

Chulalongkorn University

## Chula Digital Collections

---

Chulalongkorn University Theses and Dissertations (Chula ETD)

---

2019

### The study of oro-dental structures and application of dental adhesives in patients affected with osteogenesis imperfecta

Oadcharawadee Nutchoey  
*Faculty of Dentistry*

Follow this and additional works at: <https://digital.car.chula.ac.th/chulaetd>

---

#### Recommended Citation

Nutchoey, Oadcharawadee, "The study of oro-dental structures and application of dental adhesives in patients affected with osteogenesis imperfecta" (2019). *Chulalongkorn University Theses and Dissertations (Chula ETD)*. 8641.

<https://digital.car.chula.ac.th/chulaetd/8641>

This Thesis is brought to you for free and open access by Chula Digital Collections. It has been accepted for inclusion in Chulalongkorn University Theses and Dissertations (Chula ETD) by an authorized administrator of Chula Digital Collections. For more information, please contact [ChulaDC@car.chula.ac.th](mailto:ChulaDC@car.chula.ac.th).

THE STUDY OF ORO-DENTAL STRUCTURES AND APPLICATION OF DENTAL ADHESIVES  
IN PATIENTS AFFECTED WITH OSTEOGENESIS IMPERFECTA



A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in Geriatric Dentistry and Special Patients Care  
Common Course  
Faculty of Dentistry  
Chulalongkorn University  
Academic Year 2019  
Copyright of Chulalongkorn University

การศึกษาลักษณะโครงสร้างช่องปากและฟันและสารยึดติดทางทันตกรรมในผู้ป่วยที่มีโรคกระดูก  
เปราะกรรมพันธุ์



น.ส.อัจฉราวดี นุชเฉย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต  
สาขาวิชาทันตกรรมผู้สูงอายุและการดูแลผู้ป่วยพิเศษ ไม่สังกัดภาควิชา/เทียบเท่า  
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย  
ปีการศึกษา 2562  
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

Thesis Title	THE STUDY OF ORO-DENTAL STRUCTURES AND APPLICATION OF DENTAL ADHESIVES IN PATIENTS AFFECTED WITH OSTEOPENIA
By	Miss Oadcharawadee Nutchoe
Field of Study	Geriatric Dentistry and Special Patients Care
Thesis Advisor	Associate Professor THANTRIRA PORNTAVEETUS, D.D.S., Ph.D.

---

Accepted by the Faculty of Dentistry, Chulalongkorn University in Partial  
Fulfillment of the Requirement for the Master of Science

..... Dean of the Faculty of Dentistry  
(Assistant Professor SUCHIT POOLTHONG, D.D.S., Ph.D.)

THESIS COMMITTEE

..... Chairman  
(Assistant Professor ANJALEE VACHARAKSA, D.D.S., Ph.D.)

..... Thesis Advisor  
(Associate Professor THANTRIRA PORNTAVEETUS, D.D.S.,  
Ph.D.)

..... External Examiner  
(Assistant Professor Paramaporn Chiewpattanakul  
Kaewmanee, D.D.S., Ph.D.)

อัญฉรวาตี นุชเฉย : การศึกษาลักษณะโครงสร้างช่องปากและฟันและสารยึดติดทางทันตกรรมใน  
ผู้ป่วยที่มีโรคกระดูกเปราะกรรมพันธุ์. ( THE STUDY OF ORO-DENTAL STRUCTURES AND  
APPLICATION OF DENTAL ADHESIVES IN PATIENTS AFFECTED WITH OSTEOGENESIS  
IMPERFECTA ) อ.ที่ปรึกษาหลัก : รศ. ทย.ดร.พันศิริรา พรทวีทัศน์

วัตถุประสงค์ เพื่อวิเคราะห์การเปลี่ยนแปลงทางพันธุกรรม ลักษณะทางคลินิกของช่องปากและฟัน โครงสร้างทางจุลภาคของฟัน และประสิทธิภาพสารยึดติดทางทันตกรรมระบบโททอลเอซท์ (ออฟติบอนด์ เอฟเอล) และระบบเซลฟ์เอซท์ (เคลียร์ฟิลล์ เอสอี บอนด์) กับฟันที่มีการสร้างเนื้อฟันไม่สมบูรณ์ในผู้ป่วยโรคกระดูกเปราะกรรมพันธุ์ เปรียบเทียบกับฟันในผู้ป่วยปกติ

วิธีการ ผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็นโรคกระดูกเปราะกรรมพันธุ์ 3 ราย ที่คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ได้รับการซักประวัติ ตรวจ ถ่ายภาพรังสี ตรวจทางห้องปฏิบัติการ และตรวจเพื่อหาความผิดปกติของสารพันธุกรรมเพื่อระบุการกลายพันธุ์อันเป็นสาเหตุของโรคด้วยวิธีการหาลำดับพันธุกรรมในเอ็กซอนและวิธแบบแซงเกอร์ ฟันที่ได้รับจากผู้ป่วยคนละ 1 ซี่ ถูกนำไปตรวจเพื่อประเมินลักษณะสีด้วยเครื่องวัดสี ศึกษาโครงสร้างและปริมาณแร่ธาตุด้วยเครื่องเอกซเรย์คอมพิวเตอร์ระดับไมโครเมตร วัดความหยาบของพื้นผิวด้วยเครื่องวัดความหยาบพื้นผิวแบบสัมผัส และตัดแบ่งฟันเพื่อทดสอบความแข็งของฟันด้วยเครื่องทดสอบความแข็งระดับจุลภาคแบบบุฟ วิเคราะห์ธาตุและโครงสร้างจุลภาคของฟันด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด หลังจากนั้นจึงนำไปทดสอบประสิทธิภาพสารยึดติดทางทันตกรรม ด้วยการยึดชิ้นเรซินคอมโพสิตกับเนื้อฟันที่ได้จากผู้ป่วย โดยใช้สารยึดติดระบบโททอลเอซท์ (ออฟติบอนด์ เอฟเอล) และระบบเซลฟ์เอซท์ (เคลียร์ฟิลล์ เอสอี บอนด์) เพื่อทดสอบหาค่าความแข็งแรงยึดติด ความแข็งแรงเฉือนและการรื้อขี้นระดับจุลภาค โดยเปรียบเทียบกับฟันกลุ่มควบคุม

ผลการศึกษา ผู้ป่วยโรคกระดูกเปราะกรรมพันธุ์ทั้ง 3 ราย พบการกลายพันธุ์ในยีน *COL1A2* แบบมิสเซนส์ การวิเคราะห์ฟันพบว่า ฟันมีสีเหลืองคล้ำจนถึงสีน้ำตาล ความหนาของชั้นเนื้อฟันพบได้ทั้งแบบบางและหนา และพบทั้งโพรงประสาทฟันขนาดใหญ่และตีบ ความหยาบผิวของฟันผู้ป่วยทั้งสามรายแตกต่างกัน ชั้นเคลือบฟันของผู้ป่วยพบว่ามีความหนาแน่นของแร่ธาตุน้อยลง แต่มีความหลากหลายของความหนาแน่นของแร่ธาตุในชั้นเนื้อฟัน ฟันผู้ป่วยทุกซี่มีความแข็งของชั้นเนื้อฟันลดลงอย่างมีนัยสำคัญ เช่นเดียวกับชั้นเคลือบฟันที่มีความแข็งน้อยกว่ากลุ่มควบคุม มีปริมาณแร่ธาตุคาร์บอนมากขึ้นทั้งในชั้นเคลือบฟันและชั้นเนื้อฟัน และเมื่อส่องเนื้อฟันด้วยกล้องจุลทรรศน์แบบส่องกราดพบว่า ท่อเนื้อฟันของผู้ป่วยมีจำนวนน้อย เรียงตัวไม่เป็นระเบียบ เมื่อนำฟันไปทดสอบประสิทธิภาพสารยึดติด พบว่า ฟันของผู้ป่วยมีค่าด้านทานแรงเฉือนและแรงดึงน้อยกว่าฟันปกติ ทั้งในระบบโททอลเอซท์ (ออฟติบอนด์ เอฟเอล) และระบบเซลฟ์เอซท์ (เคลียร์ฟิลล์ เอสอี บอนด์) และสารยึดติดระบบโททอลเอซท์ที่มีค่าด้านทานแรงเฉือนและแรงดึงมากกว่าระบบเซลฟ์เอซท์

สรุปผลการศึกษา ผู้ป่วยโรคกระดูกเปราะกรรมพันธุ์ ที่มีการกลายพันธุ์ที่ยีน *COL1A2* แบบมิสเซนส์ มีผลต่อลักษณะทางคลินิกของฟัน โครงสร้างของเนื้อฟัน ความหนาแน่นของแร่ธาตุในชั้นเคลือบฟัน ความแข็งของชั้นเนื้อฟัน และคุณสมบัติในการยึดติดกับวัสดุทางทันตกรรม

สาขาวิชา	ทันตกรรมผู้สูงอายุและการดูแล	ลายมือชื่อนิสิต .....
	ผู้ป่วยพิเศษ	
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

# # 6075843832 : MAJOR GERIATRIC DENTISTRY AND SPECIAL PATIENTS CARE

KEYWORD: Genetics, Microtensile bond strength, Microshear bond strength, Dentin-adhesive interface

Oadcharawadee Nutchoey : THE STUDY OF ORO-DENTAL STRUCTURES AND APPLICATION OF DENTAL ADHESIVES IN PATIENTS AFFECTED WITH OSTEOGENESIS IMPERFECTA . Advisor: Assoc. Prof. THANTRIRA PORNTAVEETUS, D.D.S., Ph.D.

Objectives: To investigate genetic mutations, oro-dental features, tooth ultrastructures, and characteristics of total-etch adhesives (Optibond FL; Kerr) and self-etch adhesives (Clearfill SE Bond; Kuraray) applied on the teeth of the patients who affected with syndromic osteogenesis imperfecta and dentinogenesis imperfecta(DGI), compared with the controls.

Methods: Three patients were diagnosed with osteogenesis imperfecta and dentinogenesis imperfecta at Faculty of Medicine, Chulalongkorn University. Clinical, radiographic, and laboratory examination were performed. Genetic mutations were analyzed by whole-exome sequencing (WES) and Sanger sequencing. Three teeth were obtained, one tooth from each patient (DGI tooth). Each tooth sample was examined for its color by colorimeter, mineral composition by Micro-CT scan, surface roughness by profilometer, microhardness by Knoop microhardness tester, microscopic morphology by scanning electron microscope (SEM) and histology, and mineral composition by EDX. The microtensile and microshear bond strengths ( $\mu$ TBS and  $\mu$ SBS) of two dental adhesive system, total-etch adhesives (Optibond FL; Kerr) and self-etch adhesives (Clearfill SE Bond; Kuraray), were analyzed compared with the controls.

Results: WES identified that the patients with OI and DGI harbored the missense mutations in *COL1A2*. The DGI teeth were yellowish and dark. The size of dentin and pulp cavity and enamel surface roughness of DGI teeth were diverse. Mineral density of DGI enamel was lower than that of the controls while the mineral density of DGI dentin was variable. Knoop microhardness values of DGI dentin were statistically lower than those of the controls. Correspondingly, DGI enamel also showed reduced microhardness values. Carbon percentage was increased in both DGI enamel and dentin. SEM revealed irregular arrangement and reduced number of dentinal tubules in DGI teeth. Dental adhesive analyses showed that  $\mu$ TBS and  $\mu$ SBS of DGI teeth were lower than those of the controls. Optibond FL showed superior  $\mu$ TBS and  $\mu$ SBS with DGI dentin compared to Clearfil SE bond.

Conclusions: This study demonstrates that syndromic DGI teeth of OI patients possessing the missense mutations in *COL1A2* exhibited alterations in clinical characteristic, dentin ultrastructure, mineral density, and hardness of dental tissues. These variations could influence the performance of dental adhesives.

Field of Study:	Geriatric Dentistry and Special Patients	Student's Signature .....
	Care	
Academic Year:	2019	Advisor's Signature .....

## ACKNOWLEDGEMENTS

This study was supported 90th Anniversary of Chulalongkorn University, Rachadapisek Sompote Fund and Faculty of Dentistry, Chulalongkorn University

I would like to express my deep gratitude to Assoc. Prof. Dr. Thantrira Porntaveetus, my best research supervisor, for her professional guidance, active motivation, and valuable support. I would like to thank Dr. Narin Intarak, Mr. Sernporn Thaweesapphithak, Mrs. Lawan Boonprakong, Mr. Anuchart Srijunbarl and researchers at Genomics and Precision Dentistry Research Unit, Department of Physiology Faculty of Dentistry for advice and assistance on my research. I would also like to extend my thanks to my friends and all lecturers in the Master of Science Program in Geriatric Dentistry and Special Patients Care (International Program). I deeply appreciate all kind support.

Lastly, I am grateful to have my family who are caring, love, and always support me. I love you to the moon and back.

Oadcharawadee Nutchoe



จุฬาลงกรณ์มหาวิทยาลัย  
CHULALONGKORN UNIVERSITY

## TABLE OF CONTENTS

	Page
.....	iii
ABSTRACT (THAI) .....	iii
.....	iv
ABSTRACT (ENGLISH) .....	iv
ACKNOWLEDGEMENTS .....	v
TABLE OF CONTENTS .....	vi
LIST OF TABLES .....	ix
LIST OF FIGURES .....	x
Chapter I .....	1
INTRODUCTION.....	1
Background and Rationale .....	1
Research questions.....	2
Research objectives.....	2
Research hypothesis.....	2
Scope of Research .....	2
Limitation.....	3
Expected Outcomes.....	3
Keyword .....	3
Research design.....	4
Obstacles and Strategies .....	4
Conceptual framework .....	4



Chapter II .....	5
LITERATURE REVIEW .....	5
Dentinogenesis imperfecta (DGI).....	5
Dental adhesives.....	6
Chapter III .....	9
RESEARCH METHODOLOGY .....	9
Subject enrollment .....	9
Tooth collection .....	9
Whole-Exome Sequencing (WES) and Sanger sequencing .....	9
Tooth analysis.....	10
Micro CT analyses.....	10
Color measurement.....	10
Surface roughness measurement.....	11
Microhardness measurement.....	11
Gold coating and Energy-Dispersive X-ray (EDX) and Scanning Electron microscopy (SEM).....	12
Histology .....	13
Dental adhesive analyses.....	13
Microtensile bond strength ( $\mu$ TBS).....	13
Microshear bond strength ( $\mu$ SBS).....	14
Microleakage test.....	14
Statistical Analysis.....	15
Chapter IV.....	16
RESULTS.....	16

Physical characteristics.....	16
Mutation analyses.....	20
Analyses of dental ultrastructure .....	20
Tooth samples .....	20
Micro-CT analyses.....	21
Color measurement .....	25
Surface roughness.....	26
Microhardness .....	27
Energy-Dispersive X-ray (EDX).....	30
Scanning Electron Microscopy (SEM).....	30
Histology .....	31
Adhesive analyses.....	33
Microshear Bond Strength ( $\mu$ SBS).....	33
Microtensile bond strength ( $\mu$ TBS).....	35
Microleakage Test .....	4
CHAPTER V .....	6
DISCUSSION.....	6
CHAPTER VI .....	9
CONCLUSION .....	9
REFERENCES .....	10
VITA.....	15

## LIST OF TABLES

	Page
<i>Table 1 The mineral density of enamel and dentin (mg/cm<sup>3</sup>) analyzed by micro-CT scan. ....</i>	23
<i>Table 2 The mean CIE L*a*b values and delta E values of OIDI samples and the controls. ....</i>	25
<i>Table 3 Surface roughness of the enamel on buccal and lingual surfaces of DGI samples and controls shown in mean ± standard deviation (SD). ....</i>	26
<i>Table 4 Knoop microhardness of the enamel and dentin of DGI samples and their controls shown in mean and standard deviation (SD). ....</i>	28
<i>Table 5 Elemental analysis (% weight) of carbon (C), oxygen (O), phosphorus (P), and calcium (Ca), of DGI samples and controls. ....</i>	30
<i>Table 6 Microshear bond Strength of Clearfil SE bond.....</i>	33
<i>Table 7 Microshear bond Strength of Optibond.....</i>	34
<i>Table 8 Microtensile bond Strength of Clearfil SE Bond.....</i>	1
<i>Table 9 Microtensile bond Strength of Optibond.....</i>	2
<i>Table 10 The scoring category of microleakage in each specimen .....</i>	4

## LIST OF FIGURES

	Page
<i>Figure 1 Phenotypes and family pedigree of patient 1 (DGI1).</i> .....	17
<i>Figure 2 Phenotypes and family pedigree of patient 2 (DGI2).</i> .....	18
<i>Figure 3 Phenotypes and family pedigree of patient 3 (DGI3).</i> .....	19
<i>Figure 4 Chromatogram.</i> .....	20
<i>Figure 5 Stereo-microscopic imaging.</i> .....	21
<i>Figure 6 Micro-CT imaging</i> .....	22
<i>Figure 7 The column chart of mineral density (mg/cm<sup>3</sup>) of enamel and dentin</i> .....	24
<i>Figure 8 The graph of surface roughness.</i> .....	27
<i>Figure 9 The charts of Knoop microhardness.</i> .....	29
<i>Figure 10 SEM imaging of the dentin.</i> .....	31
<i>Figure 11 Histology imaging of dentin</i> .....	32
<i>Figure 12 The chart of Microshear bond strength (μSBS).</i> .....	35
<i>Figure 13 The chart of Microtensile bond strength (μTBS).</i> .....	3
<i>Figure 14 Stereo-microscopic images of Microleakage test.</i> .....	5

## Chapter I

### INTRODUCTION

#### Background and Rationale

Dentinogenesis imperfecta (DGI) is an autosomal dominant genetic disorder affecting the dentin of both primary and permanent dentitions. Three types of DGI have been proposed. DGI type I is associated with osteogenesis imperfecta while type II and III are isolated or non-syndromic. The mutations in the *COL1A1* and *COL1A2* genes have been shown to cause type I whereas mutations in the *DSPP* gene which encodes proteins related to dentin formation are the main determinant of type II and III DGI. Generally, the patient's teeth are yellow-brown opalescent in color, weak, and prone to rapid wear. Until now, the understandings of dentin defects associated with syndromic DGI and the studies about dental adhesives in DGI teeth are still limited. This study therefore aimed to study the characteristics of orodental anomalies, tooth ultrastructure, and characteristics of dental adhesives applied on syndromic DGI teeth. The mutations were identified by whole exome sequencing (WES) and Sanger sequencing. Tooth color, surface roughness, nanohardness, mineral composition, ultrastructure of the affected teeth were studied compared with the controls (tooth type-matched) obtained from healthy age and sex-matched individuals. The microtensile strength, microshear strength, microleakage, and formation of resin tags of total-etch (OptiBond FL, Kerr, California, USA) and two-step self-etch (Clearfil SE bond, Kuraray, Tokyo, Japan) applied on the DGI teeth were studied compared with the controls. The findings broaden the knowledge on clinical features, genetic variants, dental ultrastructure, and dental adhesive application of syndromic DGI teeth. These are highly valuable for the diagnosis, prognosis, and management of the disorders.

### Research questions

1. What are genetic variants associated with syndromic DGI?
2. What are the oro-dental characteristics and dental ultrastructure of DGI teeth in OI patients?
3. What are the characteristics of dental adhesives applied on the teeth affected with syndromic DGI?

### Research objectives

1. To analyze genetic mutations associated with syndromic DGI patient's phenotypes
2. To investigate oro-dental characteristic and ultrastructure of syndromic DGI teeth.
3. To investigate the characteristics of total-etch adhesives (Optibond FL; Kerr) and self-etch adhesives (Clearfill SE Bond; Kuraray) applied on DGI teeth compared to the controls.

### Research hypothesis

1. Genetic mutations affect craniofacial and dental characteristics of patients with syndromic DGI.
2. Syndromic DGI teeth have different ultrastructure compared to the controls.
3. Total-etch and self-etch adhesives applied on DGI dentin show different microtensile strength, microshear strength, and microleakage compared to the controls.

### Scope of Research

The research aimed to investigate the characteristics of oro-dental structure, ultrastructure of dental tissue, and application of dental adhesives of DGI teeth associated with OI. Three patients were diagnosed with OI at the Faculty of Medicine

and received dental treatment at the Faculty of Dentistry, Chulalongkorn University. One tooth was obtained from each patient (DGI tooth) will be included in the study. Each DGI tooth was studied compared with three sound teeth (tooth type-matched teeth) obtained from healthy individuals (age and sex-matched). Three ml. of blood samples were collected for DNA extraction and subjected for mutation analyses. The informed consents were obtained from each participant. The researcher recorded the history and performed clinical, radiographic, and laboratory examinations of the patients. The exfoliated or extracted teeth were subjected for investigations of their color, surface roughness, nanohardness, ultrastructure, and mineral composition measurements. Ultrastructures were analyzed by micro-CT, SEM, and histology. The outcomes of dental adhesives applications including the microshear strength, microtensile bond strength, and microleakage were investigated. The total-etch adhesives (Optibond FL; Kerr) and self-etch adhesives (Clearfill SE Bond; Kuraray) were included.

### **Limitation**

The number of DGI teeth obtained from OI patients were limited.

### **Expected Outcomes**

1. To improve understandings of oro-dental characteristic and dental ultrastructure of syndromic DGI teeth.
2. To identify genetic mutations and expand genetic knowledge associated with DGI and OI.
3. To expand the understanding of an application of dentin adhesives for DGI teeth which is beneficial for clinical practice, education, and research.

### **Keyword**

Genetics, Microtensile bond strength, Microshear bond strength, Dentin-adhesive interface

## Research design

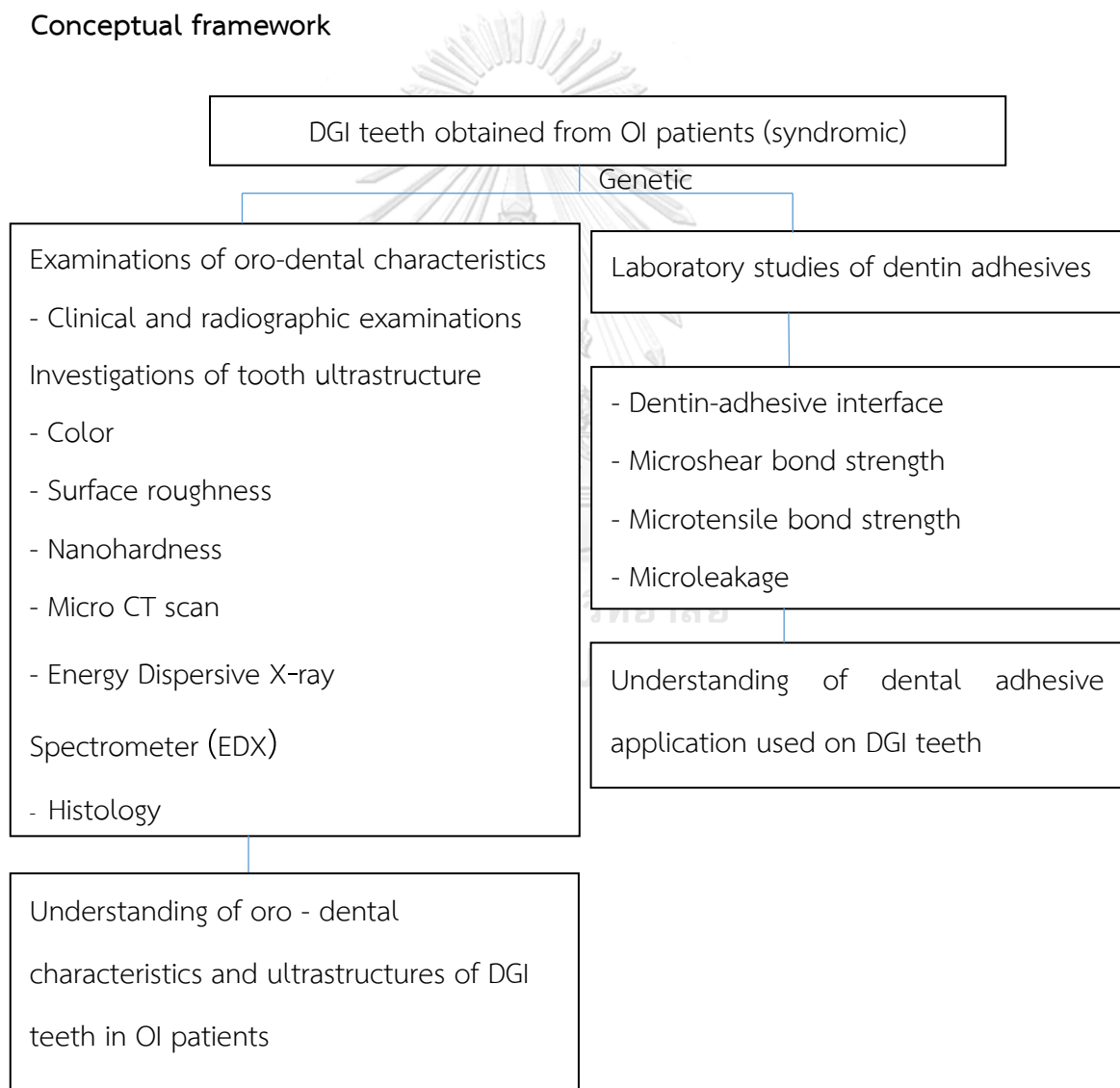
Clinical and laboratory research

## Obstacles and Strategies

Obstacle: syndromic DGI teeth were fragile and easily fractured during sectioning.

Strategy: the lowest speed of precision saw (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA) was used to prevent sample crack during sectioning.

## Conceptual framework





## Chapter II

### LITERATURE REVIEW

#### Dentinogenesis imperfecta (DGI)

DGI is an autosomal dominant genetic condition showing the disturbances of dentin formation with an incidence of 1 in 6,000 to 1 in 8,000 (1). DGI affects both primary and permanent dentitions. Shield *et al.* (1973) classified 3 types of DGI based on phenotypic variability (2). DGI Type I is associated with bone disorder, osteogenesis imperfecta. OI is mostly caused by mutations in the genes that code type 1 collagen: collagen 1A1 (*COL1A1*) and collagen 1A2 (*COL1A2*) gene (1, 3). DGI Type II is isolated and not associated with OI. Oral clinical and radiographic features are similar to DGI type I. Clinically, the DGI teeth are opalescent with the color ranging from bluish-gray to yellow-brown. The teeth are weaker than normal making them prone to rapid wear and fracture (4, 5). Radiographically, the DGI teeth show bulbous crowns with cervical constrictions, various sizes of pulp cavities, and short and narrow roots. Pulp chambers and root canals may be abnormally wide and look like “shell teeth” which can become obliterated due to dentin overproduction (6, 7). DGI type III or Brandywine type was found in the Brandywine triracial isolate in Southern Maryland. DGI type III shows variable discoloration and morphology of the teeth. Multiple pulp exposures and “shell teeth” are major characteristics of type III (8). DGI type II and III have been associated with mutations in the dentin sialophosphoprotein (*DSPP*) gene locating on chromosome 4q21. The *DSPP* encodes major non-collagenous proteins of the dentin including dentin phosphoprotein (DPP) and dentin sialoprotein (DSP). *DSPP* is expressed in a number of tissues including bone, kidney, salivary gland, and lung but its expression in dentin is hundreds of times higher than in other tissues (5). These three traditional categories of DGI are proposed based on clinical and radiographic manifestations, however, recent genetic

studies attribute that DGI type II and type III could be the same disease with phenotypic variability.

Histologically, the dentinal tubules of the circumferential dentin in DGI teeth are coarse, branched, irregular, and reduced in number. Atubular area in the dentin with reduced mineralization and reduced number of odontoblasts are commonly present. The enamel is normal but can be crack easily due to abnormal dentin and dentinoenamel junction (DEJ). The typical scallop pattern of DEJ believed to provide a mechanical lock between enamel and dentin is not present. The fractured enamel results in rapid wearing and loss of interdental contact making the DGI teeth more susceptible to caries. The DGI teeth show low hardness, elasticity, and stiffness compared to normal teeth which could lead to early failure of restorations (1-3). Porntaveetus *et al.* (2017) reported that the primary teeth with non-syndromic DGI showed opalescent color, amorphous dentin, irregular and reduced dentinal tubules, and reduced mineral density compared to the controls (9). Wieczorek *et. al.* observed that the enamel affected with DGI type II had decreased calcium and magnesium and increased phosphorus composition compared to the controls. The DGI teeth showed reduced calcium-phosphorus ratio, modulus of elasticity, and hardness. The phosphorus content was increased in the enamel. The Vicker's hardness of DGI type II was significantly less than that of the control(10, 11). Kerebel *et. al.* (1981) reported that DGI dentin showed decreased calcium and phosphorus while its calcium-phosphorus ratio and water content were increased (12).

### **Dental adhesives**

Dental adhesives are solutions of resin monomers that attach the resin to dental substrates. Mechanisms of dental bonding are both mechanical and chemical adhesion (13). The dental adhesives can be divided into 2 major categories: etch-and-rinse adhesives (total-etch) and self-etch adhesives. The total-etch adhesives are

classified into three-step and two-step adhesives. The self-etch adhesives are classified into two-step and one-step (14).

The techniques of total-etch adhesives generally comprise an application of phosphoric acid etching of enamel and dentin, followed by application of hydrophilic resins. To clarify, three-step or conventional system has separate steps of etching, priming, and adhesive (15). The phosphoric acid is etched to remove the smear layer, open the dentinal tubules, and decalcify the intertubular and peritubular dentin. Hydroxyapatite crystals are dissolved leaving a collagen meshwork. The primer containing solvent such as acetone, ethanol, or resin monomers is applied after the etchant is rinsed off. The primer wets and penetrates the collagen meshwork to increase surface energy and wettability of the dentin. The bonding agent is then applied to penetrate the primed dentin. The bonding agent typically contains a hydrophobic resin such as Bis-GMA, but the hydrophilic resin such as HEMA may also be added to improve wetting of the bonding. The bonding agent copolymerizes with the primer to form an intermingled layer of collagen fibers and resin called the “hybrid layer”. The etch-primer-bond adhesives procedures are technique-sensitive (13). Therefore, the researchers and companies have developed simpler technique known as two-step etch and rinse system. This system still requires etching as the first step whereas primer and bonding agents are combined into a single solution in the second step (14). Previous studies have demonstrated that three-step etch and rinse system showed highest bond strength, followed by two-step total etch adhesives (16). A recent *in vitro* study of three-step total-etch adhesives showed that Optibond FL (Kerr) was considered to be the golden standard of its class because it provides high microtensile bond strength and low nanoleakage (13). Kensche *et al.* (2016) determined shear bond strength of Optibond and Clearfil SE bonded on normal deciduous teeth and found comparable values of strength of  $19.06 \pm 5.62$  MPa and  $17.6 \pm 6.55$  MPa respectively (17).

Due to a high incidence of postoperative sensitivity and technical sensitivity of total-etch adhesives, self-etching system has been introduced. The original self-etch system includes two-steps: an acidic self-etch primer and a bonding agent (18). The two-step self-etch adhesives have been shown to reduce clinical steps and postoperative sensitivity compared to etch and rinse technique. In self-etch system, the resin monomers penetrate the depth where the acidic monomers demineralize the tooth structure, allowing complete penetration of resin monomer into the demineralized tissue. Clearfil SE Bond (CSE, Kuraray) has been known as the golden standard for self-etch adhesives with good clinical performance and minor leakage (19). In 2003, Armstrong *et al.* reported that the nanoleakage of Optibond (89%) was higher than that of Clearfil SE Bond (55%) at the dentin-resin interface in non-carious extracted human third molars (20).

The characteristic and composition of the dentin have been shown to affect the dentin bond strength. Panighi and G'Sell (1993) studied the effect of tooth microstructure on the shear bond strength of a dental composite in human molar teeth. The results showed that the microhardness of enamel and dentin surface linearly increased with their calcium concentration and found positive correlation between shear bond strength of dental-composite and Vickers microhardness of enamel and dentin (21). Perdigão (2010) found the sclerotic dentin containing denatured collagen and hypermineralized tissue had lower adhesive strength than the controls (22).

Early diagnosis and prevention of tooth wear are essential for successful dental management for DGI patients. Tooth-colored material is now commonly used in dentistry (3). The success of composite restoration is relied on both dental adhesives and tooth structure. To date, a limited number of studies have demonstrated the use of dental adhesives on the DGI in the patients with OI leaving the outcome of dental treatment for these patients questionable.

## Chapter III

### RESEARCH METHODOLOGY

All equipment required in this project is available for use

#### Subject enrollment

Teeth in this study were collected from the patient who diagnosed with DGI associated with OI at Faculty of Medicine, Chulalongkorn University. Three patients from independent families were included in the study. One tooth was obtained from each patient. DGI1 was the primary right first molar, DGI2 was the primary left central incisor, and DGI3 was the primary right second molar. The control teeth with the same tooth type were collected from age- and sex-matched healthy individuals. Patients' informed consents were acquired. Extraoral and intraoral photographs were taken. Clinical and radiographic oral examinations were performed.

#### Tooth collection

After extraction, the teeth were rinsed in running water to remove blood and adherent tissue, placed in distilled water or 1.0% chloramine-T trihydrate bacteriostatic/bactericidal solution for maximum of one week, and stored in distilled water in a 4°C refrigerator. The storage medium was replaced at least once every week (23).

#### Whole-Exome Sequencing (WES) and Sanger sequencing

The 3 ml of peripheral blood samples from the patients were collected with informed consent. The patient's genomic DNA was extracted from peripheral blood leukocytes and sent to Macrogen Inc. (Seoul, Korea) for next-generation sequencing (24). DNA were captured on the TruSeqExome Enrichment Kit (Illumina) and subsequently sequenced on the HiSeq2000 Instrument. The raw data per exome was mapped to the human reference genome hg19 using CASAVA v1.7. Variant calling was performed using SAM tools (<http://samtools.sourceforge.net/>). The sequencing

data was mapped to NCBI37 reference human genome (the version used for 1000 Genomes project), NHLBI Exome Variant Server (EVS), and in House database. The mutations were validated by Sanger sequencing.

## **Tooth analysis**

### **Micro CT analyses**

The teeth were scanned with specimen  $\mu$ CT 35 (SCANCO Medical, Brüttisellen, Switzerland) at the Excellence Center in Regenerative Dentistry at the Faculty of Dentistry, Chulalongkorn University. All images were processed using the Image Processing Language (IPL, Scanco Medical AG). The data was available for three-dimensional (3D) reconstructions and cross-section for the measurement of tooth size and mineral density.

### **Color measurement**

A digital intraoral colorimeter was used to measure tooth shade (ShadeEye NCC Dental Chroma Meter, Shofu Inc., Kyoto, Japan). Before each measurement, the device was calibrated according to the manufacturer's recommendations. The measurement was acquired from each specimen by contacting the device tip on the smooth buccal and lingual surfaces of the enamel and dentin. The measurement was examined in the same area under the same lighting condition and be repeated for 3 times. The color in  $L^*a^*b$  values was recorded defined by the International Commission on Illumination. The lightness in  $L^*$  showed black and white scale, the saturation in  $a^*$  showed red and green scale, and the saturation in  $b^*$  showed blue and yellow scale. The color difference ( $\Delta E^*$ ) was calculated using the following equation.

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

### Surface roughness measurement

Surface roughness measurements were performed by surface Profilometer (model Talyscan 150, Taylor Hobson Ltd). Each specimen was measured 30 times randomly every 600 micrometers on Y-axis. Tracing area was at 1 mm x 1 mm; stylus speed at 1000 micrometer/second; and cut-off length at 0.025 mm. The calculation of the surface topography parameters was carried out by the TalyMap Universal program.

### Microhardness measurement

Samples were embedded in acrylic block and divided horizontal plane to expose a flat mid-coronal dentin surface using the slow-speed precision saw (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA) with diamond disc at a speed of 100 rpm under constant water cooling. Then, a cut surface was grinded with Grit#1200 Silicon-carbide paper under water irrigation until the surface was flat and parallel. The alumina powder was used to polish samples on polishing pad (10-inch MICROPAD, Pace technologies).

Once the samples were prepared, micrographs were acquired by stereomicroscopy (Olympus SZ61, Tokyo, Japan). Image of the affected teeth were analyzed and compared with the control teeth. The microhardness was determined on the systemic DGI dentin surface compared with the control group at the same areas.

The microhardness was measured using Knoop indenter microhardness tester (Mitutoya, Japan). The specimens were mounted on base with thin double side tape. All samples were indented at 100 gF loading 15 seconds force on enamel and dentine in 30 random locations. The indentation depth (micrometer) was recorded. Values of Knoop hardness were calculated for enamel and dentine according to following formula, HK value, Knoop hardness number; P value, test load (N); L value, indentation diagonal length( $\mu\text{m}$ ).

$$HK = 14229 \frac{P}{L^2}$$

The teeth were then separated into 3 pieces using a low speed diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) under running water and studied for EDX and SEM, histology, and adhesive performance.

#### **Gold coating and Energy-Dispersive X-ray (EDX) and Scanning Electron microscopy (SEM)**

The specimens were fixed in 2.5% glutaraldehyde and washed in phosphate buffered saline (PBS) 3-5 minutes for 2 times. Dehydration with ethanol series (30%, 50%, 70%, 90%, and 100% ethanol) was performed for 10 minutes each. The specimens were bathed in 100% ethanol and placed in a critical point dryer (Emitech K850, Emitech Ltd, Kent, England) where they were purged in liquid CO<sub>2</sub> until the ethanol was replaced with CO<sub>2</sub>. The samples were exposed to a critical point drying process.

Dried specimens were placed on aluminium stubs and covered with golden powder in a media of argon-cathode atomization with fine coater (JFC 1200, Tokyo, Japan) for 10 seconds. Then, extracellular matrix compounds were measured on 5 locations. Energy-dispersive X-ray (EDX) used (ISIS 300 EDX-system; Oxford Instruments, UK) to determine the elemental levels (%) of carbon (C), oxygen (O), phosphorus (P), and calcium (Ca) (Jeol, JSM 5410 LV Scanning electron microscope, Japan). Lastly, the specimens were covered with gold powder again for 110 seconds. The scanning electron microscopy (Quanta Feg 250, FEI Company, Oregon, USA) was used to evaluate the structures of dental hard tissues in cross-section between the DGI and control samples.



## Histology

The specimens were transferred into xylene solution followed by soft paraffin (melting point 46-48°C) and hard paraffin (melting point 56-58°C) bathes respectively. The tissue blocks were cut at 7 µm thickness by rotary microtome. The sections were stained with H&E and mounted on glass slides (25). The histological structure of enamel and dentin was examined using light microscope compared with the controls.

## Dental adhesive analyses

The adhesive procedures were performed by one operator. The adhesives were used according to the manufacturer's instruction. The occlusal part of the teeth was removed perpendicular to the long axis of the tooth to expose a flat mid-coronal dentin surface using a low speed diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) under running water. The peripheral enamel was removed from the mesial and distal proximal portions of the tooth.

The occlusal part was used for microshear bond strength and microleakage while the cervical part of the tooth was used for microtensile bond strength.

## Microtensile bond strength (µTBS)

The occlusal dentin surface was polished with 600-grit silicon carbide sandpaper for 60 seconds. The dentin surface was thoroughly washed with water and immediately dried with moisture-free air. Then, the specimens were randomly divided into two groups. One was treated with total-etch adhesive (Optibond FL; Kerr) and another one was treated with self-etch adhesive (Clearfill SE Bond; Kuraray). After bonding procedures, the samples were built up with resin composite at least 6 mm in height by incremental technique. All specimens stored at 37°C in water for 24 hours. Afterward, the specimens were sectioned in the long axis direction through

the resin composite and dentin. The blocks with approximately 2 mm in thickness, 2 mm in width, and 6 mm in length were obtained using the low-speed diamond saw (Isomet 1000) under water cooling (23).

The specimens were attached to a testing apparatus with cyanoacrylate adhesives and subjected to microtensile force testing with a crosshead speed of 1 mm/min. The microtensile bond strength ( $\mu$ TBS) were expressed in MPa (16, 23, 26).

#### **Microshear bond strength ( $\mu$ SBS)**

The tooth specimens were fixed in the mounting material. A template of cylinder with an internal diameter of 0.8 mm and a height of 3 mm, was mounted on the dentin perpendicular to the tooth surface. A restorative resin composite was placed into the cylinder after applying a thin layer of adhesive. The specimens were stored at room temperature for one hour prior to a removal of the mold tubing, and then kept in water at 37°C for 24 hours.

The specimens were fixed in the position where the adhesive interface was within 0.5 mm of shearing blade. They mount the apparatus in a universal testing machine. The load rate 1 mm/min crosshead speed was applied to test  $\mu$ SBS (21, 27).

#### **Microleakage test**

After microshear bond strength test, cavity was prepared at the size of 2x2 mm using the diamond round bur. The specimens were randomly divided into two groups and treated with total-etch adhesive (Optibond FL; Kerr) and self-etch adhesive (Clearfil SE Bond; Kuraray) according to the manufacturer's instruction. After bonding procedures, Nail varnish was applied to the dentin within 0.5 mm–1 mm of the adhesive interface before immersing in 2% methylene blue dye solution for 24 hours. The samples were then rinsed with running water for 5 minutes to remove dye on the surface. Each specimen was examined under light microscopy and scored

under a microscope at 10x magnification for penetration of tracer along the cavity walls. The following scoring system was used:

No penetration = 0

Penetration into the dentin/material interface, but not including the pulpal floor of the cavity =1

Penetration including the pulpal floor of cavity =2

The data of two adhesive systems was collected. Nonparametric test was used to compare between 2 dental adhesive systems.

### **Statistical Analysis**

The data obtained from surface roughness, microhardness, was analyzed with SPSS software package (version22; SPSS Inc., Chicago, IL, USA). An assessment of the normality of data was performed and calculated by Kolmogorov-Smirnov test. Normally distributed data was analyzed by ANOVA. The data with non-normal distribution was analyzed by Turkey test at a significance level at 0.05. The data of dental adhesive application was used descriptive statistic compared with the control.

## Chapter IV

### RESULTS

#### Physical characteristics

Three patients who diagnosed with OI at Faculty of Medicine, Chulalongkorn University were enrolled in this study.

The first patient was a 7 years-old Thai boy (DGI1). He was born with multiple bone fractures. His eyes were blue sclera. Intraoral examination at 1 years of age revealed the deciduous dentition with bluish brown discoloration. The primary maxillary central incisors were fractured and pulpal exposed. The primary maxillary molar had dentin exposure (Figure 1A-C). Intraoral radiographs showed thin dentin and cervical constriction. The pulp chambers were large. (Figure 1D-K). The radiograph of the lower extremity at the age of 6 years showed surgical reconstruction for femur shaft fractures (Figure 1L). His parents were healthy and had normal teeth (Figure 1M).

The second patient was a 6 years-old Thai boy (DGI2). Intra oral findings at the age of 1 year revealed the deciduous dentition with opalescent discoloration. (Figure 2A - C). He had a history of multiple bone fracture. Radiographic of lower extremity at age 1.7 years showed bowing of femurs and zebra stripe signs of pamidronate therapy (Figure 2D). His eyes showed blue sclera. His mother was diagnosed with OI (Figure 2E).

The third patient was a 13 years-old Thai boy (DGI3). Intraoral examination showed mixed dentition. The teeth color was yellowish (Figure 3A-C). From panoramic radiograph at 14 years old revealed that the pulp cavities of erupted permanent teeth became narrow while those of unerupted teeth were wider (Figure 3D). He had a history of repeated femur fractures. The imaging of the lower

extremity at 14 years-old showed right-left femur fracture and bowing of long bone (Figure 3E). No other family members were affected (Figure 3).

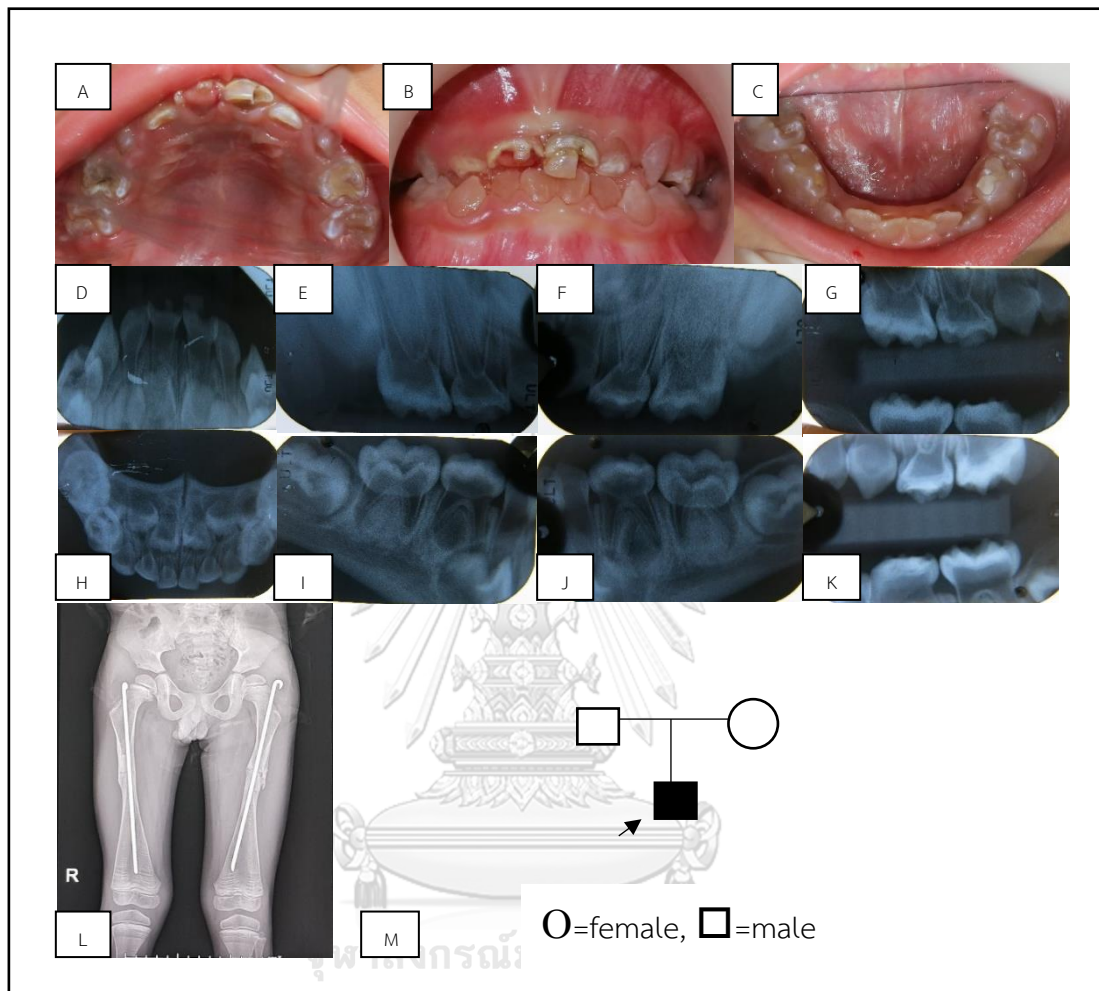


Figure 1 Phenotypes and family pedigree of patient 1 (DG11).

Upper, frontal, and lower views of intraoral photographs (A, B, C). Radiographs of upper and lower anterior teeth (D, H). Periapical radiographs of posterior teeth (E - J). Bitewing radiographs (G, K). Radiographic image showed the reconstruction of femur fractures (L). Family pedigree showed affected proband. Filled symbol indicates affected individual (M).

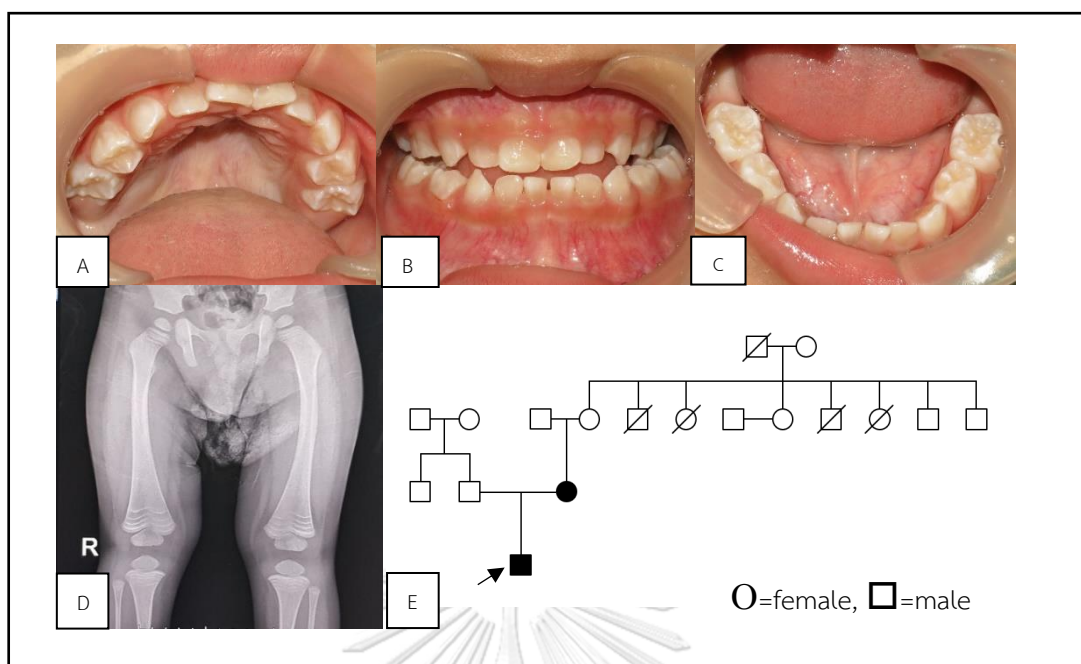


Figure 2 Phenotypes and family pedigree of patient 2 (DGI2).

Upper, frontal, and lower views of intraoral photographs of DGI2 show upper, anterior, and lower views of deciduous dentition (A - C). The teeth were opalescent. Radiograph exhibited bowing of femurs with numerous zebra lines (D). Family pedigree showed affected mother and proband (E). Filled symbols indicate affected individuals.

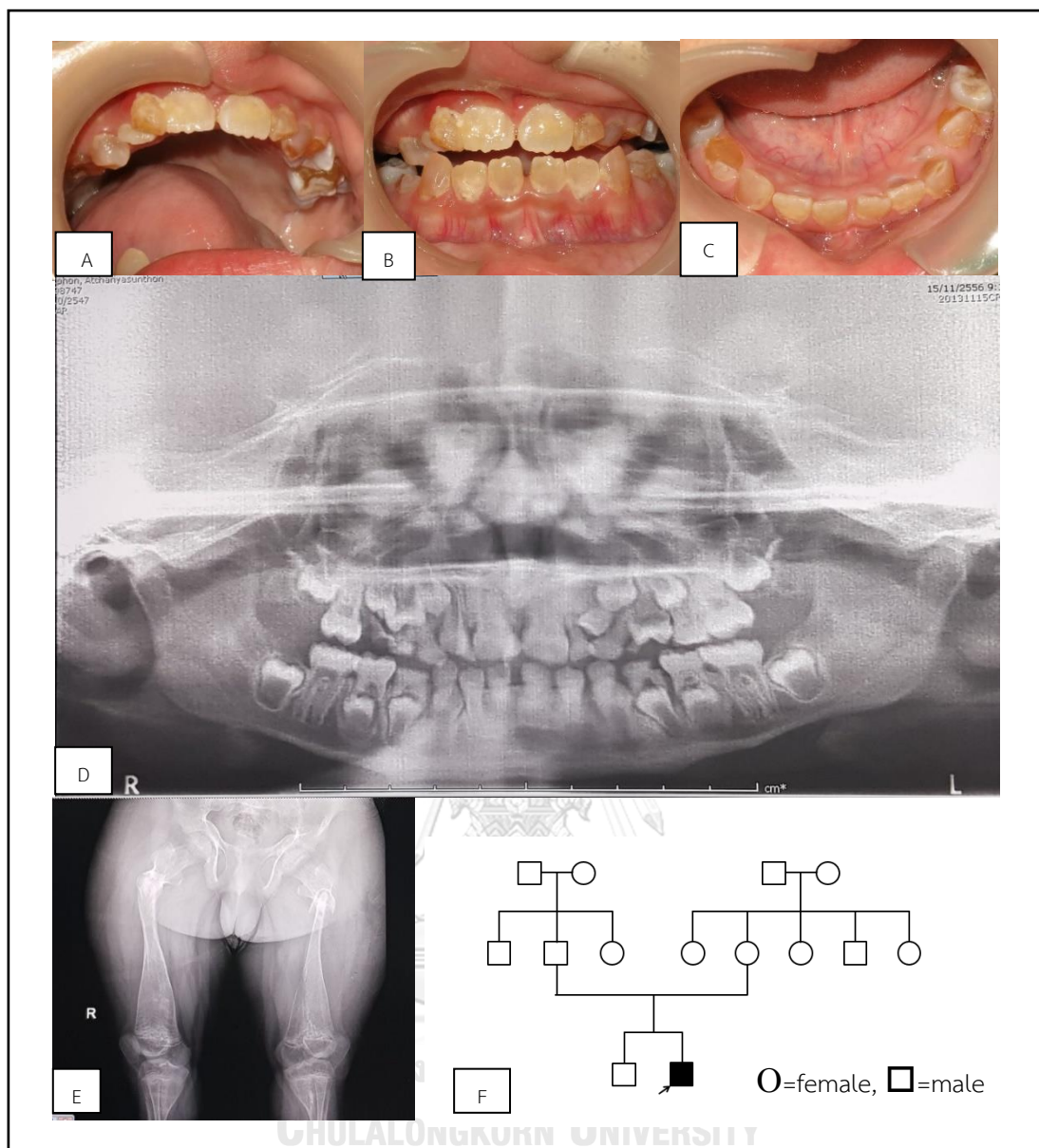


Figure 3 Phenotypes and family pedigree of patient 3 (DGI3).

Upper, frontal, lower intraoral photographs revealed mixed dentition with yellowish coloration (A - C). Panoramic radiograph showed that the erupted teeth had obliterated pulp chamber while unerupted teeth had wide pulp chamber (D). Radiograph of lower extremity showed fracture of femurs (E). Family pedigree showed affected proband (F). Filled symbol indicates affected individual.

### Mutation analyses

Whole-exome sequencing was used for mutation analysis. The mutation data was screened in to NCBI37 reference human genome (the version used for 1000 Genomes project), NHLBI Exome Variant Server (EVS), and in-House database. It was found that patient 1 harbored the missense mutation, c.1531G>T, p.G511C, in exon 26 of COL1A2 (NM\_000089.4) (Figure 4A). Patient 2 possessed the missense mutation, c.3106G>C, p.G1036R, in exon 47 of COL1A2 (Figure 4B) and patient 3 had the missense mutation, c.2027G>T, p.G676V, in exon 34 of COL1A2 (Figure 4C).

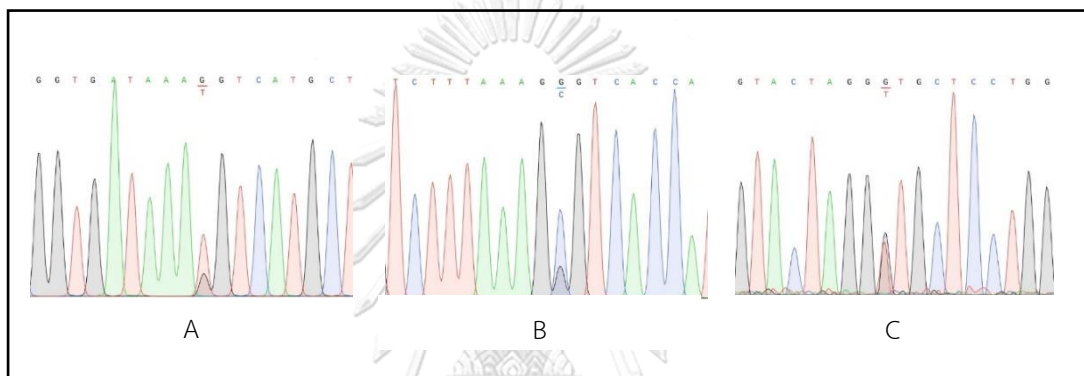


Figure 4 Chromatogram.

Chromatogram of DGI patient 1 (A), patient 2 (B) and patient 3 (C) revealed the missense mutations in exon 26, 47, and 34 in COL1A2, respectively.

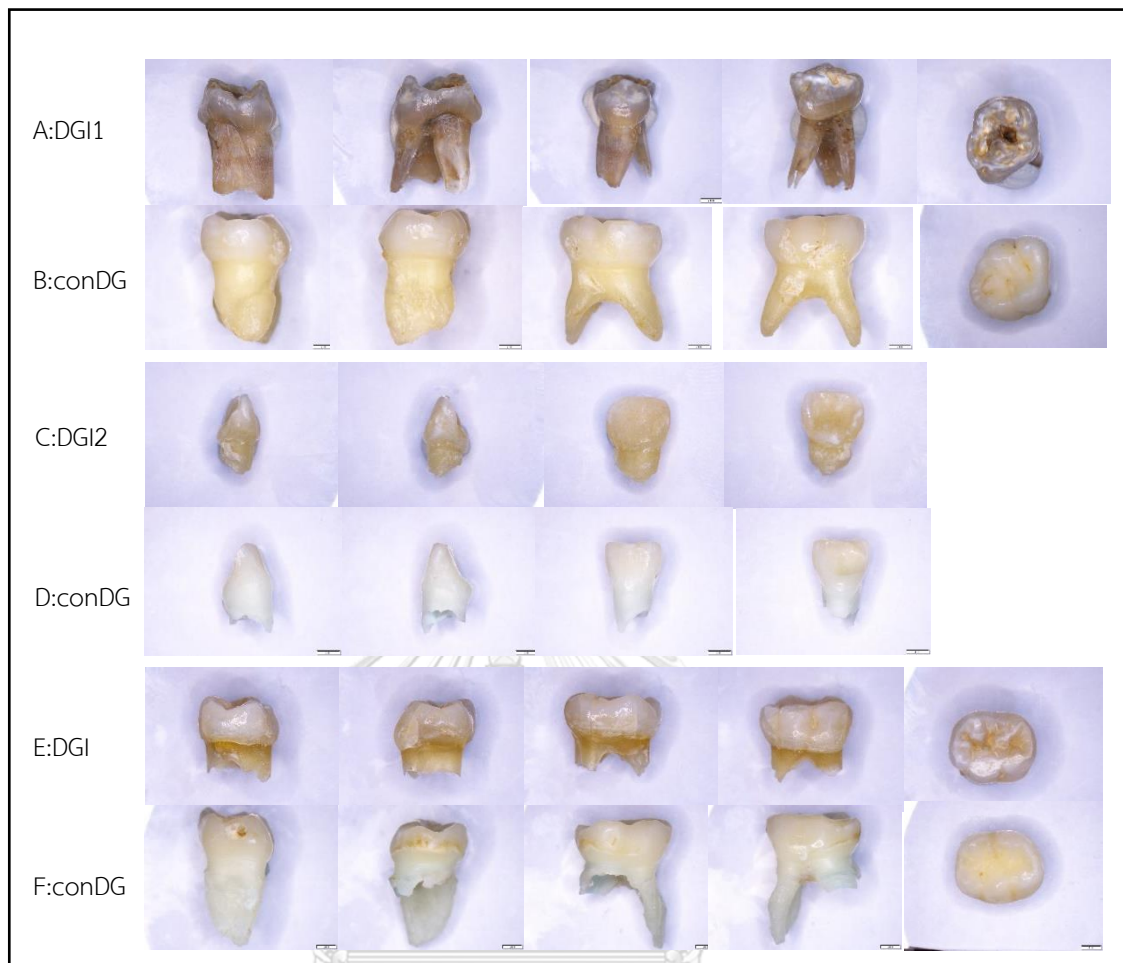
### Analyses of dental ultrastructure

#### Tooth samples

The primary maxillary right first molar was obtained from patient 1 (DGI1), the primary maxillary left central incisor from patient 2 (DGI2) and the primary mandibular right second molar from patient 3 (DGI3).

DGI1 was found to be dark brown in color and showed cervical constriction. For DGI2, the enamel surface was more irregular than that of the controls. The color was yellowish and darker. DGI3 was yellow and bluish in color (Figure 5).





*Figure 5 Stereo-microscopic imaging.*

*The image showed Stereo-microscopic images DGI1, DGI2, DGI3 and their controls (A-F).*

### **Micro-CT analyses**

The micro-CT cross-sections revealed that DGI samples had comparable density and thickness of enamel to the controls. The dentin thickness of DGI1 was thinner while its pulp cavity was wider than the controls. In contrast, pulp cavity of DGI2 was narrow and of DGI3 was obliterated (Figure 6).

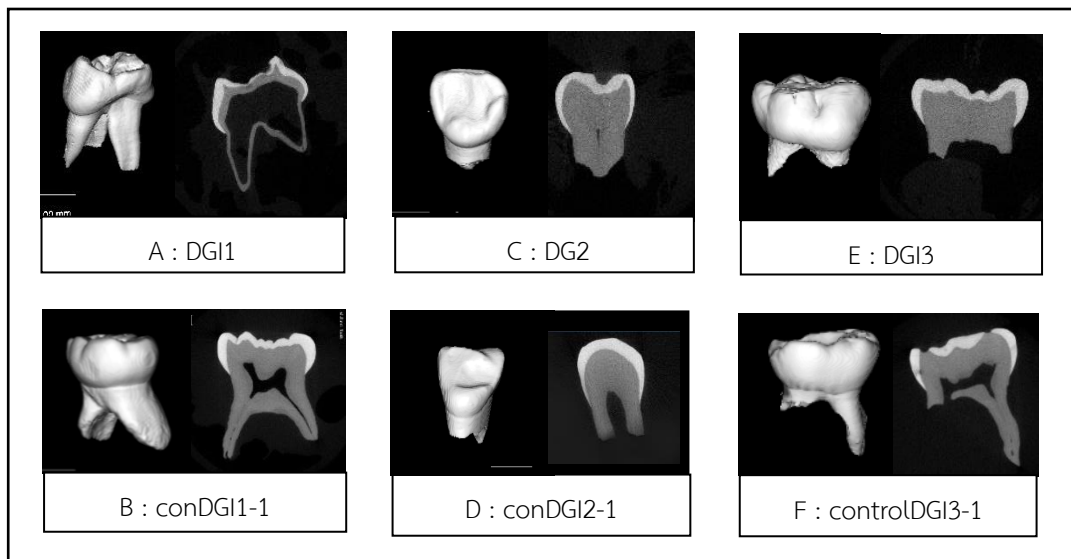


Figure 6 Micro-CT imaging

The image showed three-dimensional (3D) reconstructions and vertical cross-sections of the DGI teeth (A, C and E) and controls (B, D and F).

For DGI1, the mineral density of enamel was  $2118.96 \text{ mg/cm}^3$  (controls  $2106.19$ ,  $2232.33$ , and  $2326.25 \text{ mg/cm}^3$ ) and of dentin was  $1255.91 \text{ mg/cm}^3$  (controls  $1101.69$ ,  $1151.41$  and  $1298.88 \text{ mg/cm}^3$ ). For DGI2, the mineral density of enamel was  $2177.77 \text{ mg/cm}^3$  (controls  $2230.37$ ,  $2134.85$  and  $2214.48 \text{ mg/cm}^3$ ) and of dentin was  $1182.26 \text{ mg/cm}^3$  (control  $1211.90$ ,  $1194.27$  and  $1214.57 \text{ mg/cm}^3$ ). For DGI3, the mineral density of enamel was  $2125.02$  (controls  $2148.53$ ,  $2185.18$  and  $2207.86 \text{ mg/cm}^3$ ) and of dentin was  $1167.60 \text{ mg/cm}^3$  (control  $1297.23$ ,  $1267.19$  and  $1210.80 \text{ mg/cm}^3$ ) (Table 1).

Table 1 The mineral density of enamel and dentin ( $\text{mg}/\text{cm}^3$ ) analyzed by micro-CT scan.

Tooth	Enamel ( $\text{mg}/\text{cm}^3$ )	Dentin ( $\text{mg}/\text{cm}^3$ )
DGI1	2118.96	1255.91
conDGI1-1	2106.19	1101.69
conDGI1-2	2232.33	1151.40
conDGI1-3	2326.25	1298.88
Mean conDGI1	2221.59	1183.99
DGI2	2177.77	1182.26
conDGI2-1	2230.37	1211.90
conDGI2-2	2134.85	1194.27
ConDGI2-3	2214.48	1214.57
Mean conDGI2	2193.23	1206.91
DGI3	2125.02	1167.60
conDGI3-1	2148.53	1297.23
conDGI3-2	2185.18	1267.19
ConDGI3-3	2207.86	1210.80
Mean conDGI3	2180.52	1258.41

The enamel and dentin density of DGI less than the average of controls except dentin density of DGI1. The statistically significant differences were not observed (Figure 7).

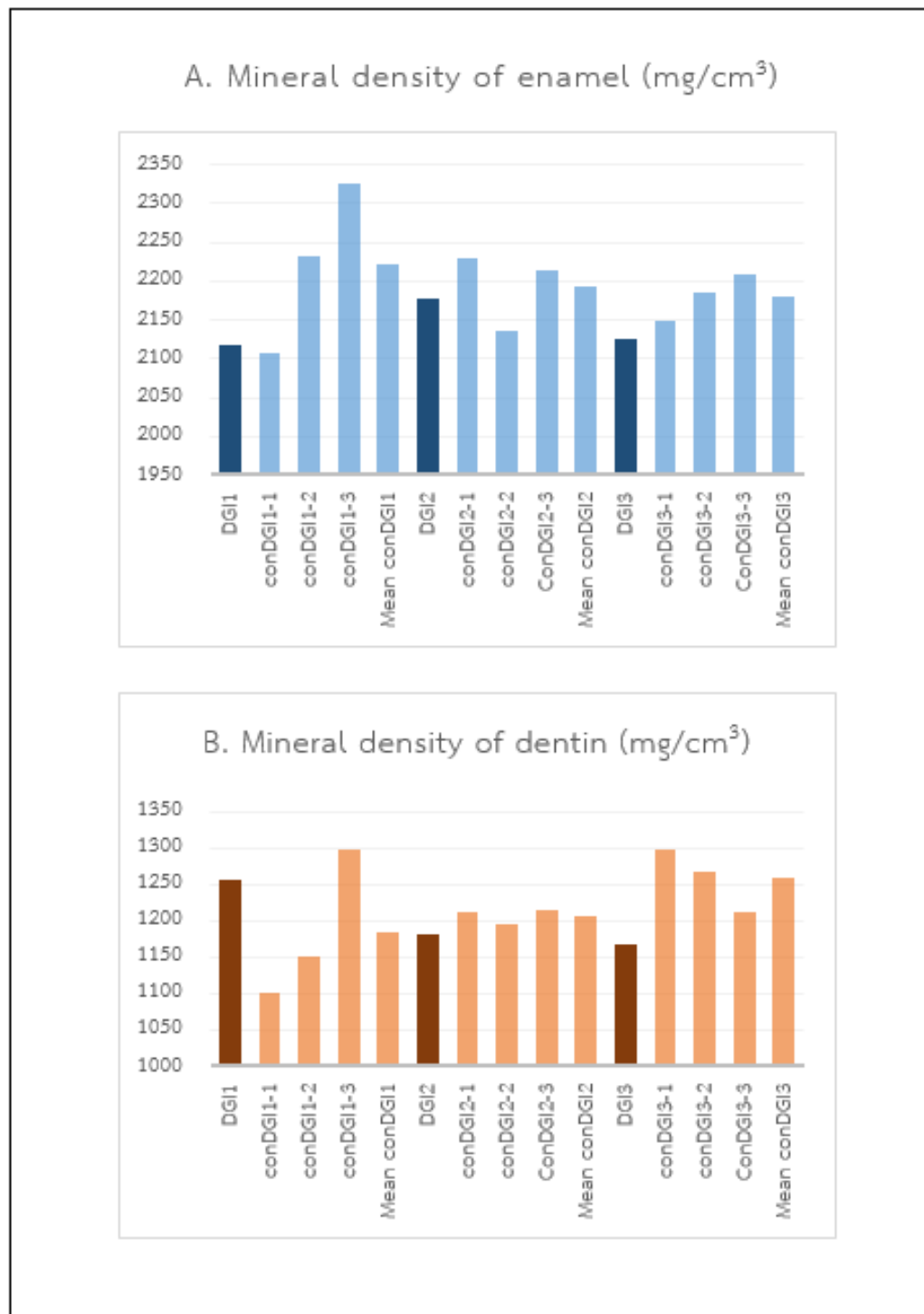


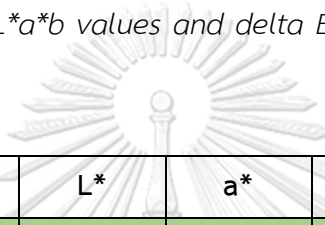
Figure 7 The column chart of mineral density ( $\text{mg}/\text{cm}^3$ ) of enamel and dentin

The column chart showed the average mineral density of enamel and dentin of DGI samples and the controls.

### Color measurement

Clinically, the DGI teeth showed grey-brownish color. The L\* values of DGI1, DGI2 and DGI3 were 38, 68 and 62 respectively, which were lower than their controls. The mean b\* values of DGI1, DGI2 and DGI3 were lower than the controls. The color difference ( $\Delta E$ ) between OIDI1, OIDI2 and OIDI3 and their corresponding controls were 50.62, 25.70 and 28.00, respectively. These suggest that the human eyes can distinguish the color difference between DGI and normal teeth (Table2).

Table 2 The mean CIE L\*a\*b values and delta E values of OIDI samples and the controls.



Sample	L*	a*	b*	$\Delta E$
OIDI1	38.00	3.15	26.15	
conOIDI1-1	90.35	3.35	43.45	58.19
conOIDI1-2	83.40	4.25	43.70	46.96
conOIDI1-3	77.25	4.95	43.10	46.71
OIDI2	68.00	2.65	27.25	
conOIDI2-1	79.6	1.40	28.15	13.48
conOIDI2-2	100.00	1.60	25.05	31.87
conOIDI2-3	96.25	1.45	25.05	31.74
OIDI3	62.00	2.20	21.55	
conOIDI3-1	93.10	0.45	31.70	32.19
conOIDI3-2	89.00	0.90	21.90	28.02
conOIDI3-3	78.25	2.20	35.25	23.78

### Surface roughness

The mean surface roughness of DGI1 was  $0.35 \pm 0.12 \mu\text{m}$  (control  $0.64 \pm 0.57$ ,  $0.34 \pm 0.23$  and  $0.74 \pm 0.55 \mu\text{m}$ ), DGI2 was  $1.47 \pm 0.59 \mu\text{m}$  (controls  $0.57 \pm 0.28$ ,  $0.64 \pm 0.25$  and  $0.68 \pm 0.54 \mu\text{m}$ ), and DGI 3 was  $0.39 \pm 0.15 \mu\text{m}$  (controls  $0.61 \pm 0.38$ ,  $0.68 \pm 0.29$  and  $0.76 \pm 0.65 \mu\text{m}$ ) (Table3).

*Table 3 Surface roughness of the enamel on buccal and lingual surfaces of DGI samples and controls shown in mean  $\pm$  standard deviation (SD).*

Surface roughness ( $\mu\text{m}$ )	Buccal enamel	Lingual enamel	Mean values
DGI1	$0.42 \pm 0.13$	$0.27 \pm 0.06$	$0.35 \pm 0.12$
conDGI1-1	$0.87 \pm 0.65$	$0.41 \pm 0.37$	$0.64 \pm 0.57$
conDGI1-2	$0.43 \pm 0.19$	$0.25 \pm 0.23$	$0.34 \pm 0.23$
conDGI1-3	$0.41 \pm 0.32$	$1.07 \pm 0.53$	$0.74 \pm 0.55$
DGI2	$1.47 \pm 0.59$	-	-
conDGI2-1	$0.57 \pm 0.28$	-	-
conDGI2-2	$0.64 \pm 0.25$	-	-
conDGI2-3	$0.68 \pm 0.54$	-	-
DGI3	$0.33 \pm 0.12$	$0.45 \pm 0.16$	$0.39 \pm 0.15$
conDGI3-1	$0.36 \pm 0.29$	$0.87 \pm 0.27$	$0.61 \pm 0.38$
conDGI3-2	$0.75 \pm 0.23$	$0.61 \pm 0.34$	$0.68 \pm 0.29$
conDGI3-3	$1.02 \pm 0.78$	$0.50 \pm 0.36$	$0.76 \pm 0.65$

The surface roughness of DGI2 was significantly higher while that of DGI1 and DGI3 was significantly lower than the average value of controls (Figure 8)

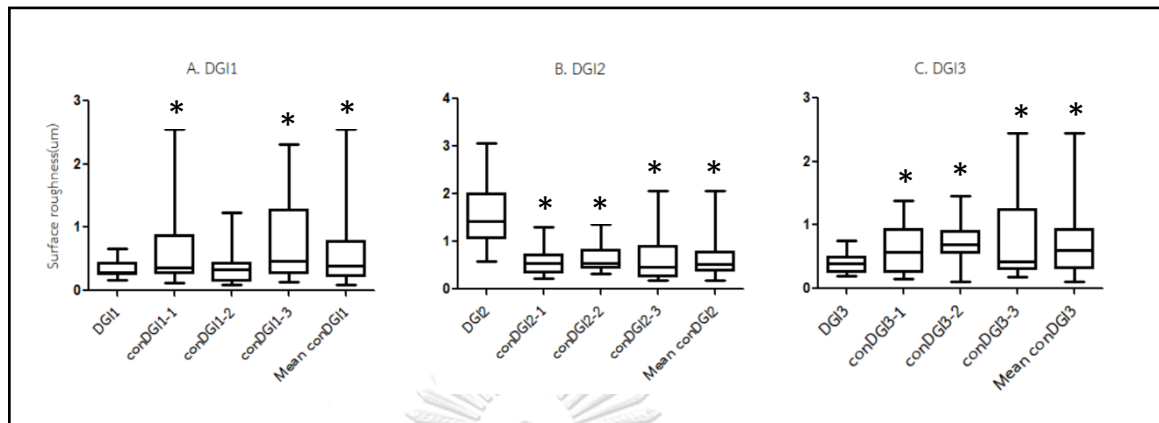


Figure 8 The graph of surface roughness.

The graph showed surface roughness of DGI samples and their controls.

\* significance at  $P < 0.05$ .

### Microhardness

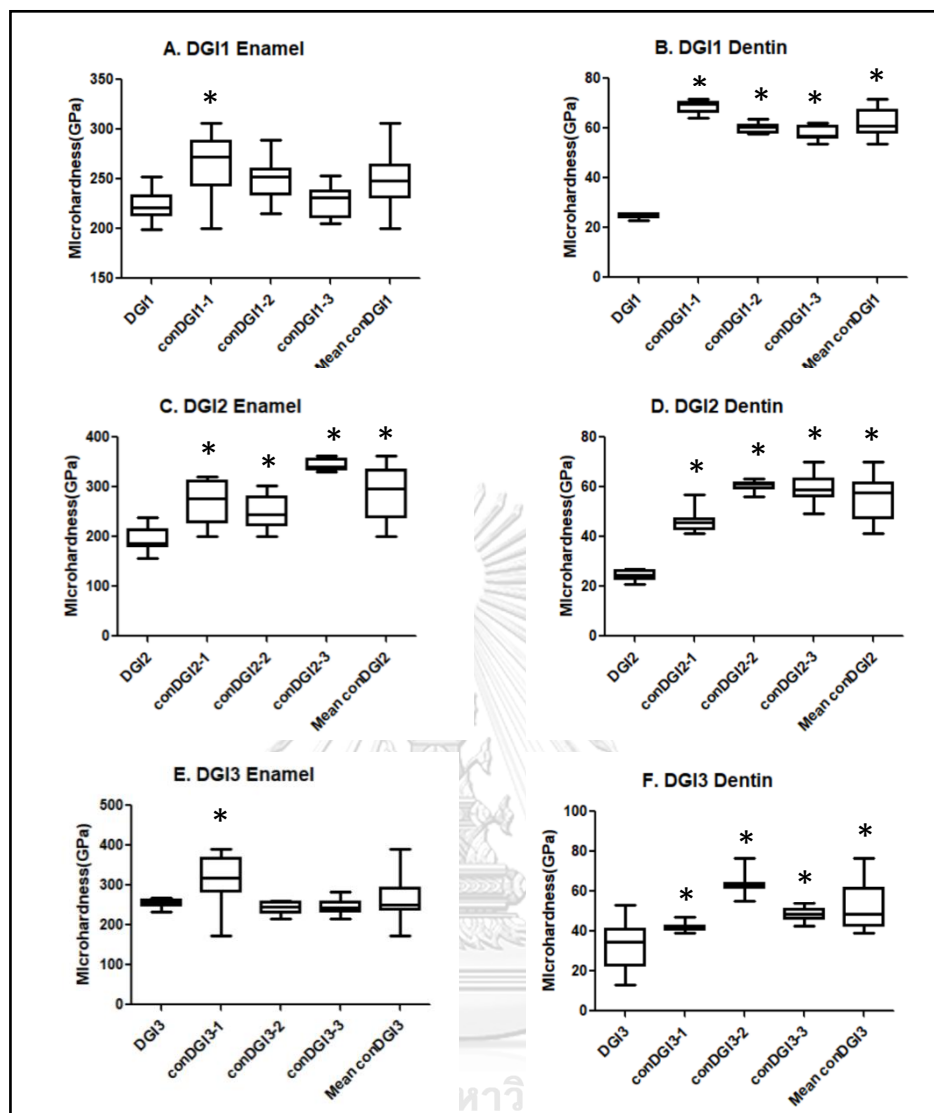
For DGI1, the mean Knoop microhardness of the enamel was  $224.04 \pm 15.50$  GPa (controls  $264.96 \pm 32.14$ ,  $250.23 \pm 20.08$  and  $228.51 \pm 16.31$  GPa) and the dentin was  $24.64 \pm 0.90$  GPa (controls  $68.71 \pm 2.50$ ,  $60.15 \pm 1.78$  and  $57.93 \pm 2.82$  GPa). For DGI2, the enamel microhardness was  $192.31 \pm 23.99$  GPa (controls  $259.89 \pm 56.12$ ,  $238.37 \pm 39.68$  and  $343.34 \pm 11.10$  GPa) and the dentin was  $24.41 \pm 2.14$  (controls  $46.11 \pm 4.33$ ,  $60.39 \pm 2.43$  and  $59.57 \pm 6.05$  GPa). And DGI3, the enamel microhardness was  $259.89 \pm 19.50$  GPa (controls  $312.46 \pm 64.86$ ,  $243.94 \pm 15.47$  and  $246.55 \pm 20.80$  GPa) and the dentin was  $33.20 \pm 13.27$  GPa (controls  $42.15 \pm 2.11$ ,  $63.29 \pm 5.36$  and  $48.44 \pm 3.42$  GPa) (Table 4).

Table 4 Knoop microhardness of the enamel and dentin of DGI samples and their controls shown in mean and standard deviation (SD).

Tooth	Enamel	Dentin
DGI1	224.04±15.50	24.64±0.90
conDGI1-1	264.96±32.14	68.71±2.50
conDGI1-2	250.23±20.08	60.15±1.78
conDGI1-3	228.51±16.31	57.93±2.82
Mean conDGI1	247.90±22.84	61.25±2.37
DGI2	192.31±23.99	24.41±2.14
conDGI2-1	259.89±56.12	46.11±4.33
conDGI2-2	238.37±39.68	60.39±2.43
conDGI2-3	343.34±11.10	59.57±6.05
Mean conDGI2	280.53±35.63	55.35±4.27
DGI3	259.89±19.50	33.20±13.27
conDGI3-1	312.46±64.86	42.15±2.11
conDGI3-2	243.94±15.47	63.29±5.36
conDGI3-3	246.55±20.80	48.44±3.42
Mean conDGI3	267.65±33.71	51.3±3.63

The Knoop microhardness values of DGI1, DGI2 and DGI3 dentin were statistically lower than those of the controls. The Knoop microhardness of DGI2 enamel was statically lower than that of the controls while the microhardness of enamel of DGI1 and DGI3 was within the values of controls (Figure 9).





CHULALONGKORN UNIVERSITY

Figure 9 The charts of Knoop microhardness.

The charts illustrated Knoop microhardness of enamel (E) and dentin (D) of DGI samples and the controls. \* significant at  $P < 0.05$ .

### Energy-Dispersive X-ray (EDX)

The enamel and dentin of DGI1 and DGI3 showed higher amount of carbon (C) but lower oxygen (O), phosphors (P), and calcium (Ca) than the controls. The dentin of DGI2 showed lower C and higher amount of O, P, Ca than the controls (Table 5).

*Table 5 Elemental analysis (% weight) of carbon (C), oxygen (O), phosphorus (P), and calcium (Ca), of DGI samples and controls.*

Tooth	Enamel				Dentin			
	C	O	P	Ca	C	O	P	Ca
DGI1	28.26	37.95	11.77	22.02	31.85	36.16	11.08	20.92
conDGI1-1	10.44	40.17	16.10	33.28	14.41	40.34	14.86	30.38
conDGI1-2	9.316	37.28	16.76	36.65	15.82	37.21	14.83	32.14
conDGI1-3	6.80	39.81	17.19	36.20	13.93	39.38	15.19	31.49
DGI2	19.02	37.17	14.10	29.71	19.71	40.70	13.10	26.49
conDGI2-1	18.74	36.07	14.27	30.92	25.23	37.55	11.59	25.63
conDGI2-2	21.44	46.63	11.13	20.79	62.36	31.61	2.20	3.82
conDGI2-3	16.58	38.13	14.73	30.57	32.57	29.70	12.04	25.69
DGI3	74.76	17.14	2.58	5.526	54.48	20.84	6.98	17.70
conDGI3-1	15.97	39.51	14.93	29.60	17.96	41.04	14.12	26.88
conDGI3-2	5.99	36.97	17.74	39.31	13.87	37.42	15.50	33.21
conDGI3-3	22.96	29.51	14.83	32.71	28.93	30.06	13.10	27.91

### Scanning Electron Microscopy (SEM)

SEM demonstrated the microstructure of dentin of DGI samples compare with the controls at magnification 5000x. The regular arrangement of dentinal tubules and smooth dentinal surface were present in the controls teeth (Figure10 B, D and F). In contrast, the dentinal tubules of DGI teeth were obscure and irregular. Voids and cracks were observed (Figure10 A, C and E).

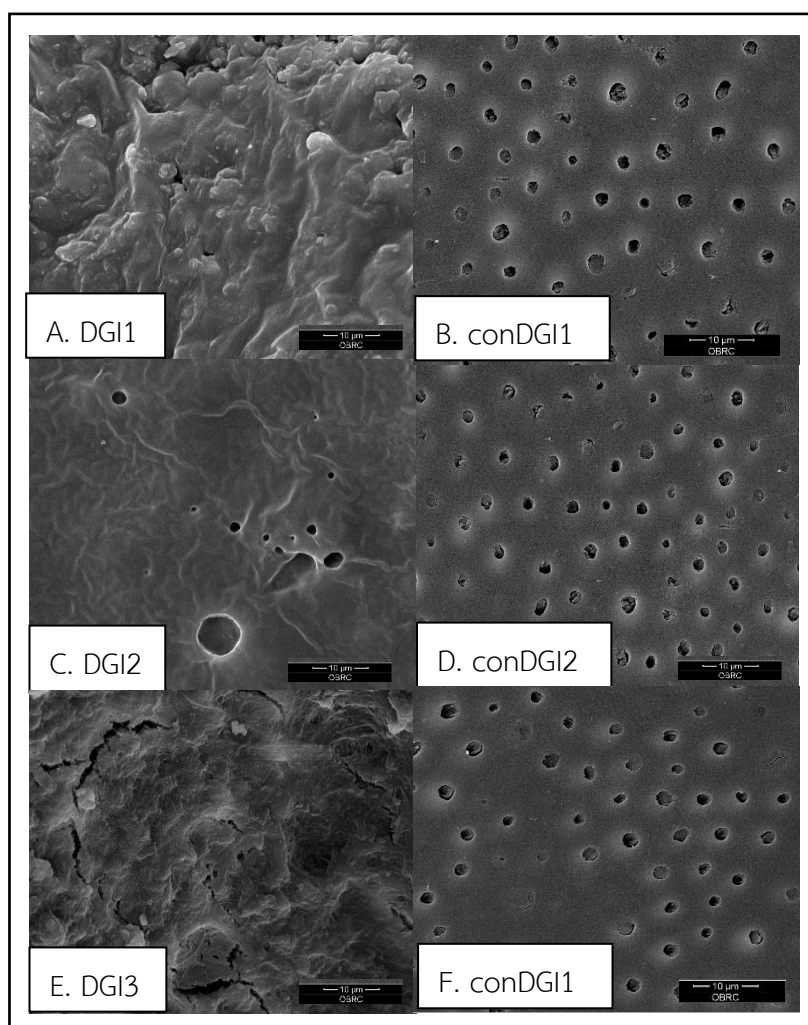


Figure 10 SEM imaging of the dentin

The SEM analysis of the dentin of DGI1, DGI2, and DGI3 and their controls at magnification 5000x.

## Histology

Histological structure of the control dentin showed a regular orientation of dentinal tubules (Figure 11 C-D, G-H, K-L). The dentinal tubules of DGI2 and DGI3 were coarse and irregular. The dentin of DGI1 was obstructed lacking the appearance of dentinal tubules. Masson's trichrome staining of DGI dentin (Figure 11 B, F and J) showed that the collagen fibers were decreased in number and abnormal in size and shape compared with the controls (Figure 11 D, H and J).

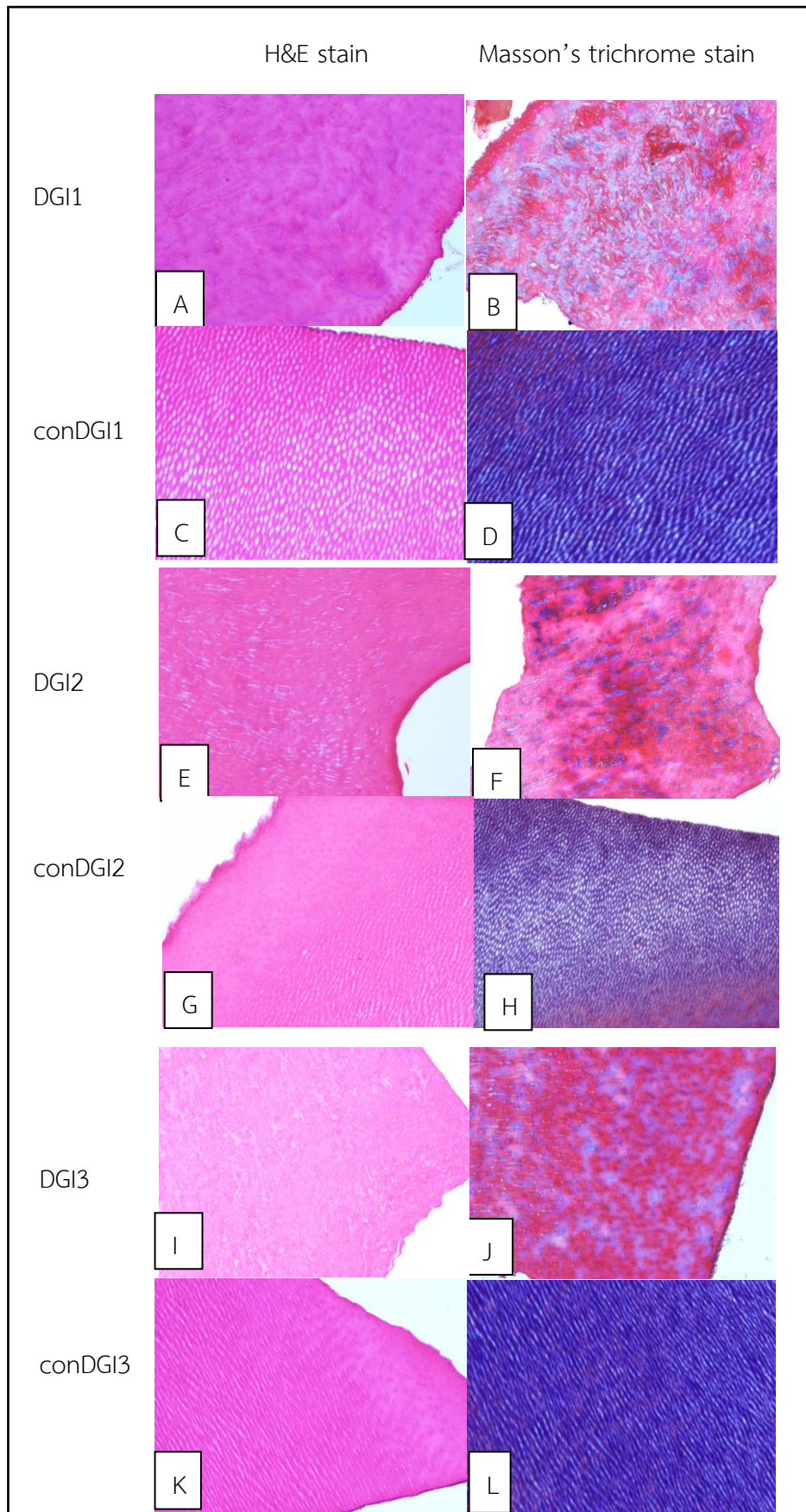


Figure 11 Histology imaging of dentin

The histological appearances of the dentin of DGI samples and controls.

Left panel was stained with H&E and right panel with Masson's trichrome at 40x. DGI1 (A-B), DGI2 (E-F), and DGI3 (I-J). The controls of DGI1 (C-D), DGI2 (G-H), and DGI3 (K-L).

## Adhesive analyses

### Microshear Bond Strength ( $\mu$ SBS)

For Clearfil SE bond, the  $\mu$ SBS of DG1 was 2.22 MPa (control 4.61 MPa), DGI2 was 3.18 MPa (control 4.54 MPa), and DGI3 was 3.47 MPa (control 4.65 MPa) (Table 6). For Optibond, the  $\mu$ SBS of DG1 was 2.65 MPa (control 7.26 MPa), DGI2 was 3.28 MPa (control 7.37 MPa), and DGI3 was 3.61 MPa (control 7.10 MPa) (Table 6, 7).

*Table 6 Microshear bond Strength of Clearfil SE bond*

Samples	$\mu$ SBS (MPa)	Controls	$\mu$ SBS (MPa)
DGI1	2.22	1	3.69
		2	4.36
		3	6.13
		4	4.28
		mean	4.61
DGI2	3.18	1	4.46
		2	4.59
		3	4.63
		4	4.46
		mean	4.54
DGI3	3.48	1	4.44
		2	4.34
		3	4.92
		4	4.16
		5	4.77
		6	5.26
		mean	4.65

Table 7 Microshear bond Strength of Optibond

Samples	$\mu$ SBS (MPa)	Controls	$\mu$ SBS (MPa)
DGI1	3.23	1	6.11
	2.07	2	7.82
mean	2.65	3	7.26
		4	7.85
		mean	7.26
DGI2	3.28	1	7.38
		2	7.50
		3	7.29
		4	7.31
		mean	7.37
DGI3	3.70	1	7.05
	3.52	2	7.05
mean	3.61	3	7.59
		4	7.30
		5	6.77
		6	6.83
		mean	7.10

When comparing between all DGI samples and controls, the DGI samples were lower than the controls both Clearfil SE and Optibond. Regarding the difference between two types of adhesive, the average  $\mu$ SBS of Optibond were higher than Clearfil SE (Figure 12).



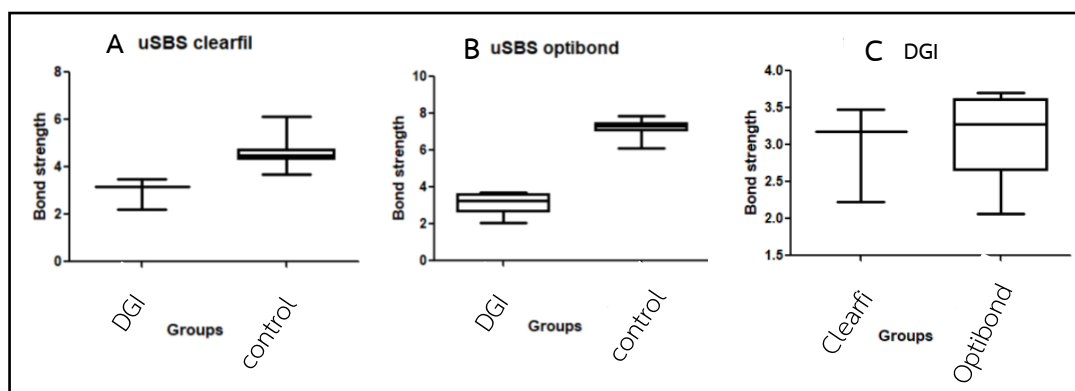


Figure 12 The chart of Microshear bond strength ( $\mu$ SBS).

For Clearfil SE,  $\mu$ SBS of DGI samples were lower than the controls (A). For Optibond,  $\mu$ SBS of DGI samples were lower than the controls (B). The average Clearfil SE was lower than Optibond for DGI samples (C).

#### Microtensile bond strength ( $\mu$ TBS)

For Clearfil SE, the  $\mu$ TBS of DGI1 was 8.19 MPa (control 21.21 MPa), DGI2 was 9.81 MPa (control 28.33 MPa), and DGI3 was 7.62 MPa (control 24.71 MPa) (Table 8). For Optibond,  $\mu$ TBS of DGI1 was 14.89 MPa (control 37.37 MPa), DGI2 was 16.41 MPa (control 51.21 MPa), and DGI3 was 17.63 MPa (control 42.81 MPa) (Table 9).

Table 8 Microtensile bond Strength of Clearfil SE Bond

Samples	$\mu$ TBS (MPa)	Controls	$\mu$ TBS (MPa)
DGI1	7.76	1	14.84
	8.62	2	20.46
Mean	8.19	3	35.27
		4	18.51
		5	22.22
		6	18.96
		7	17.26
		8	18.61
		9	22.17
		10	23.84
		Mean	21.21
DGI2	9.82	1	13.41
		2	25.51
		3	11.56
		4	12.44
		5	18.78
		6	18.83
		7	67.15
		8	45.16
		9	18.76
		10	51.66
		Mean	28.33
DGI3	6.45	1	14.91
	8.79	2	17.24
Mean	7.62	3	22.21
		4	12.79
		5	19.50
		6	10.34



		7	42.90
		8	31.52
		9	26.44
		10	49.23
		Mean	24.71

*Table 9 Microtensile bond Strength of Optibond*

Tooth	$\mu$ TBS (MPa)	Control samples	$\mu$ TBS (MPa)
DGI1	14.89	1	16.79
		2	15.46
		3	45.25
		4	40.21
		5	54.98
		6	42.83
		7	32.58
		8	53.68
		9	34.54
		10	37.36
		Mean	37.37
DGI2	16.41	1	32.14
		2	25.03
		3	29.76
		4	42.26
		5	30.03
		6	36.02
		7	18.25
		8	27.00
		9	46.26
		10	225.3846

		Mean	51.21
DGI3	20.22	1	34.81
	15.04	2	53.04
Mean	17.63	3	29.25
		4	41.46
		5	43.13
		6	76.78
		7	91.72
		8	27.72
		9	16.49
		10	13.68
		Mean	42.81

The average  $\mu$ TBS values of Clearfil SE and Optibond were lower in DGI samples compared to the controls. For DGI samples, the average  $\mu$ TBS value of Optibond were higher than Clearfil SE (Figure 13).

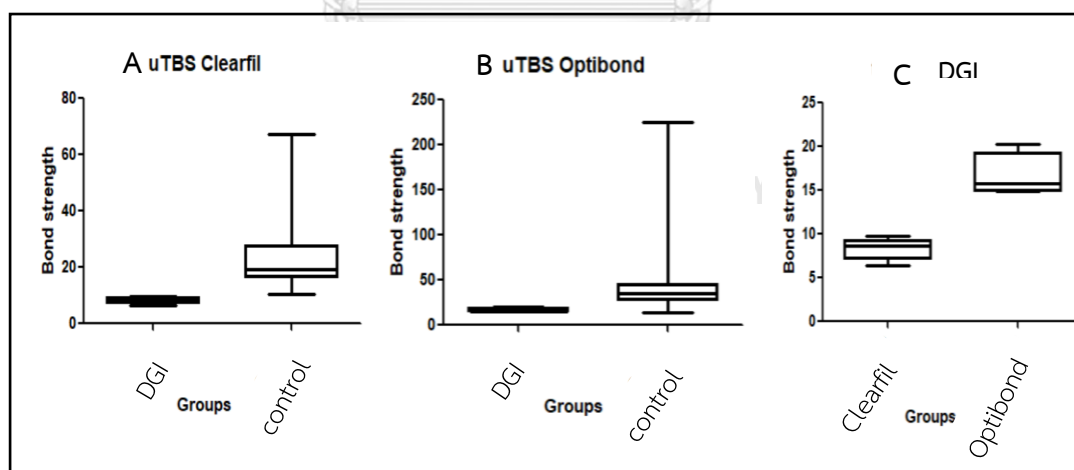


Figure 13 The chart of Microtensile bond strength ( $\mu$ TBS).

The Clearfil SE and Optibond  $\mu$ TBS of DGI samples were lower than the controls (A-B). The  $\mu$ TBS of Optibond were higher than the Clearfil SE in DGI samples (C).

### Microleakage Test

Microleakage was determined by the distribution of 2% methylene blue dye solution into the pulpal floor of cavity of DGI1 and DGI3 samples. The DGI1 and DGI3 samples showed comparable microleakage with Optibond and Clearfil SE to their controls (Table 10 and Figure 14).

*Table 10 The scoring category of microleakage in each specimen*

Group	Clearfil SE	Optibond
DGI1	2	0
conDGI1-1	2	0
conDGI1-2	2	0
DGI3	2	0
conDGI3-1	2	0
conDGI3-2	2	0

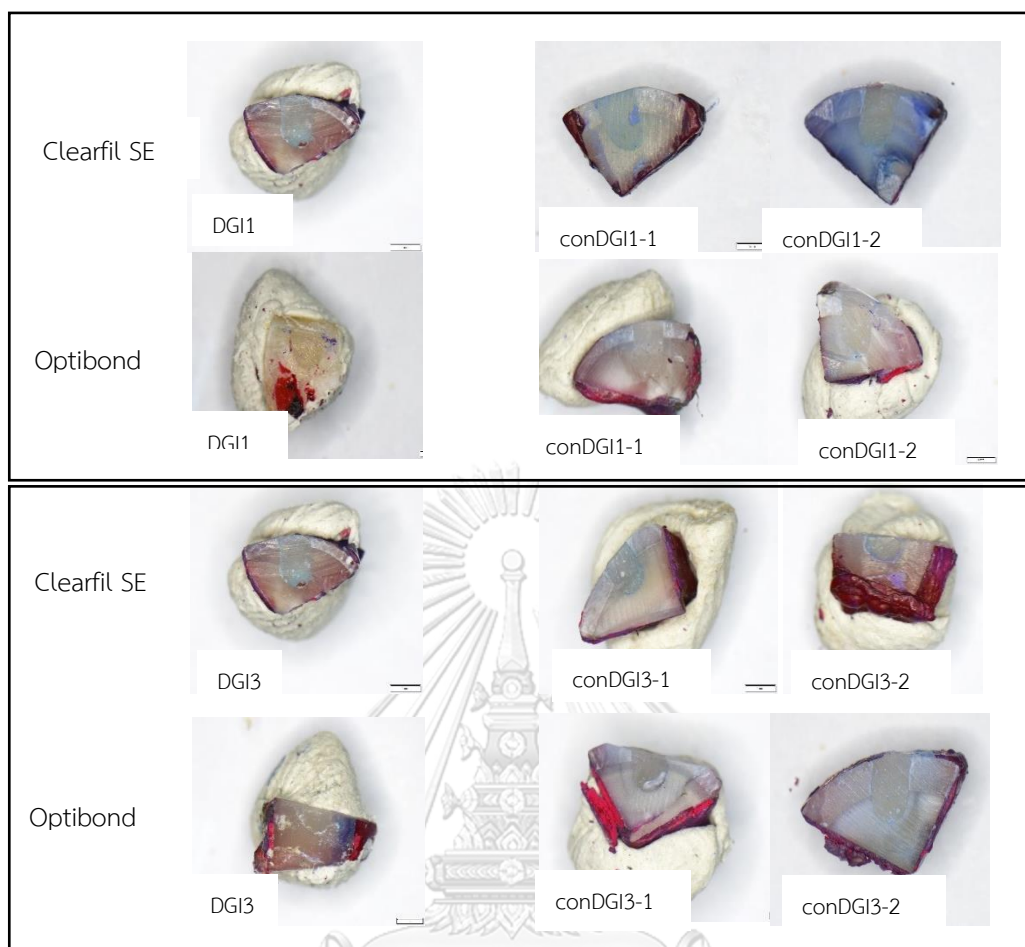


Figure 14 Stereo-microscopic images of Microleakage test.

DGI1 and DGI3 samples showed comparable penetration of dye to their controls. No penetration of dye = 0. Penetration into the dentin/material interface, but not including the pulpal floor of the cavity = 1. Penetration including the pulpal floor of cavity = 2

## CHAPTER V

### DISCUSSION

Three patients from unrelated families experienced multiple bone fractures. Physical and laboratory findings of the patients revealed deformities of extremities, blue sclerae, frontal bossing, and low bone mineral density suggesting OI. Oral examinations of three patients revealed the opalescent teeth. Radiographic and micro-CT scanning features of the teeth showed variable dentin thickness. DGI1 showed wide pulp chamber while DGI2 and DGI3 showed narrow pulp cavities. These are consistent with the feature of the dentinogenesis imperfecta (DGI).

Mutation analyses identified that three patients had glycine substitutions in *COL1A2*. Changes of glycine which is the smallest amino acid cause the disruption of collagen chain formation and defects of collagen structure. These could affect the structure of bone and teeth. Correspondingly, previous reports demonstrated that the missense mutations in *COL1A2* gene cause abnormal type I collagen (28, 29).

Regarding oro-dental manifestations, the color of DGI samples measured by colorimeter were yellowish and darker than the controls. The color difference can be perceived by human eyes. The width of dentin and pulp cavity and surface roughness were variable. The enamel of DGI samples showed higher mineral density and percentage of carbon element higher than those of the controls. Histology revealed that the dentinal tubules of DGI teeth were irregular in size and arrangement. The number of tubules were reduced. Masson trichrome staining showed that the number of collagen in DGI dentin was decreased and the shape and size of collagen fibrils were abnormal compared with the controls. Wright and Gant, 1985 showed that syndromic DGI teeth had diverse size of dentin and pulp cavity; and irregular dentinal tubules (30). It has been postulated that the irregular dentinal tubules and dentin of DGI teeth were caused by atypical odontoblasts and their odontoblastic processes (31, 32). The malformed odontoblasts were expected to malbehave and overproduce dentin causing pulp obliteration (30).

The microshear and microtensile bond strengths of total-etch adhesives (Optibond FL; Kerr) and self-etch adhesives (Clearfil SE Bond; Kuraray) applied on DGI were lower than those of the controls. The Optibond FL applied on DGI teeth had higher microshear and microtensile bond strength than Clearfil SE bond.

The Knoop microhardness values of the dentin of DGI samples were significantly decreased compared to the controls. These are consistent with previous studies. Franco *et al.*, 2006 showed that the mice with *COL1A2* mutation had reduced dentin hardness (33).

Our study showed that the enamel and dentin of DGI molar teeth had lower oxygen, phosphorus and calcium levels than the controls. The carbon percentage in the dentin and enamel of DGI samples were increased (11). Interestingly, Panighi and Sell, 1993 reported that the calcium element and microhardness of the DGI dentin had a linear correlation with bonding adhesion (21).

Dental adhesive analyses showed that the microshear and microtensile bond strengths of DGI samples were lower than those of the controls. The hybrid layer theory stating that resin in primer and bonding adhesives diffuses into the dentinal collagen fibrils and creates micromechanical interlock. The mineral composition and collagen structure of the teeth were shown to influence the bond strength of dental adhesives (14, 34, 35). Our study demonstrated that DGI teeth had decreased hydroxyapatite crystals, reduced number of dentinal tubule, malformed collagen fibers, and abnormal dentin, resulting in reduced microshear and microtensile bond strength of the adhesives. Comparing between two types of adhesives applied on DGI samples, microshear and microtensile bond strengths of Optibond were higher than those of Clearfil SE. It can explained by that Optibond contains glycerol phosphate dimethacrylate that can create chemically bonding with hydroxyapatite. It also has very high filler loading (48% by volumn) that can creates elastic shock absorber upon bonding with composite resin (34, 36). In addition, total etch adhesive has been shown to create the resin tag deeper than the self etch adhesive (37, 38).

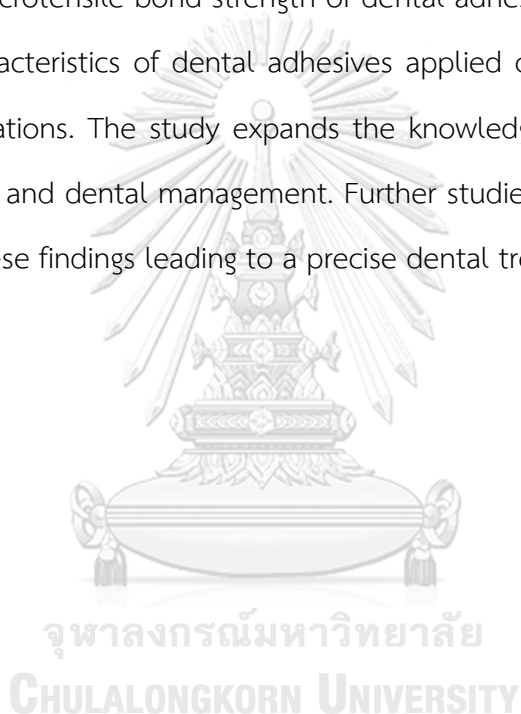
Etching of total etch bonding system can dissolve smear layer and demineralize better than acidic monomer of self etch system. These line of evidences explain that Optibond has higher bond strength than Clearfil SE applicable to both normal and DGI teeth. However, only three DGI samples were included in this study. More samples are required to obtain a solid evidence.



## CHAPTER VI

### CONCLUSION

The DGI teeth obtained from the patients affected with OI and glycine substitutions in *COL1A2* showed diverse tooth abnormalities including color, size of dentin and pulp cavity, dentinal tubules, mineral density, mineral composition, surface roughness, and microhardness. These variations affect the properties of microshear and microtensile bond strength of dental adhesives. This is the first study revealing the characteristics of dental adhesives applied on DGI teeth in OI patient with *COL1A2* mutations. The study expands the knowledge of disease phenotypes, genetic mutations, and dental management. Further studies collecting more samples would validate these findings leading to a precise dental treatment for patients with DGI and OI.





## REFERENCES



จุฬาลงกรณ์มหาวิทยาลัย  
**CHULALONGKORN UNIVERSITY**

1. Barron MJ, McDonnell ST, Mackie I, Dixon MJ. Hereditary dentine disorders: dentinogenesis imperfecta and dentine dysplasia. *Orphanet J Rare Dis*. 2008;3(1):31.
2. Shields E, Bixler D, El-Kafrawy A. A proposed classification for heritable human dentine defects with a description of a new entity. *Archives of Oral Biology*. 1973;18(4):543IN7-53.
3. Majorana A, Bardellini E, Brunelli PC, Lacaita M, Cazzolla AP, Favia G. Dentinogenesis imperfecta in children with osteogenesis imperfecta: a clinical and ultrastructural study. *International Journal of Paediatric Dentistry*. 2010;20(2):112-8.
4. Kim JW, Simmer JP. Hereditary Dentin Defects. *Journal of Dental Research*. 2007;86(5):392-9.
5. Singhal P, Arya S, Vengal M, Bhalodia M. Dentinogenesis Imperfecta Type II–A Case Report with Review of Literature. *Global Journal of Medical Research*. 2015;14(4).
6. Sapir S, Shapira J. Dentinogenesis imperfecta: an early treatment strategy. *Pediatric dentistry*. 2001;23(3):232-7.
7. Leal C, Martins L, Verli F, de Souza M, Ramos-Jorge M. Case report: Clinical, histological and ultrastructural characterization of type II dentinogenesis imperfecta. *European Archives of Paediatric Dentistry*. 2010;11(6):306-9.
8. Seow W. Developmental defects of enamel and dentine: challenges for basic science research and clinical management. *Australian dental journal*. 2014;59(s1):143-54.
9. Porntaveetus T, Osathanon T, Nowwarote N, Pavasant P, Srichomthong C, Suphapeetiporn K, et al. Dental Properties, Ultrastructure, and Pulp Cells Associated With a Novel DSPP Mutation. *Oral diseases*. 2017.
10. Wiczorek A, Loster J. Analysis of the mineral composition of permanent teeth in dentinogenesis imperfecta type II. *J Stoma*. 2012;65:404-10.
11. Wiczorek A, Loster J, Ryniewicz W, Ryniewicz AM. Dentinogenesis imperfecta: hardness and Young's modulus of teeth. *Acta of bioengineering and biomechanics*. 2013;15(3):65--9.

12. Kerebel B, Daculsi G, Menanteau J, Kerebel L. The inorganic phase in dentinogenesis imperfecta. *Journal of dental research*. 1981;60(9):1655-60.
13. Sezinando A. Looking for the ideal adhesive—a review. *Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial*. 2014;55(4):194-206.
14. Tyas M, Burrow M. Adhesive restorative materials: a review. *Australian Dental Journal*. 2004;49(3):112-21.
15. Ekambaram M, Yiu CKY, Matinlinna JP. An overview of solvents in resin–dentin bonding. *International Journal of Adhesion and Adhesives*. 2015;57:22-33.
16. Sarr M, Kane AW, Vreven J, Mine A, Van Landuyt K, Peumans M, et al. Microtensile bond strength and interfacial characterization of 11 contemporary adhesives bonded to bur-cut dentin. *Operative Dentistry*. 2010;35(1):94-104.
17. Kensche A, Dähne F, Wagenschwanz C, Richter G, Viergutz G, Hannig C. Shear bond strength of different types of adhesive systems to dentin and enamel of deciduous teeth in vitro. *Clinical oral investigations*. 2016;20(4):831-40.
18. Swift EJ. Dentin/enamel adhesives: review of the literature. *Pediatric dentistry*. 2002;24(5):456-61.
19. Hasan MMB. Self-etching Adhesive Systems in Operative Dentistry: A Literature Review Research & Reviews: *Journal of Dental Sciences*. 2017;5(2):23-32.
20. Guzman-Armstrong S, Armstrong S, Qian F. Relationship between nanoleakage and microtensile bond strength at the resin-dentin interface. *Operative dentistry*. 2003;28(1):60-6.
21. Panighi M, G'Sell C. Effect of the tooth microstructure on the shear bond strength of a dental composite. *Journal of Biomedical Materials Research Part A*. 1993;27(8):975-81.
22. Perdigão J. Dentin bonding—Variables related to the clinical situation and the substrate treatment. *Dental Materials*. 2010;26(2):e24-e37.
23. Standardization IOF. ISO 11405:2015 Dental materials: testing of adhesion to tooth structure. 2015:1-10.
24. Livingston G, Sommerlad A, Orgeta V, Costafreda SG, Huntley J, Ames D, et al. Dementia prevention, intervention, and care. *The Lancet*. 2017;390(10113):2673-734.

25. Keklikoglu N, Akinci S. Comparison of three different techniques for histological tooth preparation. *Folia histochemica et cytobiologica*. 2013;51(4):286-91.
26. Inoue S, Vargas MA, Abe Y, Yoshida Y, Lambrechts P, Vanherle G, et al. Microtensile bond strength of eleven contemporary adhesives to dentin. *Journal of Adhesive Dentistry*. 2001;3(3).
27. Standardization IOF. ISO 29022:2013 Dentistry-adhesion-notched-edge shear bond strength test. . 2013:1-12.
28. De Coster P, Cornelissen M, De Paepe A, Martens L, Vral A. Abnormal dentin structure in two novel gene mutations [COL1A1, Arg134Cys] and [ADAMTS2, Trp795-to-ter] causing rare type I collagen disorders. *Archives of oral biology*. 2007;52(2):101-9.
29. Budsamongkol T, Intarak N, Theerapanon T, Yodsanga S, Porntaveetus T, Shotelersuk V. A novel mutation in COL1A2 leads to osteogenesis imperfecta/Ehlers-Danlos overlap syndrome with brachydactyly. *Genes & diseases*. 2019;6(2):138-46.
30. Wright J, Gantt D. The ultrastructure of the dental tissues in dentinogenesis imperfecta in man. *Archives of oral biology*. 1985;30(2):201-6.
31. Ivancie GP. Dentinogenesis imperfecta. *Oral Surgery, Oral Medicine, Oral Pathology*. 1954;7(9):984-92.
32. Hursey RJ, Witkop CJ, Miklashek D, Sackett LM. Dentinogenesis imperfecta in a racial isolate with multiple hereditary defects. *Oral Surgery, Oral Medicine, Oral Pathology*. 1956;9(6):IN1-IN4.
33. Franco GL, Huang A, Camacho NP, Stone D, Blank R. Increased Young's modulus and hardness of Col1a2oim dentin. *Journal of dental research*. 2006;85(11):1032-6.
34. Van Meerbeek B, Willems G, Celis J-P, Roos J, Braem M, Lambrechts P, et al. Assessment by nano-indentation of the hardness and elasticity of the resin-dentin bonding area. *Journal of dental research*. 1993;72(10):1434-42.
35. Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P, Vanherle G. Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems. *Journal of dental research*. 1992;71(8):1530-40.

36. Loguercio A, Luque-Martinez I, Muñoz M, Szesz A, Cuadros-Sánchez J, Reis A. A comprehensive laboratory screening of three-step etch-and-rinse adhesives. *Operative dentistry*. 2014;39(6):652-62.
37. Hashimoto M, Ohno H, Yoshida E, Hori M, Sano H, Kaga M, et al. Resin–enamel bonds made with self-etching primers on ground enamel. *European journal of oral sciences*. 2003;111(5):447-53.
38. Glasspoole EA, Erickson RL, Davidson CL. Effect of enamel pretreatments on bond strength of compomer. *Dental Materials*. 2001;17(5):402-8.



## VITA

NAME	Oadcharawadee Nutchoeey
DATE OF BIRTH	16 January 1990
PLACE OF BIRTH	Nakhonsawan
INSTITUTIONS ATTENDED	Doctor of Dentistry surgery (D.D.S), Faculty of Dentistry, Nareasuan University (2008-2014) Master of science (M.Sc.), Chulalongkorn University (2017- 2019)
HOME ADDRESS	78/8 M.10 T.Watzai A.Muang Nakhonsawan
PUBLICATION	Poster presentation at THE 4th RSU National and International Research Conference On Science and Technology, Social Science and Humanities 2019