed by the clinician and can be expressed by the patient. Occlusal interference can influence the treatment provided (Day and Duggal, 2006).
- **Apical maturity.** Traumatised immature teeth have higher chances for pulp survival (Andreasen et al., 2004b, Feely et al., 2003). Also the type of fracture

healing can be affected by the stage of root development (Andreasen et al., 2004b, Cvek et al., 2001).

- **Location of the fracture.** It has been shown that the site of root fracture had a significant effect on the survival of the coronal part of the tooth (Welbury et al., 2002).
- **Sensibility test at the time of trauma injury.** There is a significant relationship between sensibility testing response and pulp survival (Andreasen et al., 2004b). Also it has been shown that the positive response to sensibility test was related to the type of healing that occurred between the fractured root parts (Cvek et al., 2001).

## 2.6 Alveolar bone fracture

- **The time between the trauma to treatment (Fixation).** It has been reported that the time between the trauma to permanent fixation was significantly associated with the risks of pulp survival (Andreasen, 1970).

## 2.7 Concussion

- **Associated crown fracture.** It has been reported that pulp survival was not significantly related to infractions trauma (Andreasen and Pedersen, 1985). Also it was reported that there was a little difference in pulp survival following concussion trauma whether it was with or without crown fracture (Day and Duggal, 2006).
- **Apical maturity.** Apical maturity has been significantly related to pulp survival (Andreasen and Pedersen, 1985, Andreasen et al., 2011).

## 2.8 Subluxation

- **Associated crown fracture.** The chance of pulp survival decreased when there was a crown fracture associated with subluxation trauma compared with subluxation trauma that was not associated with crown fracture (Day and Duggal, 2006).
- **Tenderness to percussion and Occlusion.** It has been reported that there were a significant relation between pulp necrosis and tenderness to percussion and pain on occlusion (Andreasen and Pedersen, 1985).

- **Apical maturity.** Significant reduction in pulp survival associated with the stage of root maturity was reported (Andreasen and Pedersen, 1985).

## 2.9 Extrusion

- **Associated crown fracture.** It has been reported that there was a decrease in pulp survival when there was an extrusion trauma which was associated with enamel dentine fracture compared with extrusion trauma without crown fracture and it was more pronounced when the root was closed apex **(Day and Duggal, 2006).** Pulp survival in extrusion trauma was not significantly affected with infraction injurie(Andreasen and Pedersen, 1985).
- **Degree of displacement.** It has been reported that the degree of displacement in millimetres was not significantly related to pulp necrosis in extrusion traumas (Andreasen and Pedersen, 1985, Lee et al., 2003). However, another study stated that the severity of extrusion trauma (more than 3 mm) was significantly associated with pulp necrosis (Humphreys et al., 2003).
- **Occlusal interference.** When there is occlusal interference, this will influence the treatment provided to the patient. It has been reported that pain on occlusion affects pulp survival in extrusion trauma (Andreasen and Pedersen, 1985).
- **Apical maturity.** Pulp survival was highly significantly affected by apical maturity for teeth suffered extrusion trauma (Andreasen and Pedersen, 1985, Humphreys et al., 2003).
- **Pulp canal obliteration.** Pulp canal obliteration is considered a sign of healing of the pulp and it was reported that it is related to the severity of the injury (Humphreys et al., 2003).
- **Delay in seeking treatment.** Delay in repositioning of extruded tooth (more than 3 hours) was found to be associated with residual extrusion after repositioning of the affected tooth (Humphreys et al., 2003).

## 2.10 Lateral luxation

- **Associated crown fracture.** It has been reported that pulp survival was reduced when there was crown fracture associated with lateral laxation injury (Day and Duggal, 2006). Enamel infraction was found to be associated significantly with pulp survival (Andreasen and Pedersen, 1985).

- **Degree of displacement.** It has been found that the degree of displacement was not significantly related to pulp survival for teeth affected with lateral luxation (Andreasen and Pedersen, 1985, Nikoui et al., 2003).
- **Mobility.** It has been shown that the degree of mobility of teeth affected with lateral luxation affect the chances of pulp survival (Andreasen and Pedersen, 1985).
- **Number of teeth involved.** Number of traumatised teeth in the same dental arch has been shown to be a significant sign for the development of pulp necrosis (Andreasen and Pedersen, 1985).
- **Pulp canal obliteration.** Pulp canal obliteration was common as 40% of the sample healed in this way (Nikoui et al., 2003)
- **Apical maturity**. For lateral luxated teeth, Apical maturity was reported to be a significant factor for pulpal survival (Andreasen and Pedersen, 1985). However, No association was found in another study (Nikoui et al., 2003).

## 2.11 Intrusion

- **Associated crown fracture.** It has been reported that there is a relationship between crown fracture and pulp survival in teeth affected with intrusive injuries (Ebeleseder et al., 2000, Humphreys et al., 2003).
- **Degree of displacement.** The degree of displacement in millimetre gives an idea about the extent of periodontal injury involved. Many studies reported that the degree of the tooth displacement was affecting significantly pulp, periodontal healing and tooth survival (Andreasen and Pedersen, 1985, Ebeleseder et al., 2000, Al-Badri et al., 2002).
- **Apical maturity.** It has been reported that apical maturity affect periodontal and pulp healing (Andreasen and Pedersen, 1985, Ebeleseder et al., 2000).

## 2.12 Avulsion

Factors affecting the prognosis of the avulsed tooth (Day and Duggal, 2003):

- Root development stage and the age of the patient.
- The dry time of the tooth before replantation and storage medium.
- The total time when the tooth was outside the patient mouth.
- Periodontal ligament contamination and cleaning the tooth before replantation.
- If antibiotics where give either topically or systemically.
- 

The avulsed tooth is also affected by the type and time of splinting as well as the status of the tooth apex whether it was open or closed at the time of trauma (Andersson et al., 2012). It was found that the prognosis of the avulsed tooth depends on the root maturity as well as the condition of the periodontal ligament cells. The condition of these cells depends on the time out side the patient's mouth and the storage medium used (Andersson et al., 2013).

# 3 Current guidelines for record keeping

There are two guidelines and standards that discuss patient's records; Faculty of general dental practitioner (FGDP) and General Dental Council (GDC). General Dental Council has nine standards that should be followed by the clinicians. The fourth standard of the GDC standards was to maintain and protect patients' information, which means that the clinician must:

- Make and keep complete and accurate patient records, including an up-to-date medical history, each time that you treat patients.
- Record as much detail as possible about the discussions you have with your patients, including evidence that valid consent has been obtained. You should also include details of any particular patient's treatment needs where appropriate.
- Understand and meet your responsibilities in relation to patient information in line with current legislation. You must follow appropriate national advice on retaining, storing and disposing of patient records.
- Ensure that all documentation that records your work, including patient records, is clear, legible, accurate, and can be readily understood by others. You must also record the name or initials of the treating clinician.

The Faculty of General Dental Practitioner, clinical examination and record keeping section in good practice guidelines (2016) has recommended and categorised the information that need to be recorded into three categories according to their importance. These recommendations are marked in three letters (A, B, C). A represents the gold standard, anything regarded A is included for completeness but not essential. For recommendations marked B, it means that basic or baseline information that should normally be recorded, or actions that should be undertaken unless the clinician decided not to do so due to a strong justification. In this case, the clinicians should make note of the details of their justification in the patient's clinical note. C for the recommendations that do not apply in every circumstance. Where they are relevant, actions labeled C should be considered as B Actions. These guidelines cover the collection and recording information, which enables a proper diagnosis to be made, and subsequently, the appropriate treatment planning will be provided to the patient enabling them to choose a treatment plan. Also a comprehensive patient medical and dental histories, examinations and records assist with quality assurance, audit and research. Moreover, they benefit not only the patient but also the practitioner because clear documentation is invaluable in case of query, complain or litigation. The courts and GDC accept patient's records so evidence of the detail of dental care and good and accurate records are an important part of dental professional evidence. It is important that patient's clinical records contain an adequate detail and are of an acceptable standard so any dentist can fully understand the history of patient care.

A discussion of record keeping in special situations such as dental trauma will be discussed in detail in the following section.

## 4 Record keeping for patient with dental trauma

According to good practice guidelines, FGDP, examination and record keeping for patients attending for emergency or unplanned visits such as dental trauma should focus on the identification of the cause and appropriate management of the presenting complaint. The medical history must be updated and signed. Adequate notes must be made and appropriate follow-up visit should be arranged.

The following section will be discussing the recommendations of FGDP guidelines (2016)

## 4.1 General considerations

### 4.1.1 Relevant information

In all cases, the relevant information should be obtained. These include the personal information, medical history and socio-behavioural history. The medical history should taken or updated and signed by both the patient/carer and the dentist.

### 4.1.2 Dental visits elsewhere

If the patient received dental treatment in a different hospital, dental practice the details of the treatment received should be recorded. If the patient attended Accident and Emergency department as a result of traffic accident or an assault then an appropriate dental assessment is required. However, as the initial presentation may be at the practice, the clinician should be aware of all other possible injuries that might occur after a trauma.

If loss of consciousness occurred then the patient should be advised to see the Accident and Emergency department for check-up unless this has already happened. Also the reason for patient attendance should be recorded. It is often helpful to quote the patient's own words.

The clinician should record the patient's recollection of the trauma event and should establish the nature of the incident. The details of the trauma history should be documented which include:

- The location, the time and the cause of the trauma.
- Loss of consciousness.
- Type of injury. For example, Avulsion, bone fracture, etc.
- Present complaint such as difficulty eating, drinking, occlusion.
- Extra-oral examination such as bruising, laceration and swelling.
- Patient's tetanus status if replantation of the avulsed tooth is considered or if there was an open wound/ laceration on the skin or mucosa.

In some cases, legal action can ensue after the trauma therefore, it is important to document an accurate detail of any clinical findings as it difficult to remember those finding after years of the trauma event. A simple illustration of the injuries can be helpful or clinical photographs following obtaining an informed consent are considered an excellent way for recording dental injuries.

## 4.2 Examining dental trauma

### 4.2.1 Extra-oral examination

The clinician should perform the examination with a routine protocol to help memorise and reduce the risk of omission. The examination should be recorded as it has been carried out and the presence or absence of abnormalities should be documented. The bony areas of the head and neck should be palpated; tenderness, steps or abnormalities should be noted. If fracture is suspected then the patient should be referred to an oral surgeon or maxillofacial department for further assessment. A full note of the findings should be documented and it is helpful to note if these findings were negative to gives an idea the area has been assessed. Extra-oral examination findings that should be recorded after traumatic injuries include:

- Head and face should be assessed at each examination and any abnormality should be recorded such as face asymmetry, swelling and abnormal discolouration. **B**
- Neck should be palpated for tenderness, lumps or abnormality especially swelling of lymph nodes. **B**
- Temporomandibular joint (TMJ) should be palpated at rest and during mouth opening. Any abnormal findings such clicking, deviation and limitation in movement must be documented. **B**
- Clinicians should always be attentive to the signs of non-accidental injuries, especially in children and vulnerable adults. Appropriate action must be taken. **C**
- Rest of the body. **C**
- Bony injuries. **C**
- Paraesthesia. **C**

### 4.2.2 Intra-oral examination

Screening of the soft tissue and Basic Periodontal Examination (BPE) should be performed whenever possible and recorded in the patient's clinical notes. Soft tissue screening is not recommended as a matter of routine. However, if any abnormalities detected such as a localised periodontal condition then it should be indicated in the notes.

Also, examination and detection of the cause of the patient's symptoms such as fractured crown/filling, swelling, abscess, soft tissue lesion, denture problem or detaching/ broken orthodontic appliance should be documented.

Following a dental trauma, it might be difficult to examine some areas properly because of patient co-operation or tenderness of the traumatised teeth. Sometimes limitation in mouth opening due to the presence of a swelling or trismus can limit a proper examination so this should be documented in patient's note. The patient should be advised to return again for further examination and assessment and this also should be recorded in the patient's note.

Intra-oral examination findings that should be recorded following a dental trauma:

- Soft tissues. **C**
- Charting of the teeth. **C**
- BPE. **C**
- Caries. **C**
- Defective restoration. **C**
- Mobility. **C**
- Occlusion. **C**
- Occlusal abnormalities. **C**
- Cause of symptoms. **B**
- Fractured teeth. **C**
- Paraesthesia. **C**

### 4.2.3 Special tests

It is necessary to carry out some tests to confirm the diagnosis such as palpation of the soft tissues to assess an area of swelling. The site, size and the type (fluctuant or firm) of the area should be recorded. Percussion of the relevant teeth might be required to confirm the diagnosis as well as sensibility tests of the traumatised teeth.

It may be difficult to perform some of these tests as it depends on patient's co-operation, tenderness of the teeth and symptoms. A note should be made if the clinician could not carry out these tests along with the justification.

### 4.2.4 Diagnosis

In most cases the diagnosis can be made and this should be recorded. In some cases, the diagnosis might not be clear then a list of the differential diagnosis should noted in the patient's notes with the most possible diagnosis noted first at the list.

### 4.2.5 Treatment

Following the clinical assessment and diagnosis, the patient should be informed of the findings, diagnosis and the proposed treatment options. The clinician should explain and discuss all the treatment options to the patient and explain the risk and benefits of each treatment option. Following a thorough discussion, the treatment plan should be agreed by the patient/ carer. Although a signed written treatment plan should be handed to the patient, it is recognised that it is not always practical to do so. An adequate information about the chair side discussion between the patient/ carer should be documented in the patient's clinical notes followed by the agreed treatment plan.

If the patient attends another dental practice or they will continue the follow-up care at another practice then it is important to provide the patient with a letter describing the findings, diagnosis and treatment plan or send these information to the clinician who will carry out future treatment.

It is important to remember that the first appointment following the trauma should be focused on identifying the cause of patient's symptoms and carry out the emergency treatment as a matter of priority.

### 4.2.6 Avulsed teeth

If the trauma included an avulsed tooth, the following note should be recorded:

- When, where and how the trauma happened.
- How long the tooth has been outside the patient's mouth.
- Where the avulsed tooth was place, the storage medium.
- An assessment of the amount of drying and its subsequent sequelae.
- Measurement of the root of the avulsed tooth.

### 4.2.7 Children

Dental trauma can happen anywhere and anytime so the child might attend the practice with any person such as schoolteacher. Attending the child without the parent/ carer will cause difficulties in taking an important information such as the medical history, allergies and in obtaining consent to proceed in the treatment required. All efforts should be made to contact the person who has the parental responsibility to inform them about the situation and the required treatment. The failed attempts in contacting the parent/ carer should be noted and if the contact was successfully made then this should be documented as well with adequate information. If there are no other concerns, essential emergency treatment must not be withheld if the clinician failed to contact the parent/ carer. Some children might be able to provide some helpful information about their medical history and give consent depending on their competence.

The clinician should be alert in identifying non-accidental injuries. When non-accidental injury is suspected, the clinician should sought advise and take the appropriate action.

## 5 Summary

An appropriate record keeping of dental trauma is mandatory for proper assessment, diagnosis and treatment planning. Standards for dental team, implemented by GDC in 2013, clearly mentioned to ‘ Maintain and protect patients’ information’ which stated that clinicians must make and keep contemporaneous, complete and accurate patient records and the notes can be readily understood by other clinicians. Clinicians should record as much detail as possible about any discussion with the patient/ carer and include details of any particular patient’s treatment needs where appropriate.

A trauma proforma had been developed by senior clinicians previously based on dental trauma guidelines, International Association of Dental Traumatology and FGDP recommendations, to ensure that all required information following dental trauma is recorded for patients attending the Paediatric department at Eastman Dental Hospital.

## 6 Why this audit

This audit was to assess clinicians’ compliance in using the trauma proforma that had been used in the Department of Paediatric Dentistry at Eastman Dental Hospital,

## 7 Aim

To audit the trauma proforma in dental records and ensure that all trauma information is recorded to establish baseline information for the upcoming follow up visits.

## 8 Standards

Standard was 100% completion of the proforma, in line with the Faculty of General Dental Practitioners Good practice guidelines.

## 9 Methods

- The audit was registered then approved by the local clinical effectiveness committee.
- A retrospective analysis of trauma proforma. The first cycle was from April 2016 to July 2016, and the second cycle was from November 2016 to

February 2017.

- Patients' notes were identified from trauma logbook.
- Inclusion criteria: All notes that had trauma proforma.
- Exclusion criteria: Notes where new patients form was used instead of trauma proforma.
- Standardised data collection sheet was used.
- Notes were requested for medical records.
- Notes were audited in a room where there is no access for the public.
- Simple descriptive analysis was done using Microsoft excel.

## 10 Data collection sheet

All information in trauma proforma was audited:

- Patient sticker: Placed/ not placed.
- Type of forma used: New patient form/ Trauma proforma.
- Date of attendance: Yes/ No
- Patient accompanied by: Yes/ No
- Referral Source: Yes/ No

Trauma history

- Date of injury
- Time of injury
- Location
- Cause
- Other injury
- Acute management
- Radiographs
- All teeth/ Fragments accounted for
- Avulsion
- Storage medium
- Relevant medical history
- Symptoms at present
- Previous dental History
- Social history
- Clinical photographs

Clinical examination

- Extra-oral examination
- Intra-oral examination
- Charting of teeth
- Sensibility testing
- Radiographs
- Diagnosis
- Treatment plan
- Long-term side effect discussed
- Prescription
- Advice
- Treatment next visit
- Signature

## 11 Results of first cycle

The total number of notes included was n = 34. The number of notes where the trauma proforma was used was 32 notes. Two notes were excluded (New patient form used). The standard was not met as only 43.7% (n = 14) of the notes were fully completed. The recording of the date of trauma was 94%, the time of the trauma was 97% and the location was 94% however for the cause of trauma 100%. For clinical examination, 88% of the notes has extra-oral examination fully completed, 97% for intra-oral examination, the charting was 94% and the sensibility test 69% of the notes were completed.

Results of the first cycle were shared at the departmental governance meeting. Following an open discussion, we agreed on the following action plan:

- Interviewing of the clinicians regarding the need for improving the trauma proforma.
- Nurses to place trauma proforma in the clinical notes and get it ready for the clinicians to use
- Junior staff and postgraduates training at the induction week
- A reminders and emails were sent to staff to remind them to use trauma proforma
- Re-audit in 3 months

## 12 Clinical staff interviewing and questionnaire

A total of 18 clinicians (Consultants, specialist, StR, SHO and postgraduates) were interviewed using a questionnaire to assess the need for improving the template of trauma proforma. Six questions were asked and the responses were assessed and analysed using Microsoft excel. The questions were:

1. Is it easy to follow and fill ?
2. Is it well structured ?
3. Is it covering all information needed ?
4. Do you have any extra questions to add ?
5. Do you prefer answering the questions by filling in the space or ticking boxes? (E.g. accompanied by : father, mother, legal guardian or others)
6. Do you recommend any further changes or modifications ?

The result of clinicians interviewing is demonstrated in figure 1 and 2. Most of the clinicians (n= 11) stated that the trauma proforma was too long and it required to be more structured and easy to follow. Clinicians preferred to have more tick boxes for some questions rather than fill in the space. Also we found that further addition would help in recording trauma information such as a diagram of the patient face and teeth to illustrate simply the trauma injury. Patient behaviour, Occlusion and habits were advised to be added in the trauma proforma.

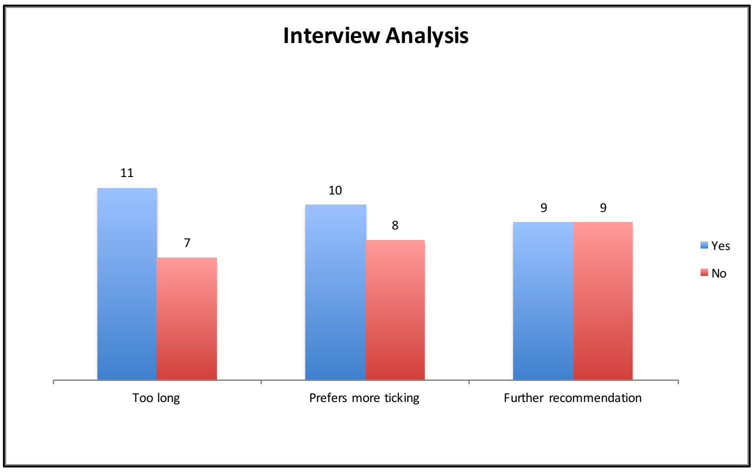



**Figure 1: Interviewing analysis**

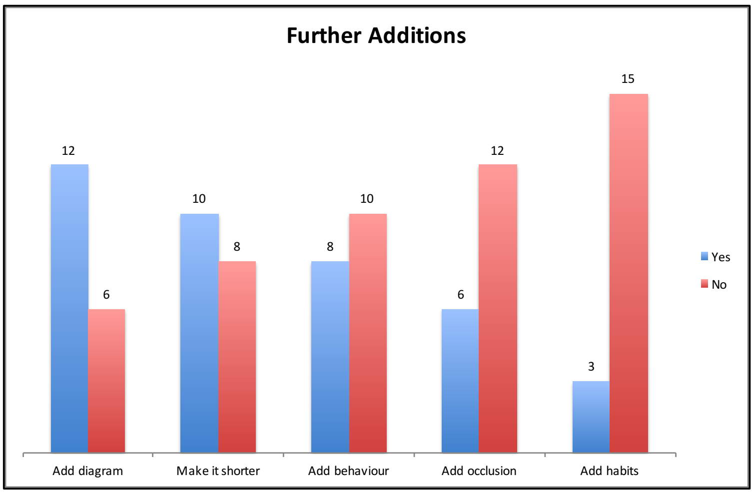



**Figure 2: Interviewing analysis**

Following the clinicians' recommendations, the trauma proforma was revised and approved to be used in the Paediatric Department. The new trauma proforma is demonstrated figure 3.

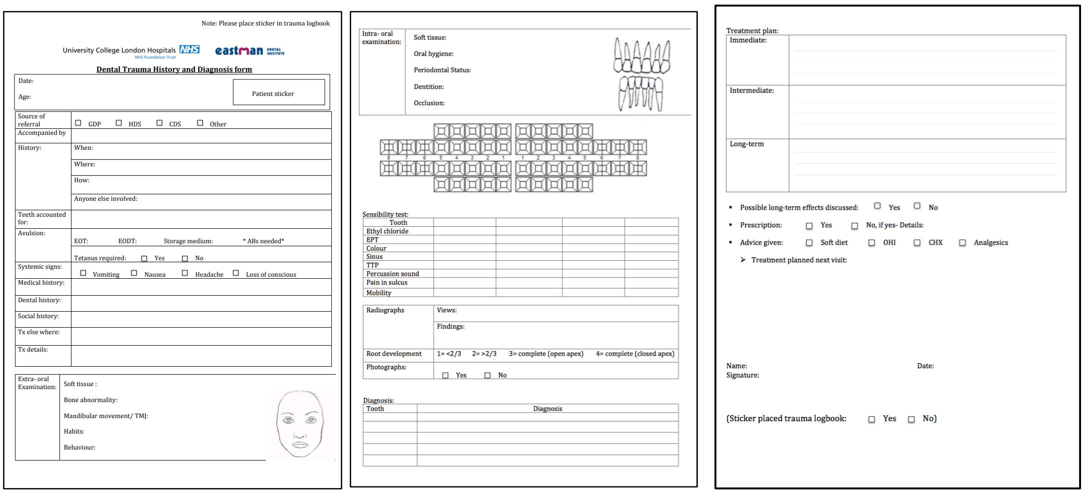



**Figure 3: The revised trauma proforma**

## 13 Results of first and second cycles

Results of record keeping in cycle one and two are demonstrated in figure 4 and 5. In the first cycle, 32 notes were audited (n=32) and 28 notes in the second cycle (n=28). The standard was not met in the first cycle only 43.7% was achieved however, an improvement in recording all the information in trauma proforma was notice in the second cycle as 96% of the notes were fully completed. Following the implementation of the action plan, the second cycle showed an overall improvement in recording all areas of trauma proforma. The recording of trauma history including the date, time, location and the cause increase in the date of the injury from 94% to 100% in the second cycle, the time of injury remained almost the same 97% in the first cycle and 96% in the second cycle, the location increased from 94% to 96% and the cause remained the same 100% in both cycles (Figure 4).

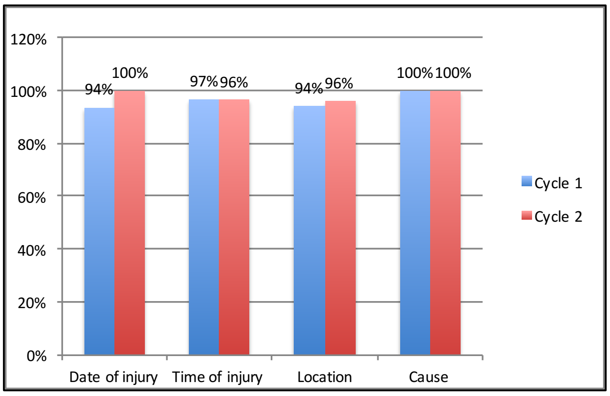



**Figure 4: Summary of the first and second cycles**

In the clinical examination part, an improvement was observed in extra-oral examination from 88% to 93%, intra-oral examination from 97% to 100%, charting of teeth remained at almost the same level 94% (first cycle) 93% (second cycle) and sensibility testing improved significantly from 69% to 96 in the second cycle (Figure 5).

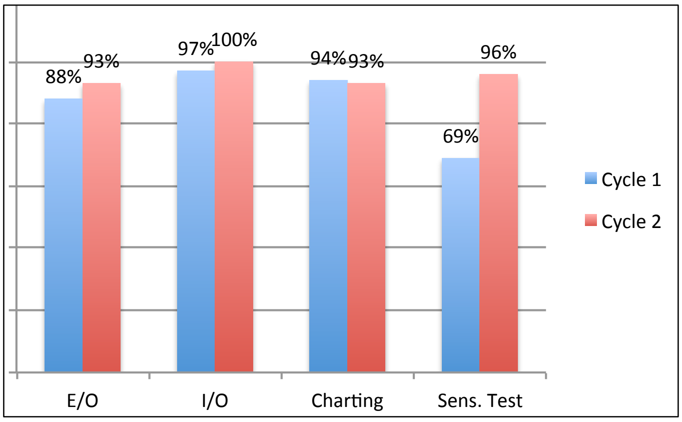



**Figure 5: Summary of results of the first and second cycles (Clinical examination)**

Table 1 shows other information that was audited in trauma proforma. An improvement was noted in recording of patient accompanied by from 75% to 95%, treatment plan from 95% to 100%, discussion of long-term side effect from 75% to 100%, advice following trauma from 88% to 95% and signature from 92% to 100%. The recording of the diagnosis was 100% in both cycles.

| **Information** | **Cycle 1** | **Cycle 2** |
|---|---|---|
| **Accompanied by** | 75% | 95% |
| **Diagnosis** | 100% | 100% |
| **Treatment plan** | 95% | 100% |
| **Long-term side effect discussed** | 75% | 100% |
| **Advice** | 88% | 95% |
| **Signature** | 92% | 100% |

**Table 1: Results of the first and second cycles (Other information)**

## 14 Discussion

Recording and Completion of the trauma proforma has improved after the first cycle and the implementation of the action plans. After revising the trauma proforma, the structure of the trauma proforma was changed based on clinicians' responses on the questionnaire and interviewing. However the gold standard was not met as 96% completion of trauma proforma was achieved. The use of tick boxes were increased and more spaces were provided to ease and facilitate documentation of trauma information.

The increase in recording the sensibility test from 69% in the first cycle to 96% in the second auditing is a point worth mentioning. It may be difficult to carry out some tests such as sensibility test at the first visit depending on symptoms, patient co-operation and condition. However a note should be made that carrying out the test was not applicable thus confirming that the clinician was not able to perform the test due to specific reasons.

The prognosis of the traumatised teeth depends on many factors such as the type of the trauma, patient's age and the severity of the trauma. A proper record keeping will provide baseline information for the clinicians, which will aid in reaching a proper diagnosis and delivering the appropriate treatment in the following visits.

Information such as post trauma advice and long- term effect should be provided to the patients. Improvements in recording whether these information were given or not has increased from 88% to 95% (Advice) and from 75% to 100% (Long-term side effect) in the second cycle. This improvement was achieved due to the use of tick boxes instead of filling in the space. The recording of patient accompanied by was also improved significantly from 75% in the first cycle to 95% in the second auditing. The reason behind this was because of the position of this question in the old trauma proforma template as it was hidden just below patient sticker on the right side of the page. Such an information is very important to check if the child was attended with the parent/ carer. Consent cannot be made if the person with the parental responsibilities was not present therefore affecting patient treatment and care. Clinicians should contact the parent/ carer whenever possible however emergency treatment must not be with held due to the absence of them.

There were two notes where new patient form was used instead of the trauma proforma. An improvement was noticed in using trauma proforma due to the use of reminders and the help of the nurses in getting the notes ready to be used by the clinicians.

As the gold standard was not met 96% a third cycle should be carried out. The following action plan was recommended:

- Reminder to clinicians regarding the importance of trauma proforma completion
- Junior staff / graduate training
- Nurses to continue placing the trauma proforma in the patients not and get it ready for the clinicians to use

## 15 References


AL-BADRI, S., KINIRONS, M., COLE, B. & WELBURY, R. 2002. Factors affecting resorption in traumatically intruded permanent incisors in children. *Dental Traumatology,* 18, pp. 73-76.

ANDREASEN, F. M. & ANDREASEN, J. O. 1985. Diagnosis of luxation injuries: the importance of standardized clinical, radiographic and photographic techniques in clinical investigations. *Dental Traumatology,* 1, pp. 160-169.

ANDREASEN, F. M. & PEDERSEN, B. V. 1985. Prognosis of luxated permanent teeth—the development of pulp necrosis. *Dental Traumatology,* 1, pp. 207-220.

ANDREASEN, J. 1970. Fractures of the alveolar process of the jaw A clinical and radiographic follow-up study. *European Journal of Oral Sciences,* 78, pp. 263-272.

ANDREASEN, J., ANDREASEN, F., MEJARE, I. & CVEK, M. 2004a. Healing of 400 intra-alveolar root fractures. 2. Effect of treatment factors such as treatment delay, repositioning, splinting type and period and antibiotics. *Dental traumatology,* 20, pp. 203-211.

ANDREASEN, J., ANDREASEN, F., SKEIE, A., HJØRTING-HANSEN, E. & SCHWARTZ, O. 2002. Effect of treatment delay upon pulp and periodontal healing of traumatic dental injuries–a review article. *Dental traumatology,* 18, pp. 116-128.

ANDREASEN JO, ANDREASEN FM.Textbook and color atlas of traumatic injuries to the teeth, 3rd edn. Copenhagen: Munksgaard; 1994.

ANDREASEN, J. O., ANDREASEN, F. M. & ANDERSSON, L. 2013. *Textbook and color atlas of traumatic injuries to the teeth*, John Wiley & Sons.

ANDREASEN, J. O., ANDREASEN, F. M., MEJÀRE, I. & CVEK, M. 2004b. Healing of 400 intra-alveolar root fractures. 1. Effect of pre-injury and injury factors such as sex, age, stage of root development, fracture type, location of fracture and severity of dislocation. *Dental Traumatology,* 20**,** pp. 192-202.

ANDREASEN, J. O., BAKLAND, L. K., FLORES, M. T., ANDREASEN, F. M. & ANDERSSON, L. 2011. *Traumatic dental injuries: a manual*, John Wiley & Sons.

ANDERSSON, L., ANDREASEN, J.O., DAY, P., HEITHERSAY, G., TROPE, M., DIANGELIS, A.J., KENNY, D.J., SIGURDSSON, A., BOURGUIGNON, C., FLORES, M.T. AND HICKS, M.L., 2012. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. *Dental Traumatology*, *28*(2), pp.88-96.

Bechtle, S., Fett, T., Rizzi, G., Habelitz, S., Klocke, A. and Schneider, G.A., 2010. Crack arrest within teeth at the dentinoenamel junction caused by elastic modulus mismatch. *Biomaterials*, *31*(14), pp.4238-4247.

CURZON, M. E. 1999. *Handbook of dental trauma: a practical guide to the treatment of trauma to the teeth*, Butterworth-Heinemann.

CVEK, M. 1978. A clinical report on partial pulpotomy and capping with calcium hydroxide in permanent incisors with complicated crown fracture. *Journal of endodontics,* 4**,** pp. 232-237.

CVEK, M., ANDREASEN, J. O. & BORUM, M. K. 2001. Healing of 208 intraalveolar root fractures in patients aged 7–17 years. *Dental traumatology,* 17**,** pp. 53-62.

DAY, P. F. & DUGGAL, M. S. 2003. A multicentre investigation into the role of structured histories for patients with tooth avulsion at their initial visit to a dental hospital. *Dental Traumatology,* 19**,** pp. 243-247.

DAY, P. F. & DUGGAL, M. S. 2006. The role for 'reminders' in dental traumatology: 3. The minimum data set that should be recorded for each type of dento-alveolar trauma–a review of existing evidence. *Dental Traumatology,* 22, pp. 258-264.

EBELESEDER, K., SANTLER, G., GLOCKNER, K., HULLA, H., PERTL, C. & QUEHENBERGER, F. 2000. An analysis of 58 traumatically intruded and surgically extruded permanent teeth. *Dental Traumatology,* 16, pp. 34-39.

FEELY, L., MACKIE, I. C. & MACFARLANE, T. 2003. An investigation of root-fractured permanent incisor teeth in children. *Dental Traumatology,* 19, pp. 52-54.

FLORES, M. T., ANDERSSON, L., ANDREASEN, J. O., BAKLAND, L. K., MALMGREN, B., BARNETT, F., BOURGUIGNON, C., DIANGELIS, A., HICKS, L. & SIGURDSSON, A. 2007. Guidelines for the management of traumatic dental injuries. I. Fractures and luxations of permanent teeth. *Dental traumatology,* 23, pp. 66-71.

FUKS, A., BIELAK, S. & CHOSAK, A. 1982. Clinical and radiographic assessment of direct pulp capping and pulpotomy in young permanent teeth. *Pediatric dentistry,* 4, pp. 240-244.

Güngör, H.C., 2014. Management of crown-related fractures in children: an update review. *Dental Traumatology*, *30*(2), pp.88-99.

HADDEN, A., 2016. Clinical examination & record-keeping: good practice guidelines.

HUMPHREYS, K., BADRI, S., KINIRONS, M., WELBURY, R., COLE, B., BRYAN, R., CAMPBELL, O. & FUNG, D. 2003. Factors affecting outcomes of traumatically extruded permanent teeth in children. *Pediatric dentistry,* 25, pp. 475-478.

LEE, R., BARRETT, E. J. & KENNY, D. J. 2003. Clinical outcomes for permanent incisor luxations in a pediatric population. II. Extrusions. *Dental Traumatology,* 19, pp. 274-279.

Love, R.M., 1996. Bacterial penetration of the root canal of intact incisor teeth after a simulated traumatic injury. *Dental Traumatology*, *12*(6), pp.289-293.

NIKOUI, M., KENNY, D. J. & BARRETT, E. J. 2003. Clinical outcomes for permanent incisor luxations in a pediatric population. III. Lateral luxations. *Dental Traumatology,* 19**,** pp. 280-285.

OLSBURGH, S., JACOBY, T. & KREJCI, I. 2002. Crown fractures in the permanent dentition: pulpal and restorative considerations. *Dental Traumatology,* 18**,** pp.103-115.

OULIS, C. & BERDOUSES, E. 1996. Dental injuries of permanent teeth treated in private practice in Athens. *Dental Traumatology,* 12**,** pp. 60-65.

RAVN, J. 1981. Follow-up study of permanent incisors with enamel fractures as a result of an acute trauma. *European Journal of Oral Sciences,* 89**,** pp. 213-217.

RAVN, J. 1982. Follow-up study of permanent incisors with complicated crown fractures after acute trauma. *European Journal of Oral Sciences,* 90**,** pp. 363-372.

ROBERTS, G. J. & LONGHURST, P. 1996. *Oral and dental trauma in children and adolescents*, Oxford University Press, USA.

ROBERTSON, A., ANDREASEN, F., ANDREASEN, J. & NOREN, J. 2000. Long-term prognosis of crown-fractured permanent incisors. The effect of stage of root development and associated luxation injury. *International journal of paediatric Dentistry,* 10**,** pp. 191-199.

WELBURY, R., KINIRONS, M., DAY, P., HUMPHREYS, K. & GREGG, T. 2002. Outcomes for root-fractured permanent incisors: a retrospective study. *Pediatric dentistry,* 24**,** pp. 98-102.

**Case report**

**Case: 1**

# Managing of caries in a young child

**In partial fulfilment of the degree**
**Clinical Doctorate in Paediatric Dentistry**

**Eastman Dental Institute**

**University College London**

**2014 - 2017**

## CASE SUMMARY

A healthy, anxious 4-years-old girl was referred by her General Dental Practitioner for management of dental caries and dental anxiety due to a traumatic past experience. She presented with multiple carious primary teeth and poor oral hygiene. A tailored prevention regime was given. Non-pharmacological behaviour management techniques (NPBMT) and inhalation sedation were provided to achieve a positive dental-attitude. Restorative treatment included fissure sealant, composite restorations, stainless steel crown, and extraction was completed using local anaesthesia. At follow-up appointments, good oral hygiene was maintained and no carious lesions were detected.

## PATIENT DETAILS

Initials: MC

Gender: Female

Age at start of treatment: 4 years 10 months

Age at last review: 5 years 11 months

### PRE-TREATMENT ASSESSMENT

### HISTORY OF PRESENTING PATIENT'S COMPLAINTS

- MC was free of pain at presentation, she had a history of an abscess two weeks previously related to LRD, no antibiotics were taken.
- She had dental anxiety due to past dental experience.

### RELEVANT MEDICAL HISTORY

- Born full-term pregnancy- natural delivery without complications.
- Medically fit and well with no known allergies.
- Not on regular medications.

### DENTAL HISTORY

- Regular attendee- every 6 months.
- One restoration had been provided under LA- traumatic experience.
- Oral hygiene (OH):
  - Brushes once daily- supervised using fluoridated toothpaste (1000 ppm fluoride)
- Diet:
  - Snacks: crisps, chocolates.
  - Drinks: mostly water and milk.

### SOCIAL HISTORY

- Lives with parents. Attends school.

**CLINICAL EXAMINATION**

**EXTRA-ORAL EXAMINATION**

- Symmetrical face
- No lymphnodes involvement
- Frankl scale: 2

**INTRA-ORAL**

- Oral hygiene (OH): Simplified plaque index SPI: 50%.
- Soft tissue: Healthy.
- Primary dentition stage.
- Hard tissue:
  - Teeth present:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| E | D | C | B | A | A | B | C | D | E |
| E | D | C | B | A | A | B | C | D | E |

  - Carious teeth:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| E | D | | | | | | | D | E |
| E | D | | | | | | | | E |

  - Composite restoration on LLD.
  - Generalised hypomineralisation incisal/coronal one-third of the crown of all primary dentition except LLB, LLA, LRA and LRB.
  - Well-aligned dental arches with teeth spacing and mild rotation of LRA and LLA.

**GENERAL RADIOGRAPHIC EXAMINATION**

- Bitewing radiographs (BWs) were taken in 26/11/2015 for to assess caries and furcation involvement.

**Figure 1: right vertical bitewing**

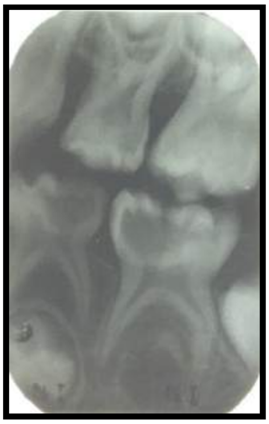


**Figure 2: left vertical bitewing**

- Radiographic findings:
  - Grade I.
  - Normal bone level.
  - Radiolucencies indicating caries affecting URE, URD, ULD, ULE, LLE, LRD and LRE.
  - Furcation involvement of LRD.

**PRE-TREATMENT PHOTOGRAPHS (01/02/2016):**

**Figure 3: Anterior view**

**Figure 4: Maxillary view**

**Figure 5: Mandibular view**

## DIAGNOSTIC SUMMARY

4 years, 10 months old girl in her primary dentition with:

1. Caries involving $URE^{O}$, $URD^{OD}$, $ULD^{OD}$, $ULE^{O}$, $LLE^{O}$, $LRD^{OD}$, $LRE^{O}$.
2. Composite restoration LLD.
3. Carious $LRD^{OD}$ with furcation involvement.
4. Dental anxiety.

**AIMS AND OBJECTIVES OF TREATMENT**

1. To improve oral hygiene through instructions and fluoride advice according the Department of Health preventive toolkit [1].
2. Restore oral health.
3. To promote a positive attitude towards dental care.

**TREATMENT PLAN**

**Initial and preventive treatment**

- Establish a preventive regimen consisting of dietary counselling and oral hygiene instruction according to the Department of Health preventive toolkit [1].
  - Use fluoridated toothpaste 1,350-1,500ppm fluoride.
  - Spitting after brushing rather than rinsing.
  - Fluoride varnish application 3-4 times annually.
  - Reduction of the frequency and amount of sugary food and drinks.

- Acclimatisation
  - Incorporation of NPBMTs and Pharmacological behaviour management technique (PBMT).
  - To consider treatment under general anaesthesia if co-operation lost.

**Intermediate and restorative treatment**

- Quadrant dentistry under IS, LA and RD with NPBMTs:
  - Maxillary right quadrant:
    - URE: Composite restoration.
    - URD: Composite restoration/ PMC.
  - Maxillary left quadrant:
    - ULE: Composite restoration.
    - ULD: Composite/ PMC.
  - Mandibular left quadrant:
    - LLE: PMC +/- pulp therapy.
    - LLD: monitor and PMC.
  - Mandibular right quadrant:
    - LRE: Composite/ PMC.
    - LRD: Extraction.

**Long- term maintenance and follow-up**

- ➢ Monitor restorations clinically and radiographically.
- ➢ Monitor the eruption of the permanent dentition.
- ➢ Fissure seal all first permanent molars once erupted.
- ➢ Reinforce preventive measures.

**TREATMENT UNDERTAKEN**

- **Behaviour management:**
  - ➢ All treatments were carried out under IS and LA in combination with NPBMTs.
  - ➢ Verbal and written consent was obtained from the mother.
  - ➢ Nitrous oxide ($N_2O$) and Oxygen ($O_2$) were titrated according to the patient's needs.
  - ➢ 100% $O_2$ was given to the patient at the end of treatment until full recovery.
- **Isolation**
  - ➢ Rubber dam (RD) isolation was used for all restorative treatment.
- **Pain control:**
  - ➢ Topical anaesthetic: 5% lidocaine gel.
  - ➢ Local anaesthetic LA: 2% Lidocaine with 1:80,000 epinephrine.

**Materials and medications used**

| Material used | Description |
|---|---|
| Prophylaxis paste | Nupro Prophylaxis Paste with Fluoride; Dentsply, USA |
| Duraphat® | Colgate Duraphat Varnish 50mg/ml dental suspension; Colgate Oral Pharmaceuticals, New York City, USA |
| Topical Anaesthesia | Xylonor gel, 5% lidocaine gel. |
| Local Anaesthesia (LA) | Lignocaine Special, 2.2 ml (2% Lidocaine, 1 :80000 epinephrine); Septodont, France |
| Intermediate restorative material | IRM; Dentsply, Milford, USA |
| Fissure sealant | Fissure Sealant Delton; Dentsply, AustraliaGC Gradia Direct®, GC corporation, USA |
| Composite material | GRADIA® Anterior, GC corporation, USA |
| Dycal® | Calcium Hydroxide Cavity Lining Material – Dentsply, USA |
| Acid etch | Phosphoric Acid Gel Etchant 37.5%; Kerr Corporation, USA |
| Bonding agent | Opti bond Solo plus, single component, Total etch bonding agent, California, USA |
| Preformed metal crowns (PMCs) | Stainless steel primary crowns; 3MTM ESPETM, Minn, USA |
| Luting cement | Aquacem®; Dentsply, Milford, USA |

**Visit 1 (26/11/15)- New patient clinic**

- Attended with parents.
- Full history and patient assessment were carried out.
- Simplified plaque index SPI: 50%.
- BWs radiographs were taken (figure 1&2).
- Thorough explanation and treatment options were discussed with parents.
- Agreed to carry out the treatment on dental chair under IS  and LA.
- Diet sheet given.
- OHI given according to the DOH toolkit [1].
- Letter to GDP.
- Behaviour: Frankl 2.

**Visit 2 (01/02/16)**

- Attended with mother.
- SPI: 44%
- IS consent was obtained.
- Introduction to IS, topical anaesthesia and RD.
- Acclimatisation: cleaning and polishing of teeth.
- Fluoride varnish application.
- Diet advice was given based on the patient diet sheet analysis.
- Pre-operative photographs were taken.
- Behaviour: Frankl 3.

**Visit 3 (15/02/16)- Maxillary right quadrant**

- OHI reinforced, SPI 40%.
- Treatment: [NPBMT, IS, LA and RD]
    - URE: complete caries removal + composite restoration + fissure sealed.
    - URD: complete caries removal + conventional PMC.
    - Post-operative instructions given.
- Behaviour: Frankl 4.

**Visit 4 (22/02/16)- Maxillary left quadrant**

- OHI reinforced, SPI 32%
- Treatment: [NPBMT, IS, LA and RD]
    - ULE: complete caries removal + composite restoration + fissure sealed.
    - ULD: complete caries removal + conventional PMC.
    - Post- operative instructions given.
- Behaviour: Frankl 4.

**Visit 5 (07/03/16)- Mandibular right quadrant**

- Chief complain: pain lower right quadrant.
- I/O: tenderness on sulcus LRD.
- Treatment: [NPBMT, IS, LA and RD]
    - Inspected mesial surface of LRE directly by drilling distal surface of LRD, which was planned to be extracted. LRE was not cavitated and arrested.
    - LRE: complete caries removal + composite restoration.
    - LRD: Extracted using forceps.
    - Haemostasis achieved.
- Post operative instructions given.
- Frankl 3.

**Visit 6 (14/3/2016)- Mandibular left quadrant**

- OHI, SPI: 15%
- I/O: enamel fracture of LLA, no history of trauma or pain given.
- Treatment: [NPBMT, IS, LA, RD]
    - LLE: partial caries removed + indirect pulp cap, placed Dycal and IRM.
    - LLE: tooth prepared then PMC cemented with Aquacem.
    - LLD: PMC cemented with Aquacem.
    - LLA: smoothed sharp edge with soflex discs.
- Post-operative photographs taken.
- Frankl 4.

**Post-treatment photographs figure 6-7-8 (14/03/16)**

Figure 6: Anterior view

Figure 7: Maxillary view

Figure 8: Mandibular view

**Visit 7 (09/06/2016)- 1ST review**

- Good oral hygiene was maintained, SPI: 10%.
- Prevention:
  - ➢ Reinforced OHI and diet advice.
  - ➢ Fluoride varnish applied and flossed in interproximal surfaces.
- Vertical BWs radiographs Figure 9-10 (09/06/16):

Figure 9: Right vertical BWs

Figure 10: left vertical BWs

- **Radiographic findings:**
  1. Grade I.
  2. Normal bone levels.
  3. No furcation involvement.
  4. No new carious lesions.
  5. Intact restorations and PMC's.
  6. LL6 erupting against PMC of LLE (monitor).

**Visit 8 (20/12/16)- 2nd review:**

- Frankl scale: 4.
- Oral hygiene: SPI: 5%
- I/O: partially erupted UL6 and LL6.
- Prevention:
  - ➢ Fluoride varnish application.
  - ➢ Reinforcement of preventive advice.
  - ➢ Clinical photographs figure 13-14-15.
  - ➢ Radiographic assessment Figure 11-12.

**Post treatment photographs (20/12/2016)**

**Figure 13: Anterior view (20/12/16)**

**Figure 14: Maxillary view (20/12/16)**

**Figure 15: Mandibular view (20/12/16)**

**POST-OPERATIVE RADIOGRAPHS (20/12/2016)**

Figure 11: Right vertical BWs (20/12/16)

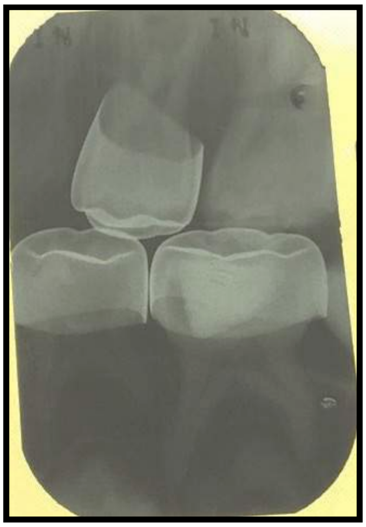


Figure 12: left vertical BWs (20/12/16)

Radiographic findings:

- Grade II.
- Normal bone levels.
- Intact restorations and well adapted PMC's.
- No new/ recurrent caries lesions detected.
- Eruption of LL6.

**LONG TERM TREATMENT PLAN AND FUTURE CONSIDERATION:**

MC maintained good oral hygiene and did not have any new carious lesions over a period of 7 months. Therefore, the following long-term plan was constructed:

1. Regular clinical reviews for reinforcement of prevention advice and fluoride varnish application.
2. Fissure seal first permanent molars once erupted.
3. Radiographs if indicated.
4. Discharge to GDP.

## DISCUSSION AND REFLECTIONS ABOUT THE CASE PRESENTED

My main goals for MC were to maintain good oral hygiene and to build a positive attitude towards dental treatment. She was considered at 'high caries risk' due to her caries experience, inadequate fluoride exposure and daily sugar consumption. When MC first presented, she showed limited cooperation because of a previous dental experience however her treatment was successfully managed under IS, LA, RD and appropriate NPBMTs.

### Behaviour management

Inhalation sedation with nitrous oxide has a favourable outcome when treating children with mild to moderate anxiety. It showed high success rate therefore, it was used to treat MC [2].

### Prevention

The preventive advice was formulated according to DoH toolkit [1]. A realistic diet advice was given as well as oral hygiene instruction. These were reinforced throughout her treatment. At the end of her treatment, MC showed an improved oral hygiene.

### Restoration

The choice of restoration depends on patients' age, extent and location of caries lesion as well as sign and symptoms associated. Composite restorations were chosen for the occlusal cavities in URE, ULE, and LRE.

Caries in ULD and URD was removed completely then restored with PMC. Preformed metal crowns showed a success rate of 97% over 7 years [6]. LLD was previously restored with composite restoration, the tooth showed no signs and symptoms. Therefore, PMC was placed to preserve tooth structure and restoration integrity.

### Indirect pulp capping

It was found that in deep lesions, partial caries removal is preferable to complete caries removal to minimise the risk of carious pulpal exposure [6]. BSPD guidelines in 2006 concluded that the appropriate selection and treatment of primary teeth with indirect pulp capping can show more than 90% clinical success rate at 3 years follow up [5].

### Review

MC was monitored on regularly according to her caries risk [4]. Her oral hygiene was improved and no new/recurrent caries was detected.

**I have learnt:**

1. The appropriate application of pharmacological and non-pharmacological behaviour management techniques can be used to re-establish a good rapport with anxious child with previous traumatic dental treatment. Parents support and cooperation contributed in achieving a successful outcome.
2. A tailored diet-advice and preventive measures have a great effect in controlling the disease.

## REFERENCES


1. Department of Health and the British Association for the Study of Community Dentistry. Delivering Better Oral Health, an evidence-based toolkit for prevention - third edition. London: Crown Copyright; 2014.

2. Hosey, M.T. and UK National Clinical Guidelines in Pediatric Dentistry, 2002. Managing anxious children: the use of conscious sedation in paediatric dentistry.

3. Uribe, S., 2006. Partial caries removal in symptomless teeth reduces the risk of pulp exposure. *Evidence-based dentistry*, *7*(4), pp.94-94.

4. NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE (2004). Dental recall: recall interval between routine dental examinations. www.nice.org.uk/CG019NICEguideline

5. Rodd, H., et al., *Pulp therapy for primary molars.* International Journal of Paediatric Dentistry, 2006. **16**(s1): p. 15-23.

6. ROBERTS JF, ATTARI N, SHERRIFF M (2005). The survival of resin modified glass ionomer and stainless steel crown restorations in primary molars, placed in a specialist paediatric dental practice. British Dental Journal; 198, 427-31.

# Case report

## Case: 2

## Special Care Dentistry for the child and adolescent

**In partial fulfilment of the degree**
**Clinical Doctorate in Paediatric Dentistry**

**Eastman Dental Institute**

**University Collage London**

**2014 – 2017**

## CASE SUMMARY

KS, a 6-year-old girl, referred by a paediatric haematologist for the management of carious and malformed dentition. She was diagnosed with Haemophagocytic lymphohistiocytosis when she was two months of age. She was treated with chemotherapy and stem-cell transplant, which led to dental anomalies in primary and permanent dentition.

KS was a regular dental attendee and temporary restorations had been placed without local anaesthesia at a specialised centre. KS has a diagnosis of MolarRoot-Incisor Malformation-(MRIM) and dental caries. After liaising with her medical team, treatment included Hall crowns, composite restorations performed in conjunction with Non-Pharmacological Behaviour Management Techniques.

## PATIENT DETAILS

Initials: KS

Gender: Female

Age at start of treatment: 6 years, 2 months

Age at last review: 7 years, 3 months

## PRE-TREATMENT ASSESSMENT

## HISTORY OF PRESENTING PATIENT'S COMPLAINTS

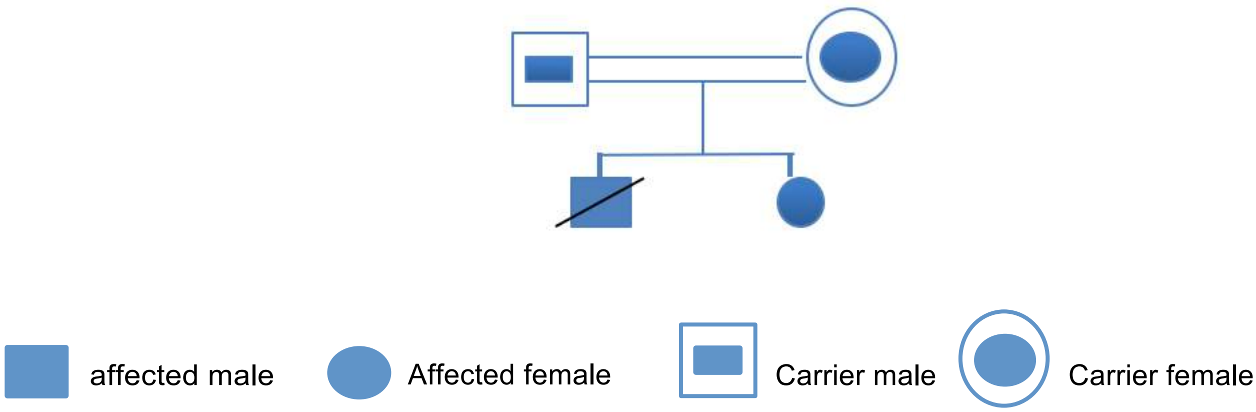
- KS complained of the appearance of her teeth
- Food packed between her back teeth.

## RELEVANT MEDICAL HISTORY

- Haemophagocytic lymphohistiocytosis (HLH)
  - Diagnosed when she was 2 months old.
  - Life threatening condition if not treated.
- Stem-cell transplant in June 2010 when she was 8 months old.
- Hypertension
- Chronic kidney disease
- Asthma
- At risk of transfusion-associated-graft-versus-host disease.
- Medications:
  - Penicillin 250mg/5ml twice daily.
  - Alfacalcidol 0.25 microgram/ml once a day.
  - Montelukast 5 mg once a day (chewable tablets).
  - Dalivit 0.6 ml once a day (Drops)
  - Seretide 50 inhaler using spacer 2 puffs twice a day.
  - Salbutamol 100 mcg inhaler using spacer as needed.
  - Enalapril 2.5 mg once a day.
- No known allergies.
- Full-term pregnancy and normal delivery.

**SOCIAL AND FAMILY HISTORY**

- Attends school.
- One brother who had the same medical condition and passed away due to medical complications during infancy. Parents were not able to provide further family history. Family tree as the following:




**DENTAL HISTORY**

- Regular attendee.
- Had temporary restorations done without local anaesthesia at a dental unit of a specialised hospital.
- Oral hygiene: brushes twice daily with 1000ppm fluoridated toothpaste and electric toothbrush- supervised.

## CLINICAL EXAMINATION

## EXTRA-ORAL EXAMINATION

- No facial asymmetry.
- No lymphadenopathy.
- Behaviour: Frankl scale: 3

## INTRA-ORAL EXAMINATION

- **Soft tissue:** Healthy
- **Oral hygiene:** Simplified plaque index (SPI): 55%.
- **Dentition:**
  - Teeth present:

| | |
|---|---|
| 6 E D C 1 | 1 C D E 6 |
| 6 E D C 2 1 | 1 2 C D E 6 |

  - Hypoplasia of all erupted permanent teeth and URE, URD, ULD, ULE, LLD, LRD, LLE and LRE.
  - Extrinsic Staining of URE and ULE.
  - Dental caries: URE, ULE, LLE and LRE.
  - LRE: Fuji Triage.
  - LLE: Glass ionomer restoration.
  - Tooth wear: all C's.
- **Occlusion:**
  - Class I molar relationship.
  - Crowded mandibular labial segment.
  - Potential crowding of maxillary labial segment.
- Basic periodontal examination (BPE):

| | | |
|---|---|---|
| 1 | 0 | 1 |
| 1 | 0 | 1 |

## RADIOGRAPHIC EXAMINATION (22.12.15)

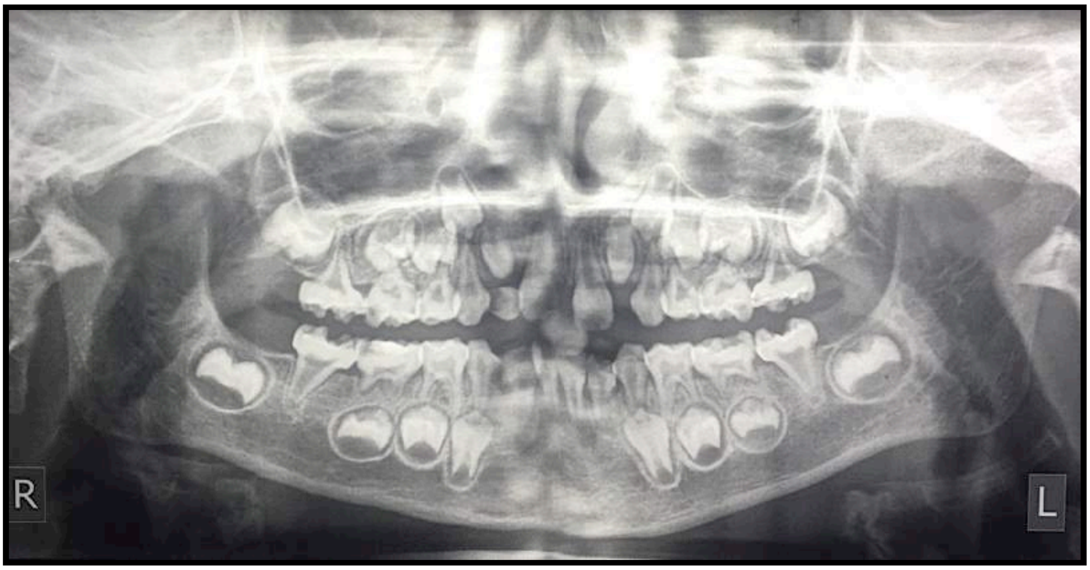



**Figure 1: Orthopantogram (OPG)**

**OPG: Grade II**

- Teeth present:

| | |
|---|---|
| 6 E D C B 1 | 1 C D E 6 |
| 6 E D C 2 1 | 1 2 C D E 6 |

- Developing un-erupted teeth:

| | |
|---|---|
| 7 5 4 3 2 | 2 3 4 5 7 |
| 7 5 4 3 | 3 4 5 7 |

- Malformed UR6, URE, ULE, UL6, LL6, LLE, LRE, LR6.
- Single root UR6, UL6, LL6 and LR6.
- Possible short roots of LR2, LR1, LL1 and LL2.
- Microdontia: UR2 and UL2.

**Figure 2: Right horizontal bitewing**

**Figure 3: Left horizontal bitewing**

**BWs: Grade I**

- Coronal radiolucency indicating caries/hypoplasia on URE, ULE, LLE and LRE.
- Malformed roots of UR6, URE, ULE, UL6, LL6, LLE, LRE and LR6.

**PRETREATMENT PHOTOGRAPHS (26/01/2016)**

**Figure 4: Anterior view of dentition in occlusion**

**Figure 5: Maxillary view**

**Figure 6: Mandibular view**

**DIAGNOSIS**

- Molar Root-Incisor Malformation (MRIM) affecting permanent and primary dentition.
- Dental caries

**AIMS AND OBJECTIVES OF TREATMENT**

- Prevention.
- Restore function and aesthetics.
- Promote positive attitude towards dental care.

**TREATMENT PLAN**

## Prevention and behaviour management

- Prevention according to Delivering Better Oral Health Toolkit [1]:
  - Brush twice daily using 1,350-1,500ppm fluoridated toothpaste.
  - Spit, not rinse.
  - The frequency and the amount of sugar intake should be reduced.
  - Liaise with medical team to request sugar free medication if possible.
- Acclimatisation to dental treatment.

## Intermediate term

- Flowable composites UR6, UL6, LL6 and LR6.
- Composite restorations on UR1, UL1, LL2, LL1, LR1, LR2.
- Preformed metal crown (PMC) on URE, ULE, LLE and LRE.

## Long term

- Clinical review every 3-4 months to monitor oral health, dental development and restorations according to the Royal Collage of Surgeons guidelines for patients who have received chemotherapy and a bone marrow transplant 2012 [2].
- Reinforcement of dietary advice and OHI.
- Patient to be reviewed regarding long term prognosis of 6's.

## TABLE OF MATERIALS USED

| Material Used | Description |
|---|---|
| Flowable composite | 3M ESPE, Dental Products, USA |
| Duraphat | Colgate Duraphat Varnish 50mg/ml dental suspension; Colgate Oral Pharmaceuticals, New York City, USA |
| Acid etch | Phosphoric Acid Gel Etchant 37.5%; Kerr Corporation, USA |
| Bonding agent | Opti bond Solo plus, single component, Total etch bonding agent, Kerr Co., California, USA |
| Composite | GC Gradia Direct®, GC corporation, USA |
| Preformed metal crowns (PMCs) | Stainless steel primary and permanent crowns; 3MTM ESPETM, St Paul, Minn, USA |
| Luting cement | Aquacem®; Dentsply, Milford, DE, USA |
| Dry Dam | DIRECTA INC, Newtown, USA |

## TREATMENT UNDERTAKEN

- Behaviour management:
  - Non-Pharmacological Behaviour Management Techniques (NPBMT):
    - Tell-show-do.
    - DVD-eye-glasses.
    - Positive reinforcement.
    - Distraction.

**Key stages in treatment progress:**

- Liaised with Paediatric Haematology Consultant before commencing treatment. Provisional treatment plan was conveyed and checked to ensure it was acceptable.
- Latest medical update was requested and obtained.
- Medical team stated not to use local anaesthesia until KS has Echocardiography (ECHO) done. However, they stressed on the importance of maintaining good oral hygiene.

**Visit 1 (22/12/15)**

- A complete medical and dental history was taken together with a clinical examination.
- Radiographs were taken.
- A provisional treatment plan was formulated, discussed and agreed by parents.
- Simplified plaque index SPI: 55%.
- Oral hygiene and diet advice were given.
- 3-day diet sheet given.
- Liaised with medical team.
- Behaviour: Frankl scale: 3.

**Visit 2 (26/01/16)**

- Medical team report received.
- URB: exfoliated.
- SPI: 39%
- Acclimatisation.
- Teeth polished with prophylactic paste using a rubber cup in a slow handpiece.
- Cotton rolls isolation.
- Flowable composite placed and cured on UR6, UL6, LL6 and LR6.
- Fluoride varnish applied and flossed interproximally.
- Diet sheet discussed and tailored diet advice was given.
- Behaviour: Frankl 4.
- Photographs taken.
- Next visit:
  - Composite restorations on UR1 and UL1.
  - Separators for Hall crowns on LRE and LLE.

**Visit 3 (02/02/16)**

- SPI: 33%
- Under dry dam isolation:
  - Composite restorations on UR1 and UL1.
- Separators placed mesial and distal to LRE and LLE.
- Frankl 4.
- Next visit:
  - Hall crowns on LRE and LLE, and to assess distal surface of LRD and URD before placement of PMC
  - Separators on URE and ULE.

**Visit 4 (16/02/16)**

- OH improved, SPI: 28%
- Separators removed, intact distal surfaces of LRD and LLD
- PMC cemented with Aquacem on LRE and LLE.
- LLE: PMC tipped lingually.
- Separators placed mesial and distal to URE and ULE.
- Post-operative instructions given.
- Frankl 4
- Next visit: Hall crowns- URE and ULE.

**Visit 5 (23/02/16)**

- SPI: 17%.
- Separators removed.
- PMC cemented with Aquacem on URE and ULE.
- Frankl 4
- Next visit: composite restorations - LL2, LL1, LR1 and LR2.

**Visit 6 (08/03/16)**

- SPI: 10%.
- Under dry dam isolation:
  - Composite restorations: LL2, LL1. LR1 and LR2.
  - Restorations finished and polished.
- Post-operative photographs taken.
- Frankl 4.
- Review in 3 months

**POST-OPERATIVE TREATMENT PHOTOGRAPHS (08/03/16)**

**Figure 7: Anterior view in occlusion**

**Figure 8: View of maxillary arch.**

**Figure 9: View of mandibular arch**

**Visit 7- first review (16/06/16)**

- SPI:10%.
- All previous restorations and PMCs were intact.
- Bitewings were taken to assess caries and presence of pathology (Figure 10-11)


Figure 10: Right BWs


Figure 11: left BWs

**Radiographic findings:**

- Grade II.
- Well-adapted PMCs.
- LLE PMC tipped lingually.
- No new caries detected.

**Visit 8- second review (10/10/16)**

- No complaints.
- Good oral hygiene: SPI: 10%
- BPE:

| | | |
|---|---|---|
| 0 | 0 | 0 |
| 0 | 0 | 0 |

- Prevention:
    - ➢ Teeth polished with prophylactic paste.
    - ➢ Fluoride varnish application.
    - ➢ OHI reemphasised.
    - ➢ Reinforced diet advice.

**Visit 9 - third review (16/01/2017)**

- No complaints.
- SPI 8%.
- Fluoride varnish application
- Post treatment photographs (figure 12-13-14).
- Prevention and OHI reinforced.
- Review in 3-4 months according to the Royal College of Surgeons Guidelines for patients who have received chemotherapy and a bone marrow transplant 2012 [2]

**POST OPERATIVE RADIOGRAPHS (16/01/2017)**

**Figure 12: Anterior view**

**Figure 13: Maxillary view**

**Figure 14: Mandibular View**

## DISCUSSION AND REFLECTION ABOUT THE CASE PRESENTED

KS was born with Haemophagocytic lymphohistiocytosis (HLH) which is a rare autosomal recessive disorder that occurs mostly during infancy with a rapidly fatal outcome if left untreated [3]. She had a very difficult time during the first few years of her life and she requires lifelong medication to control her illness.

<u>Medical condition and dental implications</u>

A childhood illness is one of the predisposing factors for the presence of dental anomalies. KS was diagnosed with HLH in 2010 when she was 2 months old following which she was treated with chemotherapy and a stem cell transplant in June 2010. The systemic upset occurring during this period may have affected the dental development of both the primary and permanent dentitions. Due to KS medical condition and its complications, local anaesthetic was not used and her dental treatment formulated based on the Paediatric haematologist report.

A Molar Root-Incisor Malformation (MRIM) is a newly described dental phenotype with molar root malformation and an incisor crown defect affecting maxillary and mandibular central incisors as well as second primary molars. These abnormalities may cause clinical problems such as impaction, early tooth loss, space loss and poor incisor aesthetics [3].

<u>Prevention</u>

KS's oral hygiene was fair but she was considered to be a high caries risk patient due to the presence of enamel defects, the frequency of sugar intake and the complexity of her medical condition. Reinforcement of her oral hygiene and diet habits was formulated according to the Department of Health tool kit [1].

<u>Behaviour management</u>

KS was initially anxious in the dental environment due to the large number of medical procedures she had undergone in different hospitals for the management of HLH. Treatment was carried out using Non- Pharmacological Behaviour Management techniques. Her treatment started with acclimatization, simple restorations and fluoride varnish.

<u>Restorations</u>

Enamel hypoplasia on all first permanent molars, maxillary central incisors, mandibular central and lateral incisors had created non- cleansable surfaces making the affected teeth more susceptible to plaque retention and caries. Hypoplastic defects were restored with composite restorations as it provides a cleansable surface, decreases caries risk and improves aesthetics.

For restoring first permanent molars (FPMs), we chose to restore the teeth with flowable composites as an intermediate treatment. KS's FPMs are considered of a poor long term prognosis, liaising with orthodontic consultant and further radiographic assessment of her premolars, second permanent molars and third permanent molars is needed in the future to consider extraction. Also the crown deformity makes providing a well fitting PMC difficult and risk of creating an unintentional overhang increases the risk for plaque retention, gingival inflammation, infection and caries

ULE, URE, LLE and LRE were restored using the Hall technique as no local anaesthesia or tooth preparation is required [4].

Maintenance and reviews

KS is on regular follow up for preventive oral care at 3-4 months according to the Royal College of Surgeons guidelines for patients who have undergone chemotherapy and a bone marrow transplant 2012 [2].

Lesson learnt:

1. The importance of liaising with the medical team and confirming the proposed treatment plan prior to commencing dental treatment.
2. The proper use of Non Pharmacological Behaviour Management Techniques can lead to a successful outcome in dental treatment.

**References**

1. Department of Health and The British Association for the Study of Community Dentistry, *Delivering Better Oral Health, an evidence-based toolkit for prevention. third edition. London: Crown Coyright; 2014*. 2014: London.

2. The Oral Management of Oncology Patients Requiring Radiotherapy, Chemotherapy and / or Bone Marrow Transplantation -Updated 2012- The Royal College of Surgeons of England / The British Society for Disability and Oral Health

3. Wright, J.T., et al., *Molar root-incisor malformation: considerations of diverse developmental and etiologic factors.* Oral surgery, oral medicine, oral pathology and oral radiology, 2016. **121**(2): p. 164-172.

4. Innes, N., D. Evans, and D. Stirrups, Sealing caries in primary molars randomized control trial, 5-year results. Journal of dental research, 2011. **90**(12): p. 1405-1410.

# Case report

# Case: 3

# Management of Dento-alveolar Trauma

# (Paediatric Dentistry Advanced Clinical Care)

**In partial fulfillment of the degree**

**Clinical Doctorate in Paediatric Dentistry**

**Eastman Dental Institute**

**University College London**

**2014 – 2017**

## CASE SUMMARY

A healthy 9-year-old boy was referred by his General Dental Practitioner (GDP) for management of dental trauma. He presented with compromised oral hygiene (OH), subluxated of UR2, avulsed UR1 and intruded UL1. The avulsed UR1 had been replanted and splinted with orthodontic wire and brackets at A&E. UR1 was extirpated and dressed with non-setting calcium hydroxide to reduce the risk of resorption and infection. However, UR1 became ankylosed hence decoronation was performed and a removable-partial-denture was provided. UR2 and UL1 were monitored. Long-term management was planned and orthodontic consultation was obtained. He was reviewed for 16 months post-operatively.

## PATIENT DETAILS

Initials: GM

Gender: Male

Age at start of treatment: 9 years, 11 months.

Age at last review: 11 years, 3 months.

**PRE-TREATMENT ASSESSMENT**

**HISTORY OF PRESENTING PATIENT'S COMPLAINTS (22/09/2015)**

- Patient's complaint
  - Pain and tenderness related to his maxillary anterior teeth.
  - Unable to eat and bite after the trauma.
- History of dental trauma:
  - When: 18.08.2015.
  - Where: Swimming pool in Spain.
  - How: Slipped and hit his front teeth on the edge of the pool.
  - Signs of head injuries: No loss of consciousness/vomiting.
  - At A&E:
    - UR1 was replanted 3 hours after the trauma and splinted with orthodontic wire and brackets at A&E.
    - Extra-oral dry time: 3 hours- stored in water.
    - Amoxicillin 250mg was prescribed by A&E.

**RELEVANT MEDICAL HISTORY**

- Fit and well.
- Immunised.

**DENTAL HISTORY**

- Regular attendee.
- No history of dental treatment.
- Brushes twice daily with adults' fluoridated toothpaste.

**SOCIAL HISTORY**

- Lives with his parents and little sister- attends school.

## CLINICAL EXAMINATION

## EXTRA-ORAL EXAMINATION

- No abnormalities detected.
- Healing laceration on upper lip.
- Behaviour: Frankl scale 2.

## INTRA-ORAL EXAMINATION

**Soft tissue:**

- Gingival inflammation around maxillary anterior teeth.

**Hard tissue:**

- Teeth present:

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | E | D | C | 2 | 1 | 1 | 2 | C | D | E | 6 |
| 6 | E | D | C | 2 | 1 | 1 | 2 | C | D | E | 6 |

- Mixed dentition stage.
- Orthodontic wire, brackets and composite splint from UR2 to UL1.
- No caries.
- Composite restoration UR1.
- Hypomineralisation of incisal/coronal edge of UR6, UR2, UL1, UL2, ULC, UL6, LL6, LRC, LR6.
- Occlusion: Class I molar relationship.
- Sensibility tests were performed for traumatised teeth except electric-pulp-test.

## PRE-TREATMENT PHOTOGRAPH

**Figure 1: Anterior view (22/09/2015)**

## GENERAL RADIOGRAPHIC EXAMINATION

**Radiographs taken (22/09/2015):**

- Periapical radiographs (LCPA) and upper standard occlusal were taken to assess root development, root fractures and any periapical radiolucency. (Figures 2, 3 and 4).

**Radiographic findings:**

- Grade I.
- No alveolar bone fracture/root fracture.
- UR1: periapical radiolucency and widening of periodontal space.
- UL1: loss of periodontal space.
- Apecies of UR2, UR1, UL1, UL2 almost closed.

**Figure 2: USO**

**Figure 3: Right LCPA**

**Figure 4: left LCPA**

## DIAGNOSTIC SUMMARY

- Laceration of upper lip.
- Plaque induced gingivitis of maxillary and mandibular anterior region.
- UL1: Mild intrusion- 2mm
- UR1: Avulsion and enamel-dentine fracture.
- UR2: Subluxation.

**AIMS AND OBJECTIVES OF TREATMENT**

- Improve OH and prevent dental disease.
- To restore function and aesthetics of traumatised incisors.
- Prevent infection and possible root resorption by extirpating UR1.
- Monitor pulp sensibility and periodontal ligament healing of UR2, UL1 and UL2.
- Manage anxiety by promoting positive attitude towards dental care.
- Provide a multidisciplinary approach to optimize management.

**TREATMENT PLAN**

**Immediate term**

- Initiate root canal treatment of UR1 and removal of splint.
- Monitoring pulp sensibility of UR2, UL1 and UL2.
- Advise regarding soft diet and analgesics as needed.
- Prevention as outlined by the Department of Health Evidence Based Toolkit.
    - ➢ Brush twice daily with fluoridated toothpaste of 1450 ppm.
    - ➢ Spitting, no rinsing after brushing.
    - ➢ The frequency and the amount of sugar intake should be reduced.
- Discuss treatment options and long-term prognosis.

**Intermediate term**

- Monitor signs of trauma sequelae and manage accordingly.
- Continue root canal treatment of UR1.
- Monitor UR2 and UL1.

**Long term**

- Reinforce prevention and oral hygiene instructions (OHI).
- Explain the possible replacement options of UR1 if lost.

## TABLE OF MATERIALS USED

| Material used | Description |
|---|---|
| Electric pulp test | Kerr; Analytic Technology Corp, Redmond, WA |
| Ethyl-chloride test | Endo Cold Spray, Henry Schein, UK |
| Topical anaesthesia | Xylonor gel, 5% lidocaine gel |
| Local Anaesthetic (LA) | Lignospan special, 2.2ml (2% lidocaine 1:80000 epinephrine); Septodont, Saint-Maur-des- Fosses, France |
| Sodium-Hypochlorite NaOCl | 2.5% Sodium-Hypochlorite, Milton; Proctor & Gamble Australia, Parramatta, NSW |
| Non-setting calcium hydroxide $Ca(OH)_2$ | Ultracal®; Ultradent; South-Jordan |
| Intermediate restorative material (IRM) | IRM®; Dentsply, Milford, DE, USA |
| Alginate impression material | Orthodontic alginate Orthoprint, Zhermack, Italy |
| Suture | 3-0 vicryl dissolvable suture |

**Treatment undertaken:**

- **Behaviour management:**
  - All treatments were carried out under inhalation sedation (IS), topical anaesthetic and local anaesthesia (LA).
  - Consent was obtained.
  - Nitrous-oxide and Oxygen were titrated to patient's needs.
  - 100% Oxygen was given at the end of treatment until full recovery.
- **Isolation:**
  - Dry-dam isolation for RCT prior to removal of splint then rubber dam isolation for the rest of RCT.

**Visit 1 (22/09/15)**

- Full assessment including trauma history, radiographs and clinical pictures.
- GM was anxious. Frankl 2
- Advised analgesics and soft diet.
- Treatment plan was discussed and IS consent was obtained.
- Acclimatisation to IS.
- UR1: Extirpated pulp and dressed with non-setting $Ca(OH)_2$.
- Working-length PA (figure 5).
- Orthodontic wire removed.
- Letter to GDP
- N/V: Brackets removal, as patient was tired.

Grade I
File size 30 at 22.5 mm

**Figure 5: LCPA (22/9/2015)**

**Visit 2 (25/09/15)**

- Trauma review, sensibility tests gave positive response to UR2, UL1 and UL2.
- OH: Simplified Plaque index (SPI): 66%
- Brackets removal.
- OHI and prevention reinforced.

**Visit 3 (26/10/15)**

- Trauma review, UR2, UL1, UL2 gave positive response to sensibility test.
- UL1: Re-erupted by 2mm.
- Oral hygiene improved, SPI: 44%
- Basic periodontal Examination (BPE):

| | | |
|---|---|---|
| 1 | 0 | 1 |
| 1 | 0 | 1 |

- OHI and prevention reinforced.
- UR1: redressed with $Ca(OH)_2$.

**Visit 4 (26/02/16)**

- OH: SPI: 33%
- Sensibility tests were positive for adjacent teeth.
- UR1: Metallic sound indicative of ankylosis.
- UR1: Canal irrigated with NaOCl and re-dressed with $Ca(OH)_2$ (figure 6).
- GM referred to Orthodontic-Paediatric clinic for long term planning of UR1.
- OHI reinforced.

Figure 6: LCPA (26/02/16)

- Grade I.
- Good $Ca(OH)_2$ placement.
- No periapical infection.
- No root resorption.

**Visit 5 (20/05/16)**

- OH improved, SPI: 22%.
- Sensibility tests were positive for adjacent teeth.
- UR1:
  - Gingival discrepancy 3mm (Figure 7).
  - Metallic sound.
  - No signs of infection or pain.
- Patient was seen on Orthodontic-Paediatric joint clinic (21/04/16), two options were suggested:
  - Extract UR1 and provide removable partial denture (RPD) to preserve central line.
  - Decoronate UR1 to maintain alveolar bone width and RPD.
- Thorough discussion regarding both options, agreed on decoronation and replacing the crown with RPD.
- Consent obtained.
- Alginate impressions were taken to construct immediate partial denture.
- Radiographs for assessment (Figure 8- 9).
- GM was upset regarding the loss of UR1 crown.
- Consent was obtained.

**Figure 7: Anterior view (20/05/2016)**

**Figure 8: Right LCPA (20/05/2016)**

**Figure 9: Left LCPA (20/05/2016)**

- UR1: Replacement resorption, PDL not identified, $Ca(OH)_2$ resorbed.
- UL1: intact PDL.
- No Periapical pathology.

**Visit 6 (01/06/16):**

- Decoronation of UR1 under topical anaesthesia, LA and IS:
  - Flap was raised with two vertical incisions.
  - Gingival tissue was elevated using muco-periosteal elevator.
  - Crown sectioned with straight hand-piece.
  - IRM and cotton pledget removed.
  - Reduced root to 1mm below crestal bone.
  - Canal cleaned and bleeding initiated by file size 50.
  - Flap sutured.
  - Haemostasis achieved and post-operative instructions given.
  - RPD checked and delivered.
- Review healing and complications in a week.
- Frankl scale 3

**Visit 7 (09/06/16)**

- OH: SPI 22%
- Good healing of soft tissues.
- RPD was broken and pontic had detached and lost.
- Alginate impression was taken to construct new RPD.
- OHI re-emphasised.

**Visit 8 (15/6/16)**

- RPD checked and delivered.
- BPE:

| | | |
|---|---|---|
| 0 | 0 | 1 |
| 0 | 1 | 0 |

- Post-operative photographs taken (figure 10-11-12).
- Review in 3 months.

**Visit 9-First three months review (20/09/16)**

- OH: SPI 17%.
- Good fitted RPD.
- Positive response of sensibility tests for adjacent.
- Reinforced OH.
- Review after 6 months.

**Visit 10-Second six months review (20/01/17)**

- BPE:

| | | |
|---|---|---|
| 0 | 0 | 0 |
| 0 | 2 | 0 |

- Good fit of RPD.
- Radiographs were taken (figure 13-14).
- Review in 6 month to check RPD fit and continue monitoring adjacent teeth.

Figure 13: Right LCPA

Figure 14: Left LCPA

- Grade I.
- Continued root development of UR2, UL1 and UL2.
- No infection.
- Replacement resorption of remaining root of UR1.

## POST-OPERATIVE PHOTOGRAPHS (15/06/2016)

**Figure 10: Anterior view**

**Figure 11: Maxillary view**

**Figure 12: Mandibular view**

## LONG TERM TREATMENT PLAN AND FUTURE CONSIDERATIONS

- To monitor sensibility of adjacent teeth and manage accordingly.
- Reinforce prevention in conjunction with GDP.
- Consider Resin-retained bridge/ implant in the future.

## DISCUSSION AND REFLECTIONS ABOUT THE CASE PRESENTED

GM, a nine-year-old boy was referred by GDP regarding traumatised maxillary incisors 4 weeks before presentation. Emergency treatment was provided at A&E. Trauma was severe, comprising avulsion, intrusion and subluxation injuries; as well as lip laceration.

### Avulsion injuries

Avulsion of permanent teeth is one of the most serious dental injuries. Appropriate and prompt management is crucial for good prognosis. In most situations, replantation is the treatment of choice [1].

In GM's case, UR1 was stored in water for 3 hours before replantation. According to International Association of Dental Traumatology (IADT), this considered of an unfavourable outcome with likely ankylosis and root resorption. A metallic sound was noted 6 months after replantation indicating ankylosis.

The treatment aims are to extirpate UR1 as soon as possible to prevent inflammatory root resorption, and remove the splint, 4 weeks for avulsed tooth with >60 mins dry time, to allow periodontal and pulpal healing as per IADT guidelines [1].

### Subluxation and intrusive injuries

Subluxation injury is defined as injury to the supporting apparatus of the tooth causing mobility with no displacement of the injured tooth. While tooth intrusion is another type of dental traumas that displacement of a tooth further into the alveolar bone and its prevalence is 1.9% [2] [3].

### Management in clinic

The treatment provided depended on root maturity, extra-oral dry time, and storage medium of the avulsed tooth. In this case, the root was considered immature with delayed replantation, which suggests a poor prognosis of traumatised tooth [1].

### Endodontic Treatment

According to IADT guidelines, root canal treatment is indicated for delayed replantation >60 mins with immature apex is recommended as it is not expected to heal [1]. In this case, due to late presentation, root canal treatment was carries out during the first visit.

**Decoronation**

Decoronation is a surgical procedure for management of ankylosed teeth in children and adolescents. This procedure involves removing the crown and root canal filling to leave the root in situ covered with a mucoperiosteal flap. The advantage of this method is to preserve alveolar bone, especially in buccopalatal width and vertical height to facilitate optimal implant placement in the future with good aesthetic crown [4].

In children, ankylosis is associated with infra-occlusion of the tooth, the extent of infraposition depends on patient's age and the pubertal growth spurt at time of ankylosis [4].

In GM's case, he was 10 years of age when ankylosis was diagnosed. The patient had not reach his pubertal growth spurt. Therefore, decoronation was performed then the crown was replaced with RPD. Replacement of crown with fixed bridge is postponed until full eruption of canines [4].

**Management of Intrusion and subluxation injuries**

UL1 was intruded by 2mm so it was allowed to re-erupt without intervention following IADT guidelines [5]. The subluxated tooth was monitored. UL1 and UR2 were monitored for a year and 4 months with no complications.

**Lessons learnt**

- The prompt and proper management of an avulsed tooth is important for a good prognosis.
- The need to maintain/ preserve bone of a growing child.
- Attention should be paid to the psychological impact after severe dental trauma.

## REFERENCES


1. Andersson, L., et al., *International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth.* Dental Traumatology, 2012. **28**(2): p. 88-96.
2. Andreasen, J.O., et al., *Traumatic intrusion of permanent teeth. Part 1. An epidemiological study of 216 intruded permanent teeth.* Dental Traumatology, 2006. **22**(2): p. 83-89.
3. Piskorowski, J., *Traumatic intrusion of a tooth: a case report.* Dentistry today, 2006. **25**(2): p. 98.
4. Malmgren, B., *Decoronation: how, why, and when.* J Calif Dent Assoc, 2000. **28**(11): p. 846-54.
5. DiAngelis, A.J., et al., *International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 1. Fractures and luxations of permanent teeth.* Dental Traumatology, 2012. **28**(1): p. 2-12.

# CASE REPORT

**Case: 4**

**Complex restorative treatment**

**(Managing the developing child)**

**In partial fulfillment of the degree**

**Clinical Doctorate in Paediatric Dentistry**

**Eastman Dental Institute**

**University College London**

**2014 – 2017**

**Case summary**

N.S is a 13 years old girl who was referred by her (GDP) to the Eastman Dental Hospital (EDH), Department of Paediatric Dentistry for management of defects that affect permanent teeth.

Diagnosis of Amelogenesis Imperfecta was made based on the history and clinical examination. She has a class I complicated with upper lateral incisors in edge to edge contact with the lower lateral incisors.

Her main complains when she first presented were the sensitivity and the colour associated with her permanent dentition.

She was born prematurely. N. is medically fit and well with no history of any early childhood illnesses or systemic disorders. She was a regular dental attendee to her GDP and had previous dental treatment with LA.

Upon examination, she presented with generalized hypoplastic amelogenesis imperfect affecting permanent dentition. The teeth were small and spaced due to very thin enameland the gingiva was inflamed especially in the anterior region The enamel surface of her teeth look yellowish brownish in colour. The LR6, was root canal treated with occlusal onlay. Radiographic examination revealed the presence of all permanent dentition, LR6 root canal treatmented with occlusal onlay.

Treatment was carried mainly using non–pharmacological behaviour management (NPBM) techniques. Treatment provided:

- Prevention and acclimatization
- Oral hygiene instructions (OHIs).
- Dietary advice.
- Liaison with orthodontic department
- Restorations:
  - Composite veneers on UR5, UR4, UR3, UR2, UR1, UL1, UL2, UL3, UL4, UL5, LL5, LL4, LL3, LL2, LL1, LR1, LR2, LR3, LR4, LR5.
  -Preformaed metal crown on LR6, LR7, LL6, UL6, UL7, UR6.

**Pre-operative photographs:**

Anterior View

Maxillary view

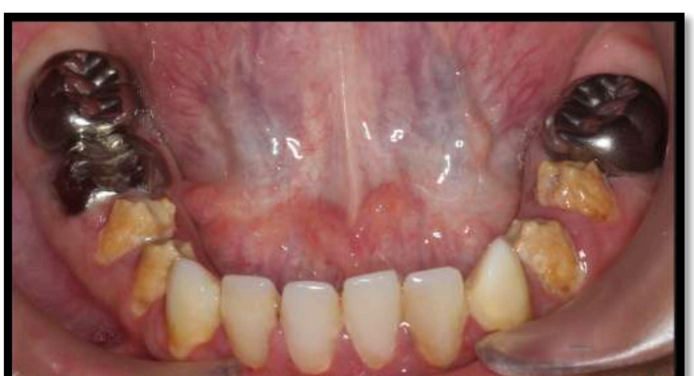
Mandibular view

**Inter-mediate post operative photographs**

**Frontal view**

**Maxillary view**

**Mandibular view**



**Personal data:**

- Name: N.S
- DOB: 11/02/2002
- Age: 13 years
- Sex: Female

**Reason for attendance:**

She was referred by his GDP to manage the deffected permanent teeth. Chief complaint (C/O):

- N and her mother complained of sensitive teeth with discoloration.
- food trapping from spacing and difficulty cleaning her teeth.
- She is also getting teased at school due to the appearance of the teeth.

**History of chief complaint:**

- N's mother noticed the brown discoloration in the newly erupted anterior teeth.
- Sensitivity in the back teeth associated with brushing and change in temperature.
- Does not disturb eating and sleeping.
- Does not require analgesics.

**Medical History (MH):**

- Medically fit and well with no relevant medical problems.
- No current medication.
- No known allergy.
- N. was born prematurely.
- No history of severe illness during the first three years of life.
- Immunisations up-to-date

**Social and Family History:**

- Lives with her parents, no siblings.
- Father was affected with defected teeth.
- Attends school.

**Dental History:**

- Regular attendee to GDP every 6 month.
- Had previous check-ups.
- Had root canal treatment and restorations done with local anaesthsia

**Dietary History:**

- Good appetite, eats a variety of food.
- Drinks mostly water.
- Limited sugar intake

**Oral Hygiene:**

- Brushes twice daily with adult toothpaste using regular manual tooth brush.

**Clinical Examination**

**Extra-oral Examination:**

- No facial asymmetry.
- No lymphadenopathy.
- Normal mouth opening with no Temporomandibular joint abnormality

**Intra-oral Examination:**

- Soft tissue (ST): Healthy soft tissue.
- Oral hygiene (OH): Fair- plaque index (PI): 62%
- Dentition: permanent dentition

| | |
|---|---|
| 6 5 4 3 2 1 | 1 2 3 4 5 6 7 |
| 7 6 5 4 3 2 1 | 1 2 3 4 5 6 |

- Upper lateral incisors in edge to edge contact with lower lateral incisors.
- Chipped lower lateral incisors.
- GIC on LR7 and LR5
- Composite restorations on UR6, UL6 and LL6.
- Occlusion:
    - Class I molar relationship.
    - Edge to edge contact of upper lateral incisors with lower lateral incisors.
- Enamel defect : Hypoplastic and hypoclacified phenotype affecting permanent dentition.

**Pre-operative radiograph (8.9.2015):**

**Orthopatogram**


Bitewings

Right side

Left side

Findings:

- All permanent dentition present.
- Reduced enamel thickness affecting all permanent dentition.
- caries on UR6, UL6 and LLL6.
- Taurodontism affected permanent molars.
- RCT on LR6

**Diagnosis**

- Combination hypoplastic-hypocalcified type Amelogenesis Imperfecta affecting permanent dentition.
- Dental caries affecting UR6, UL6 and LL6.
- Dental anxiety

**Treatment Objectives**

- To relieve the symptoms associated with permanent teeth.
- To stabilise dentition until definitive treatment can be done.
- To promote oral preventive measures.
- To restore function and aesthetic.
- Manage anxiety and enhance positive attitude towards dental care.

Provisional Treatment Plan

Prevention Treatment:

- Fluoride varnish application every 4 months.
- Oral hygiene education.
- Dietary education.

Behaviour Management:

- To use Non-Pharacological behavior management (NPBM) techniques.

Restorations and Extractions:

- Referral to Orthodontic department for orthodontic assessment and treatment planning.
- Restorative:
  - Restorations of UR6, UL6 and LL6 with Preformed metal crowns
  - Composite restorations on

| | |
|---|---|
| 5 4 3 2 1 | 1 2 3 4 5 |
| 5 4 3 2 1 | 1 2 3 4 5 |

Maintenance and Follow up:

- Clinical review every 4 months.
- Reinforcement of dietary advice and OHI.
- Monitor composite restorations and restored 6's.
- Radiographic review every 6-12 months.

**Treatment progress and Dental management**

**Visit 1 (8.9.2015)**

- Attended with her mother.
- A complete medical and dental history with clinical and radiographic examination taken.
- A provisional treatment plan was discussed and agreed by mother and patient.
- Orthodontic consultation regarding bonding of orthodontic brackets, agreed to restore UL3 to UR3 and LL3 to LR3 then decide whether to maintain or extract first permanent molars.
- Pre-operative clinical photographs.
- Oral hygiene instructions:
    - Brush twice daily
    - Adult toothpaste with 2500PPM Fluride.
    - Spitting after brushing.
    - Use a fluoride mouth rinse daily (0.05% NaF-) at a different time to brushing.
    - Diet education

Behavior: quiet but co-operative.

Next visit: composite build up of UR1 and UL1.

**Visit 2 (5.11.2015)**

- Attended with mother.
- MH: No change.
- C/O: sensitivity.
- E/O: no change.
- I/O: no change

Treatment (Tx):

UL1 and UR1

- Topical anesthesia 20% benzocaine
- Buccal infiltration, 2% Lidocaine 1:80,000 with adrenaline.
- Rubber dam isolation.
- Teeth were cleaned using prophy paste and brush using slow speed.
- Etched, bond, composite resin shade A1 body and XW enamel (filtek) using crown form.
- Finished and polished with soflex discs.

Behavior: co-operative.

Next visit: Composite build up on LL1 and LR1.

**Visit 3 (10.11.2015)**

- Attended with her mother.
- MH: No change
- E/O: No change
- I/O: Redness of the gingiva at LL7 region.
- C/O: Mild Pain in lower left quadrant due to erupting LL7.

Treatment (Tx):

LL1 and LR1

- Topical anesthesia applied 20% benzocaine.
- 2% lidocaine 1:80.000 was given as infiltration.
- Rubber dam isolation.
- Used prophy paste and brush with slow speed hand-piece.
- Itching, bonding, composite restoration with shade A1 body and Shade XW enamel (Filtek) using crown form.
- Finished and polished with soflex discs.

Behavior: Co-operative.

Next visit: composite build up of UL2 and UR2.

**Visit 4 (15.12.2015)**

- Attended with her mother
- MH: no change
- E/O: Nil
- I/O: Nil
- C/O: Discomfort with biting on first day but managed well later.

Treatment (Tx):

UL2 and UR2:

- ➢ Topical Anesthesia appiled.
- ➢ 2% lidocaine 1:80.000 adrenlaine was given as infiltration.
- ➢ Rubber dam isolation.
- ➢ Etching, bonding, composite resin restoration with shade A1 and XW (Filtek).
- ➢ Finished and polished with soflex discs.

Behavior: Co-operative.

Next visit: composite build-up of LL2 and LR2.

**Visit 5 (2.2.2016)**

- Attended with her mother.

Treatment:

LL2 and LR2:

- ➢ Topical anesthesia (20% Benzocaine).
- ➢ Local anesthesia, 2% lidocaine with 1:80.000 adrenaline was given as infiltration.
- ➢ Rubber dam isolation
- ➢ Etching, bonding, composite restoration shade A1 and XW (Filtek) using crown forms.
- ➢ Finished and polished with soflex discs.

Behavior: Co-operative.

Next visit: composite restorations on UL3 and UR3.

**Visit 6 (1.3.2016)**

- Attended with her mother.

Treatment:

UL3 and UR3:

- Topical anesthesia (20% Benzocaine)
- Local anesthesia, 2% lidocaine with 1:80.000 adrenaline was given as infiltration.
- Rubber dam isolation.
- Etching, bonding, composite restoration shade A1 and XW (Filtek) using crown forms.
- Finished and polished.

Behavior: Co-operative.

Next visit: Composite build-up on LL3 and LR3.

**Visit 7 (19.4.2016)**

- Attended with her mother
- MH: No change.

Treatment:

LL3 and LR3:

- Topical anesthesia (20% benzocaine)
- Local anesthesia, 2% lidocaine with 1:80.000 with adrenaline was given as infiltration.
- Rubber dam isolation.
- Etching, bonding, composite resin shade A1 and XW (Filtek).
- Finished and polished.

Behavior: co-operative.

Next visit: composite restoration of UR4.

**Visit 8 (3.5.2016)**

- Attended with her mother.

Treatment:

UR4

- ➢ Topical anesthesia (20% Benzocaine).
- ➢ Local anesthesia, 2% lidocaine with 1:80.000 adrenaline given as infiltration.
- ➢ Rubber dam isolation.
- ➢ Etching, bonding, composite restoration with shade A1 and XW (Filtek) using crown form.
- ➢ Finished and polished.

- Orthodontic consultation: thorough discussion regarding the importance of maintaining good oral hygiene and starting orthodontic treatment. Patient agreed not to commence orthodontic treatment at present and if the patient is concerned about upper segment alignment then removable appliance can be considered in the future.

Next visit: Preformed metal crown UL6 and UR6.

**Visit 9 (15.5.2016)**

- Attended with her mother.
- MH: No change
- C/O: Sensitivity of her back teeth.

Treatment: UL6 and UR6:

- ➢ Topical anesthesia (20% Benzocaine)
- ➢ Local anesthesia, 2% Lidocaine with 1:80.000 adrenaline.
- ➢ Cleared contact with mesial and distal slice using long tapered bur.
- ➢ Preformed metal crown size 5, trimmed and crimped.
- ➢ Cemented with aquacem on UL6 and UR6.
- ➢ Removed excess cement.

Behavior: co-operative.

Next visit: Preformed metal crown LL6 and LR6.

**Visit 10 (7.6.2016)**

- Attended with her mother

Treatment:

LL6 AND LR6:

- ➢ Topical anesthesia (20% Benzocaine).
- ➢ Local anesthesia, 2% Lidocaine with 1:80.000 adrenaline.
- ➢ Cleared contact with mesial slice using long tapered finishing bur.
- ➢ Preformed metal crown size 3, trimmed and crimped.
- ➢ Cemented with aquacem on UL6 and UR6.
- ➢ Removed excess cement
- ➢ Post- operative instructions given.

Behavior: co-operative.

Next visit: composite restoration UL4 and UL5.

**Visit 11 (14.6.2016)**

- Attended with her mother

  UL4 AND UL5

  Treatment:

  - ➢ Topical anesthesia (20% Benzocaine).
  - ➢ Local anesthesia, 2% Lidocaine with 1:80.000 adrenline.
  - ➢ Rubber dam placed.
  - ➢ Prophylactic paste and brush used to clean the tooth.
  - ➢ Celluloid crown form trimmed and adjusted.
  - ➢ Etching, bonding, composite restoration with shade A1 and XW (Filtek) using crown form.
  - ➢ Finished and polished.
  - ➢ Occlusion checked.
  - ➢ POIG.

  Next visit: composite restoration UR5.

**Visit 12 (21.6.2016)**

- Attended with her mother.

  Treatment: UR5

  - Topical anesthesia (20% Benzocaine).
  - Local anesthesia, 2% lidocaine with 1:80.000 adrenaline given as infiltration.
  - Rubber dam isolation.
  - Etching, bonding, composite restoration with shade A1 and XW (Filtek) using crown form.
  - Finished and polished.
  - Checked the occlusion.
  - Occlusion checked.
  - POIG.

  Next visit: Composite restoration of LR4 and LR5.

**Visit 13 (4.8.2016)**

- Attended with her mother.

  C/O: NIL.

  Treatment: LR4 and LR5

  - Topical anesthesia (20% Benzocaine).
  - Local anesthesia, 2% lidocaine with 1:80.000 adrenaline given as infiltration.
  - Rubber dam isolation.
  - Etching, bonding, composite restoration with shade A1 and XW (Filtek) using crown form.
  - Finished and polished.
  - Checked the occlusion.
  - Occlusion checked.
  - POIG.

  Next visit: Composite restoration of LL4 and LL5.

**Visit 15 (14.12.2016)**

- Attended with her mother.

  Treatment: LL4 and LL5

  - Topical anesthesia (20% Benzocaine).
  - Local anesthesia, 2% lidocaine with 1:80.000 adrenaline given as infiltration.
  - Rubber dam isolation.
  - Etching, bonding, composite restoration with shade A1 and XW (Filtek) using crown form.
  - Finished and polished.
  - Checked the occlusion.
  - Occlusion checked.
  - POIG.

Next visit: review in 3 months

**Visit 16 (15.2.2017)**

- Attended with her mother.
- C/O: pain in UL6

Radiograph:

- Right and left bitewings:
  - SSC of the UL6 seated on the distal side of UL5.
  - SSC of the UR6 seated on the distal side of UR5.

Treatment: Replace SSC of the UL6

- Topical anesthesia.
- Local anesthesia, 2% Lidocaine with 1:80.000 adrenaline given as infiltration.
- SSC was sectioned and removed.
- Separator was placed between UL5 and UL6 (separator was left until second appointment at the afternoon.
- Separator removed.
- UL5 re-restored with composite filling to cover the distal part of the tooth.
- SSC size 3 was cemented with aquacem on UL6.

- Proximal surfaces checked with dental floss.
- POIG.

Next visit: replace SSC of the UR6 AND Composite restoration of UR5.

**Visit 17 (11.7.2017)**

- Attended with her mother.

Treatment: replace SSC UR6 and composite restoration on UR5.

- Topical anesthesia.
- Local anesthesia, 2% Lidocaine with 1:80.000 adrenaline given as infiltration.
- SSC was sectioned and removed.
- Separator was placed between UR5 and UR6 (separator was left until second appointment at the afternoon.
- Separator removed.
- UL5 re-restored with composite filling to cover the distal part of the tooth.
- SSC size 3 was cemented with aquacem on UR6.
- Proximal surfaces checked with dental floss.
- POIG.
- Oral hygiene instruction given.
- Post-operative photograph was taken.

Post- operative photograph

Appraisal and discussion

N.S, a 14 years old female, was born prematurely at 36 weeks of gestation. Many studies revealed a strong relationship between premature infants and enamel defects. The prevalence of enamel defects of premature infants in both primary and permanent dentitions were 78% and 83%, respectively.

N. was diagnosed with a combination of hypoplastic and hypomineralised Amelogenesis Imperfecta based on history, clinical presentation and radiographic examination. AI is defined as ''hereditary enamel defect affecting the structure and clinical appearance of the enamel of all or nearly all the teeth in a more or less equal manner and which may be associated with morphologic or biochemical changes elsewhere in the body'' ( Crawford et al , 2007).

Aims of the treatment were to relieve dental sensitivity and to restore function and aesthetic of permanent dentition. Also to protect the teeth from further breakdown and caries.

Behavior management

Treatment was carries out using Non- pharmacological behavior management (NPBM) techniques as she was willing to sit for dental examination. N. and her mother were keen to restore her teeth. They attended all the appointments and were always on time.

Prevention

N. was considered a high-risk patient due to defective enamel. It was cited that enamel hypoplasia is associated with plaque accumulation and therefore dental caries (Eastman 2003). Her oral hygiene was fair possibly due to sensitivity and difficulty in maintaining good oral hygiene. Oral hygiene instructions were formulated according to Department of Health tool kit.

Restorations

Composite restoratios were used to restore function, aesthetic and relieve sensitivity. Also to protect her teeth from further breakdown. Composite restorations are considered conservative and offer a satisfactory long-term outcome. Layering of dentine and enamel shades was used to simulate natural color gradations.

Molars were restored using preformed metal crowns (PMC). Stainless steel crowns have proved to be very successful solution to preserve posterior molars with developmental defects until an appropriate time to place more permanent restorations (Hanlin et al., 2015)

Orthodontic treatment

N. was assessed in orthodontic department and after thorough discussion, agreed to not carry out orthodontic treatment as N. is finding difficulties to maintain good oral hygiene. Commencing orthodontic treatment at present might further complicate teeth brushing therefore plaque accumulation and caries risk will be increase. Agreed to provide removable appliance after completion of restorative treatment to align upper labial segment.

Maintenance and reviews

N. will be reviewd every 3-4 months to reinforce OHI and dietary advice (Department pf Health, 2009), also to monitor composite restorations. Radiographic review will be every 6-12 months as needed (SDCEP, 2010).

## References

Crawford, P., Aldred, M., & Bloch, A. (2007). Amelogenesis Imperfecta. Orphanet Journal of Rare Diseases, Volume 2.2:12 dor 10.1186/1172-2-17.

Department of Health, 2015. Delivering Better Oral Health An evidence-based toolkit for prevention - third edition.

Eastman, D. L. (2003). "Dental outcomes of preterm infants." Newborn and Infant Nursing Reviews 3(3): 93-98. Fadavi, S., S. Adeni, et al. (1991). "The oral effects of orotracheal.

Hanlin, S., Burbridge, L., Drummond, B. 2015. Restorative Management of Permanent Teeth Enamel Defects in Children and Adolescents. Planning and Care for Children and Dolescent with Dental Enamel Defects. 11, 139-155.

Scottish Dental Clinical Effectiveness Programme, 2010. Prevention and Management of Dental Caries in Children4