รายงานวิจัยฉบับสมบูรณ์

โครงการ การทดสอบประสิทธิภาพการยึดติดและคุณสมบัติวัสดุทางทันตกรรม ด้วยวิธีการที่มีการพัฒนาขึ้นใหม่ mini-(interfacial) fracture toughness

Validating the mini-(interfacial) fracture toughness testing on bonding effectiveness and biomedical materials

โดย **ผศ.ดร.ทพ.พงศ์ พงศ์พฤกษา**

29 พฤษภาคม 2563

สัญญาเลขที่ RSA6080090

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ผศ.ดร.ทพ.พงศ์ พงศ์พฤกษา

สังกัด ภาควิชาทันตกรรมหัตถการและวิทยาเอ็นโดดอนต์

คณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย และมหาวิทยาลัยมหิดล

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว. และ มหาวิทยาลัยมหิดล ไม่จำเป็นต้องเห็นด้วยเสมอไป)

กิตติกรรมประกาศ

โครงการวิจัยนี้สำเร็จได้ด้วยดี จากการได้รับการสนับสนุนจากสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ที่ให้การสนับสนุนเงินทุนการวิจัยตลอดโครงการวิจัย การสนับสนุนจากเจ้าหน้าที่ของสำนักงานกองทุนสนับสนุนงานวิจัย ที่ให้คำปรึกษาการบริหารจัดการทุนวิจัย การจัดการเอกสารต่างๆ ให้เรียบร้อยอย่างดี การได้เป็นส่วนหนึ่งของผู้วิจัยที่ได้รับการสนับสนุนจาก สกว. เป็นความภาคภูมิใจของนักวิจัยไทยทุกคน การได้ร่วมกิจกรรมประจำปี และได้เห็นงานวิจัยที่หลากหลายในประเทศ ทำให้เกิดแรงบันดาลใจในการทำงานที่หลากหลาย และยังได้ติดต่อประสานงานรวมถึงคำแนะนำจากนักวิจัยต่างสาขา ต่างสถาบัน ซึ่งเป็นช่องทางให้เกิดการสร้างเครือข่ายความร่วมมือได้เป็นอย่างดี

โครงการวิจัยนี้ได้รับการสนับสนุนเงินทุนการวิจัยร่วมกับมหาวิทยาลัยมหิดล การสนับสนุนการปฏิบัติงาน โดยเฉพาะสำนักวิจัย และ รศ.ดร.ฤดี สุราฤทธิ์ รองคณบดีฝ่ายวิจัย คณะทันแพทยศาสตร์ มหาวิทยาลัยมหิดล ที่ให้คำปรึกษา สนับสนุนเครื่องมือวิจัย และสถานที่วิจัย รวมถึงเจ้าหน้าที่สำนักวิจัย ที่มีส่วนช่วยจัดการเอกสาร ให้เรียบร้อย ซึ่งช่วยให้นักวิจัยสามารถปฏิบัติงานได้อย่างราบรื่น ลดความยุ่งยากและซับซ้อนของระบบลงได้

การทำงานวิจัยจำเป็นต้องได้รับการสนับสนุนและความร่วมมือ จากผู้ร่วมงานในภาควิชาทันตกรรมหัตถการ และวิทยาเอ็นโดดอนต์ คณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล มิเช่นนั้น โครงการวิจัยนี้จะไม่สามารถสำเร็จลงได้ ซึ่งอาจารย์และผู้ร่วมงานทุกท่านในภาควิชาฯ ได้ให้ความร่วมมือกันเป็นอย่างดี ช่วยส่งเสริมและสนับสนุนให้เกิดงานที่มีคุณภาพมากขึ้น

นอกจากการสนับสนุนภายในคณะฯ แล้ว โครงการวิจัยนี้ยังได้รับการสนับสนุนและคำปรึกษาจากอาจารย์และนักวิจัย จากภายนอก อาจารย์คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย อาจารย์คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล อาจารย์คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย และ นักวิจัยจากศูนย์เทคโนโลยีโลหะและวัสดุแห่งชาติ (MTEC) ที่มีส่วนกับความสำเร็จของโครงการวิจัยนี้

นอกจากการสนับสนุนภายในประเทศแล้ว การสนับสนุนจากอาจารย์ที่ปรึกษา Prof.Bart Van Meerbeek และเพื่อน Dr.Jan De Munck เป็นส่วนสำคัญกับความสำเร็จของโครงการวิจัยอย่างมาก

ขอบคุณ นักศึกษาหลังปริญญา ที่ช่วยเป็นมดงาน เพื่อให้ได้ข้อมูลและผลงานวิจัยที่สมบูรณ์ ซึ่งช่วยให้โครงการสำเร็จไปได้ตามระยะเวลาที่กำหนด

สุดท้าย ขอขอบคุณภรรยา ทพญ.เพ็ญดิถี ชัยผาติกานต์ ที่คอยเป็นให้กำลังใจ เข้าใจ ช่วยคิดแก้ปัญหา และสนับสนุน ทั้งการทำงาน และการทำวิจัยมาโดยตลอด

บทคัดย่อ

รหัสโครงการ: RSA6080090

ชื่อโครงการ: การทดสอบประสิทธิภาพการยึดติดและคุณสมบัติวัสดุทางทันตกรรมด้วยวิธีการที่มีการพัฒนาขึ้นใหม่ mini-(interfacial) fracture toughness

ชื่อนักวิจัย: ผศ.ดร.ทพ.พงศ์ พงศ์พฤกษา ภาควิชาทันตกรรมหัตถการและวิทยาเอ็นโดดอนต์ คณะทันตแพทยศาสตร์ มหาวิทยาลัยมหิดล

E-mail Address: pong.pon@mahidol.ac.th

ระยะเวลาโครงการ: 36 เดือน

การศึกษาประสิทธิภาพสารยึดติดทางทันตกรรมด้วยวิธีการ mini-interfacial fracture toughness (mini-iFT) โดยทดสอบสารยึดติดที่มีความเข้มข้นของสารก่อตัวต่างกัน พบว่าวิธีการใหม่นี้สามารถแสดงความแตกต่างของประสิทธิภาพสารยึดติดได้ดี (ภาคผนวก 1) **การศึกษา**และพัฒนา การเติม bioactive glass หรือ zinc oxide ในวัสดุคอมโพสิต เพื่อผลการต้านแบคทีเรีย และการป้องกันฟันผุ การทดสอบคอมโพสิต ด้วยวิธี mini-FT, fractural strength การดูดน้ำ การละลายตัว ความลึกการบ่มตัว และการต้านแบคทีเรีย ผลการศึกษาพบว่า วิธี mini-FT ได้ผลในทางเดียวกันกับวิธี fracture strength สารที่เติมในคอมโพสิตทำให้ประสิทธิภาพคอมโพสิตลดลง และคุณสมบัติการต้านแบคทีเรียยังไม่เป็นที่พอใจ (ภาคผนวก 2) **การศึกษา**ผลของไอออนต่อความแข็งผิวของวัสดุ เพื่อศึกษาปัจจัยที่ทำให้วัสดุมีความแข็งผิวเพิ่มขึ้นหลังจากการบูรณะในผู้ป่วย โดยนำวัสดุชนิดต่างๆ มาแช่น้ำเทียบกับน้ำลายเทียม และทดสอบค่าความแข็งผิวที่เวลา 1 วัน ถึง 42 วัน พบว่าวัสดุกลาสไอโอโนเมอร์มีความแข็งผิวเพิ่มขึ้นเรื่อยๆ เมื่อแช่ในน้ำลายเทียม เนื่องจากไอออนของ ฟอสฟอรัส แคลเซียม โปแตสเซียม และแมคนีเซียม มีการเกาะเพิ่มขึ้นที่ผิวของวัสดุ (ภาคผนวก 3) **การศึกษา**ปัจจัยที่มีผลต่อความสำเร็จและความล้มเหลวในการบูรณะฟันในคลินิก โดยศึกษาความสำเร็จของการบูรณะฟันด้วยคอมโพสิตบริเวณคอฟันร่วมกับสารยึดติด พบว่าความสำเร็จในการบูรณะฟันด้วยคอมโพสิตมากกว่าร้อยละ 95 ที่เวลา 5 ปี ปัจจัยที่มีผลต่อความล้มเหลวของการบูรณะมากที่สุด คือ สารยึดติด คอมโพสิตไม่มีผลต่อทั้งความสำเร็จและความล้มเหลวในการบูรณะ (ภาคผนวก 4)

โครงการวิจัยที่สำเร็จไปแล้วนั้น ช่วยให้มั่นใจในการทดสอบวัสดุและประสิทธิภาพการยึดติดด้วยวิธี mini-iFT เพื่อต่อยอดการพัฒนาวัสดุให้มีประสิทธิภาพมากยิ่งขึ้น การศึกษาสารตัวเติมและการเพิ่มขึ้นของความแข็งผิว มีผลต่อการพัฒนาวัสดุอุดฟัน และการนำไปใช้งานในคลินิกต่อไป

คำหลัก: Mini-FT, ชีววัสดุทางทันตกรรม, สารยึดติดทางทันตกรรม, ทันตวัสดุศาสตร์, ความสำเร็จในการบูรณะทางคลินิก, ความล้มเหลวในการบูรณะทางคลินิก

ABSTRACT

Project Code: RSA6080090

Project Title: Validating the mini-(interfacial) fracture toughness testing on bonding effectiveness and biomedical materials

Investigator: Asst.Prof.Dr.Pong Pongprueksa, Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University

E-mail Address: pong.pon@mahidol.ac.th

Project Period: 36 months

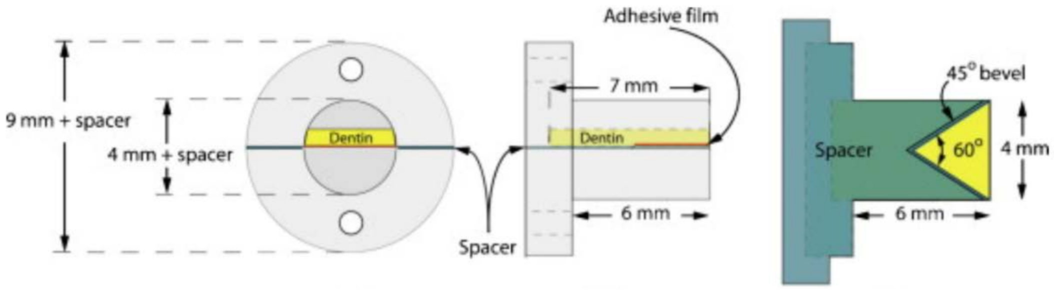
The first study evaluated the bonding effectiveness of dental adhesive with various initiator concentrations using the mini- interfacial fracture toughness test. The result found that the higher photoinitiator increased the higher mini-iFT and the different photoinitiator showed the different polymerization kinetic. (Appendix 1) **The second study** was the influence of bioactive glass and zinc oxide on resin composite. The experimental composite property was test using in mini-FT, flexural strength, and anti-bacterial. The result showed that the mini-FT and flexural strength were correlated, however, the anti-bacterial property was absent. (Appendix 2) **The third study** was the ion-releasing influence to the material surface hardness. The surface microhardness was investigated on the material surface, which storing in either deionized water or artificial saliva for 42d. This study found that the microhardness of glass ionomer cement gradually increased in artificial saliva storage at 42d. Phosphorus, calcium, potassium, and magnesium element in artificial saliva influence to the surface microhardness of material. (Appendix 3) **The last study** was the retrospective clinical study on the success and failure on Class V composite restoration. This study found an impressive clinical success at 95% in 5 years. The adhesive was the most factor influence to the clinical failure, while the composite was not influence to the clinical success and failure. (Appendix 4)

This project has been successfully on the validating the mini-(interfacial) fracture toughness of materials in the reliable and valuable technique. The technique is an essential step to the development of an innovative dental materials, which consequently to the successfully used in clinical practice.

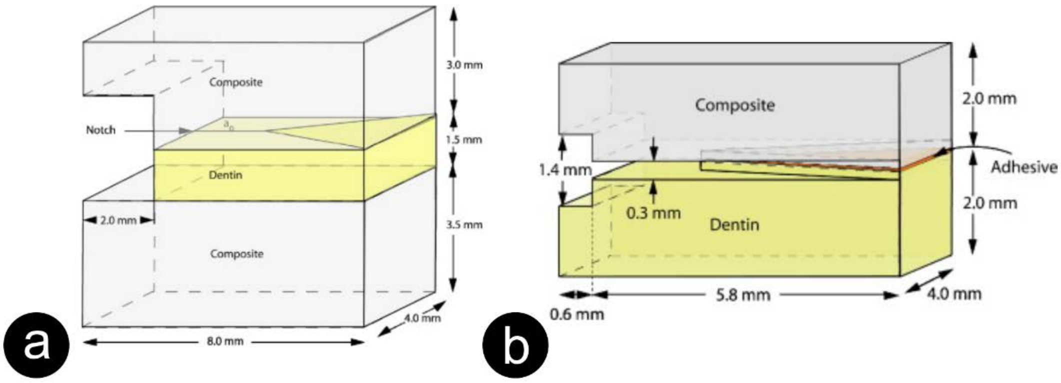
Keywords: Mini-FT, Dental bioactive material, Dental adhesive, Clinical success, Clinical failure

บทนำ

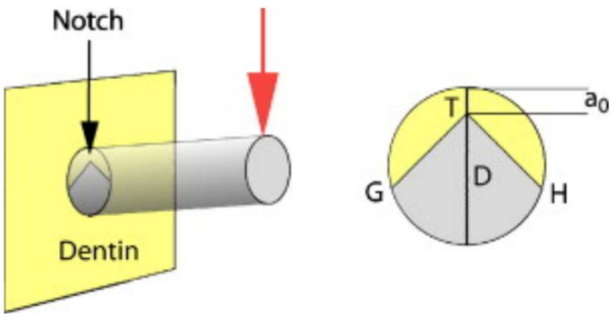
Mini-interfacial fracture toughness (mini-iFT) เป็นวิธีทดสอบประสิทธิภาพการยึดติดของสารยึดติดที่นำเสนอครั้งแรกในปี 2016 (Pongprueksa et al. 2016b) เพื่อใช้ในการทดสอบประสิทธภาพสารยึดติดที่มีขนาดเล็ก สามารถใช้ทดสอบบนเนื้อฟัน (Pongprueksa et al. 2016b) และบนเคลือบฟัน (Pongprueksa et al. 2016a) วิธีทดสอบ mini-interfacial fracture toughness พัฒนามาจากวิธี conventional fracture toughness ได้แก่ Chevron notch beam fracture toughness (De Munck et al. 2013; De Munck et al. 2015) และ single gradient notched beam fracture toughness (Wan et al. 2009) โดยวิธีแบบดั้งเดิมอื่นๆ ไม่ได้รับความนิยม เนื่องจากชิ้นทดสอบมีขนาดใหญ่ ขั้นตอนการเตรียมชิ้นทดสอบมีความยุ่งยาก ต้องอาศัยประสบการณ์และการฝึกเตรียมชิ้นทดสอบที่ยุ่งยาก ดังแสดงภาพประกอบที่ 1 - 5 (Tam and Pilliar 1993; Armstrong et al. 1998; Far and Ruse 2003; Scherrer et al. 2010; Soderholm 2010; De Munck et al. 2013) การพัฒนาวิธี mini-iFT ให้ดีกว่าแบบดั้งเดิม โดย ชิ้นทดสอบที่มีขนาดเล็ก การเตรียมชิ้นทดสอบที่ง่ายขึ้น และใช้เวลาการเตรียมชิ้นทดสอบน้อยลง ตามภาพประกอบที่ 6 และเมื่อเทียบกับวิธีทดสอบประสิทธิภาพการยึดติดที่ได้รับความนิยม micro-tensile bond strength พบว่า mini-iFT มีความเที่ยงตรงกว่า มีค่าเบี่ยงเบนมาตรฐานน้อยกว่า ความยุ่งยากในการเตรียมชิ้นทดสอบไม่ต่างกันมาก และ mini-iFT แสดงผลการยึดติดที่รอยต่อการยึดติดมากกว่า (Pongprueksa et al. 2016b) วิธีการทดสอบนี้เริ่มได้รับการยอมรับเพื่อทดสอบประสิทธิภาพการยึดติดของสารยึดติด อย่างไรก็ตามการใช้วิธี mini-FT เพื่อทดสอบคุณสมบัติของวัสดุทางทันตกรรมยังไม่เป็นที่ยอมรับ ซึ่งจำเป็นต้องศึกษาเพื่อให้แน่ใจว่าสามารถใช้ทดสอบประสิทธิภาพของวัสดุทางทันตกรรมที่มีขนาดเล็กต่อไป



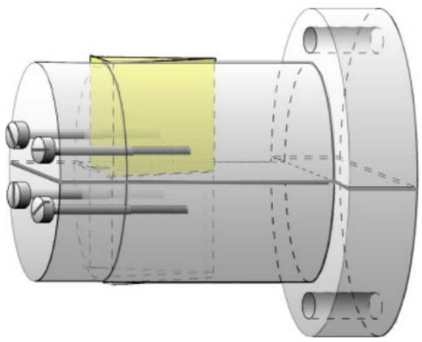
ภาพประกอบที่ 1 ภาพแสดงชิ้นตัวอย่างทดสอบ interfacial fracture toughness แบบ short-rod interfacial fracture toughness โดย Tam and Pilliar ในปี 1993 (adapted from Söderholm, 2010)



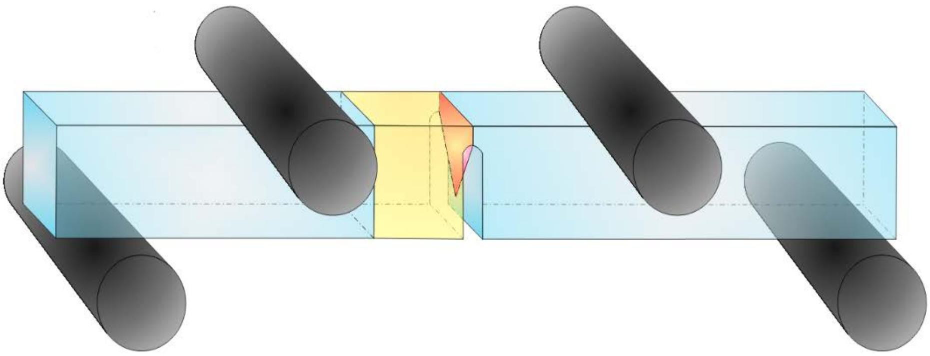
ภาพประกอบที่ 2 ภาพแสดงชิ้นตัวอย่างทดสอบ interfacial fracture toughness **(a)** Chevron-notched short bar interfacial fracture toughness specimen โดย Lin and Douglas ในปี 1994. **(b)** Plane-strain chevron-notched short bar fracture toughness specimen โดย Armstrong *et al.* ในปี 1998. (adapted from Söderholm, 2010)



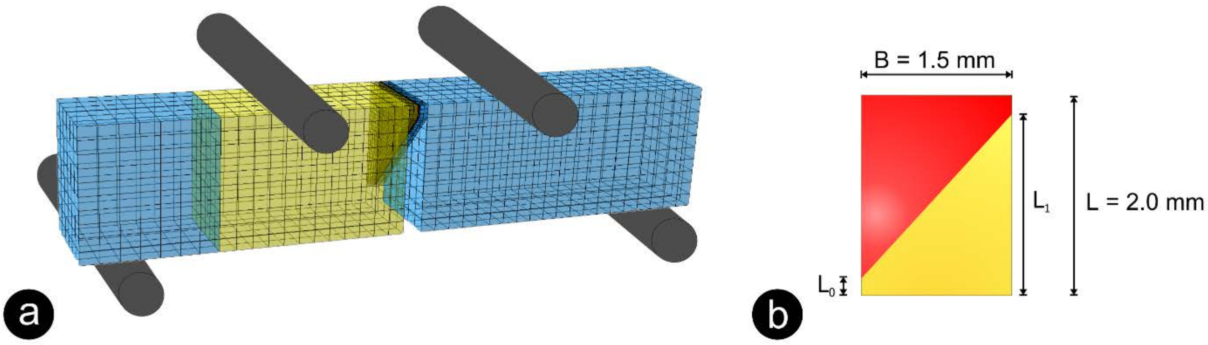
ภาพประกอบที่ 3 ภาพแสดงชิ้นตัวอย่างทดสอบ interfacial fracture toughness โดย Cheng *et al.* ในปี 1999 (adapted from Soderholm, 2010)



ภาพประกอบที่ 4 ภาพแสดงชิ้นตัวอย่างทดสอบ Notchless triangular prism interfacial fracture toughness specimen โดย Far and Ruse ในปี 2003 (adapted from Söderholm, 2010)



ภาพประกอบที่ 5 ภาพแสดงชิ้นตัวอย่างทดสอบ Chevron notched beam interfacial fracture toughness specimen โดย De Munck et al. ในปี 2013 (adapted from De Munck et al., 2013)



ภาพประกอบที่ 6 ภาพแสดงชินตัวอย่างทดสอบ Mini-interfacial fracture toughness specimen design. **(a)** ภาพ mini-iFT specimen สร้างจากโปรแกรม Finite Elements Analysis; สีฟ้าแสดง composite, สีเหลืองแสดง dentin และสีแดงที่รอยต่อแสดง adhesive resin. **(b)** ภาพตัดขวางบริเวณรอยบากของชิ้นทดสอบ mini-iFT; สีแดงแสดง adhesive interface (adapted from Pongprueksa et al., 2016).

วัสดุบูรณะฟันคอมโพสิต (composite) ได้รับการพัฒนาและยอมรับเพื่อใช้งานทางทันตกรรม จากคุณสมบัติ ความแข็งแรง ทนทาน ความสวยงาม และสามารถยึดกับฟันได้เมื่อใช้ร่วมกับสารยึดติดทางทันตกรรม (dental adhesive) การพัฒนาวัสดุคอมโพสิตในปัจจุบัน มีการพัฒนาอย่างรวดเร็ว ในหลายปัจจัยขึ้นกับการนำใช้งาน อย่างไรก็ตาม แนวโน้มการพัฒนาคอมโพสิตที่น่าสนใจ ได้แก่ การพัฒนาความเข้ากันกับเนื้อเยื้อ (biocompatibility) (Khvostenko et al. 2013b; Khvostenko et al. 2016) การต้านแบคทีเรีย (anti-bacterial) (Chatzistavrou et al. 2014) และการลดความเป็นกรดของเพื่อป้องกันการผุ (buffering property) (Nedeljkovic et al. 2016) โดยสารตัวเติมที่น่าสนใจ และนำมาใช้เพื่อเพิ่มประสิทธิภาพดังกล่าว ได้แก่ bioactive glass (Chatzistavrou et al. 2015; Hench and Jones 2015) และ ซิงค์ออกไซด์ (zinc oxide) (Wanitwisutchai et al. 2019) ซึ่งการพัฒนาคอมโพสิตที่มีประสิทธิภาพดังกล่าว คาดหวังเพื่อช่วยลดการผุซ้ำหลังการอุดคอมโพสิตและช่วยให้วัสดุคงอยู่ในช่องปากได้นานขึ้น

วัสดุบูรณะในช่องปากจำเป็นต้องต้านทานต่อสภาวะในช่องปากที่ซับซ้อนได้ โดยสภาวะที่ซับซ้อนได้แก่ สภาวะที่มีน้ำ น้ำลาย เอมไซด์ ความเป็นกรด และแร่ธาตุต่างๆ (Crisp et al. 1976; Ilie 2018; Klauer et al. 2019; van Dijken et al. 2019) ซึ่งความแข็งแรงบริเวณผิวของวัสดุ (surface microhardness) เป็นสิ่งสำคัญที่ต้องคำนึงถึง โดยความแข็งผิวจะเกี่ยวข้องกับการต้านทานสภาวะที่ซับซ้อนในช่องปาก และต้านทานการสึกกร่อนบริเวณผิววัสดุ (McCabe and Rusby 2004) โดยพบว่า ความแข็งผิวของวัสดุบูรณะส่วนใหญ่ลดลง ตามระยะเวลา (Okada et al. 2001; Mayworm et al. 2008) อย่างไรก็ตาม พบว่าวัสดุบางชนิดมีความแข็งผิวเพิ่มขึ้นหลังจากการแช่ในน้ำหรือในน้ำลาย (Okada et al. 2001; Aliping-McKenzie et al. 2003; Shiozawa et al. 2014; Ilie and Stawarczyk 2016) โดยการศึกษาการเปลี่ยนแปลงค่าความแข็งผิวนี้ จะทำให้เข้าใจถึงปัจจัยที่มีผลต่อความแข็งผิว และจะเป็นประโยชน์ต่อการศึกษาและพัฒนาวัสดุทางทันตกรรมต่อไป

การพัฒนาวัสดุทางทันตกรรมนอกจากจะพัฒนาคุณสมบัติของวัสดุให้ดีขึ้นแล้ว ความสำเร็จของการใช้งานในคลินิกเป็นสิ่งสำคัญที่ต้องคำนึง เนื่องจากการใช้งานในคลินิกมีปัจจัยที่เกี่ยวข้องมากมาย ทั้งปัจจัย

ภายในผู้ป่วยเอง (Sawlani et al. 2016; Wierichs et al. 2018; Correia et al. 2020) ได้แก่ พฤติกรรมการดูแลสุขภาพของผู้ป่วย ตำแหน่งของฟัน การใช้งานจากการบดเคี้ยว และปัจจัยภายนอก (Giachetti et al. 2007; Scotti et al. 2016) ได้แก่ ทันตแพทย์ และวัสดุที่ใช้ ดังนั้น การศึกษาปัจจัยที่มีผลต่อความสำเร็จและความล้มเหลวของการรักษา เป็นส่วนสำคัญในการพัฒนาวัสดุเพื่อให้ใช้งานได้ในคลินิก

วัตถุประสงค์ของโครงการวิจัยประกอบด้วย

1. ทดสอบและนำวิธี mini-(interfacial) fracture toughness ไปใช้ทดสอบประสิทธิภาพการยึดติดและความแข็งต้านการแตกหักของวัสดุทางทันตกรรม
2. พัฒนาวัสดุทางทันตกรรมให้มีคุณสมบัติที่เข้ากันได้ดีกับเนื้อเยื้อและต้านแบคทีเรียได้
3. ศึกษาปัจจัยที่ทำให้วัสดุบูรณะทางทันตกรรมมีความทนทาน และต้านทานการใช้งานในช่องปาก
4. การศึกษาความสำเร็จและความล้มเหลวในการบูรณะฟันในคลินิกเพื่อเป็นแนวทางในการศึกษาวัสดุทางทันตกรรมในผู้ป่วยต่อไป

วิธีการทดลอง

การทดสอบประสิทธิภาพการยึดติดของสารยึดติดที่มีความเข้มข้นของสารก่อตัวต่างชนิดกันด้วยวิธี mini-iFT *(ภาคผนวก 1)*

เตรียมสารยึดติดที่มีความเข้มข้นของสารก่อตัวต่างกันสองชนิด ได้แก่ CQ/amine และ TPO โดยส่วนประกอบและความเข้มข้นของสารก่อตัวได้แสดงใน ตารางที่ 1 ทาสารยึดติดลงบนฟันและอุดด้วยคอมโพสิต ฉายแสงด้วยเครื่องฉายแสงที่มีความเข้มแสง 1,100 mW/cm^2 ตามคำแนะนำของบริษัท จากนั้นเก็บฟันไว้ในน้ำเป็นเวลา 1 สัปดาห์ นำฟันที่ได้ไปตัดให้ได้ชิ้นทดสอบแบบแท่งขนาด (หน้าตัด1.5x2.0 มม. ยาว 16-18 มม.) จากนั้นนำชิ้นทดสอบไปเตรียมรอยตัดด้วยแผ่นตัดเพชรขนาดบางพิเศษ (M1DO8; Struers) และนำไปทดสอบที่เครื่องทดสอบ universal testing machine (Instron 5848 Micro Tester, MA, USA) แบบสัมผัสสี่จุด (4-point-bending) ภายหลังการทดสอบวัดขนาดของหน้าตัดชิ้นทดสอบและนำไปตรวจสอบพื้นผิวด้วยกล้องจุลทรรศน์อิเลคตรอนชนิดส่องกราด (scanning electron microscope; JSM-6610LV, JEOL, Tokyo, Japan) นำสารยึดติดทั้งหมดไปทดสอบการบ่มตัว (degree of conversion) และ การบ่มตัวทางจลศาสตร์ (polymerization kinetic) ด้วย micro-Raman spectroscopy (Senterra; BrukerOptik, Ettlingen, Germany) เปรียบเทียบการบ่มตัวบนเนื้อฟันและการบ่มตัวบนแผ่นกระจก (glass slide)

ตารางที่ 1 แสดงส่วนประกอบของสารยึดติด LUB ที่มีสารกระตุ้นการก่อตัวที่ต่างกันในความเข้มข้นที่ต่างกัน

Adhesive	Composition[1]	Initiators[1]
LUB-CQ/amine_high	15 wt% Bis-GMA, 30 wt% HEMA, 10 wt% MDP, 15 wt% TEGDMA, 10 wt% colloidal silica, 15 wt% ethanol, 15 wt% water, stabilizers (minute amount)	2.0 wt% CQ, 2.0 wt% EDMAB
LUB-CQ/amine_low		0.35 wt% CQ, 0.35 wt% EDMAB
LUB-TPO_high		2.0 wt% TPO
LUB-TPO_low		0.35 wt% TPO
Clearfil S^{3} Bond Plus[2]	15-35 wt% Bis-GMA, 10-35 wt% HEMA, MDP, hydrophilic aliphatic dimethacrylate, hydrophobic aliphatic methacrylate, colloidal silica, <0.1 wt% sodium fluoride, <20 wt%, water ethanol	dl-camphorquinone, accelerators, initiators

[1]Abbreviations: Bis-GMA: bisphenol A glycidyl dimethacrylate; CQ: camphorquinone; EDMAB: ethyl 4-(dimethylamino)benzoate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogenphosphate; TEGDMA: triethylene glycol dimethacrylate; TPO: diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide; [2]Commercially available, precise composition not released by Kuraray Noritake (Lot: 00012A).

การศึกษาผลการเติม bioactive glass และ ซิงค์ออกไซด์ ต่อประสิทธิภาพคอมโพสิต *(ภาคผนวก 2)*

เตรียมคอมโพสิตโดยใช้ 15wt% 45S5 bioactive glass หรือ 15wt% S53P4 bioactive glass หรือ 1wt% zinc oxide ร่วมกับสารตัวเติม (filler) ชนิดซิลิคอนไดออกไซด์ ให้ได้ปริมาณ 25wt% เทียบกับวัสดุที่มีซิลิคอนไดออกไซด์อย่างเดียว นำสารตัวเติมดังกล่าวผสมกับเรซินที่ผสมระหว่าง UDMA:TEGDMA ที่อัตราส่วน 80:20 และผสมสารก่อตัว (initiator) ชนิด CQ, TPO และ EDMEB ส่วนประกอบของคอมโพสิตแต่ละชนิดแสดงใน ตารางที่ 2 นำคอมโพสิตที่ได้มาทดสอบ mini-fracture toughness (mini-FT), flexural strength, flexural modulus, water sorption, water solubility, depth of curing และ การกำจัดแบคทีเรีย

ตารางที่ 2 แสดงส่วนประกอบของคอมโพสิตในแต่ละกลุ่มการทดลอง

Composition[a]	45S5	S53P4	ZnO	SiO_2	Systemp onlay
BAG-45S5	15	-	-	-	-
BAG-S53P4	-	15	-	-	-
ZnO powder	-	-	1	-	-
SiO_2	10	10	24	25	-
Highly dispensed SiO_2, silanized	-	-	-	-	19.4
Prepolymerized dimethacrylate	42	42	42	42	42.7
UDMA	25.2	25.2	25.2	25.2	-
Polyester urethane dimethacrylate	-	-	-	-	29.4
TEGDMA	6.3	6.3	6.3	6.3	-
Ethyl triglycol methacrylate CAS:39670-09-2	-	-	-	-	7.5
CQ	0.5	0.5	0.5	0.5	-
EDMAB	0.5	0.5	0.5	0.5	-
TPO	0.5	0.5	0.5	0.5	-
Catalysts, stabilizers and triclosan	-	-	-	-	1.0
Pigments	-	-	-	-	<0.1

Abbreviation: ***BAG-45S5***, 45%SiO_2, 6%P_2O_5, 24.5%Na_2O, 24.5%CaO; ***BAG-S53P4***, 53%SiO_2, 4%P_2O_5, 23%Na_2O, 20%CaO; ***ZnO***, 40nm Zinc Oxide, CAS:1314-13-2; ***SiO_2***, 0.7µm silanized glass filler, CAS:65997-17-3; ***UDMA***, urethane dimethacrylate, CAS:72869-86-4; ***TEGDMA***, triethylene glycoldimethacrylate, CAS:109-16-0; ***CQ***, Camphorquinone, CAS:10373-78-1; ***EDMAB***, ethyl 4-(dimethylamino)benzoate, CAS:10287-53-3; ***TPO***, diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide, CAS:75980-60-8.

การศึกษาปัจจัยที่มีผลต่อความแข็งผิววัสดุหลังแช่ในน้ำหรือน้ำลายเทียม *(ภาคผนวก 3)*

วัสดุบูรณะฟัน 6 ชนิด ได้แก่ Equia Forte Fil, Ketac Universal, Beutifil II, Fuji II LC, Activa BioActive และ Filtek Z350XT ส่วนประกอบของวัสดุแต่ละชนิดแสดงใน ตารางที่ 3 นำวัสดุบูรณะมาเตรียมให้ได้รูปร่างลักษณะแผ่นขนาดเส้นผ่านศูนย์กลาง 5 มม. ลึก 2 มม. เตรียมตามคำแนะนำของบริษัทผู้ผลิต นำวัสดุทั้งหมดแยกแช่ในน้ำปราศจากไอออน (deionized water) หรือในน้ำลายเทียม (artificial saliva) โดยส่วนผสมของน้ำลายเทียมแสดงไว้ใน ตารางที่ 4 นำวัสดุไปทดสอบความแข็งผิว (surface microhardness) ด้วยเครื่องทดสอบความแข็งผิว (microhardness tester, FM700; Future Tech, Tokyo,Japan) ที่แรงกด 100 กรัม ในวันที่ 1, 7, 21 และ 42 นำชิ้นตัวอย่างที่ 1 วัน และ ที่ 42 วัน ในน้ำปราศจากไอออนและในน้ำลายเทียม มาตรวจสอบแร่ธาตุที่ผิวด้วย กล้องจุลทรรศน์อิเลคตรอนแบบส่องกราด (SEM-EDX, JSM610LV; JEOL, Tokyo Japan) นำน้ำปราศจากไอออนหลังแช่ชิ้นทดสอบที่ 1, 21 และ 42 วัน มาตรวจสอบปริมาณไอออนในน้ำด้วยเครื่อง Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES; ACTIVA M, Horiba Jobin Yvon, France) โดยไอออนที่ศึกษาได้แก่ โซเดียม ซิลิคอน แคลเซียม อลูมิเนียม แมคนีเซียม และฟอสฟอรัส

ตารางที่ 3 แสดงส่วนประกอบของวัสดุแต่ละชนิดที่ใช้ในการทดสอบค่าความแข็งผิว

Materials	**Composition**[a]
EQUIA® Forte Fil (capsule) (Batch no. 1606221) GC Corporation, Tokyo, Japan	Powder; Ultrafine highly reactive FAS glass, high molecular weight polyacrylic acid powder, Iron(III) oxide(CASRN: 1309-37-1) Liquid; Distilled water, Polybasic carboxylic acid, Tartaric acid(CASRN: 87-69-4)
Ketac™ Universal Aplicap™ (capsule) (Batch no. 637544) 3M, ESPE, St. Paul, MN, USA	Powder; Oxide glass chemicals(CASRN: 65997-17-3) Liquid; Water(CASRN: 7732-18-5), Copolymer of acrylic acid-maleic acid(CASRN: 29132-58-9), Tartaric acid(CASRN: 87-69-4), Benzoic acid(CASRN: 65-85-0)
Filtek™ Z350XT (Batch no. N827976) 3M, ESPE, St. Paul, MN, USA	Silane treated ceramic(CASRN: 444758-98-9), Silane treated silica(CASRN: 248596-91-0), Silane treated zirconia, UDMA, Bis-GMA, TEGDMA, Bis-EMA(6), PEGDMA
Beautifil II (Batch no. 121496) Shofu, The Alpha, Science Park II Singapore	Bis-GMA, TEGDMA, UDMA, Bis-MPEPP, Aluminofluoro-borosilicate glass(CASRN: 65997-18-4), Al_2O_3 (CASRN: 1344-28-1), DL-Camphorquinone(CASRN: 10373-78-1),
GC Fuji II LC ® (capsule) (Batch no. 1508211) GC Corporation, Tokyo, Japan	Powder; (Fluoro) alumino silicate glass (FAS Glass) Liquid; Distilled water(CASRN: 7732-18-5), UDMA, HEMA, 2-hydroxy-1,3 dimethacryloxypropane(CASRN:1830-78-0), Polyarcylic acid(CASRN: 9003-01-04), Tartaric acid(CASRN: 87-69-4), Camphorquinone(CASRN: 465-29-2),
Activa BioActive RESTORATIVE (Batch no. 150212) Pulpdent Corporation, Watertown, MA USA	UDMA, Bis (2-(Methacryloyloxy) Ethyl) phosphate, Barium glass, Ionomer glass, Polyacrylic acid/maleic acid copolymer, Dual-cure chemistry, Amorphous silica, Sodium fluoride

[a] Abbreviations: UDMA: Diurethane dimethacrylate (CASRN: 72869-86-4); Bis-GMA: Bisphenol A diglycidyl ether dimethacrylate (CASRN: 1565-94-2); TEGDMA: Triethylene glycol dimethacrylate (CASRN: 109-16-0); HEMA: 2-Hydroxyethyl methacrylate (CASRN: 868-77-9); Bis-EMA(6): Bisphenol A polyethylene glycol diether dimethacrylate (CASRN: 41637-38-1); PEGDMA: Polyethylene glycol dimethacrylate (CASRN: 25852-47-5).

ตารางที่ 4 แสดงส่วนประกอบของน้ำลายเทียมที่ใช้ในการศึกษา

Composition	**Weight/Volume**
Sodium chloride (NaCl)	0.798 grams
Potassium chloride (KCl)	1.2 grams
Calcium chloride ($CaCl_2$)	0.147 grams
Potassium dihydrogen phosphate (KH_2PO_4)	0.272 grams
Magnesium chloride hexahydrate ($MgCl_2.6H_2O$)	0.093 grams
Deionised water	990 millilitres

การศึกษาปัจจัยที่มีผลต่อความสำเร็จและความล้มเหลวในการบูรณะฟันแบบที่ 5 *(ภาคผนวก 4)*

ศึกษาจากผู้ป่วยที่ได้รับการบูรณะและกลับตามตรวจเป็นประจำที่คลินิกทันตกรรมหัตการ คณะทันตแพทยศาสตร์ โดยบันทึกผู้ป่วยที่ได้รับการรักษาจากนักศึกษาทันตแพทย์และทันตแพทย์หลังปริญญาสาขาทันตกรรมหัตถการ ระหว่างวันที่ 1 พฤษภาคม 2561 ถึง วันที่ 31 ตุลาคม 2561 การศึกษานี้ได้ผ่านการรับรองจริยธรรมในการศึกษาวิจัยในมนุษย์เลขที่ COA MU-DT/PY-IRB 2018/025.3004 ผู้ป่วยได้รับการตรวจจากนักศึกษาหลังปริญญา 2 ท่าน เพื่อเปรียบเทียบ โดยปัจจัยที่บันทึกได้แก่ เพศ อายุ ตำแหน่งฟันที่บูรณะ การบดเคี้ยว ความเสี่ยงการเกิดฟันผุ ทันตแพทย์ผู้ให้การรักษา และวัสดุที่ใช้บูรณะ โดยความสำเร็จและความล้มเหลวดูจาก การคงอยู่ของวัสดุ การผุซ้ำ การติดสีที่ขอบวัสดุ และความแนบสนิทของวัสดุ โดยใช้เกณฑ์ของ modified USPHS ดังแสดงใน ตารางที่ 5 จากนั้นนำผลที่บันทึกไว้มาคำนวณทางสถิติ

ตารางที่ 5 เกณฑ์การประเมินความสำเร็จในการบูรณะฟันทางคลินิก ตามเกณฑ์ของ modified USPHS

Retention	A	Good retention
	B	Partially dislodged restoration
	C	Totally dislodged restoration
Caries	A	No caries present
	C	Caries present
Marginal discoloration	A	No discoloration
	B	Discoloration without axial penetration, can be removed by polishing
	C	Discoloration with penetration in pulpal direction, cannot be removed by polishing
Marginal integrity	A	The restoration appeared to adapt closely to the tooth
	B	Explorer penetrates, edge of the restoration does not adapt closely to the tooth structure, dentin or base are not exposed
	C	Explorer penetrates, crevice in which dentin is exposed

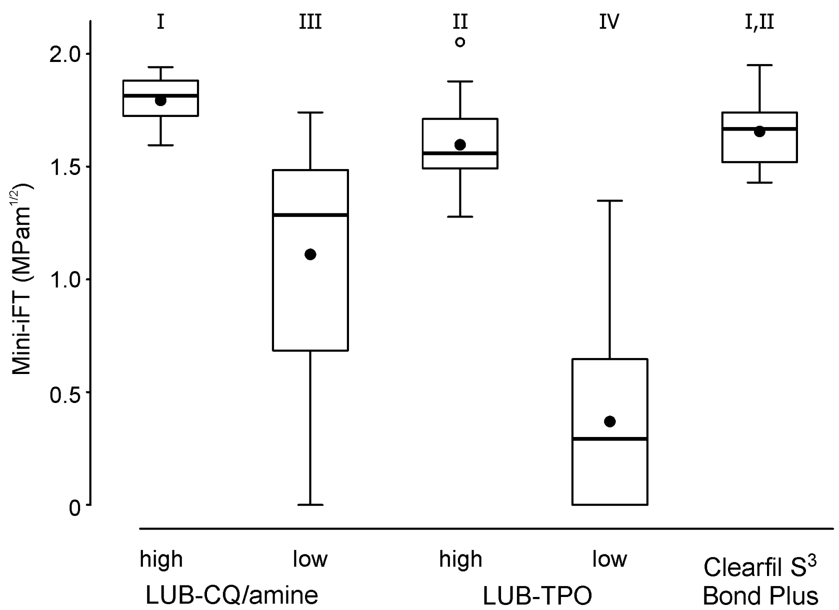
A=Alpha, B=Bravo, C=Charlie

ผลการทดลอง

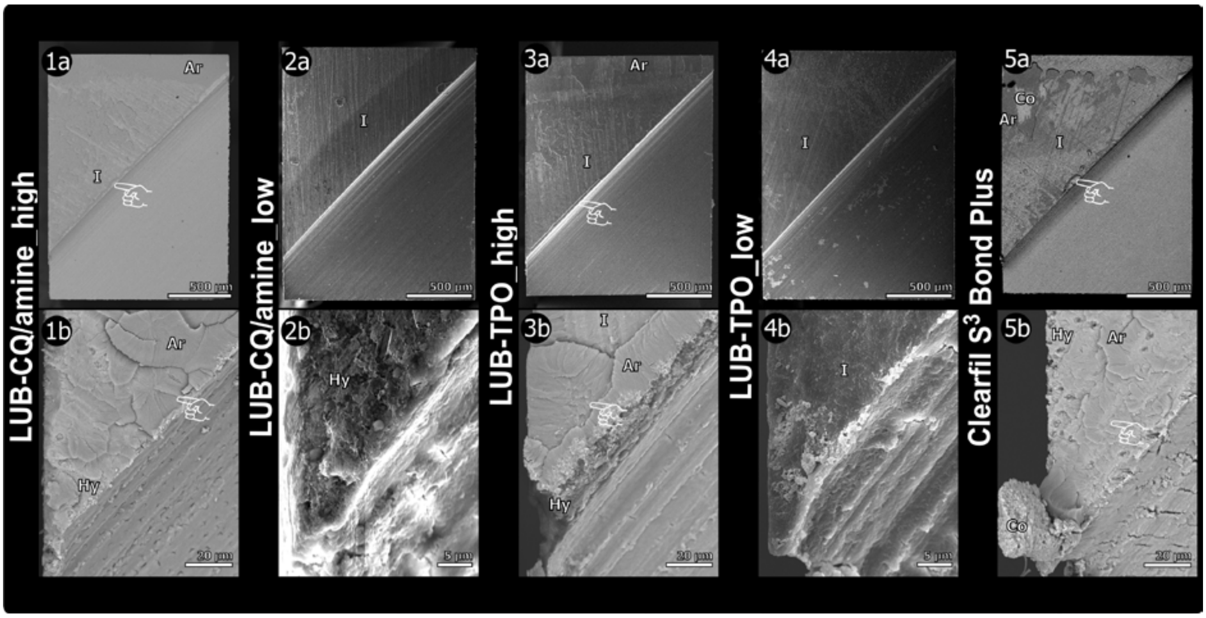
ประสิทธิภาพการยึดติดของสารยึดติดที่มีความเข้มข้นของสารก่อตัวต่างชนิดกัน *(ภาคผนวก 1)*

ประสิทธิภาพการยึดติดของสารยึดติดที่มีความเข้มข้นของสารก่อตัวมากมีประสิทธิภาพการยึดติดของ mini-iFT มากกว่าสารยึดติดที่มีความเข้มข้นของสารก่อตัวน้อย และมีค่าไม่ต่างจากสารยึดติดที่เติมสารก่อตัวจากบริษัท โดยประสิทธิภาพการยึดติด mini-iFT ของสารยึดติดทั้งสองชนิด CQ/amine และ TPO ที่มีความเข้มข้นสูง มีประสิทธิภาพการยึดติดไม่ต่างกัน ดังแสดงในภาพประกอบที่ 7 และ ลักษณะชิ้นทดสอบหลังการทดสอบ mini-iFT แสดงอยู่ในภาพประกอบที่ 8

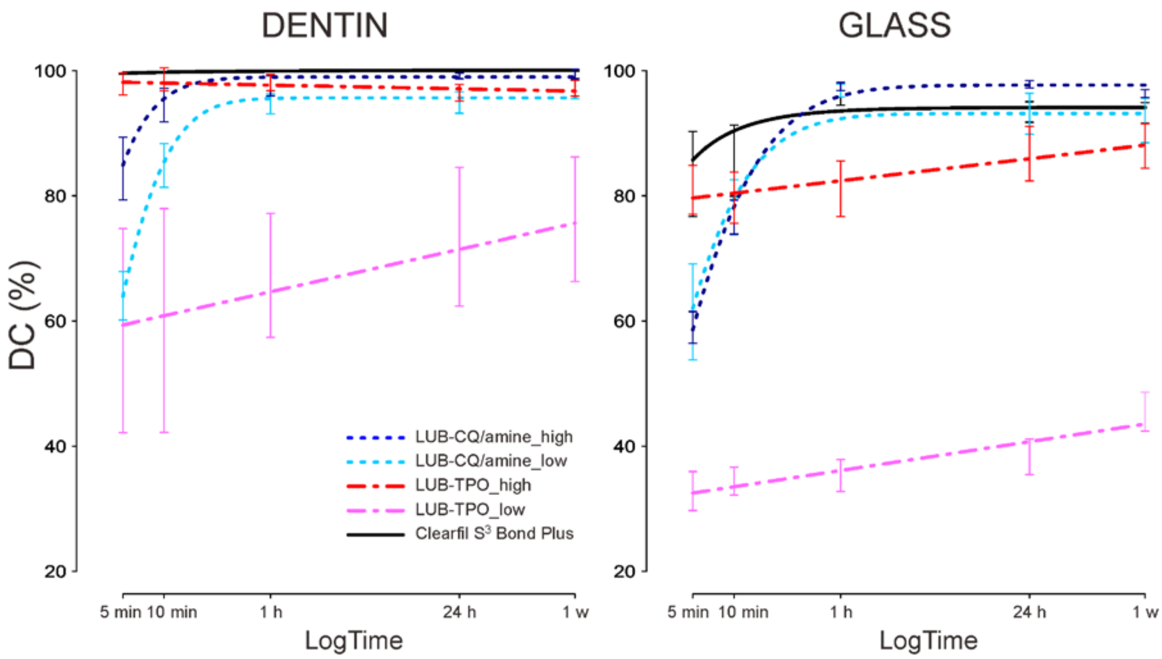
การบ่มตัวทางจลศาสตร์ (polymerization kinetic) บนพื้นผิวเนื้อฟันและบนแผ่นกระจกมีความแตกต่างกัน โดยการก่อตัวบนเนื้อฟันมีการบ่มตัวทางจลศาสตร์ สูงกว่าการก่อตัวบนแผ่นกระจก การบ่มตัวทางจลศาสตร์ของสารก่อตัวทั้งสองชนิดมีลักษณะแตกต่างกันโดย TPO มีการเพิ่มขึ้นของการก่อตัวหลังการฉายแสงน้อย ในขณะที่การก่อตัวหลังการฉายแสงของ CQ/amine มีการดำเนินต่อไปมากกว่า ดังภาพประกอบที่ 9



ภาพประกอบที่ 7: แผนภูมิ Box plots แสดงค่า mini-iFT จากสารยึดติดที่มีความเข้มข้นสารกระตุ้นการก่อตัวที่ต่างกัน; ตัวเลขโรมันที่เหมือนกันแสดงค่าที่ไม่แตกต่างกันทางสถิติ



ภาพประกอบที่ 8 ภาพจากกล้องจุลทรรศอิเลคตรอนแบบส่องกราด แสดงภาพชิ้นทดสอบหลังทดสอบประสิทธิภาพการยึดติดด้วยวิธี mini-iFT



ภาพประกอบที่ 9 กราฟแสดงการเปลี่ยนแปลงการบ่มตัว (polymerization kinetic) ของสารยึดติดที่มีสารกระตุ้นการก่อตัวต่างกันที่มีความเข้มข้นต่างกัน

ค่า mini-fracture toughness, flexural strength, และ flexural modulus แสดงในตารางที่ 6 แสดงให้เห็นประสิทธิภาพและคุณสมบัติของวัสดุไปในแนวทางเดียวกัน โดยวัสดุที่มีส่วนผสมของ ซิลิคอนไดออกไซด์ หรือ ซิงค์ออกไซด์ มีค่าสูงที่สุดโดยวัสดุทั้งสองตัวมีค่าไม่ต่างกัน และมีค่ามากกว่าวัสดุคอมโพสิตที่ผสม bioactive glass ทั้งสองชนิด โดยที่วัสดุคอมโพสิตที่ผสม bioactive glass ทั้งสองชนิดมีค่าไม่ต่างกัน

ตารางที่ 6. ค่า flexural strength, flexural modulus and fracture toughness ของคอมโพสิตที่ทดสอบ

Material	Flexural strength			Flexural modulus (GPa)	Fracture toughness		
	B^1 (m)	η^2	Characteristic strength³		B^1 (m)	η^2	Characteristic strength³
45S5	15.3	36.5	$36.5(34.8\text{-}38.5)^B$	$1.5\ (0.1)^b$	5.9	1.6	$1.6(1.4\text{-}1.8)^{II}$
S53P4	12.0	35.8	$35.8(33.7\text{-}38.3)^B$	$1.5\ (0.1)^b$	14.1	1.6	$1.6(1.5\text{-}1.7)^{II}$
ZnO	14.5	82.7	$82.7(78.5\text{-}87.3)^A$	$2.5\ (0.2)^a$	26.2	2.5	$2.5(2.4\text{-}2.6)^{I}$
SiO_2	12.4	85.6	$85.6(80.6\text{-}91.2)^A$	$2.2\ (0.3)^a$	7.0	2.8	$2.8(2.5\text{-}3.1)^{I}$
Systemp onlay	5.9	11.7	$11.7(10.3\text{-}13.3)^C$	$0.1\ (0.01)^c$	-	-	-

Different letters indicate statistical differences within a column ($p<0.05$).
[1] Beta, shape, slope or modulus of Weibull parameter.
[2] Eta, Characteristic life or scale of Weibull parameter.
[3] 95% confidence interval at Characteristic strength (=63.2% unreliability); groups with the same superscript letter are statistically not different.

ค่าการดูดน้ำ (water sorption) ค่าการละลายน้ำ (water solubility) และค่าความลึกของการบ่มตัว (depth of curing) แสดงในตารางที่ 7 ค่าการดูดน้ำ มีค่าสูงสุดในวัสดุคอมโพสิตที่ผสม 15wt% 45S5 โดยที่ค่าการดูดน้ำของ วัสดุผสมซิงค์ออกไซด์ และซิลิคอนไดออกไซด์ มีค่าไม่แตกต่างกัน ค่าการละลายน้ำ พบมีค่าเป็นลบในวัสดุคอมโพสิตที่ผสม 15wt% S53P4 ค่าความลึกของการบ่มตัว พบว่าวัสดุที่สามารถบ่มตัวได้ลึกที่สุดคือ คอมโพสิตผสมซิลิคอนไดออกไซด์ ที่ 3.9 มม. โดยวัสดุที่มีค่าบ่มตัวตื้นที่สุดได้แก่วัสดุคอมโพสิตผสมซิงค์ออกไซด์ ที่ความลึก 2.7 มม. โดยวัสดุทั้งหมดไม่มีประสิทธิภาพต้านแบคทีเรีย

ตารางที่ 7 ค่า water sorption, solubility and depth of cure ของวัสดุที่ใช้ทดสอบ

Material	Water sorption ($\mu g/mm^3$)	Water solubility ($\mu g/mm^3$)	Depth of cure (mm)
45S5	63.2 (2.5)^{A}	5.5 (1.7)^{a,b}	3.0 (0.2)^{II,III}
S53P4	56.5 (3.3)^{B}	-10.1 (0.9)^{c}	3.2 (0.3)^{II}
ZnO	20.1 (1.1)^{C}	2.1 (0.8)^{a,b,c}	2.7 (0.2)^{III}
SiO_2	20.8 (1.5)^{C}	0.5 (0.7)^{b,c}	3.9 (0.3)^{I}
Systemp onlay	20.4 (1.7)^{C}	27.9 (2.8)^{a}	4.4 (0.5)^{I}

Different letters indicate statistical differences within a column (*x*0.05).

ปัจจัยที่มีผลต่อความแข็งผิววัสดุหลังแช่ในน้ำหรือน้ำลายเทียม *(ภาคผนวก 3)*

ค่าความแข็งผิวของวัสดุที่แช่ในน้ำปราศจากไอออน และวัสดุที่แช่ในน้ำลายเทียมแสดงไว้ในตารางที่ 8, และ 9 ตามลำดับ ค่าเปรียบเทียบความแข็งผิวของวัสดุที่แช่ในน้ำปราศจากไอออน และน้ำลายเทียม ที่เวลาต่างๆ เทียบกับความแข็งผิววันแรก แสดงในภาพประกอบที่ 10 ค่าความแข็งผิวของ กลาสไอโอโนเมอร์ ที่แช่ในน้ำปราศจากไอออนมีค่าคงที่ที่ 42 วัน เมื่อเทียบกับค่าความแข็วผิวที่ 1 วัน โดยที่ค่าความแข็งผิวของ กลาสไอโอโนเมอร์ ที่แช่ในน้ำลายเทียมมีค่าเพิ่มขึ้น วัสดุชนิดอื่น มีค่าความแข็งผิวลดลงทั้งที่แช่ในน้ำปราศจากไอออนและน้ำลายเทียม โดยค่าความแข็งผิวของวัสดุที่แช่ในน้ำลายเทียมมีค่ามากกว่าวัสดุที่แช่ในน้ำปราศจากไอออน

ตารางที่ 8 แสดงค่าความแข็งผิว (Hv in kgf/mm^2±SD) ของวัสดุชนิดต่างๆ ที่แช่ไว้ในน้ำปราศจากไอออน ที่เวลา 1, 7, 21, และ 42 วัน

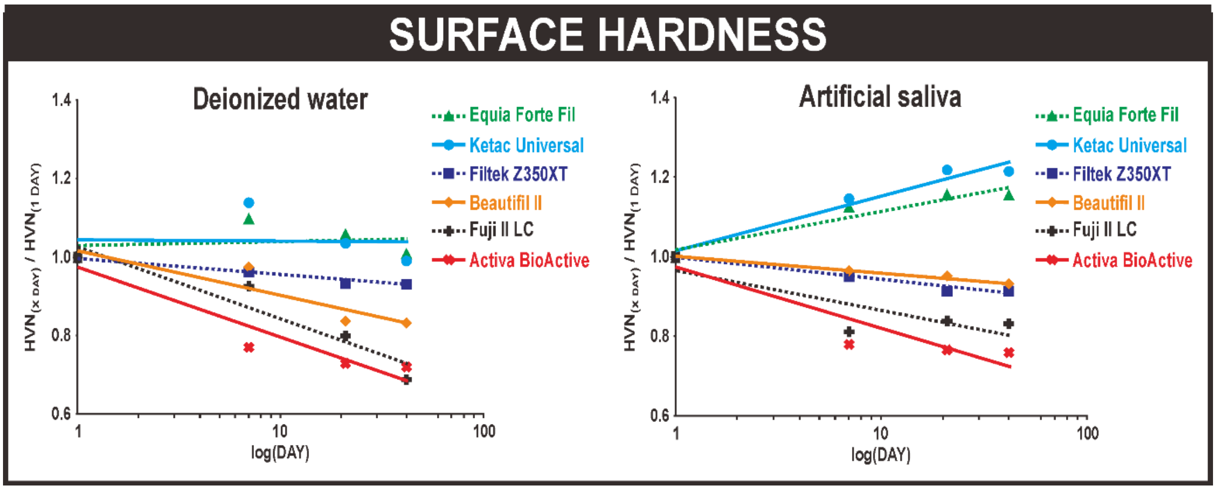
Materials	**Time**			
	1 day	**7 days**	**21 days**	**42 days**
Equia Forte Fil	79.2 ± 3.4^{Bb}	86.9 ± 6.0^{Ab}	$83.8\pm5.6A^{ABa}$	79.6 ± 2.8^{ABa}
Ketac Universal	83.8 ± 2.1^{Bb}	95.4 ± 1.8^{Aa}	86.7 ± 2.5^{Ba}	83.1 ± 3.0^{Ba}
Filtek Z350XT	90.3 ± 1.4^{Aa}	87.2 ± 1.7^{Bb}	84.5 ± 1.4^{Ca}	82.1 ± 1.5^{Ca}
Beautifil II	81.5 ± 1.5^{Ab}	79.6 ± 2.3^{Ac}	68.3 ± 10.5^{Bb}	68.0 ± 6.0^{Bb}
Fuji II LC	66.6 ± 4.1^{Ac}	61.6 ± 1.8^{Ad}	53.3 ± 5.2^{Bc}	46.0 ± 2.0^{Cc}
Activa BioActive	28.4 ± 1.9^{Ad}	22.0 ± 0.6^{Be}	20.8 ± 0.9^{Bd}	20.5 ± 0.3^{Bd}

[1]Different superscript capital or small letter indicates a statistically significant difference in the rows and columns, respectively.

ตารางที่ 9 แสดงค่าความแข็งผิว (Hv in $kgf/mm^2 \pm SD$) ของวัสดุชนิดต่างๆ ที่แช่ไว้ใน<u>น้ำลายเทียม</u> ที่เวลา 1, 7, 21, และ 42 วัน

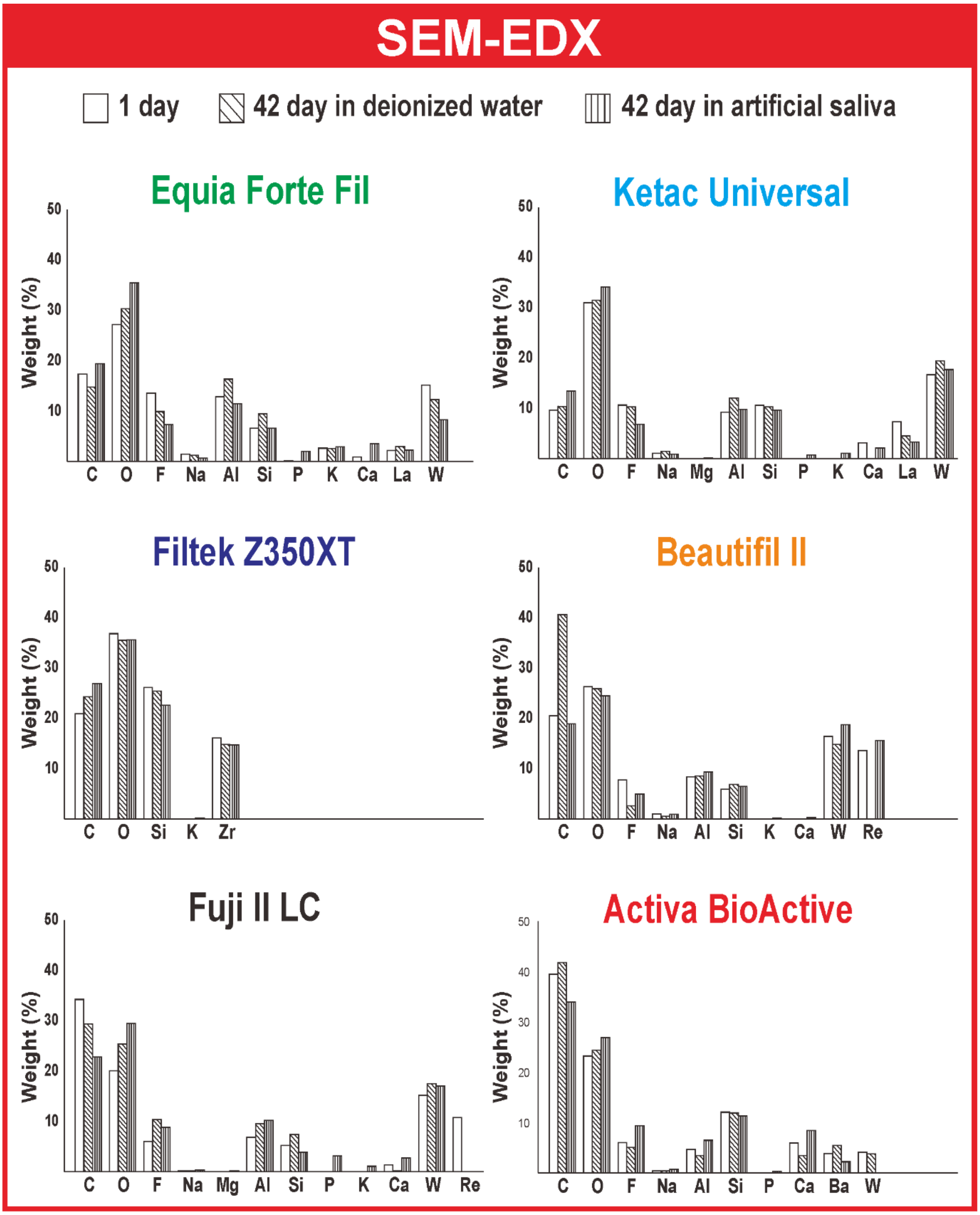
Materials	Time			
	1 day	7 days	21 days	42 days
Equia Forte Fil	77.4 ± 3.5^{Bc}	86.9 ± 1.7^{Ab}	89.5 ± 4.3^{Ab}	89.1 ± 2.4^{Ab}
Ketac Universal	85.2 ± 3.2^{Bb}	97.7 ± 6.5^{Aa}	103.6 ± 5.3^{Aa}	103.3 ± 4.2^{Aa}
Filtek Z350XT	90.2 ± 0.9^{Aa}	85.5 ± 2.0^{Bb}	82.3 ± 1.1^{Cc}	83.3 ± 1.7^{BCc}
Beautifil II	81.3 ± 1.6^{Abc}	78.0 ± 1.7^{Bc}	77.4 ± 1.8^{Bc}	75.7 ± 1.5^{Bd}
Fuji II LC	70.6 ± 4.3^{Ad}	57.3 ± 3.1^{Bd}	59.3 ± 3.9^{Bd}	58.6 ± 4.9^{Be}
Activa BioActive	28.4 ± 1.2^{Ae}	22.2 ± 0.4^{Be}	21.8 ± 1.0^{Be}	21.6 ± 1.4^{Bf}

[1]Different superscript capital or small letter indicates a statistically significant difference in the rows and columns, respectively.

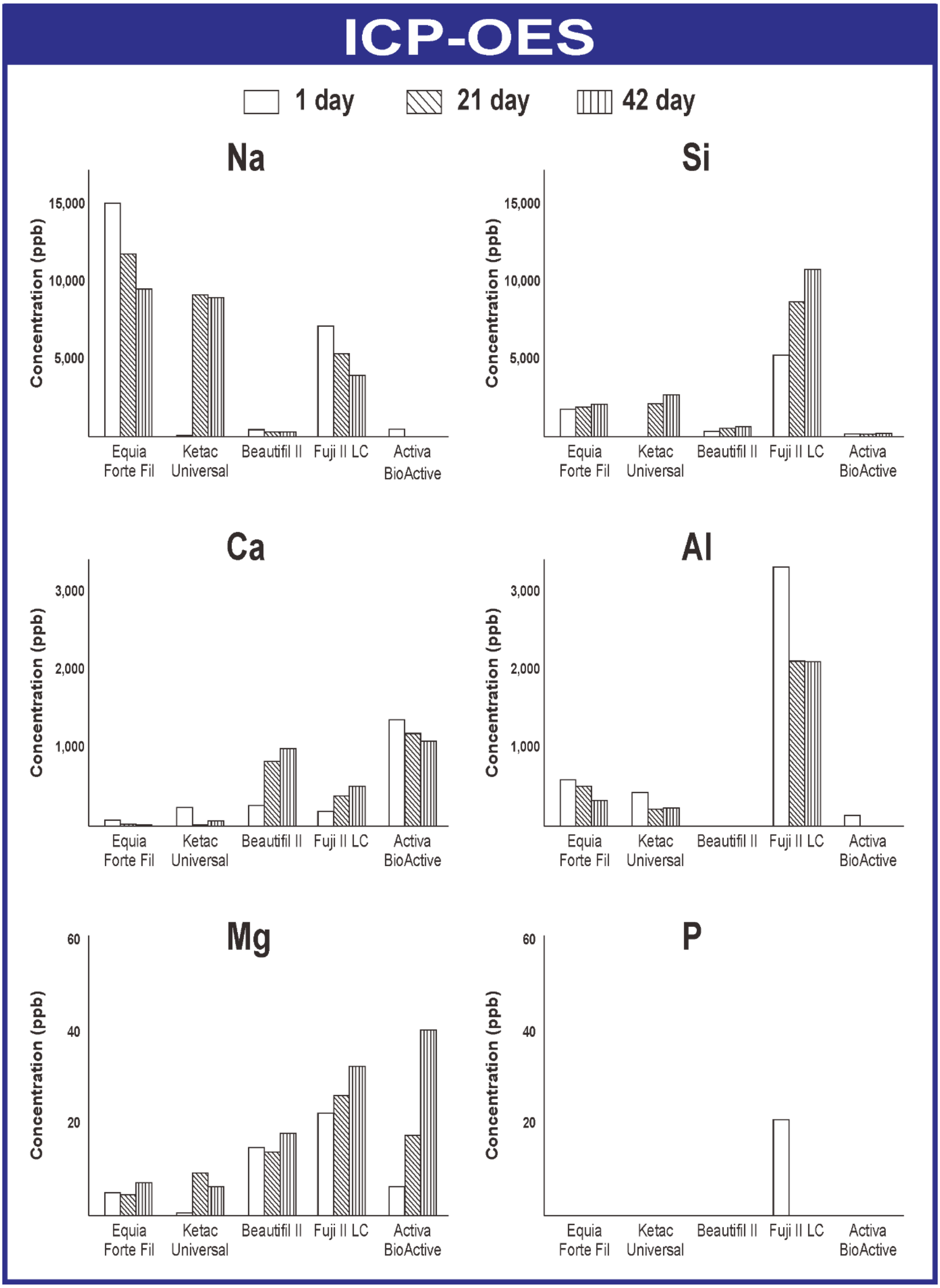


ภาพประกอบที่ 10: ค่าความแข็งผิวของวัสดุต่างๆ ที่เปลี่ยนแปลงไปเมื่อเทียบกับความแข็งผิวในวันแรกหลังแช่ไว้ในน้ำปราศจากไอออน และในน้ำลายเทียม The calculated fitting lines were plotted for each material

ผลการศึกษาปริมาณแร่ธาตุที่ผิววัสดุ เปรียบเทียบที่ 1 วัน และที่ 42 วัน ในน้ำปราศจากแร่ธาตุ และน้ำลายเทียม ดังแสดงในภาพประกอบที่ 11 เมื่อเปรียบเทียบแร่ธาตุบนผิว กลาสไอโอโนเมอร์ ที่แช่ในน้ำปราศจากไอออนและน้ำลายเทียมที่ 42 วัน พบการเพิ่มขึ้นของธาตุ ฟอสฟอรัส แคลเซียม โปแทสเซียม และแมกนีเซียม บนผิวกลาสไอโอโนเมอร์ ที่แช่ในน้ำลายเทียม โดยไม่พบแร่ธาตุดังกล่าวบนวัสดุที่แช่ในน้ำปราศจากไอออน ผลการศึกษาปริมาณไอออนในน้ำปราศจากไอออน หลังแช่วัสดุไว้ 21 และ 42 วัน แสดงในภาพประกอบที่ 12 พบไอออนของธาตุ แคลเซียม และแมกนีเซียม ในปริมาณที่น้อยในน้ำที่แช่ กลาสไอโอโนเมอร์ เมื่อเทียบกับวัสดุอื่นๆ และ ไอออนของธาตุ ซิลิคอน และ อลูมิเนียม ในปริมาณมากในน้ำที่แช่ resin-modified glass ionomer (RMGIC) เมื่อเทียบกับ กลาสไอโอโนเมอร์ ซึ่ง RMGIC มีค่าความแข็งผิวลดลง



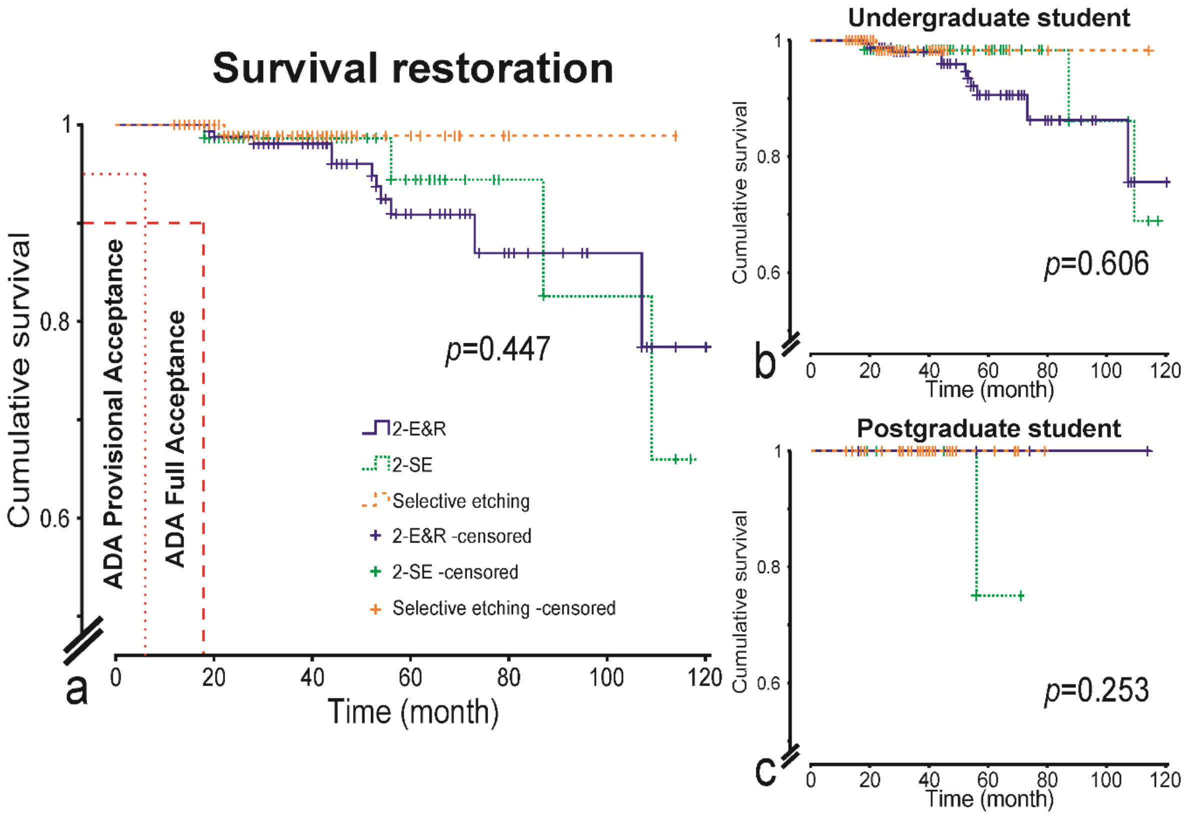
ภาพประกอบที่ 11 แผนภูมิแท่งแสดงปริมาณแร่ธาตุบนผิววัสดุ โดยใช้เครื่อง SEM-EDX ที่ 1 วัน เทียบกับที่ 42 วัน ในน้ำปราศจากแร่ธาตุและน้ำลายเทียม



ภาพประกอบที่ 12 แผนภูมิแท่งแสดงปริมาณไอออนในน้ำปราศจากแร่ธาตุ หลังจากการแช่วัสดุไว้ 1, 21 และ 42 วัน

ปัจจัยที่มีผลต่อความสำเร็จและความล้มเหลวในการบูรณะฟันแบบที่ 5 *(ภาคผนวก 4)*

ความสำเร็จทางคลินิกในการบูรณะฟันแบบที่ 5 ด้วยคอมโพสิต ไม่ขึ้นกับปัจจัย ของสารยึดติด และทันตแพทย์ที่ทำการรักษา โดยที่ความสำเร็จของการรักษาอยู่ที่ร้อยละ 95 ที่เวลา 5 ปี โดยความสำเร็จของการรักษาจะลดลงเรื่อยๆ ตามเวลา ดังแสดงในภาพประกอบที่ 13



ภาพประกอบที่ 13 กราฟแสดงความสำเร็จในการบูรณะด้วยคอมโพสิต Kaplan-Meier survival analysis เทียบกับสารยึดติดชนิดต่างๆ (a) กราฟแสดงความสำเร็จในการบูรณะโดยรวม (b) กราฟแสดงความสำเร็จในการบูรณะเมื่อเทียบสารยึดติดที่ต่างกัน ในนักศึกษาทันตแพทย์ (c) กราฟแสดงความสำเร็จในการบูรณะเมื่อเทียบสารยึดติดที่ต่างกัน ในนักศึกษาเฉพาะทางหลังปริญญา

ความล้มเหลวทางคลินิกของการบูรณะฟันแบบที่ 5 ด้วยคอมโพสิต ในความล้มเหลวรูปแบบต่างๆ แสดงในตารางที่ 10 และ 11 ปัจจัยภายนอกที่มีผลต่อความล้มเหลวทางคลินิกมากที่สุดได้แก่ ชนิดและการใช้สารยึดติด ตามด้วยทันตแพทย์ที่ให้การรักษา โดยปัจจัยเรื่องคอมโพสิตที่ใช้อุดไม่มีผลต่อความล้มเหลวของการบูรณะ นอกจากนี้ปัจจัยภายในที่มีผลมากที่สุดได้แก่ ปัจจัยของการพบรอยสึกบนด้านบดเคี้ยวของฟันที่บูรณะ ความล้มเหลวที่พบมากที่สุดคือความแนบของวัสดุบูรณะ

ตารางที่ 10 แสดงความล้มเหลวในการบูรณะในลักษณะ restoration loss, partial retention loss and caries failure ที่เกี่ยวข้องกับปัจจัยต่างๆ ผลที่ได้นำเสนอในรูปแบบร้อยละ และจำนวนภายในวงเล็บ

Factor		**Teeth**	**Restoration loss (C)**	***p*-value**	**Partial retention loss (B+C)**				**Caries**	***p*-value**
					Occlusal	***p*-value**	**Gingival**	***p*-value**		
Gender	Male	**36.1**(166)	**3.6**(6)	0.905	**9.6**(16)	0.124	**13.9**(23)	0.005*	**0.6**(1)	0.544
	female	**63.9**(294)	**3.4**(10)		**5.8**(17)		**6.1**(18)		**1.0**(3)	
Age	15-59 years	**50.0**(230)	**3.5**(8)	1.0	**7.0**(16)	0.857	**7.8**(18)	0.413	**0.9**(2)	0.688
	≥60 years	**50.0**(230)	**3.5**(8)		**7.4**(17)		**10.0**(23)		**0.9**(2)	
Arch	Upper	**53.0**(244)	**2.0**(5)	0.075	**5.3**(13)	0.103	**7.0**(17)	0.12	**0.8**(2)	0.641
	Lower	**47.0**(216)	**5.1**(11)		**9.3**(20)		**11.1**(24)		**0.9**(2)	
Position	Q1	**28.0**(129)	**2.3**(3)	0.317	**7.0**(9)	0.144	**6.2**(8)	0.156	**1.6**(2)	0.263
	Q2	**25.7**(118)	**1.7**(2)		**3.4**(4)		**8.5**(10)		**0**(0)	
	Q3	**23.5**(108)	**5.6**(6)		**7.4**(8)		**7.4**(8)		**0**(0)	
	Q4	**22.8**(105)	**4.8**(5)		**11.4**(12)		**14.3**(15)		**1.9**(2)	
Tooth type	Anterior	**28.1**(129)	**6.2**(8)	0.107	**8.5**(11)	0.354	**10.9**(14)	0.387	**1.6**(2)	0.49
	Premolar	**54.1**(249)	**2.0**(5)		**5.6**(14)		**7.2**(18)		**0.8**(2)	
	Molar	**17.8**(82)	**3.7**(3)		**9.8**(8)		**11.0**(9)		**0**(0)	
Occlusion	Denture	**12.7**(59)	**3.4**(2)	0.926	**6.8**(4)	0.716	**15.3**(9)	0.174	**1.7**(1)	0.830
	Fixed	**8**(37)	**2.7**(1)		**2.7**(1)		**13.5**(5)		**0**(0)	
	Natural	**76.1**(354)	**3.7**(13)		**7.6**(27)		**7.3**(26)		**0.8**(3)	
	Edentulous	**2.2**(10)	**0**(0)		**10.0**(1)		**10.0**(1)		**0**(0)	
Wear facets	Yes	**61.5**(283)	**4.9**(14)	0.03*	**8.8**(25)	0.081	**12.4**(35)	0.001*	**1.1**(3)	0.501
	No	**38.5**(177)	**1.1**(2)		**4.5**(8)		**3.4**(6)		**0.6**(1)	
Caries risk	High	**7.0**(32)	**6.3**(2)	0.652	**15.6**(5)	0.133	**12.5**(4)	0.117	**0**(0)	0.813
	Moderate	**75.6**(348)	**3.2**(11)		**6.9**(24)		**9.5**(33)		**0.9**(3)	
	Low	**17.4**(80)	**3.8**(3)		**5.0**(4)		**5.0**(4)		**1.3**(1)	
Operator	Undergraduate	**81.1**(378)	**4.0**(15)	0.16	**8.3**(31)	0.05*	**9.9**(37)	0.081	**0.8**(3)	0.569
	Postgraduate	**18.9**(87)	**1.1**(1)		**2.3**(2)		**4.6**(4)		**1.1**(1)	
Adhesive	2 step E&R	**40.2**(185)	**5.9**(11)	0.017*	**11.4**(21)	0.003*	**12.4**(23)	0.087	**1.1**(2)	0.6
	2 step SE	**20.2**(93)	**4.3**(4)		**8.6**(8)		**7.5**(7)		**0**(0)	
	Selective etching	**39.6**(182)	**0.5**(1)		**2.2**(4)		**6.0**(11)		**1.1**(2)	
Composite	Filtek Z350	**70.4**(324)	**2.8**(9)	0.22	**6.2**(20)	0.378	**9.0**(29)	0.419	**0.3**(1)	0.081
	Estelite Sigma	**26.1**(120)	**5.8**(7)		**10.0**(12)		**10.0**(12)		**2.5**(3)	
	Filtek Z250	**3.5**(16)	**0**(0)		**6.3**(1)		**0**(0)		**0**(0)	
Overall	Total	**100**(460)	**3.5**(16)		**7.2**(33)		**8.9**(41)		**0.9**(4)	

*Association between the factor of the restoration and the parameter failures; retention and caries (*p*-value<0.05, Pearson chi-square or Fisher's exact test)

ตารางที่ 11 แสดงความล้มเหลวในการบูรณะในลักษณะ marginal discoloration and marginal integrity ที่เกี่ยวข้องกับปัจจัยต่างๆ ผลที่ได้นำเสนอในรูปแบบร้อยละ และจำนวนภายในวงเล็บ

Factor		**Marginal discoloration (B+C)**				**Marginal Integrity (B+C)**			
		Occlusal	***p*-value**	**Gingival**	***p*-value**	**Occlusal**	***p*-value**	**Gingival**	***p*-value**
Gender	Male	**15.1**(25)	0.721	**15.1**(25)	0.01*	**47.6**(79)	0.09	**59.6**(99)	0.004*
	Female	**16.3**(48)		**7.5**(22)		**39.5**(116)		**45.6**(134)	
Age	15-59 years	**16.1**(37)	0.898	**9.6**(22)	0.644	**38.7**(89)	0.109	**48.3**(11)	0.305
	≥60 years	**15.7**(36)		**10.9**(25)		**46.1**(106)		**53.0**(122)	
Arch	Upper	**14.3**(35)	0.341	**7.8**(19)	0.067	**43.9**(107)	0.5	**49.6**(121)	0.628
	Lower	**17.6**(38)		**13.0**(28)		**40.7**(88)		**51.9**(112)	
Position	Q1	**15.5**(20)	0.819	**8.5**(11)	0.429	**42.6**(55)	0.817	**53.5**(69)	0.426
	Q2	**13.6**(16)		**7.6**(9)		**45.8**(54)		**44.1**(52)	
	Q3	**16.7**(18)		**12.0**(13)		**39.8**(43)		**52.8**(57)	
	Q4	**18.1**(19)		**13.3**(14)		**41.0**(43)		**52.4**(55)	
Tooth type	Anterior	**16.3**(21)	0.917	**13.2**(17)	0.15	**34.9**(45)	0.091	**55.0**(71)	0.433
	Premolar	**15.3**(38)		**10.4**(26)		**46.6**(116)		**49.8**(124)	
	Molar	**17.1**(14)		**4.9**(4)		**41.5**(34)		**46.3**(38)	
Occlusion	Denture	**22.0**(13)	0.537	**15.3**(9)	0.172	**35.6**(21)	0.46	**71.2**(42)	0.002*
	Fixed	**13.5**(5)		**2.7**(1)		**45.9**(17)		**48.6**(18)	
	Natural	**15.3**(54)		**9.9**(35)		**42.7**(151)		**48.3**(171)	
	Edentulous	**10.0**(1)		**20.0**(2)		**60.0**(6)		**20.0**(2)	
Wear facets	Yes	**18.0**(51)	0.11	**13.8**(39)	0.001*	**42.8**(121)	0.841	**59.4**(168)	<0.001*
	No	**12.4**(22)		**4.5**(8)		**41.8**(74)		**36.7**(65)	
Caries risk	High	**25.0**(8)	0.074	**25.0**(8)	0.007*	**59.4**(19)	0.114	**65.6**(21)	<0.001*
	Moderate	**16.7**(58)		**10.5**(35)		**40.5**(141)		**53.7**(187)	
	Low	**8.8**(7)		**5.0**(4)		**42.4**(35)		**31.3**(25)	
Operator	Undergraduate	**16.9**(63)	0.215	**11.8**(44)	0.021*	**45.6**(170)	0.004*	**54.4**(203)	0.001*
	Postgraduate	**11.5**(10)		**3.4**(3)		**28.7**(25)		**34.5**(30)	
Adhesive	2-step E&R	**16.2**(30)	<0.001*	**13.5**(25)	0.01*	**46.5**(86)	<0.001*	**57.8**(107)	0.016*
	2-step SE	**28.0**(26)		**14.0**(13)		**58.1**(54)		**51.6**(48)	
	Selective etching	**9.3**(17)		**4.9**(9)		**30.2**(55)		**42.9**(78)	
Composite	Filtek Z350	**13.3**(43)	0.059	**9.0**(29)	0.381	**43.2**(140)	0.826	**49.1**(159)	0.384
	Estelite Sigma Quick	**21.7**(26)		**13.3**(16)		**40.0**(48)		**55.8**(67)	
	Filtek Z250	**25.0**(4)		**12.5**(2)		**43.8**(7)		**43.8**(7)	
Overall	Total	**15.9**(73)		**10.2**(47)		**42.4**(195)		**50.7**(233)	

*Association between the factor of the restoration and the parameter failures; marginal discoloration, and marginal integrity (*p*-value<0.05, Pearson chi-square or Fisher's exact test)

บทวิจารณ์

ประสิทธิภาพการยึดติดของสารยึดติดที่มีความเข้มข้นของสารก่อตัวต่างชนิดกัน *(ภาคผนวก 1)*

การศึกษานี้เป็นการศึกษาผลของสารก่อตัวสองชนิดที่ต่างกันที่ความความเข้มข้นต่างกัน ในสารยึดติดที่มีส่วนประกอบเดียวกันกับ Clearfil S^3 Bond Plus (Kuraray Noritake) โดยศึกษาประสิทธิภาพการยึดติดด้วยวิธี mini-iFT และศึกษาจลศาสตร์การบ่มตัว (polymerization kinetic) เมื่อทาสารยคดติดลงบนเนื้อฟันหรือบนแผ่นกระจก จากการศึกษาพบการเปลี่ยนแปลงการบ่มตัว ขึ้นกับปัจจัยของสารก่อตัว และความเข้มข้นของสารก่อตัว ซึ่งส่งผลถึงประสิทธิภาพการยึดติดของสารยึดติด และพบความสัมพันธ์ระหว่างการบ่มตัวและประสิทธิภาพการยึดติดด้วย

การศึกษาจลศาสตร์การบ่มตัว พบการเปลี่ยนแปลงค่าการบ่มตัวเพิ่มขึ้นหลังจากการบ่มตัวระยะแรกจนถึง 1 สัปดาห์ ซึ่งสัมพันธ์กับการศึกษาก่อนหน้านี้ (Guo et al. 2009; Zhang and Wang 2013; Luhrs et al. 2014; Inokoshi et al. 2016) การศึกษานี้พบว่าลักษณะจลศาสตร์การบ่มตัวที่เกิดจากสารก่อตัวต่างชนิดกันมีลักษณะต่างกัน กล่าวคือ TPO มีการบ่มตัวที่เร็วกว่าและให้ค่าที่สูงในระยะแรก ซึ่งต่างจาก CQ/amine ที่มีการบ่มตัวไม่มากในระยะแรก แต่มีการเพิ่มขึ้นของการบ่มตัวหลังจากมีการกระตุ้น และอาจเพิ่มขึ้นสูงกว่าการบ่มตัวด้วย TPO ในบางความเข้มข้น ดังแสดงในภาพประกอบที่ 9 ซึ่งผลการศึกษานี้สัมพันธ์กับการศึกษาก่อนหน้าที่พบว่า TPO ไม่มีการบ่มตัวภายหลังการกระตุ้นด้วยแสง ในขณะที่ CQ/amine มีการบ่มตัวต่อเนื่องหลังการกระตุ้นด้วยแสงไปแล้ว (Liu et al. 2016)

การศึกษานี้ พบว่าประสิทธิภาพการยึดติดของสารยึดติดมีค่าสูงเมื่อสารยึดติดมีความเข้มข้นของสารก่อตัวสูง ดังแสดงในภาพประกอบที่ 7 ซึ่งสัมพันธ์กับการศึกษาก่อนหน้านี้ที่พบว่า ค่าการยึดติดไมโครเทนไซด์เพิ่มขึ้นเมื่อความเข้มข้นสารก่อตัวสูงขึ้น (Van Landuyt et al. 2009) นอกจากนี้ ยังพบว่าการเพิ่มความเข้มข้นของสารก่อตัวทำให้ค่าเบี่ยงเบนมาตรฐานลดลงด้วย

ผลการเติม bioactive glass และ ซิงค์ออกไซด์ ต่อประสิทธิภาพคอมโพสิต *(ภาคผนวก 2)*

ค่า flexural strength, flexural modulus, and fracture toughness มีผลไปในทางเดียวกันคือคอมโพสิต SiO_2 และ ซิงค์ออกไซด์ มีค่าไม่ต่างกันและมีค่าสูงกว่า คอมโพสิตที่ผสม 45S5 and S53P4 ดังผลในตารางที่ 6 ซึ่งผลดังกล่าวสัมพันธ์กับการศึกษาก่อนหน้าที่ การเติมซิงค์ออกไซด์ในปริมาณร้อยละ 1-5 ไม่มีผลต่อค่า flexural strength และ flexural modulus ของคอมโพสิต (Hojati et al. 2013)นอกจากนี้ การเติม bioactive glasses (45S5 and S53P4) มีผลต่อการลดลงของ flexural strength และ flexural modulus นั้นสัมพันธ์กับการศึกษาก่อนหน้า (Yang et al. 2013; Par et al. 2019b) โดยปริมาณ bioactive glass ที่เพิ่มขึ้นที่ ร้อยละ 5-40 โดยน้ำหนัก ส่งผลโดยตรงต่อการลดลงของคุณสมบัติทางกล (mechanical property) ของวัสดุ (Par et al. 2019b) ทั้งนี้ มีการศึกษาพบว่า หากเพิ่มปริมาณ bioactive glass ที่ร้อยละ 10 (Par et al. 2019b) หรือร้อยละ 15 (Khvostenko et al. 2013a) จะไม่ส่งผลกับคุณสมบัติทางกลของคอมโพสิต.

ค่าการดูดน้ำและการละลายน้ำ ขึ้นกับปริมาณน้ำที่วัสดุซึมซับเข้ามาพร้อมกับการละลายตัวซึ่งทั้งสองอย่างจะเกิดขึ้นพร้อมกัน (Ferracane 2006) ขึ้นกับองค์ประกอบของวัสดุ การบ่มตัว และตัวทำละลายที่ใช้ศึกษา (Mese et al. 2008; Van Landuyt et al. 2011) การดูดน้ำของคอมโพสิตที่มีส่วนผสมของ bioactive glass มากกว่าคอมโพสิตกลุ่มอื่น ดังแสดงในตารางที่ 7 สัมพันธ์กับการศึกษาก่อนหน้านี้ (Par et al. 2019a) ซึ่งอธิบายสาเหตุไว้ว่า bioactive glass มีความชอบน้ำ (hydrophilic) ทำให้ซับน้ำขึ้นมามากกว่าคอมโพสิตที่ประกอบไปด้วย ซิลิกอนไดออกไซด์ อย่างเดียว

การละลายน้ำสัมพันธ์กับการบ่มตัว (polymerization) ของวัสดุ ซึ่งวัสดุที่มีการบ่มตัวต่ำ มักมีการหลุดหรือปลดปล่อยส่วนประกอบออกมามากกว่า (Pongprueksa et al. 2014) ซึ่งจะสัมพันธ์กับการละลายตัวของวัสดุ การศึกษานี้พบค่าการละลายตัวเป็นลบของ คอมโพสิตที่มี S53P4 โดยค่าที่เป็นลบหมายถึงการที่วัสดุซับน้ำเอาไว้และไม่ปล่อยน้ำออกมาขณะทำให้วัสดุแห้ง ซึ่งลักษณะดังกล่าวไม่ได้หมายความว่าวัสดุไม่มีการละลาย แต่หมายถึงวัสดุซับน้ำเอาไว้มากกว่าการละลายตัวออกไป โดยค่าการละลายน้ำเป็นลบสามารถอธิบายได้โดย อาจเกิดจาก bioactive glass ทำปฏิกิริยาเคมีกับน้ำทำให้เกิดผิวที่เรียกว่า gel formation โดยมากลักษณะดังกล่าวมักเกิดกับ กลาสไอโอโนเมอร์ (Gladys et al. 1997; Toledano et al. 2006) หรืออาจเกิดกับ bioactive glass (Renno et al. 2013) ได้ โดยปกติ bioactive glass สามารถเกิดปฏิกิริยาเคมีกับ simulated body fluid solution (SBF) สร้างชั้นที่เรียกว่า hydroxylcarbonate apatite formation (Hench 1998) สาเหตุอื่นอาจเกิดจากการที่ เรซินที่ใช้มีความไม่ชอบน้ำสูงเมื่อน้ำแทรกซึมเข้าไปในร่างแหของเรซินแล้ว น้ำอาจจะไม่สามารถระเหยกลับออกมาได้ (Wei et al. 2011)

การศึกษาก่อนหน้านี้พบว่า ซิงค์ออกไซด์ มีประสิทธิภาพต้านแบคทีเรียได้ ในความเข้มข้นต่ำ (Sergi et al. 2019; Wanitwisutchai et al. 2019) อย่างไรก็ตามปริมาณความเข้มข้นร้อยละ 1 ของซิงค์ออกไซด์ในการศึกษานี้ไม่สามารถต้านแบคทีเรียได้ ซึ่งต่างกับการศึกษาก่อนหน้านี้ (Hojati et al. 2013) อาจเกิดจากการบ่มตัวของคอมโพสิตที่สูงในการศึกษานี้ ทำให้ ซิงค์ออกไซด์ ไม่สามารถมีประสิทธิภาพพียงพอต่อการต้านแบคทีเรีย

การศึกษานี้พบว่าการตัวเติมทั้ง bioactive glass และ ซิงค์ออกไซด์ ไม่สามารถแสดงประสิทธิภาพได้อย่างที่ตั้งใจ อาจเนื่องมาจากปริมาณความเข้มข้นของสารตัวเติมที่น้อยเกินไป หรือการบ่มตัวของเรซินที่สูงทำให้สารตัวเติมไม่สามารถแสดงประสิทธิภาพอย่างเพียงพอ การศึกษาต่อไปอาจเพิ่มความเข้มข้นของสารตัวเติม หรือลดปริมาณการบ่มตัวของคอมโพสิต นอกจากนี้ อาจทำการศึกษาสารที่มีฤทธิ์ต้านแบคทีเรียและส่งเสริมการคืนกลับแร่ธาตุของฟัน ชนิดอื่นๆ ต่อไป

ปัจจัยที่มีผลต่อความแข็งผิววัสดุหลังแช่ในน้ำหรือน้ำลายเทียม *(ภาคผนวก 3)*

การศึกษานี้ปัจจัยทั้งสามอย่างได้แก่ ตัววัสดุบูรณะ สารที่ใช้แช่วัสดุ และเวลาในการแช่วัสดุ มีผลต่อค่าความแข็งผิวของวัสดุบูรณะที่นำมาศึกษา โดยพบว่า กลาสไอโอโนเมอร์ มีค่าความแข็งผิวของคงที่เมื่อแช่ในน้ำปราศจากไอออน และมีค่าความแข็งผิวเพิ่มขึ้นเมื่อแช่ในน้ำลายเทียมที่ 42 วัน ซึ่งได้ผลต่างจากวัสดุชนิดอื่นที่ค่าความแข็งผิวลดลงหลังแช่ในน้ำปราศจากไอออนและน้ำลายเทียม ดังแสดงในตารางที่ 8 และ 9

ค่าความแข็งผิวของกลาสไอโอโนเมอร์ ที่คงที่หลังจากแช่ไว้ในน้ำปราศจากไอออนนั้น สัมพันธ์กับการศึกษาก่อนหน้า ที่พบค่าความแข็งผิวคงที่เมื่อแช่ในน้ำเปล่าที่เวลา 1 ปี (Shiozawa et al. 2014) และการศึกษาอื่นๆ ที่พบค่าความแข็งผิวเพิ่มขึ้นทั้งการแช่ในน้ำเปล่าและน้ำลายเทียม (Okada et al. 2001; Aliping-McKenzie et al. 2003; Shiozawa et al. 2014; Ilie and Stawarczyk 2016) ค่าความแข็งผิวที่เพิ่มขึ้นหลังจากวัสดุแข็งตัวไปแล้วของกลาสไอโอโนเมอร์ เกิดจากการปรับตัวภายหลังการผสมวัสดุ (maturation) ของกลาสไอโอโนเมอร์ (Ilie 2018) ภายหลังการเกิดปฏิกิริยาการบ่มตัวแบบกรดเบส (acid-base reaction) ระหว่าง กรดอคลิลิกและผลึกแก้วในกลาสไอโอโนเมอร์ ยังมีการดำเนินต่อไปภายหลังการบ่มตัวที่ 24 ชั่วโมง ความแข็งแรงที่เพิ่มขึ้นเกิดจากการแลกเปลี่ยนไอออน เช่น อลูมิเนียม และฟลูออไรด์บริเวณ hydrogel matrix นอกจากนี้การแทรกซึมของไอออนจากสิ่งแวดล้อมมีส่วนช่วยให้เกิดโครงสร้างที่แข็งแรงขึ้นได้ (Crisp et al. 1974; Crisp and Wilson 1974; Nicholson 1998; Sidhu and Nicholson 2016) ซึ่งเป็นเหตุผลในการอธิบายเรื่องค่าความแข็งผิวของกลาสไอโอโนเมอร์ ที่เพิ่มขึ้นเมื่อแช่วัสดุในน้ำลายเทียมเทียบกับการแช่วัสดุในน้ำปราศจากไอออน ที่ 42 วัน

ผลการศึกษาการกระจายแร่ธาตุบริเวณผิวของกลาสไอโอโนเมอร์เทียบวัสดุที่แช่ในน้ำปราศจากไอออนและน้ำลายเทียม ดังภาพประกอบที่ 11 พบการเพิ่มขึ้นของแร่ธาตุ โปแทสเซียม แคลเซียม แมกนีเซียม และฟอสฟอรัส ในพื้นผิว กลาสไอโอโนเมอร์ ที่แช่ในน้ำลายเทียม ซึ่งมีความเป็นไปได้ว่ากรดอคลิลิกที่หลงเหลืออยู่บนผิววัสดุ (Crisp et al. 1974) ทำปฏิกิริยาเคมีกับไอออนของ โปแทสเซียม แคลเซียม และแมกนีเซียม ในน้ำลายเทียม (Hopkins 1955) ทำให้ค่าความแข็งผิวกลาสไอโอโนเมอร์สูงขึ้น

นอกจากนี้มีการศึกษาพบว่า สารละลาย แคลเซียมครอไรด์ ในน้ำลายเทียมทำให้ค่าความแข็งผิวของกลาส ไอโอโนเมอร์เพิ่มขึ้น (Shiozawa et al. 2014) เนื่องจาก แคลเซียมไอออน ทำปฏิกิริยากับ กรดคาร์บอกซิลิก ในเมทริกซ์ (matrix) เกิดเป็น แคลเซียมโพลีอคลิลิกเมทริกซ์ ขึ้น

การศึกษานี้พบว่า โปแทสเซียม แคลเซียม และแมกนีเซียม ในน้ำลายเทียมมีส่วนสำคัญต่อค่าความแข็งผิวที่เพิ่มขึ้น นอกจากนี้การละลายตัวที่ต่ำของ แคลเซียม แมกนีเซียม และฟอสฟอรัส ในน้ำปราศจากไอออน ของกลาสไอโอโนเมอร์ ช่วยคงความแข็งผิวของ กลาสไอโอโนเมอร์ ได้

ปัจจัยที่มีผลต่อความสำเร็จและความล้มเหลวในการบูรณะฟันแบบที่ 5 *(ภาคผนวก 4)*

การศึกษานี้ แสดงให้เห็นถึงปัจจัยที่มีผลต่อความสำเร็จและความล้มเหลวของการบูรณะโพรงฟันแบบที่ 5 ด้วยคอมโพสิต เปรียบเทียบการรักษาระหว่างนักศึกษาทันตแพทย์ และนักศึกษาหลังปริญญาหลักสูตรทันตกรรมหัตถการ ซึ่งพบว่าความสำเร็จของการบูรณะ ไม่ได้ขึ้นกับทันตแพทย์และชนิดของสารยึดติด ซึ่งต่างจากความล้มเหลวของการบูรณะ ขึ้นกับปัจจัยหลายปัจจัย โดยเฉพาะ ทันตแพทย์ การใช้สารยึดติด และการพบการสึกบริเวณด้านบดเคี้ยวของฟันที่ได้รับการบูรณะ (present of wear facets)

การศึกษานี้พบว่าความสำเร็จของการบูรณะไม่ขึ้นกับทันตแพทย์ ซึ่งต่างจากการศึกษาก่อนหน้านี้ที่พบว่า ประสบการณ์ของทันตแพทย์มีส่วนสำคัญต่อความสำเร็จในการบูรณะ (Giachetti et al. 2007; Scotti et al. 2016) อย่างไรก็ตามการศึกษาส่วนมากศึกษาจากทันตแพทย์จำนวนไม่มาก (Van Meerbeek et al. 2005; Giachetti et al. 2007; Peumans et al. 2015; Scotti et al. 2016) ซึ่งอาจจะไม่ได้เป็นตัวแทนของทันตแพทย์ในแต่ละกลุ่ม

ปัจจัยเรื่องทันตแพทย์ผู้ให้การรักษา ทั้งนักศึกษาทันตแพทย์ และนักศึกษาหลังปริญญา ซึ่งเป็นตัวแทนของผู้รักษาที่มีประสบการณ์การให้การรักษาที่ต่างกัน ปัจจัยนี้มีผลต่อความล้มเหลวของการบูรณะโพรงฟันแบบที่ 5 ด้วยคอมโพสิต เรื่องการหลุดของวัสดุบูรณะบางส่วน การติดสีตามขอบวัสดุ และความแนบสนิทของวัสดุบูรณะตามขอบ จากข้อมูลที่ได้รวบรวมมาพบว่าการเลือกใช้สารยึดติดของทันตแพทย์มีความแตกต่างกัน โดย นักศึกษาทันตแพทย์ เลือกใช้สารยึดติดชนิด 2-step etch&rinse adhesive (2-E&R) ร้อยละ 46.9 เลือกใช้สารยึดติด selective etching ร้อยละ 30.8 และเลือกใช้การยึดติดชนิด 2-step self etching adhesive (2-SE) ร้อยละ 22.3 ในขณะที่ นักศึกษาหลังปริญญาเลือกใช้สารยึดติดชนิด selective etching มากที่สุดที่ ร้อยละ 77.0 และเลือกใช้สารยึดติดชนิด 2-E&R และ 2-SE เท่ากันที่ร้อยละ 11.5 จากข้อมูลที่ได้ พบว่า นักศึกษาทันตแพทย์ เลือกใช้สารยึดติดชนิด 2-E&R มากที่สุด ซึ่งจากการศึกษาก่อนหน้าพบว่า การใช้สารยึดติดแบบ 2-E&R มีโอกาสเกิดการปนเปื้อนน้ำและน้ำลายบริเวณขอบโพรงฟันด้านเหงือกมากกว่าการใช้สารยึดติดแบบ selective etching (Nair et al. 2017) นอกจากนี้นักศึกษาหลังปริญญา มี

ประสบการณ์การป้องกันการปนเปื้อนของน้ำลายตามขอบเหงือกได้ดีกว่า น่าจะทำให้พบการล้มเหลวในการบูรณะตามขอบน้อยกว่า ซึ่งตรงกับการศึกษาก่อนหน้า ที่พบความสำเร็จในการบูรณะของทันตแพทย์ที่มีประสบการณ์มากกว่า (Giachetti et al. 2007; Scotti et al. 2016)

สารยึดติดเป็นปัจจัยสำคัญที่สุดที่ทำให้เกิดความล้มเหลวในการบูรณะในการศึกษานี้ โดยพบว่า ไม่มีความแตกต่างกันของความล้มเหลวของการบูรณะจากการใช้สารยึดติดชนิด 2-SE และ 2-E&R ซึ่งต่างจากการศึกษาก่อนหน้านี้ที่พบว่าการใช้สารยึดติด 2-SE เกิดความล้มเหลวน้อยกว่าการใช้ 2-E&R (van Dijken 2010; Peumans et al. 2014) ผลดังกล่าวน่าจะเกิดจากสารยึดติดที่ใช้ในแต่ละการวิจัยมีความแตกต่างกัน โดยพบว่า สารยึดติดชนิด 2-E&R ที่บันทึกในการศึกษานี้ ให้ผลที่ดีเมื่อเทียบกันสารยึดติดอื่นๆ (Peumans et al. 2014) การศึกษานี้พบว่า สารยึดติดชนิด selective enamel etching เกิดความล้มเหลวของการบูรณะน้อยที่สุด เมื่อเทียบกับอีกสองชนิดในการศึกษานี้ เมื่อเทียบสารยึดติดระหว่าง selective enamel etching และ 2-E&R พบว่า selective enamel etching มีความล้มเหลวน้อยกว่า ซึ่งน่าจะมาจากผลของการใช้กรดฟอสฟอริก ในสารยึดติด 2-E&R บริเวณเนื้อฟัน ที่มีความรุนแรงกว่าการใช้ 10-MDP ใน Clearfil SE bond (Frankenberger et al. 2008) ซึ่ง 10-MDP นั้นมีความรุนแรงน้อยกว่าและยังสามารถเกิดปฏิกิริยาการยึดติดทางเคมีกับเนื้อฟันได้ ซึ่งมีส่วนช่วยให้เกิดการยึดติดที่ทนทานมากกว่า (Van Meerbeek et al. 2020) ผลจากการศึกษาพบว่า การใช้ selective enamel etching พบความล้มเหลวบริเวณขอบโพรงฟันด้านบดเคี้ยวน้อยกว่าการใช้สารยึดติดชนิด 2-SE ซึ่งสัมพันธ์กับการใช้กรดฟอสฟอริกเฉพาะที่บริเวณเคลือบฟันที่โพรงฟันด้านบดเคี้ยว ทำให้ได้การยึดติดและความแนบสนิทบริเวณด้านบดเคี้ยวที่ดีกว่า โดยพบว่าการศึกษาก่อนหน้าที่ระยะเวลาศึกษาไม่นานจะพบกว่าการใช้สารยึดติดทั้งสองชนิดไม่มีความแตกต่างกัน (Van Meerbeek et al. 2005; Peumans et al. 2007) ในขณะที่การศึกษาในระยะยาวพบว่า selective enamel etching ให้ผลที่ดีกว่า 2-SE (Peumans et al. 2015; Szesz et al. 2016)

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารนานาชาติ

1.1 **Pongprueksa Pong**, De Munck Jan, Inokoshi Masanao, Van Meerbeek Bart. Polymerization efficiency affects interfacial fracture toughness of adhesives. Dental Materials. 2018 34(4):684-692. **(Published)** (ภาคผนวก 1)

1.2 Saengnil Wanchanok, Anuntasainont Munlika, Srimaneekarn Natchalee, Miletic Vesna and **Pongprueksa Pong**. A clinical study on factors influencing success and failure of NCCLs restorations. **(Submitted for publication in an international journal)** (ภาคผนวก 2)

1.3 Intra Watcharapon, Lapirattanakul Jinthana, Traiphol Nisanart, Monmaturapoj Naruporn, **Pongprueksa Pong**. Influence of bioactive glasses and zinc oxide in light-curing composite. **(Submitted for publication in an international journal)** (ภาคผนวก 3)

1.4 Tararatsatid Natta, Vongphan Nataya, Intranont Noramon, Rojanathanes Rojrit, **Pongprueksa Pong**. Ion-releasing influences to surface microhardness of bioactive filling materials. **(on the final proof for submitting in an international journal)** (ภาคผนวก 4)

2. การนำผลงานวิจัยไปใช้ประโยชน์

2.1 เชิงสาธารณะ

โครงการวิจัยนี้ ทำให้เกิดการสร้างเครือข่ายความร่วมมือทางด้านวัสดุศาสตร์ วัสดุ bioactive glass และอื่นๆ เพื่อพัฒนานำวัสดุต่างๆ มาใช้ใน ทันตวัสดุศาสตร์ จากหลายหน่วยงาน ได้แก่ นักวิจัยจากศูนย์เทคโนโลยีโลหะและวัสดุแห่งชาติ อาจารย์ภาควิชาวัสดุศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล อาจารย์ภาควิชาวัสดุศาสตร์ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย และอาจารย์คณะวิศวกรรมศาสตร์ มหาวิทยาลัยพระจอมเกล้าธนบุรี

2.2 เชิงวิชาการ

ผลงานวิจัยเรื่องการทดสอบ mini-iFT ช่วยปรับปรุงวิธีการทดสอบวัสดุทางทันตกรรมให้มีประสิทธิภาพ และมีความแม่นยำมากขึ้น เพื่อประโยชน์ในการพัฒนาวัสดุทางทันตกรรมต่อไป

ผลงานวิจัยเรื่องการเติมสาร bioactive glass และ ซิงค์ออกไซด์ ในคอมโพสิต ช่วยให้เข้าใจการออกฤทธิ์ของสารตัวเติม และการทำงานของสารตัวเติมร่วมกับเรซินมากขึ้น และเกิดความร่วมมือกันทางทันตวัสดุศาสตร์

ผลงานวิจัยเรื่องไอออนที่มีผลต่อค่าแข็งผิวของวัสดุบูรณะ ทำให้เข้าใจถึงไอออนที่มีผลต่อความแข็งแรงที่เพิ่มขึ้น รวมถึงความสมดุลของเรซินที่มีผลต่อค่าความแข้งผิวของวัสดุมากขึ้น และยังสามารถเป็นแนวทางการศึกษาสมดุลของไอออน ที่ผิวของวัสดุได้

ผลงานวิจัยเรื่องปัจจัยที่มีผลต่อความสำเร็จและความล้มเหลวของการบูรณะฟันด้วยคอมโพสิต โดยเฉพาะทำให้ทราบถึงปัจจัยที่มีผลต่อความล้มเหลวในการบูรณะ ทำให้เกิดความรู้การเพิ่มความระมัดระวัง และการพัฒนาการเรียนการสอนนักศึกษา รวมถึงการรักษาผู้ป่วยมากขึ้น นอกจากนี้การศึกษาทางคลินิกมีข้อควรระวังและมีความละเอียดสูง ซึ่งการศึกษานี้จะเป็นแนวทางเพื่อใช้ในการศึกษาผลการรักษาทางคลินิกต่อไปในอนาคต

3. การเสนอผลงานในที่ประชุมวิชาการ

3.1 Saengnil Wanchanok, Anuntasainont Munlika, Srimaneekarn Natchalee, **Pongprueksa Pong**. 2019. Evaluation of Non-carious Cervical Lesion Restorations on Different Operator Experience and Adhesives: A Retrospective Clinical Study. CED IADR, 18-21 September 2019, Madrid, SPAIN

3.2 **Pongprueksa Pong**, De Munck Jan, Inokoshi Masanao, Van Meerbeek Bart. 2019 Polymerization efficiency affects interfacial fracture toughness of adhesives. ประชุมวิชาการ “TRF-OHEC Annual Congress 2019 (TOAC 2019)” ครั้งที่ 18 ณ โรงแรมเดอะรีเจ้นท์ ชะอำ บีช รีสอร์ท จังหวัดเพชรบุรี วันที่ 9-11 มกราคม 2562

3.2 Tararatsatid Natta, Vongphan Nataya, Intranont Noramon, Rojanathanes Rojrit, **Pongprueksa P**. 2018. Ion-releasing influences to surface hardness of bioactive filling material, IADR General Session/ IADR-PER, 25-28 July 2018, London, ENGLAND

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Polymerization efficiency affects interfacial fracture toughness of adhesives

Pong Pongprueksa[a,b], Jan De Munck[a], Masanao Inokoshi[a,c], Bart Van Meerbeek[a,*]

[a] KU Leuven (University of Leuven), Department of Oral Health Sciences, BIOMAT & UZ Leuven (University Hospitals Leuven), Dentistry, Kapucijnenvoer 7, blok a – bus 7001, B-3000 Leuven, Belgium

[b] Mahidol University, Faculty of Dentistry, Department of Operative Dentistry and Endodontics, 6 Yothi Road, Ratchathewi, Bangkok 10400, Thailand

[c] Tokyo Medical and Dental University, Graduate School of Medical and Dental Sciences, Department of Gerodontology and Oral Rehabilitation, 1-5-45 Yushima, Bunkyo, Tokyo 113-8510, Japan

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ABSTRACT

Objective. To evaluate the effect of the kind and concentration of photo-initiator on the degree of conversion (DC) of adhesives on dentin/glass substrates and their mini-interfacial fracture toughness (mini-iFT) to dentin.

Methods. We tested the adhesive Clearfil S^3 Bond Plus and 4 derived experimental 'LUB' ('Leuven Univesity Bond') adhesives (all from Kuraray Noritake), namely 'LUB-CQ/amine_high', 'LUB-CQ/amine_low', 'LUB-TPO_high', and 'LUB-TPO_low', respectively containing 2.0 wt% camphorquinone (CQ) and 2.0 wt% EDMAB (amine), 0.35 wt% CQ and 0.35 wt% amine, 2.0 wt% TPO, and 0.35 wt% TPO. For DC, each adhesive was applied onto glass or dentin prior to being cured (Bluephase 20i; Ivoclar Vivadent: "high mode") for 10 s. DC was measured at 5 min, 10 min, 1 h, 24 h and 1 week using micro-Raman spectroscopy (SENTERRA; BrukerOptik). For mini-iFT, each adhesive was bonded to 320-grit SiC-paper ground dentin and covered with composite (Z100; 3M ESPE). The restored teeth were cut in sticks ($1.5 \times 2.0 \times 16$ mm), after which a single-gradient notch was prepared at the adhesive-dentin interface using a 150-µm diamond blade. The micro-specimens were loaded until failure in a 4-point bending test and the mini-iFT in term of K_{QvM} was calculated.

Results. DC was higher on dentin than on glass. All adhesives were adequately polymerized at 1 week, except for LUB-TPO_low. DC at 5 min was significantly higher for LUB-TPO_high than for both CQ/amine-based adhesives. The highest and most reliable mini-iFT was measured for LUB-CQ/amine_high, despite its 5-min DC was relatively low. No correlation between DC and mini-iFT was found.

Significance. Curing of TPO-based adhesives is faster, but the dark cure of the CQ/amine-containing adhesives is more efficient. The differences in curing profiles do affect the mechanical properties of the resultant interfaces at dentin.

* Corresponding author.
E-mail address: bart.vanmeerbeek@kuleuven.be (B. Van Meerbeek).

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1. Introduction

The photoinitiator kind and its concentration are of importance to dental adhesives and their eventual bonding effectiveness [1,2]. Reaching a sufficiently high degree of conversion (DC) not only contributes to the adhesive's bond strength and durability [2–4], but it is also associated with biocompatibility issues caused by monomer and/or photoinitiator elution [4–6]. Most commonly used photoinitiators in dental adhesives are camphorquinone combined with tertiary amine (CQ/amine) and diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO).

We recently introduced a mini-interfacial fracture toughness (mini-iFT) test to assess bonding effectiveness of adhesives to tooth tissue [7,8]. This mini-iFT test enabled to discriminate adhesives better on their bonding effectiveness and, more importantly, the mini-iFT was shown to better reflect the mechanical strength of the actual interface than the today more commonly used micro-tensile bond-strength (μTBS) test [9]. A general consensus exists indeed in literature to preferentially use a fracture toughness approach to assess bonding effectiveness [10,11], despite the specimen-preparation methodology for interfacial fracture toughness testing is generally thought to be more technique-sensitive and labor-intensive. Several interfacial fracture toughness test protocols have been introduced, among which the most common are a 'short rod chevron notch' [12], a 'chevron-notched short bar' [13], a 'plane-strain chevron-notched short bar' [14], an 'interfacial fracture toughness related to the energy release rate' [15], a 'notchless triangular prism' [16] and a 'chevron notch beam' [17,18]. The major advantages of our newly developed mini-iFT test are that it is more accurate, more reproducible, less test dependent and that it reveals the interfacial properties better than a μTBS test. In addition, the mini-iFT test is less laborious and less time-consuming than a conventional interfacial fracture toughness. It has proven to be a valid and reliable laboratory test to determine bonding effectiveness to human dentin and enamel [7,8].

Since the mini-iFT test was shown to better assess the mechanical strength of the adhesive-tooth interface itself, we believed that this method would also suit well to investigate the effect of the kind and concentration of photoinitiator on the bonding effectiveness to dentin. Experimental adhesive formulations that differed for the kind of photoinitiator (CQ/amine versus TPO) and the photoinitiator concentration were tested. DC was measured over time when the adhesives were applied on dentin and glass in order to evaluate any correlation that may exist between DC and mini-iFT.

2. Materials and methods

2.1. *Materials used*

Five different adhesive formations, consisting of the commercially available adhesive Clearfil S^3 bond Plus (Kuraray Noritake, Tokyo, Japan) and 4 experimental derivatives (also provided by Kuraray Noritake), being referred to as 'LUB-CQ/amine_high', 'LUB-CQ/amine_low', 'LUB-TPO_high' and 'LUB-TPO_low', with a photoinitiator concentration of, respectively, 2.0 wt% CQ and 2.0 wt% EDMAB (amine), 0.35 wt% CQ and 0.35 wt% amine, 2.0 wt% TPO, and 0.35 wt% TPO, were used in this study (Table 1). The experimental LUB adhesives were provided by Kuraray Noritake without photoinitiator. The photoinitiators CQ in combination with the co-initiator EDMAB and TPO were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie, Steinheim, Germany). The respective amounts of CQ and EDMAB, and of TPO were measured on an analytical balance with a 0.01-mg accuracy (AB304-S' analytic balance; Mettler-Toledo, Greifensee, Switzerland); the photoinitiator was added to the LUB adhesive in light-shielding amber vials that were extra wrapped with aluminum foil to shield protect the solution from light. The photoinitiator was dissolved in the adhesive using a closed container that was immersed in an ultrasonic bath (Bandelin Sonorex; Bandelin Electronic, Berlin, Germany) for 1 min and then homogeneously mixed using a rotating machine (Rotator; Agar Scientific, Essex, United Kingdom) for 24 h, this following the recipe described in detail in the previous study [4].

2.2. *Mini-iFT*

Fifty non-carious human third molars (collected following informed consent approved by the Commission for Medical Ethics of KU Leuven under the file number S57622), stored in 0.5% chloramine T/water at 4 °C, were used within three months after extraction. The occlusal third of the crown was removed with a diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA), exposing a flat mid-coronal dentin surface, which was wet-sanded with 320-grit SiC paper (Buehler-Met II SiC wet grinding paper; Buehler, Lake Bluff, IL, USA) to produce a standard smear layer resembling that produced by a regular diamond bur. All dentin surfaces were carefully examined for remaining enamel and pulp tissue using a stereo-microscope (Stemi 2000 CS; Carl Zeiss, Jena, Germany). Each adhesive was applied following the instructions of Kuraray Noritake for the commercial adhesive Clearfil S^3 Bond Plus by actively rubbing the adhesive onto the dentin surface for 10 s, followed by 5 s gentle air-drying until the adhesive no longer moved. The adhesive formulations were next light-cured for 10 s using a polywave LED light-curing unit (Bluephase 20i; Ivoclar Vivadent, Schaan, Liechtenstein), employed in 'high mode', with an output of around 1100 mW/cm^2; the radiant exposure was 2.9 J/cm^2 up to 420 nm and 14 J/cm^2 above 420 nm, as was measured by a MARC Resin Calibrator (BlueLight Analytics, Halifax, NS, Canada). A composite build-up (Filtek Z100; 3M ESPE, Seefeld, Germany: shade A2, lot N459523) was made in layers using a polytetrafluoroethylene mold ($8 \times 8 \times 10$ mm). The root of the tooth was removed 3 mm below the adhesive-dentin interface and a similar composite build-up was made at the root side using the self-etch adhesive Clearfil SE Bond (Kuraray Noritake). After 1 week water storage at 37 °C, the specimens were sectioned perpendicular to the interface using a semi-automatic high-speed diamond saw (Accutom-50; Struers, Ballerup, Denmark: feed speed of 0.075 mm/s, wheel speed at 4000 rpm) with a water-cooled diamond blade with a diameter of 102 mm and a thickness of 300 μm (M1D10; Struers) to obtain rectangular sticks (micro-specimens of 1.5×2.0 mm wide and 16–18 mm

Table 1 – Composition of the experimental LUB adhesive derivatives that differ for the photoinitiator added in various concentrations and of Clearfil S[3] Bond Plus (Kuraray Noritake).

Adhesive	Composition[a]	Initiators[a]
LUB-CQ/amine_high	15 wt% Bis-GMA, 30 wt% HEMA, 10 wt% MDP, 15 wt% TEGDMA, 10 wt% colloidal silica, 15 wt% ethanol, 15 wt% water, stabilizers (minute amount)	2.0 wt% CQ, 2.0 wt% EDMAB
LUB-CQ/amine_low		0.35 wt% CQ, 0.35 wt% EDMAB
LUB-TPO_high		2.0 wt% TPO
LUB-TPO_low		0.35 wt% TPO
Clearfil S[3] Bond Plus[b]	15–35 wt% Bis-GMA, 10–35 wt% HEMA, MDP, hydrophilic aliphatic dimethacrylate, hydrophobic aliphatic methacrylate, colloidal silica, <0.1 wt% sodium fluoride, <20 wt%, water ethanol	dl-camphorquinone, accelerators, initiators

[a] Abbreviations: Bis-GMA: bisphenol A glycidyl dimethacrylate; CQ: camphorquinone; EDMAB: ethyl 4-(dimethylamino)benzoate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl dihydrogenphosphate; TEGDMA: triethylene glycol dimethacrylate; TPO: diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide.
[b] Commercially available, precise composition not released by Kuraray Noritake (Lot: 00012A).

long). Per experimental group, 2 sticks originating from 10 different teeth (n = 20) were obtained.

Mini-iFT specimen preparation has been detailed before [7,8]. By firmly fixing each stick (micro-specimen) to the diamond saw holder (Accutom-50; Struers) using an Accutom holder specimen, a single notch mini-iFT notch tip was prepared under a stereo-microscope with a magnification of 40x (Leica M715, Wetzlar, Germany), precisely at the adhesive-dentin interface, using a water-cooled ultra-thin 150-μm diamond blade (M1DO8; Struers: feed speed of 0.015 mm/s, wheel speed at 1,000 rpm). The notch thickness was smaller than 0.3 mm and included the complete adhesive-dentin interface for the full length of the notch (from the tip to the end of the notch). The angle of the mini-iFT notch tip was 45° in respect to the long side of the specimen. The tip of the single mini-iFT notch was located on the long side of the specimen at 0.24-0.48 mm from the bottom corner. The opposite part of the notch did end at less than 0.2 mm from the upper corner. The actual specimen geometry was schematically detailed in the previous studies [7,8]. All specimens were stored in 0.5% chloramine T solution at 37 °C and were tested 1 wk later. The specimens were transferred to a universal testing machine (Instron 5848 Micro Tester, MA, USA), putting the specimen upside down in the test fixture [7,8]. The specimens were tested in a 4-point bending test setup with a crosshead speed of 0.05 mm/min; the outer and inner span were 10 and 5 mm, respectively. After testing, all fractured surfaces were processed for scanning electron microscopy (SEM; JSM-6610LV, JEOL, Tokyo, Japan) using common specimen processing [19], including fixation, dehydration and HMDS drying prior to gold-sputter coating, this to determine the fracture location, crack propagation and any possible specimen imperfections. Finally, the exact dimensions of the mini-iFT notch were measured using a measuring optical microscope (400-NRC; Leitz, Wetzlar, Germany) at 250x magnification, after which the conditional plane strain interfacial fracture toughness, K_{QvM}, was calculated in MPa $m^{1/2}$; this according to the method described in detail previously [7,20].

2.3. DC

Another set of thirty non-carious human third molars were used to prepare mid-coronal dentin discs using a diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA). Each adhesive formulation was applied as described in detail above for the mini-iFT test, but now onto dentin discs as well as glass slides (Microscope Slides; VWR, Leuven, Belgium), this prior to being squeezed to a thickness of approximately 100 μm with a microscope cover glass (VWR, Leuven, Belgium) and two other cover glasses employed as spacer. The adhesive formulations were cured likewise as for the mini-iFT test. DC of six specimens per experimental group (n = 6) was measured using micro-Raman spectroscopy (μRaman; Senterra, BrukerOptik, Ettlingen, Germany) through the microscope cover glass to avoid oxygen inhibition; the specimen was excited with a near-infrared (785 nm) laser of 100 mW and analyzed through a 100× microscope and 50-μm pin-hole aperture. The collected spectra ranged from 50 to 3500 cm^{-1} with a resolution of around 9–15 cm^{-1}. The integration time was set to 20 s with 2 co-additions. The CCD detector to obtain the μRaman spectra possessed a 1024 × 256 pixel resolution and was cooled down thermo-electrically to a temperature of −65 °C. The data were processed using OPUS 7.1 software (Bruker). Each specimen was measured twice at 5 min, 10 min, 1 h, 24 h and 1 week. In between measurements, the specimens were kept dry in the dark at 37 °C. DC was calculated as the ratio of peak intensities of the carbon-aliphatic 1639 cm^{-1} and the carbon-aromatic 1609 cm^{-1} peaks measured in the cured and uncured materials.

2.4. Statistics and correlation analysis

The mini-iFT data were statistically analyzed using a linear model, taking into account the data variance, as we observed that the experimental groups with the low photoinitiator-concentrated adhesive formulations revealed a higher standard deviation than those with the high photoinitiator-concentrated adhesives. In this model, two fixed effects, 'photoinitiator' and 'photoinitiator concentration', and their interaction term were included. In addition, specific contrasts were calculated to assess the effect of photoinitiator at different concentrations on the mini-iFT.

For each DC specimen, a function was fitted to express DC in function of the log-transformed time in min. Because of the different curing behavior, a linear function was applied for the TPO-based adhesives, while the Avrami function ($Y = 1 - \exp(-Kt^n)$) was used for the CQ/amine-based adhesives [21]. From these fitted functions, maximum DC (DC_{max})

Table 2 – Mini-interfacial fracture toughness (mini-iFT) and degree of conversion (DC) of the different adhesive formulations investigated.

Adhesive	Mini-iFT[1] (SD)	ptf/n[2]	Dentin		Glass		Glass vs. Dentin
			DC_{max} in %[3] (SD)	DC_{90} in min[4] (SD)	DC_{max} in %[3] (SD)	DC_{90} in min[4] (SD)	p-value[5]
LUB-CQ/amine_high	1.79 (0.11)^{I}	0/15	99.0 (0.7)^{A}	7.0 (2.4)	97.7 (0.5)^{a}	18.9 (3.9)	0.976
LUB-CQ/amine_low	1.13 (0.46)^{III}	1/16	95.7 (1.3)^{B}	11.2 (2.4)	93.2 (4.8)^{a,b}	13.9 (4.9)	0.743
LUB-TPO_high	1.60 (0.20)^{II}	0/16	98.2 (0.9)^{A,B}	<5 min	88.1 (6.2)^{b}	20.4 (25.9)	<0.001
LUB-TPO_low	0.45 (0.35)^{IV}	8/18	75.9 (2.9)^{C}	1092 (1463)	43.9 (3.1)^{c}	749.5 (722)	<0.001
Clearfil S^3 Bond Plus	1.66 (0.16)^{I,II}	0/14	100.0 (0)^{A}	<5 min	94.1 (1.7)^{a,b}	8.3 (9.4)	<0.035

[1] Mini-interfacial fracture toughness K_{QvM} in $MPam^{1/2}$; the same superscript Roman figure indicates that the mean mini-iFTs are statistically not different.
[2] Number of pre-testing failures (ptf) per total specimen number (n).
[3] Maximum DC in %; the same superscript letter indicates statistically not different.
[4] Time in min needed to reach 90% of maximum DC_{max}.
[5] p-value for difference in DC_{max} measured onto dentin vs. glass for each adhesive formulation with statistical significance considered from $p < 0.05$.

and the time needed to reach 90% of maximum DC (DC_{90}) were deduced. Maximum DCs were compared by one-way ANOVA and planned contrasts to assess the effect of substrate ('dentin' versus 'glass') for each adhesive formulation. All tests were performed at a critical value of $\alpha = 0.05$ using a statistical software package (R3.1.0 and nlstools package, R Foundation for Statistical Computing, Vienna, Austria). Pearson's correlation coefficients were calculated to assess potential correlation between DC at various time points and mini-FT.

3. Results

3.1. Mini-iFT

The results of the mini-iFT test are graphically presented in box plots in Fig. 1; the mean mini-iFT and corresponding standard deviation (SD) are detailed for the different adhesive formulations in Table 2.

The mini-iFT of the low photoinitiator-concentrated adhesive formulations was significantly lower ($p < 0.0001$) than that of the high photoinitiator-concentrated adhesive formulations and the commercial adhesive Clearfil S^3 Bond Plus. The TPO-based adhesives presented with a significantly lower mini-iFT than the CQ/amine-based adhesives at high ($p = 0.0018$) as well as low concentration ($p < 0.0001$).

SEM failure analysis of representative mini-iFT specimens are presented in Fig. 2. Overall, failure analysis disclosed that the mini-iFT specimens always failed at the notch tip along the adhesive-dentin interface. For the high photoinitiator-concentrated adhesives and Clearfil S^3 Bond Plus, the crack propagated along the adhesive-dentin interface and mostly deviated to the adhesive layer near the end of the notch. Along the notch edge, often some adhesive remnants were observed (Fig. 2: 1a, 3a and 5a). At higher magnification, a brittle-like failure pattern with lots of hackle was disclosed within the adhesive layer near the top of hybrid layer (Fig. 2: 1b, 3b and 5b). Regarding the low photoinitiator-concentrated adhesives, the crack propagated almost exclusively along the adhesive-dentin interface with only very little adhesive remnants remaining at the fractured surface (Fig. 2: 2a and 4a).

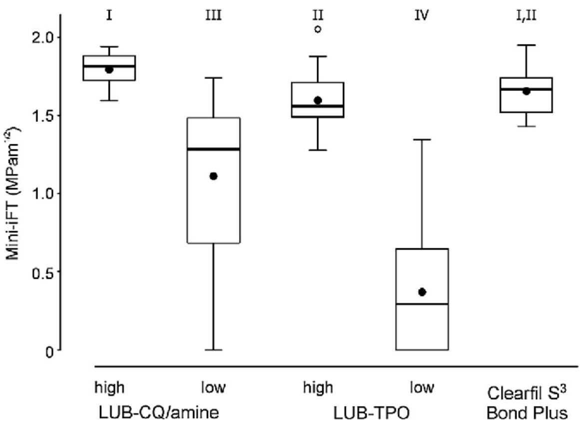

Fig. 1 – Box plots presenting the mini-interfacial fracture toughness (mini-iFT) for the different adhesive formulations investigated; the same superscript Roman figure indicates that the mean mini-iFTs are statistically not different. The thick horizontal line in the box represents the median DC; the boxes represent the first quartile (Q1) to the third quartile (Q3); the whiskers represent the lower and the upper quartile; the closed dot represents the mean value and the open dot represents an outlier data point.

3.2. DC

The progression of DC in function of time is graphically presented in Fig. 3 for the different adhesive formulations applied on dentin and glass, respectively. Table 2 mentions DC_{max} and DC_{90} for the different adhesive formulations applied on dentin and glass, respectively, along with potential statistical difference in DC_{max} at dentin versus glass.

When applied on dentin (Table 2), DC_{max} of the high photoinitiator-concentrated adhesives was not statistically different from DC_{max} of the commercial adhesive Clearfil S^3 Bond Plus. A significantly lower DC_{max} was measured for LUB-CQ/amine_low (though still being $95.7 \pm 1.3\%$), of

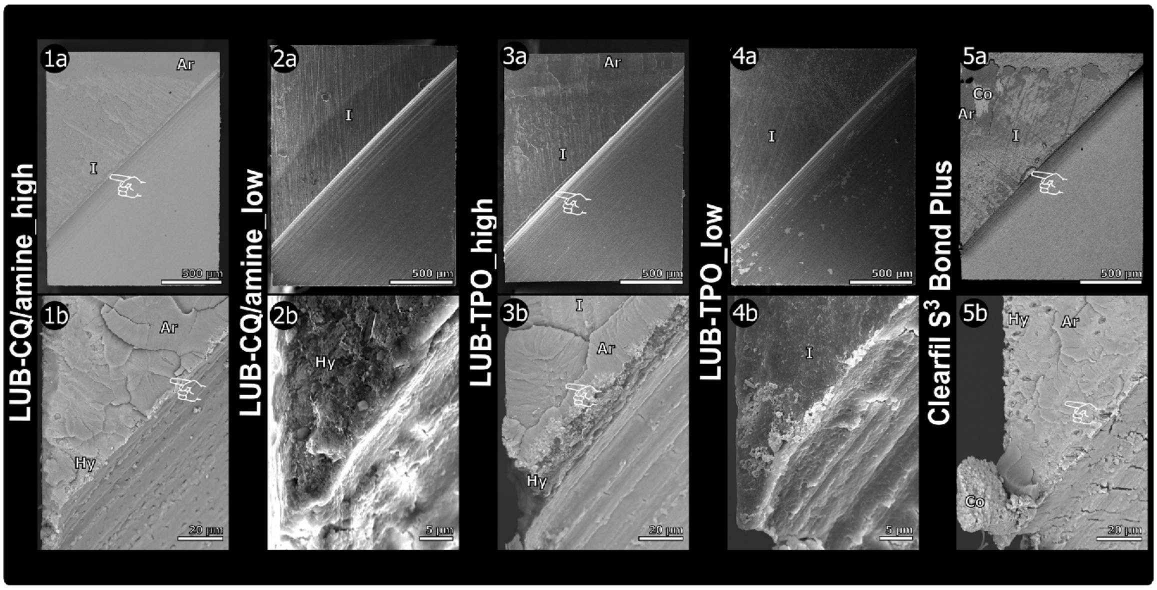

Fig. 2 – SEM fracture analysis of representative mini-iFT specimens of the different adhesive formulations investigated. (1a) Overview photomicrograph of the fractured surface of a LUB-CQ/amine_high specimen using backscatter electron (BSE) imaging. Although the entire specimen failed close to the adhesive-dentin interface (I), the crack propagated inside the adhesive resin (Ar) towards the edge of the specimen. Some adhesive resin remnants can be observed along the inner notch edge (hand pointer). (1b) High-magnification photomicrograph of the notch tip, BSE imaged. The notch tip is sharp and the fracture initiated exactly at the adhesive-dentin interface. The hybrid layer (Hy) and adhesive resin (Ar) can be distinguished. In the adhesive resin, some radiating crystallite formations were observed (hand pointer), suggesting a semi-crystalline nature that resulted in a more brittle-like fracture mode. (2a) Overview photomicrograph of the fractured surface of a LUB-CQ/amine_low specimen. Almost the entire surface failed at the adhesive-dentin interface. (2b) High-magnification photomicrograph of the notch tip. No adhesive remnants, nor evidence indicative of a brittle fracture can be seen. (3a) Overview photomicrograph of the fractured surface of a LUB-TPO_high specimen. Although the crack initiated within the adhesive resin with adhesive remnants remaining along the notch edge (hand pointer), it immediately propagated to and further along the adhesive-dentin interface until the middle part of the notch, where it deviated towards the adhesive resin. (3b) High-magnification photomicrograph of the notch tip, BSE imaged from an oblique angle at 20°. Viewed under this angle, the crack can be seen to have immediately propagated from the adhesive resin towards the adhesive-dentin interface. Failure occurred along the top of the hybrid layer. (4a) Overview photomicrograph of the fractured surface of a LUB-TPO_low specimen. The entire surface failed along the adhesive-dentin interface. Scratches induced by the SiC-paper can be observed. (4b) High-magnification photomicrograph of the notch tip, imaged from an oblique angle at 30 degrees. Dentin at the actual interface is still covered by some adhesive resin with the surface being rather smooth and uniform. (5a) Overview photomicrograph of the fractured surface of a Clearfil S^3 Bond Plus specimen using BSE imaging. Note the presence of small adhesive resin remnants along the notch edge (hand pointer). (5b) High-magnification photomicrograph of the notch tip using BSE imaging from an oblique angle at 20 degrees. Despite the notch tip was positioned at the interface, a very small amount of composite (Co) can be observed at the tip. Thanks to the favorable loading conditions, the crack returned immediately towards the adhesive-dentin interface. Note again the presence of radiating crystallite formations (handpointer), suggesting a semi-crystalline nature.

which its DC_{max} was significantly higher than that measured for LUB-TPO_low (that reached only 75.9 ± 2.9%). With respect to polymerization kinetics, DC_{90} was reached within 5 min for the high TPO-concentrated adhesive (LUB-TPO_high) as for the commercial adhesive Clearfil S^3 Bond Plus. A longer time was needed for both the CQ/amine adhesives; somewhat faster was DC_{90} reached for the high-concentrated (LUB-CQ/amine_high) than the low-concentrated (LUB-CQ/amine_low) adhesive formulation, but this was much shorter than the time needed for LUB-TPO_low to reach DC_{90}. The DC-progression curves in Fig. 3 (dentin) confirm that DC_{max} was reached within 5 min for LUB-TPO_high and Clearfil S^3 Bond Plus, while more than 10 min was needed for both the CQ/amine adhesives (with CQ/amine_high being faster than CQ/amine_low, and the latter reaching a lower DC); the concentration of TPO in LUB-TPO_low was clearly too low to reach a sufficiently high DC.

When applied on glass (Table 2), a relatively similar trend was observed regarding DC_{max} with the significantly highest DC_{max} reached for LUB-CQ/amine_high and Clearfil S^3

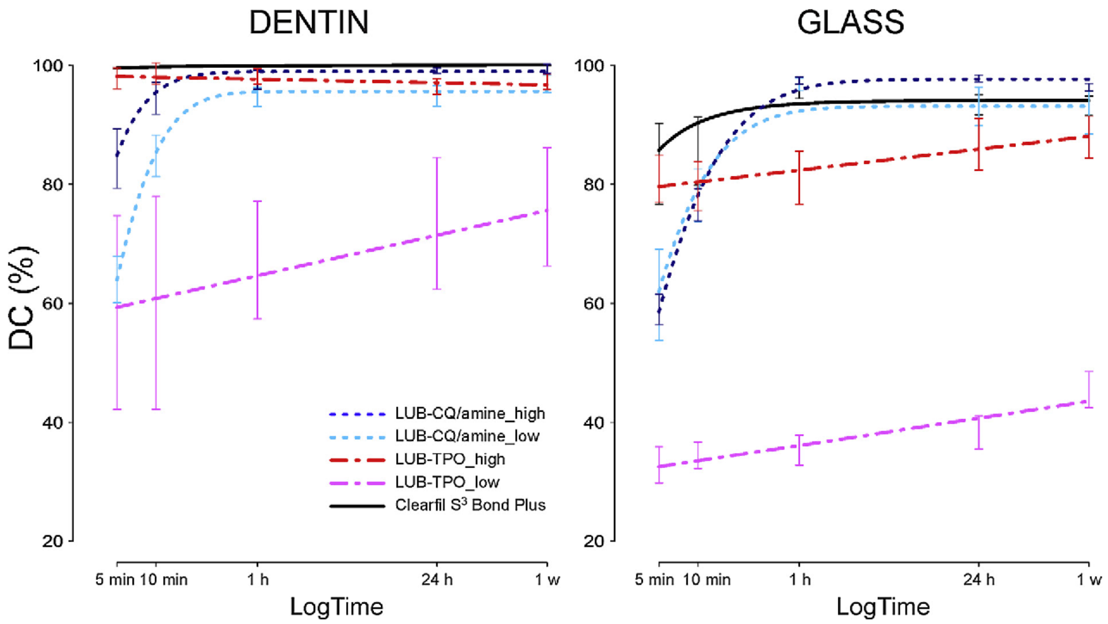

Fig. 3 – Progression in degree of conversion (DC) upon application of the adhesive formulations onto dentin versus glass. Error bars represent the 95% confidence intervals.

Bond Plus, but also for LUB-CQ/amine_low. DC_{max} of LUB-TPO_high was significantly lower (though still reaching 88.1%), while DC_{max} of LUB-TPO_low reached only 43.9%. All DC_{max} percentages were lower when the adhesives were applied on glass than on dentin, with significant differences measured for both TPO-based adhesives and Clearfil S^3 Bond Plus. Likewise, the time to reach DC_{90} was longer when the adhesives were applied on glass than on dentin, with the shortest time recorded for Clearfil S^3 Bond Plus, followed in order by LUB-CQ/amine_low ≤ LUB-CQ/amine_high < LUB-TPO_high < LUB-TPO_low. These differences in polymerization kinetics among the different adhesive formulations investigated are also reflected in the DC-progression curves in Fig. 3 (glass); they resemble those recorded at dentin with the differences that DC_{max} was somewhat later reached for Clearfil S^3 Bond Plus, that the polymerization kinetics of both CQ/amine adhesives were similar with the exception that a higher DC_{max} was reached for LUB-CQ/amine_high, and that DC_{max} of both the TPO-based adhesives were lower than those reached at dentin.

Overall, higher and faster polymerization rates were reached at dentin than on glass. The higher the photoinitiator concentration, the higher DC_{max}. Noteworthy is also that the TPO-based adhesives and Clearfil S^3 Bond Plus reached DC_{max} faster than the CQ/amine-based adhesives.

3.3. *DC and mini-iFT correlation analysis*

A positive, significant correlation was found between mini-iFT and DC at 24 h on dentin ($r^2 = 0.8364$, $p < 0.001$) as well as on glass ($r^2 = 0.8864$, $p < 0.001$) (Fig. 4). These correlations are however skewed by the low DC and mini-iFT data recorded for the LUB-TPO_low adhesive formulation. When this LUB-TPO_low adhesive was eliminated from the analysis, a relatively low, but significant correlation ($r^2 = 0.4978$, $p < 0.001$) was recorded between mini-iFT and DC at 24 h on dentin, but no correlation was found when applied on glass (Fig. 4).

4. Discussion

This study investigated the effect of two different photoinitiators added in a low and high concentration to an experimental one-step self-etch adhesive formulation. These formulations are derivatives of the commercially available one-step self-etch adhesive Clearfil S^3 Bond Plus (Kuraray Noritake). Bonding effectiveness was measured using an innovative mini-iFT that appeared capable of more accurately measuring interfacial bond strength than the today more popular μTBS approach [7]. Results were reported as the conditional plane strain interfacial fracture toughness K_{QvM} rather than the more common K_{Ic}. Because of the complex multi-layer specimen design, some mode II and mode III loading must have been present along the interface tested. Therefore, the conditional K_{QvM} fracture toughness is assumed a conservative estimate of the plane-strain K_{Ic} fracture toughness [14]. The effect of the today two most commonly used photoinitiators and their concentration on the mini-iFT was measured, as well as the according polymerization kinetics were analyzed when the adhesives were applied on two different substrates, namely dentin and glass. Overall, DC increased with time following the DC-progression rate to reach maximum DC depending on the photoinitiator used and its concentration. This different curing behavior did affect the resultant mechanical proper-

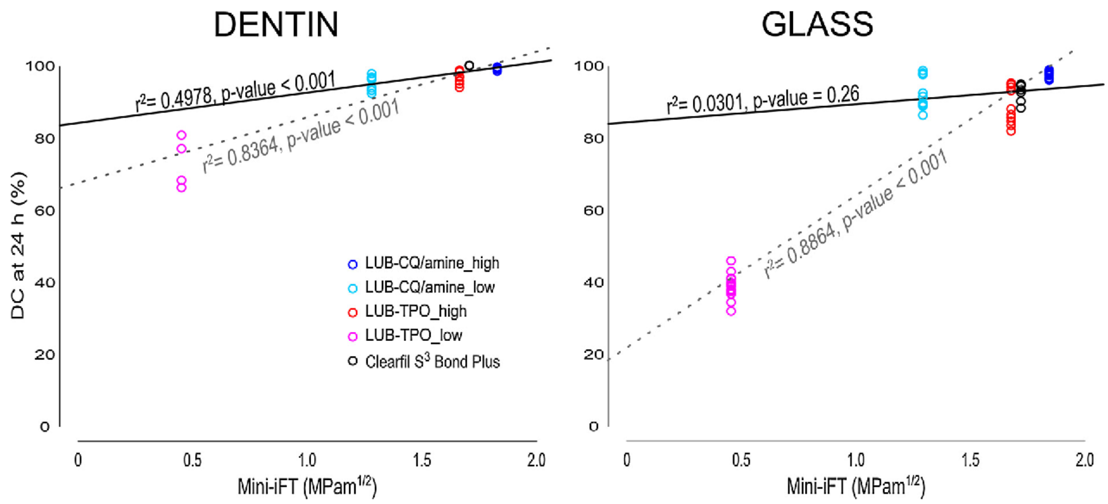

Fig. 4 – Correlation plots between mini-interfacial fracture toughness (mini-iFT) and degree of conversion (DC) at 24 h for the different adhesive formulations applied onto dentin and glass, respectively. The dotted regression line represents the correlation for all adhesives and the solid regression line represents the correlation when the LUB-TPO_low adhesive was omitted.

ties at the interface, as a significant positive correlation was found between DC and mini-iFT.

We measured DC using μRaman spectroscopy. Crucial in the assessment of polymerization under laboratory conditions is to control various environmental parameters that may affect polymerization; more specifically, ambient light, temperature and oxygen directly influence the curing efficiency and kinetics of dental adhesives. To exclude ambient light effects, the specimens were stored in the dark and all manipulations in the μRaman device were performed under orange-filtered light. To control ambient temperature at a clinically relevant condition, all specimens were continuously stored at 37 °C. Since oxygen inhibits free-radical polymerization of dental adhesives [22], oxygen was excluded by pressing a microscopy cover glass on the adhesive film (this also to reach a clinically relevant film thickness). In addition, DC was measured at the specimen center, to exclude areas near the specimen periphery, where the adhesive may potentially have cured improperly [23]; this proofed effective as for some groups a 100% conversion rate was observed. Previous studies investigating the polymerization efficiency and kinetics of composite cements [24,25] indeed demonstrated that without the cover glass or when the covering glass was removed after light-curing, the cements stopped to cure further in the dark because of oxygen inhibition.

According to the curing profiles of the adhesive formulations investigated, a significant increase of DC with time was recorded, for some even up to 1 week. This finding corroborates previous research that also observed this time-depending increase of DC [24–29]. This study revealed clearly different curing profiles for the CQ/amine- and TPO-based adhesives. TPO-based adhesives polymerized much faster, so that a higher DC was reached earlier. CQ/amine-based adhesives, on the other hand, exhibited more dark cure, so to reach eventually a higher DC than their TPO-based counterparts. This result corroborated a recent study demonstrating that a TPO-based adhesive exhibited no dark cure at all [30], while DC of CQ/amine-based adhesives increased considerably up to one hour after light curing. More research is warranted to explore if the initially faster conversion rate for TPO not only affects the ultimate conversion rate, but also influences the cross-link density and other related properties [31].

The adhesive formulations investigated polymerized faster and reached a higher DC_{max} on dentin than when applied on a glass substrate. This difference should primarily be attributed to the different nature of both substrates with hydroxyapatite (HAp) and water being present at the dentin surface. It is very well known that DC of CQ/amine-based adhesives is affected by acidic functional monomers due to inactivation of the amine co-initiator through acid-base reaction [32,33]. During bonding, functional monomers of self-etch adhesives, such as the commonly employed 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate), chemically interact with HAp. Recent research showed that the latter interaction of the functional monomer with tooth tissue and thus with HAp reduced the interference of the functional monomer with polymerization [33]. In addition, HAp acts as a buffer and so increases the local pH of a self-etch adhesive [34,35]. With increasing pH, the photoinitiator activity and especially that of the co-initiator increases as well [34,36]. Therefore, the presence of HAp promotes the polymerization process, this in particular when the acidic monomer interacts sufficiently intense with HAp [37]. Furthermore, the small amount of residual water in dentin helps acidic monomers to ionize and so to enhance the above-mentioned buffering action and polymerization. Obviously, excessive water will negatively affect resin polymerization [36,38]. In this respect, the polarity of the photoinitiator may also have influenced the curing profile of the adhesive [29,39]; we used in this study TPO, which is known to be more water soluble than CQ/amine. Moreover, TPO on its own was shown to increase DC of resin mixtures that contain besides 10% ethanol also 10% water, this in contrast to CQ/amine [39].

The commercial adhesive Clearfil S^3 Bond Plus, used in this study, contains CQ/amine as photoinitiator and exhibited the fastest and highest polymerization rate. One may expect that this very efficient polymerization should be related to an optimized concentration of photoinitiator and co-initiator and/or optimal photoinitiator/co-initiator combination. Unfortunately, the manufacturer did not disclose this information. In the present study, the same experimental adhesive formulations and photoinitiator concentrations were used as in our previous study [40]. The low 0.35 wt% CQ plus 0.35 wt% amine photoinitiator concentration was selected as this concentration was found to be minimally needed to enable the one-step self-etch adhesive to achieve an adequate micro-tensile bond strength to dentin [1]. The high 2 wt% CQ plus 2 wt% amine photoinitiator concentration was also used in previous research [2,5,40]. The combined 4 wt% CQ/amine photoinitiator concentration was found to produce a comparable quantity of free radicals as 2 wt% TPO. The photoinitiator concentration in an adhesive should definitely be optimized to achieve favorable physical properties, among which DC, but also in light of biocompatibility [5].

Mini-iFT is a simplified and miniaturized version of the single gradient notched beam (SGNB) fracture toughness test [20]. This mini-iFT technique was recently documented to be a valid method to assess bonding effectiveness to dentin with a better discriminative power than the today very popular μTBS test [7]. Mini-iFT data are less variable and reflect better the interfacial mechanical properties than common bond-strength tests [7]. In this study, the mini-iFT was higher for the high photoinitiator-concentrated adhesives than the low photoinitiator-concentrated adhesives. These data correspond to a previous study that revealed the μTBS to increase with an increased concentration of CQ [1]. In this study, the mini-iFT varied less with a higher photoinitiator concentration, rendering these adhesives more reliable than their low photoinitiator-concentrated counterparts. Apart from DC_{max}, also the polymerization rate is important to obtain optimal bonding effectiveness. While for the adhesive formulations with a high photoinitiator concentration DC on dentin did not differ much, the mini-iFT of the adhesive formulations containing CQ/amine and TPO as photoinitiator at high concentration was significantly different. Interesting in this respect is the observation of a more favorable cross-linking density for CQ/amine- than for TPO-based adhesives [31]. Hence, DC as measure of polymerization efficiency may not be the only factor influencing the mini-iFT; other factors like cross-linking density may be important as well and warrant further research.

5. Conclusion

Curing of TPO-based adhesives is faster, but the dark cure of the CQ/amine-containing adhesives is more efficient. The differences in curing profiles do affect the mechanical properties of the resultant interfaces at dentin, as was measured in this study in terms of mini-iFT.

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ภาคผนวก 2

(Appendix 2)

Influence of bioactive glasses and zinc oxide in light-curing composite

Watcharapon INTRA[1], Jinthana LAPIRATTANAKUL[2], Nisanart TRAIPHOL[3,4], Naruporn MONMATURAPOJ[5], Pong PONGPRUEKSA[1]

[1]Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, Bangkok, Thailand;

[2]Department of Oral Microbiology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand;

[3]Laboratory of Advanced Chromic Materials, Department of Materials Science, Faculty of Science, Chulalongkorn University, Bangkok, Thailand;

[4]Center of Excellence on Petrochemical and Materials Technology, Chulalongkorn University, Bangkok, Thailand;

[5]Assistive Technology and Medical Devices Research Center, National Science and Technology Development Agency, Pathumtani, Thailand

Running title: Composite with bioactive glass and ZnO.

Keywords: Bioactive glass, Zinc oxide, Provisional composite, Fractural strength, Mini fracture toughness

Corresponding author: Pong PONGPRUEKSA, Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, 6 Yothi Rd., Ratchathewi, Bangkok 10400, Thailand; E-mail: pong.pon@mahidol.edu, TEL: +66-96-745-9977,

ABSTRACT

This study aims to evaluate the influencing of additional bioactive glasses (45S5, S53P4) and Zinc oxide filler in resin composite on mechanical, physical, and antibacterial property. Composite incorporating with various additive fillers (15wt% 45S5, 15wt% S53P4, and 1wt% ZnO) was evaluated comparing to SiO_2 composite and Systemp onlay. The mechanical property (flexural strength, flexural modulus, and fracture toughness), physical property (water sorption, water solubility), depth of curing, and antibacterial property was evaluated. The 15wt% bioactive glasses composites were lower the flexural strength, flexural modulus, and fracture toughness to 1wt% ZnO and SiO_2 composite. The 15wt% 45S5 composite was the highest water sorption. Systemp onlay was the highest water solubility, while the 15%wt S53P4 composite was indicated a negative solubility. Systemp onlay was the deepest depth of cure, while ZnO composite was the lowest depth of cure. All experimental composites were not shown an antibacterial property against *Streptococcus mutans.* In conclusion, 15wt% bioactive glasses decreased the mechanical property, physical property, and depth of cure of composite, while 1wt% ZnO significantly decreased the depth of cure of composite. Besides, all experimental composites were absent for the antibacterial property.

Introduction

The visible light-cured composite has recently developed and increased popularity in dentistry. It has been developed to improve the mechanical property, biocompatibility and antibacterial property, which depended on various kinds of dental work. The provisional composite has been evolved in the material development and presented several advantages regarding the handing properties, controllable working time [1], incorporate antimicrobial agents, easily placed and readily removed in one piece [2].

The antibacterial composite has been focused as an innovative material, which incorporation of antibacterial agents into the resin composite [3]. Triclosan is a common additive agent used as a broad-spectrum antimicrobial activity with a favourable safety profile. Conversely, the evidence suggested that Triclosan has been linked to hormonal disruption and increased the risk of breast cancer [4].

Bioactive glass has widely been studied and used in dentistry for oral surgery and bone replacement. The advantage of bioactive glass is biocompatibility, antibacterial, and dentin remineralization [5]. A recent study has shown a stable mechanical property and inhibited bacterial growth when incorporating bioactive glass to composite [6]. Alternatively, zinc oxide nanoparticle is one of the most critical metal oxide particles, which has received interest in various fields of study. This metal oxide ion has shown antibacterial and disinfecting property, which it has widely used in various kinds of medicine, temporary dental filling and dental root canal sealer [7].

In this study, the experimental composite corporating bioactive glasses (45S5 and S53P4) and Zinc oxide particle were evaluated comparing to the SiO_2 composite and Systemp onlay. Therefore, the purpose of this study was to evaluate the influence of additive bioactive glasses and zinc oxide in composite on the mechanical, physical, and antibacterial property.

MATERIALS & METHODS

Four experimental light-curing composites contained various fillers as 15wt% 45S5 bioactive glass (45S5), 15wt% S53P4 bioactive glass (S53P4), and 1wt% zinc oxide (ZnO) corporating with silicon dioxide (SiO_2; 0.7µm silanized glass filler, Esstech, Essington, PA, USA) to the total 25wt% filler. The filler was mixed with 42wt% of 62µm prepolymerized dimethacrylate (UDMA). A resin matrix was prepared with an 80:20 mixture of UDMA:TEGDMA, including the photo-initiator of CQ, TPO, and EDMAB, the experimental composite composition is shown in Table 1. All monomers and photo-initiators were purchased from Sigma-Aldrich (Steinheim, Germany). The experimental composite with 25wt% SiO_2 filler and the commercially available antibacterial composite Systemp onlay (Ivoclar-Vivadent, Schaan, Liechtenstein) were used as control.

Flexural strength and flexural modulus

The provisional composite was placed into a polyethylene mold with the dimension of 2x2x25 mm bar-shaped (n=10) according to ISO 4049:2008 [8], the mold was covered with a transparent celluloid strip, and a glass plate was attached tightly to the mold surface, excessive material was removed. The specimen was light-cured for 20s using a polywave LED curing unit (Bluephase N; Ivoclar Vivadent, Schaan, Liechtenstein) at high mode with an output of around 1100 mW/cm^2, and following with a light furnace device (Lumamat 100; Ivoclar Vivadent, Schaan, Liechtenstein) for 60s. The specimen was incubated in water storage at 37°C, for 1wk.

The specimen dimension was measured using a digital calliper (Mitutoyo, Tokyo, Japan). The specimen was fractured with a three-point bending test on a universal testing machine (Model LF Plus; AMETEK Lloyd Instrument, Hampshire, UK), a supporting bar distance was 20mm at a crosshead speed of 1mm/min. Flexural strength (FS) and flexural modulus (FM) were calculated following equations:

$$\mathrm{FS} = \frac{3\mathrm{F_{max}l}}{2\mathrm{wh}^2} \qquad \mathrm{FM} = \frac{\mathrm{F_{lin}l}^3}{4\mathrm{d_{lin}wh}^3}$$

Where; F_{max} = ultimate force (N), l = distance between support bars (mm), w = width of the specimen (mm), h = height of the specimen (mm), F_{lin} = force in the linear part of the stress-strain curve (N) and d_{lin} = deflection at F_{lin} (mm).

Fracture toughness

A rectangular bar 2x1.5x16 mm of the composite specimen was prepared (n=10). The specimen was light-cured for 20s using Bluephase N and 60s using Lumamat 100, then the specimen was stored in water at 37°C, for 1wk. Mini Fracture Toughness (mini-FT) notch was prepared using a 150-μm ultra-thin diamond blade (M1DO8; Struers, Ballerup, Denmark) at a feed speed of 0.015mm/s and a wheel speed of 1000rpm water-cooling under a stereo-microscope following the previous study protocol [9]. The specimen was then transferred to the universal testing machine, putting the specimen tip down in the test fixture. The mini-FT (K_{Ic}) was tested using a 4-point bending test setup with a crosshead speed of 0.05 mm/min [9]. After testing, all fractured specimens were processed for SEM and measuring the exact dimensions of the mini-FT notch using an Image-Pro Plus (Media Cybernetics, MD, USA), after which K_{Ic} was calculated according to following equations from the previous study [9]:

$$K_{Ic} = \frac{F(S_o - S_i)}{BW^{3/2}} \times \frac{Y^{*}min}{\sqrt{1000}}$$

with the minimum stress intensity factor coefficient Y*min being calculated from the following function:

$$Y^{*}min = [2.92 + 4.52(l_0/W) + 10.14(l_0/W)^2]\sqrt{\frac{(l_1/W) - (l_0/W)}{1 - (l_0/W)}}$$

where K_{Ic} is the fracture toughness value in ($MPa.m^{1/2}$), F is the total force (N) (maximum force, F_{max}, plus tare force, F_{Tare}), S_o is the outer span (mm) or 10.0, S_i is the inner span (mm) or 5.0, B is the specimen thickness (mm) or 1.50±0.1, W is the specimen width (mm) or 2.00±0.1, Y*min is the stress intensity factor coefficient, l_0 is the position of the tip (mm) with $0.12<l_0/W<0.24$, and l_1 is the position of the bottom part (mm) with $0.90<l_1/W<1.00$.

Water sorption and solubility testing

The rectangular bar was made by using a mold with 2x2x25 mm (n=7), and then the specimen was light-cured for 20s using Bluephase N, following with 60s using Lumamat 100. The cured specimen was dry stored in a desiccator at 37°C for 24 hours, and the individual specimen was weighed to a constant mass (m1) at an accuracy of ±0.1mg. The specimen was deposited in distilled water at 37°C for 7d. After which, the specimen was removed, dried in the air, and weighed 1min to verify mass after saturation with water (m2). The specimen was then placed in the desiccator again until a constant dry mass (m3) was obtained[10].

The volume (V) of each specimen was calculated based on the following equation:

$$V = length\ x\ heigth\ x\ width$$

Water sorption (WS) and water solubility (SL), given in μg/mm³, were calculated as follows:

$$WS = \frac{m2 - m3}{V}$$

$$SL = \frac{m1 - m3}{V}$$

Depth of cure testing

The solvent dissolution technique was used for testing the depth of cure. The cylindrical samples were prepared in a mold with 5mm in diameter, 10mm in depth (n=6), the specimen was then light-cured through a glass slide at the top of specimen for 20s using Bluephase N at high mode with an output of around 1100 mW/cm^2. The cured sample was immersed immediately in Tetrahydrofuran (Ajax Finechem, Inc., Auckland, New Zealand) and dark stored at 22ºC for 48h. After this, the specimen was dried, and the maximum length of remaining material was measured using a digital calliper and divided by two according to the ISO/DIS 4049 (2008) standard [8, 11].

Antibacterial property (Direct contact)

Antibacterial properties of materials against two concentrations ($1x10^5$CFU/ml and $1x10^7$CFU/ml) of a cariogenic pathogen *Streptococcus mutans* was evaluated following the previous technique with some modifications [12, 13]. The material was prepared in a mold of 10mm diameter, 1mm thick, and light-cured for 20s (both top and bottom surfaces) using Bluephase N at high mode with an output of around 1100mW/cm^2, and then the cured disk specimen was sterilized by ultraviolet light.

S. mutans strain UA159 was used and grown in brain heart infusion (BHI) broth for 24h. The 500µl of the bacterial suspension was transferred into fresh BHI broth (5ml) and incubated at 37°C, 5% CO_2 to obtain the exponential phase of growth (optical density at 550nm; OD_{550nm}=0.40). This *S. mutans* suspension was diluted with BHI broth to yield the numbers of viable cells at $1x10^5$CFU/ml and $1x10^7$CFU/ml.

Four disks of each composite were placed in a 24-well plate (Thermo Fisher Scientific, Jiangsu, China) and 20µl of bacterial suspension prepared was placed on the surface of the specimen. The same amount of *S. mutans* suspension was also added into four blanked wells as the control. Besides, the disks covered with sterilized BHI broth were included to test the sterilization. To ensure direct contact between the bacterial cells and the tested surfaces, the evaporation of suspension to obtain a thin layer of bacteria on disk was performed by opening the 24-well plate containing specimens in a laminar flow clean bench (NuAire, Inc., Plymouth, USA) for 1 h. After that, BHI broth (520µl per well) was added and gently mixed for 10min. After mixing, 20µl solution from each well was transferred to a new 24-well plate containing BHI broth (480µl per well). These two 24-well plates, i.e., plates with and without the specimens were incubated at 37°C, 5% CO_2 for 18h, and the optical density at 590nm (OD_{590nm}) was determined by a microplate reader (BioTek ELx800; BioTek Instruments, Inc., Vermont, US). All procedures were independently performed three times to obtain average data of OD_{590nm} for each composite.

Statistical analysis

The data were subjected to the Shapiro-Wilk test for normal distribution and the Levene's test for homogeneity of variances. Flexural modulus, water sorption, and depth of cure were analyzed by one-way ANOVA and Tukey's HSD test at 95% confidence interval ($p<0.05$). Water solubility and antibacterial were analyzed by Kruskal Wallis test. The statistical analysis was carried out using SPSS statistics version 18. Flexural strength and fracture toughness were analyzed by Weibull distribution (95% confidence bounds as calculated from R version 3.6.0 with WeibullR and Weibulltools packages).

RESULTS

Flexural strength, flexural modulus, and fracture toughness

The flexural strength, flexural modulus, and fracture toughness are summarized in Table 2. The flexural strength was significantly higher for SiO_2 and ZnO composite than the bioactive glass composites. The lowest flexural strength was significantly for Systemp onlay ($p<0.001$). The flexural modulus was shown a similar trend to the flexural strength, which was significantly higher for SiO_2, and ZnO than bioactive glass composite and the lowest flexural modulus was significantly for Systemp onlay ($p<0.001$). The mini-fracture toughness was significantly higher for SiO_2 and ZnO composite to the bioactive glass composites (45S5 and S53P4). The mini-FT of

Systemp onlay was unable to evaluate as the specimen was not broken. The SEM failure analysis of the representative mini-FT specimen is presented in Figure 1.

Water sorption, water solubility, and depth of cure

The water sorption, water solubility, and depth of cure are shown in Table 3. The highest water sorption was found for 45S5 composite, which significantly higher than S53P4, SiO_2, Systemp onlay, and ZnO composite, respectively. The latter three composites were not statistically different in the water sorption. The highest water solubility was found for Systemp onlay, and the negative water sorption was indicated for the S53P4 composite. The deepest depth of cure was found for Systemp onlay at 4.4mm, while the lowest depth of cure was ZnO composite at 2.7mm depth.

Antibacterial properties

The concentration of bacterial cells in suspension was assessed by the optical density measurement. This method is performed by measuring the absorbance value of a liquid medium at 590nm. The growth of *S. mutans* in BHI broth after direct contact with the tested materials made a cloudy appearance of the medium. Hence the measurement of bacterial turbidity in BHI medium was stated as optical density.

The optical density regarding the direct contact between 10^5CFU/ml of *S. mutans* suspension and experimental composites is presented in Table 4, for both presence (A) and absence (B) of composite specimens. Although the optical density of all composites was lower than the control, no significant difference was observed ($p>0.05$). Similar findings were found for the direct contact test using 10^7CFU/ml of *S. mutans* suspension. There was no significant difference in the optical density between all composites and the control.

Discussion

This study was evaluated the influence of three kinds of additive filler (BAG-45S5, BAG-S53P4, and Zinc oxide nanoparticle) in experimental composites comparing to SiO_2 composite and Systemp Onlay on mechanical property, physical property, depth of cure, and antibacterial property. The null hypotheses were rejected for the mechanical, physical, and depth of curing as the flexural strength, flexural modulus, fracture toughness, water sorption, water solubility, and depth of cure were significantly different among the composites. However, the null hypothesis was accepted for the antibacterial property as there was no significant different antibacterial among the composites. Therefore, the different additive fillers influenced the mechanical, physical, and depth of curing properties of the composite.

The mechanical property on flexural strength, flexural modulus, and fracture toughness were the same trend as the SiO_2 and ZnO composite were significantly higher mechanical property than 45S5 and S53P4 composite, besides, the Systemp onlay was the lowest mechanical property (Table 2). The SiO_2 and ZnO composite were not significantly different on mechanical properties which correlated to a previous study, that the flexural strength and flexural modulus were not influenced by increasing concentration of ZnO nanoparticle at 0-5wt% [14]. The higher mechanical properties of SiO_2 and ZnO composite to the bioactive glasses (45S5 and S53P4) composite associated with the previous studies [15, 16] that the flexural strength was declined corresponding to the bioactive glass filler proportion increased in the composite. Besides, the negative linearly correlation was found, when the amount of bioactive glass increased from 5-40wt%, the mechanical properties (flexural strength and flexural modulus) decreased [15]. However, the flexural strength was unchanged when the 10wt% bioactive glass was included [15]. Besides, a previous study showed that flexural strength and fracture toughness were unaffected by increasing concentration up to 15wt% of bioactive glass [6].

The lowest mechanical property (flexural strength and flexural modulus) was Systemp onlay. Besides, the fracture toughness was unable to perform as the specimen was bendable and unbreakable. The fracture toughness test is a potential significance of indentation-induced cracking to characterize the toughness of brittle materials [17]. Besides, the main different mechanical property of the composite depended on the diverse compositions (filler, monomer, and initiators). The Systemp onlay contains 62.1% filler concentration, while the other experimental composites contain 67% filler concentration. In general, a resin composite with higher filler volume results in higher flexural strength and flexural modulus values [18]. According to the manufacturer, Systemp onlay composed of a dimethacrylate monomer (polyester urethane dimethacrylate) and a monomethacrylate monomer (monofunctional ethyl triglycol methacrylate), while the experimental composite composed of two dimethacrylate monomers (urethane dimethacrylate and triethylene glycoldimethacrylate). In general, the dimethacrylates showed higher mechanical property than the monomethacrylates, in which the dimethacrylates performed a cross-linked structure, while the monomethacrylate produces a linear structure [19]. Therefore, these different kinds of monomers resulted in unique characteristics of Systemp onlay, which was a highly flexible material. Furthermore, the use of initiators in Systemp onlay was around 1wt% including catalyst, stabilizer, and Triclosan, while the experimental composite contained 1.5wt% initiators and co-initiator (CQ, Lucerin TPO, and EDMAB). In the present study, these different types and concentration of initiators were added to increase the initial polymerization of provisional composite, which, however these photo-initiators influenced the higher monomer polymerization and resulted in higher mechanical properties [20, 21].

Water sorption and solubility of the composite were related to the water uptake and dissolution of the material components at the same time [22]. The water sorption and water solubility of the composite depended on filler (type, content, concentration, particle size, nature of filler), the coupling agent, monomer (hydrophobicity and hydrophilicity), polymerization and the type of solvent [23, 24].

The water sorption was significantly higher for bioactive glass composites than the other composites. This result corresponded to the previous study which the higher concentration of bioactive glass related to the higher water sorption [25], which the explanation was a highly hydrophilic bioactive glass filler increasing water sorption compared to the SiO_2 composite [25].

The water solubility was significantly higher for Systemp onlay than other composites. These can be associated with the polymerization as previously discussed on the mechanical properties and the initiator concentration. Thus, a lower degree of polymerization results in more leachable monomer components [26], which related to a higher solubility. However, the low polymerization did not necessarily mean that residual monomers were free to leach, but most likely that there were many unreacted groups pendant from the network [27]. In this study, the water solubility indicated a negative value for the S53P4 composite. The negative water solubility value means that water was not completely removed during the dry storage, however, it did not mean nothing eluted from the material. The possible explanation was that glass particles and ions participate in water formed the silica gel formation, which the silica gel formation often occurred in glass ionomer-based materials [28, 29] and bioactive glass materials [30]. Besides, the hydroxylcarbonate apatite formation can be structured at the surface of bioactive glass, when the bioactive glass exposes to the simulated body fluid solution [31]. Although the 45S5 could form the silica gel formation at the particle surface, the 45S5 shown a positive value of water solubility. These could be the higher dissoluble of 45S5 particle than the S53P4 particle [32-34]. Another possible explanation was that partial water absorbed into a highly hydrophobic composite, which the water was bounded in the resin matrix and entrapped in the resin network [35].

The depth of cure of composite depended on several factors, such as chemical composition (filler and monomer), curing intensity, and curing time [36]. In the present study, monomers, curing intensity, and curing time were controlled, while the filler was varied. These filler and monomer compositions were directly related to the material opacity, which associated with the refractive index of each composition. The mismatched refractive index among filler and resin monomer influenced to increase the material scattering at the filler/resin interface [37]. On the other hand, the well matching refractive index among filler and resin monomer increased the material translucency and improved the light transmittance of the composite [38]. Besides, the material refractive indexes in this study were TEGDMA (1.461), UDMA (1.485), Silanized SiO_2 (1.553), and ZnO powder (1.989) (Wang et al. 2017). In this study, the depth of cure was higher for Systemp onlay and SiO_2 composite to the other composites, the commercial composite might be a well matching refractive index. The bioactive glasses composite was shown a lower depth of cure compared to SiO_2 composite, which collaborated to previous studies that bioactive glass fillers reduced the curing efficiency at depth in composite [39, 40]. The lowest depth of cure was the ZnO composite, which related to the big different refractive index between ZnO and resin monomer. The result cooperated with the previous study as the higher concentration of ZnO filler reduced the depth of cure of composite [14] and significantly increased the refractive index of the composite [41, 42].

The antibacterial activity against *S. mutans* at 10^5CFU/ml and 10^7CFU/ml showed that the optical density results indicating bacterial growth were lower in the composites compared to the control. However, there was no statistical difference. Such observation was found from either group A (presence of tested materials) or group B (absence of tested materials). Besides, adding bioactive glasses or ZnO to the composite did not influence antibacterial property. The antibacterial behaviour of the materials depends on their degradation and ion release. The previous study on the antibacterial property of Ag-doped bioactive glass reported the composite containing Ag_2O (2.1wt%) showed an antibacterial property regarding the Ag-ion releasing, and it was not depended on the pH value change [3]. Another reason related to the absence of antibacterial activity of experimental composites in this study might be the composite polymerization, thus the high polymerization relating to the low releasing material components, which previously discussed. Therefore, the experimental composites in this study might have a high polymerization, resulting in a small amount of active component releasing and consequently to the absence of an antibacterial effect.

Many studies support that ZnO has shown an inhibitory effect on oral bacteria [43, 44]. However, the composites containing 1wt% ZnO nanoparticle found the absence of antibacterial activity, which in contrast to a previous study that 1wt% flowable composite containing ZnO resulted in reducing a colony-forming unit of *S. mutans*, while 4wt% ZnO performed an entirely inhibiting antibacterial effect [14].

Systemp onlay is a composite containing Triclosan as to reduce bacterial growth on the restoration. The composite containing Triclosan was reported to perform an inhibitory effect on *S. mutans* for 7d [45]. Although the direct contact method seemed to stimulate this principle, Systemp onlay did not show the antibacterial property in this study, which contrast to the previous study that showed the antibacterial activity of System onlay against *S. mutans* suspension [45]. These might be utilized the dissimilarity of procedures regarding the bacterial preparation and result measurement.

Therefore, increasing the amount of the additive fillers bioactive glasses or ZnO might lower mechanical and physical property, which it might be possible for provisional composites. These additional fillers may achieve the antibacterial property of composite.

CONCLUSION

The 15wt% bioactive glasses (45S5, S53P4) composite decreased the mechanical property, physical property, and depth of cure, while the 1wt% ZnO experimental composite was not influencing the mechanical and physical property, although it significantly decreased the depth of cure. Besides, all experimental composites and Systemp onlay were not shown the antibacterial property.

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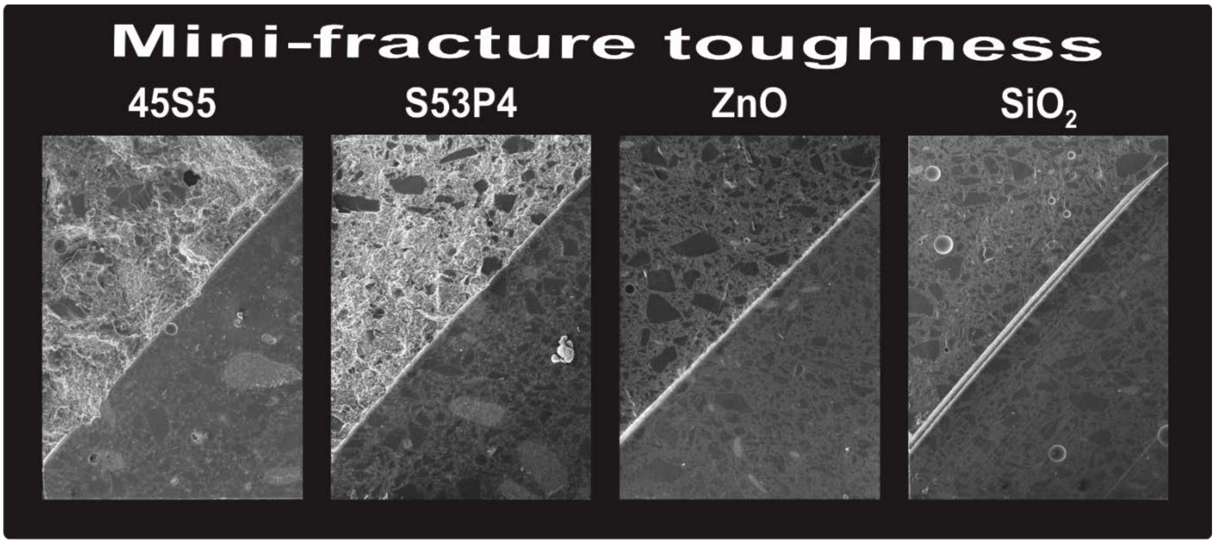
FIGURE LEGENDS

Figure 1. SEM fractured surface analysis of representative mini-interfacial fracture toughness specimens.



TABLES

Table 1. Composition of the experimental provisional resins used in this study and a commercially provisional resin (Systemp onlay).

Composition[a]	45S5	S53P4	ZnO	SiO_2	Systemp onlay
BAG-45S5	15	-	-	-	-
BAG-S53P4	-	15	-	-	-
ZnO powder	-	-	1	-	-
SiO_2	10	10	24	25	-
Highly dispensed SiO_2, silanized	-	-	-	-	19.4
Prepolymerized dimethacrylate	42	42	42	42	42.7
UDMA	25.2	25.2	25.2	25.2	-
Polyester urethane dimethacrylate	-	-	-	-	29.4
TEGDMA	6.3	6.3	6.3	6.3	-
Ethyl triglycol methacrylate CAS:39670-09-2	-	-	-	-	7.5
CQ	0.5	0.5	0.5	0.5	-
EDMAB	0.5	0.5	0.5	0.5	-
TPO	0.5	0.5	0.5	0.5	-
Catalysts, stabilizers and triclosan	-	-	-	-	1.0
Pigments	-	-	-	-	<0.1

Abbreviation: ***BAG-45S5***, 45% SiO_2, 6% P_2O_5, 24.5% Na_2O, 24.5% CaO; ***BAG-S53P4***, 53% SiO_2, 4% P_2O_5, 23% Na_2O, 20% CaO; ***ZnO***, 40nm Zinc Oxide, CAS:1314-13-2; ***SiO_2***, 0.7µm silanized glass filler, CAS:65997-17-3; ***UDMA***, urethane dimethacrylate, CAS:72869-86-4; ***TEGDMA***, triethylene glycoldimethacrylate, CAS:109-16-0; ***CQ***, Camphorquinone, CAS:10373-78-1; ***EDMAB***, ethyl 4-(dimethylamino)benzoate, CAS:10287-53-3; ***TPO***, diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide, CAS:75980-60-8.

Table 2. Flexural strength, flexural modulus and fracture toughness results.

Material	Flexural strength			Flexural modulus (GPa)	Fracture toughness		
	B[1] **(m)**	η[2]	**Characteristic strength**[3]		***B***[1] **(m)**	η[2]	**Characteristic strength**[3]
45S5	15.3	36.5	36.5(34.8-38.5)^{B}	1.5 (0.1)^{b}	5.9	1.6	1.6(1.4-1.8)^{II}
S53P4	12.0	35.8	35.8(33.7-38.3)^{B}	1.5 (0.1)^{b}	14.1	1.6	1.6(1.5-1.7)^{II}
ZnO	14.5	82.7	82.7(78.5-87.3)^{A}	2.5 (0.2)^{a}	26.2	2.5	2.5(2.4-2.6)^{I}
SiO_2	12.4	85.6	85.6(80.6-91.2)^{A}	2.2 (0.3)^{a}	7.0	2.8	2.8(2.5-3.1)^{I}
Systemp onlay	5.9	11.7	11.7(10.3-13.3)^{C}	0.1 (0.01)^{c}	-	-	-

Different letters indicate statistical differences within a column ($p<0.05$).
[1] Beta, shape, slope or modulus of Weibull parameter.
[2] Eta, Characteristic life or scale of Weibull parameter.
[3] 95% confidence interval at Characteristic strength (=63.2% unreliability); groups with the same superscript letter are statistically not different.

Table 3. Values of water sorption, solubility and depth of cure of materials studied.

Material	Water sorption ($\mu g/mm^3$)	Water solubility ($\mu g/mm^3$)	Depth of cure (mm)
45S5	63.2 (2.5)^{A}	5.5 (1.7)^{a,b}	3.0 (0.2)^{II,III}
S53P4	56.5 (3.3)^{B}	-10.1 (0.9)^{c}	3.2 (0.3)^{II}
ZnO	20.1 (1.1)^{C}	2.1 (0.8)^{a,b,c}	2.7 (0.2)^{III}
SiO_2	20.8 (1.5)^{C}	0.5 (0.7)^{b,c}	3.9 (0.3)^{I}
Systemp onlay	20.4 (1.7)^{C}	27.9 (2.8)^{a}	4.4 (0.5)^{I}

Different letters indicate statistical differences within a column (*x*0.05).

Table 4. Antibacterial properties of materials against two concentrations of *S. mutans*.

Material	Optical density (OD_{590nm})			
	S. mutans 10^5 CFU/ml.		*S. mutans* 10^7 CFU/ml.	
	presence of tested materials	absence of tested materials	presence of tested materials	absence of tested materials
45S5	0.25 ± 0.02 (0.25)	0.25 ± 0.03 (0.26)	0.30 ± 0.01 (0.29)	0.30 ± 0.02 (0.30)
S53P4	0.25 ± 0.01 (0.25)	0.25 ± 0.01 (0.25)	0.29 ± 0.01 (0.29)	0.30 ± 0.01 (0.30)
ZnO	0.25 ± 0.01 (0.25)	0.25 ± 0.01 (0.25)	0.29 ± 0.01 (0.30)	0.30 ± 0.01 (0.30)
SiO_2	0.27 ± 0.02 (0.26)	0.26 ± 0.01 (0.26)	0.30 ± 0.02 (0.30)	0.30 ± 0.03 (0.29)
Systemp onlay	0.27 ± 0.01 (0.28)	0.27 ± 0.01 (0.27)	0.30 ± 0.003 (0.30)	0.31 ± 0.02 (0.31)
Control	0.30 ± 0.02 (0.30)	0.29 ± 0.03 (0.30)	0.31 ± 0.01 (0.31)	0.32 ± 0.003 (0.32)

mean ± SD (median)

ภาคผนวก 3

(Appendix 3)

ION-RELEASING INFLUENCES TO SURFACE MICROHARDNESS OF BIOACTIVE FILLING MATERIALS

Natta Tararatsatid[1], Nataya Vongphan[1], Noramon Intranont[2], Rojrit Rojanathanes[3], Pong Pongprueksa[1]

[1]Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, Thailand; [2]Environment Research Unit, National Metal and Materials Technology Center (MTEC), Thailand; [3]Department of Chemistry, Faculty of Sciences, Chulalongkorn University, Thailand

Corresponding author: **Pong Pongprueksa**, Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, Thailand, 6 Yothi Rd., Ratchathewi, Bangkok 10400, Thailand. TEL: +66-96-745-9977, E-mail: **pong.pon@mahidol.edu**

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ABSTRACT

Objective: The purpose of this study was to investigate the restorative material surface microhardness, surface chemical analysis, and ion-releasing of bioactive restorative material.

Methods: Twelve specimens of each material (Equia Forte Fil, Ketac Universal, Beautifil II, GC Fuji II LC, Activa BioActive, and Filtek Z350XT) were prepared in disc-shaped (5mm diameter, 2mm height). The specimen was stored in 100% humidity at 37°C for 24h, and then six specimens of each material were randomly divided and stored either in deionised water or artificial saliva at 37°C for 42d. The storage medium was changed weekly. The Vickers surface microhardness test was evaluated on the top of the specimen at 1d, 7d, 21d and 42d. Chemical analysis at the specimen surface was performed in two specific periods at 1d and 42d in both deionised water and artificial saliva using SEM-EDX. Ion-releasing from the material was analysed from the deionised water storage medium at 1d, 21d and 42d using ICP-OES. The surface microhardness data were statistically analysed using three-way ANOVA and Tukey multiple comparison test ($\alpha = 0.05$).

Results: The surface microhardness was influenced by all factors (materials, storage medium and storage time) at $p<0.05$. The surface microhardness increased after 42d for Equia Forte Fil and Ketac Universal in artificial saliva, while other materials gradually decreased with time in both mediums. SEM-EDX revealed the increasing element of P,K, and Ca for GICs and RMGIC after storing in artificial saliva. ICP-OES revealed that the Na ion releasing was high for GICs and RMGIC, while Si and Al ion releasing was high for RMGIC. Besides, Ca and Mg ion releasing was low for GIC.

Conclusion: The surface microhardness of conventional glass ionomer was relatively constant in deionised water and significantly increased in artificial saliva after 42d. Therefore, the deposition element of P, K, Ca, and Mg on the material surface from artificial saliva and the low ions releasing of Ca and Mg could influence to maintain the material surface microhardness.

INTRODUCTION

Restorative material needs to be withstanding for long term durability in the oral cavity, which the complex oral environment contains saliva, water, enzyme, and several elements [1-4]. Surface hardness is an essential property of restorative materials, which correlates to compressive strength and abrasive resistance [5]. The surface hardness of restorative material is the resistance of a material to plastic deformation, usually by indentation. Besides, the surface hardness changing depends on several factors such as the degradation from the solution, the effect of acidic food and drink, type of materials, type of storage medium and time [6].

Several direct restorative materials have been developed and used in dentistry. Composite has widely been accepted for a long term restorative, esthetics and excellent adhesion to tooth substrate with an adhesive [7]. Glass ionomer cement (GIC) has been used as its principal property on fluoride-releasing, fluoride recharging from the oral environment [8, 9] and including the chemical adhesion to the tooth substrate [10]. Resin-modified glass ionomer cement (RMGIC) has been used with the resin is improved mechanical property to GIC and remaining the potential of chemical interaction to the tooth substrate [11].

Recently the dental bioactivity materials have been introduced and developed to optimise their properties; [12] as ion-releasing promoting remineralisation [13] or stimulating hydroxyapatite formation [14]. The bioactivity material is including bioactive calcium silicate cement (white ProRoot MTA, Biodentine), bioactive calcium aluminate cement (Ceramir), and bioglass-reinforced glass ionomer restorative cement (Activa™ BioActive) [15]. The latter material was developed and claimed to use as filling material. According to the manufacturers' information, the bioactive property consideration mimics the ion exchange process (calcium, phosphate and fluoride ions) between the material, saliva and tooth structure, which stimulates apatite formation that fills gaps and seals margins against microleakage [16].

The present study focused on the surface microhardness of restorative materials, which related to the element distribution on the surface and the ion-releasing from the material. Therefore, the purposed of this study was to study the changing surface microhardness of restorative material in deionised water and artificial saliva. Secondly, to observe the element distribution on the surface between deionised water and artificial saliva and the ion-releasing of material in deionised water storage.

MATERIALS & METHODS

Six commercial restorative materials consisted of two conventional glass ionomers (Equia Forte Fil; GC, Tokyo, Japan, Ketac Universal; 3M ESPE, MN, USA), a Giomer (Beautifil II; Shofu, Kyoto, Japan), a resin-modified glass ionomer (Fuji II LC; GC, Tokyo, Japan), a bioactive material (Activa BioActive Restorative; Pulpdent, MA, USA), and a nanohybrid composite (Filtek Z350XT, 3M ESPE, MN, USA). The material composition is shown in Table 1. Twelve disc-shaped specimens of each material were prepared according to the manufacturers' instructions. Equia Forte Fil and Ketac Universal were mixed with amalgamator (Silamat S6, Ivoclar-Vivadent, Schaan, Liechtenstein) for 10 seconds. The material was placed in the Polytetrafluoroethylene mold (5mm diameter and 2mm height), which was placed between two glass slides. Equia Forte Fil and Ketac Universal were kept between the glass slide for 2:30 min and 3:40 min, respectively, which is the material setting time. Activa BioActive, Beautifil II, Fuji II LC and Filtek Z350XT were placed in the mold and cured with LED light-curing unit (Bluephase N, Ivoclar-Vivadent, Schaan, Liechtenstein) for 20s at an intensity of 1,100 mW/cm^2. All specimens were kept in the incubator (Shel Lab Incubator, Sheldon Manufacturing, OR, USA) at 37°C, 100 % humidity for 24h.

Vickers microhardness

After the material maturation for 24h, the top surface of the specimen was polished with silicon carbide paper no.1000 (CarbiMet PSA 1000, Buehler, IL, USA) under running water, and followed by alumina polishing powder 0.05 µm (Micropolish II, Buehler, IL, USA). Twelve specimens of each material were randomly divided into two groups, which either stored in deionised water or artificial saliva. The artificial saliva was prepared following the previous study composition [17] as shown in Table 2. Each specimen was kept in 15 ml microtube containing with 2 ml of storage medium at 37°C and the medium was changed weekly. The surface microhardness was measured on the top surface using microhardness tester (FM 700, Future Tech, Tokyo, Japan) under the load of 100 grams for 15s at 1, 7, 21 and 42 days. The specimen was blotted dry with a filter paper prior to the surface microhardness test. Five indentations were performed on each specimen surface.

Chemical analysis on the specimen surface

Two specimens of each material at 1d and 42d in both deionised water and artificial saliva were randomly selected. The specimen surface was coated with a gold sputter coating machine (Balzers SCD040, Balzer Union, Wallruf, Germany). The element distribution was analysed using a scanning electron microscope with energy-dispersive x-ray spectrometer (SEM-EDX, JSM-610LV; JEOL, Tokyo, Japan). The qualitative analysis was performed for the surface element distribution.

Ion-releasing in deionised water

Ion-releasing of the material in deionised water was analysed using Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES; ACTIVA M, Horiba Jobin Yvon, France). Sodium (Na), silicon (Si), calcium (Ca), aluminium (Al), magnesium (Mg), and phosphorus (P) ion were determined with the wavelengths of 589.59 nm, 251.61 nm, 393.30 nm, 396.15 nm, 279.55 nm, 213.61 nm, respectively. The ion-releasing was analysed at 1, 24 and 42 d in triplicate, and the element was calibrated with the pure deionised water. The qualitative data analysis was performed for the ion-releasing in deionized water.

Statistical analysis

The surface microhardness data were statistically analysed by Shapiro-Wilk test, Levene's test, three-way ANOVA to analyse three factors (material, storage medium, and storage time) and Tukey's multiple comparison test (α=0.05).

RESULTS

The surface microhardness of restorative material in deionised water or artificial saliva at different periods are shown in Table 3 and 4 and the microhardness changing comparing to the microhardness at 1d ($Hv_{(x\ day)}/Hv_{(1\ day)}$) is presented in Figure 1. The statistical analysis of three-way ANOVA revealed that type of materials, storage medium and storage time factor were significant influence the surface microhardness. In general, the material microhardness decreased after storage in both mediums for all restorative materials except for the two conventional GICs (Equia Forte Fil and Ketac Universal). The surface microhardness of both conventional GICs were constant after 42d storage in deionised water, while the surface microhardness of GIC gradually increased after 42d storage in artificial saliva. Moreover, the surface microhardness of GIC in artificial saliva was significantly higher than in deionised water storage at 42d (Table 3 and 4).

In deionised water storage, the highest surface microhardness was Filtek Z350XT, and the lowest surface microhardness was Activa BioActive in both 1d and 42d, besides the surface microhardness of Filtek Z350XT, Equia Forte Fil and Ketac Universal were not significantly different at 42d. The surface microhardness of Activa BioActive, Beautifil II, Fuji II LC and Filtek Z350XT were decreasing from 1d to 42d. In contrast, the surface microhardness of conventional GIC (Equia Forte Fil and Ketac Universal), reached to the maximum microhardness at 7d and the surface microhardness relatively decreased and constant at 42d comparing the microhardness at 1d.

In artificial saliva storage, the highest surface microhardness was Filtek Z350XT, and the lowest surface microhardness was Activa BioActive in 1d, however, the surface microhardness of Equia Forte Fil and Ketac Universal were significantly higher than Filtek Z350XT at 42 d. The surface hardness of Activa BioActive, Beautifil II, Fuji II LC and Filtek Z350XT were decreasing from 1d to 42d, while the surface microhardness of conventional GIC was significantly increasing to 42d comparing to the microhardness at 1d.

Chemical analysis on the specimen surface

The qualitative analysis of the chemical element on the specimen surface at 1d was comparable to the materials composition according to the manufacturers' information, as shown in Figure 2 and Table 1. The elements analysis on the specimen surface revealed some additive elements from the artificial saliva storage at 42d comparing to in deionised water at 42d, as shown in Figure 2. The Equia Forte Fil was found an increased element of phosphorus, and calcium. The Ketac Universal and Fuji II LC were found an increased of phosphorus, calcium, potassium, and magnesium. The Activa BioActive was found an increased of phosphorus. The Filtek Z350XT was found an increase in potassium.

Nevertheless, the SEM-EDX disclosed other elements further from the manufacturers' information as to which; Sodium was found in Equia Forte Fil, Ketac Universal, Fuji II LC. Phosphorus was found in Equia Forte Fil. Lanthanum was found in Equia Forte Fil, Ketac Universal, Tungsten was found in Equia Forte Fil, Ketac Universal, Beautifil II, Fuji II LC, and Activa BioActive. Rhenium was found in Beautifil II and Fuji II LC.

Ion releasing in deionised water

The limit of quantitation (LOQ) of each ion analytic is the standard deviation of analysed ten duplicated blank solution samples and multiplied by the factor of ten, which the LOQ of each ion was on the following; Na (1.6 ppb), Si (1.5 ppb), Ca (2.8 ppb), Al (1.9 ppb), Mg (0.2 ppb), and P (13.9 ppb).

The qualitative analysis of ions releasing from ICP-OES is presented in Figure 3. The ions-releasing revealed that the Na ions releasing was high for GICs and RMGIC, while Si and Al ion releasing was high for RMGIC. Ca and Mg ions releasing was low for GIC. P ion releasing was low in all materials, except for Fuji II LC at 1d.

DISCUSSION

In this study, the surface microhardness of different bioactive materials stored in either deionised water or artificial saliva on various storage time up to 42d were investigated. The surface microhardness of GIC was constant after 42d in deionised water, while other materials decreased. Although the surface microhardness of GIC gradually increased after 42d in artificial saliva, other materials decreased. The null hypotheses that there was no difference in surface microhardness among the materials in different storage media and storage times must be rejected. The ANOVA statistical analysis revealed that all the factors among the material, the storage media and the storage time were significantly different to surface microhardness material, besides the statistic shown a significant interaction among all three factors. The present study was performed under the loading of 100g, which indicating microhardness only at the surface of the material.

The two conventional GICs surface microhardness were relatively constant after 42d in deionised water, while the surface microhardness increased significantly after 42d in artificial saliva. The result cooperated to the previous study that the surface hardness of GIC was stable for one year [18], which contrast to previous studies that the surface hardness of conventional GIC was increasing with the time either in distilled water and human saliva storage [6, 12, 19, 20]. The higher surface hardness of GIC could be the reason for material maturation [2]. The setting reaction of GIC is an acid-base reaction between polyacrylic acid and glass powder. The initial setting reaction occurs rapidly by forming of calcium polymatrix and continues for an extended time after initial setting. Both GICs stored in deionized water, the surface hardness values at 7d reached the maximum value, which was significantly higher than the surface hardness values at 1d. The maximum value at 7d can be explained by the hardening phase occurring after gelation phase that involves the continual formation of aluminium polymatrix. The hydrogel matrix consists of several ions such as aluminium, fluoride and polyacrylate ions which might be active in the setting process. The diffusion of ion on the surface and the ion attachment from the solution. The soluble ions from the surrounding solution can diffuse into the GIC structure [21-24]. Therefore, the deionized water was used in this study, the ion reattachment from the solution was absent, these might be a reason for the microhardness of GIC gradually decreased and constant in deionized water at 42d.

The higher surface microhardness of both GICs in artificial saliva storage than in deionised water was probably the result of artificial saliva components. SEM-EDX analysis revealed that the higher element including potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P) were found higher in artificial saliva than in deionised water on the surface of GICs. Thus, unreacted carboxylic acid remained in the cement matrix after the GIC setting [23]. The cations (K^{1+}, Ca^{2+}, and Mg^{2+}) from the artificial saliva could create a chemical bond with the unreacted carboxylic acid remaining in the matrix. Beside, polyacrylic acid forms crosslink with divalent metal ions [25], and calcium polyacrylate participated in the cross-linking of GIC structure can strengthen the GIC. Another study showed that calcium chloride in artificial saliva caused a considerable increase in the surface hardness of GIC [19], which calcium ion reacted with the carboxylic acid in the cement matrix and formed calcium polyacrylate matrix. Moreover, magnesium was found on the surface of Ketac Universal and Fuji II LC at the low amount after 42d in artificial saliva storage, which magnesium can also form a polymer with a polyacrylic acid group [25]. However, there was no previous study focusing on the role of magnesium on GIC surface and structure.

The surface microhardness of materials containing resin as in Filtek Z350XT, Beautifil II, and Fuji II LC decreased after 42d storage in both deionised water and artificial saliva, which the decreasing microhardness was correlated to previous studies on composite [6, 26] and RMGICs [27]. The main reason contributing to the surface microhardness reduction was the resin composition, which the water interacts with the inter-polymer chain [28]. The resin monomer in direct restorative material has never fully polymerized, which correlated to salivary and water sorption [29], and it is degrading with time. Thus, the water absorption in the resin, especially on the unpolymerized monomer, decreased the surface microhardness of material. Beautifil II and Filtek Z350XT contained Bis-GMA and TEGDMA as hydrophobic monomers, and these monomers enhanced the rate and degree of water sorption [30]. Bis-GMA is a standard monomer used in dentistry, however, Bis-GMA showed the lowest degree of conversion and highest water sorption among TEGDMA, UDMA, and Bis-EMA [31].

Besides, Fuji II LC contains HEMA as a hydrophilic monomer, the polar functional group in the HEMA polymeric chain is sensitive to water uptake [32]. This water uptake of RMGICs did not reach their equilibrium after one year [32]. Hence, water absorption affects the physical properties, including the surface hardness of RMGIC [27]. Moreover, HEMA monomer replacing water in liquid component affected the conformation of polyacrylic acid and possessed a lower dielectric constant than water [22], which retard the ion formation in the acid-base reaction [33]. These result in an inhibiting acid-base reaction [33, 34] and influence the hardening and strengthening of material. Moreover, the pre-reacted glass filler within Giomer structure produced an osmotic effect that influenced water sorption [5], which can affect the lower surface hardness. The previous study showed a significant increase in hardness value of Beautifil Bulk from 24h to 3m and maintained the hardness value up to 1 y in distilled water storage [12]. This contrary result could be the different hardness objective and the setting parameter as the present study was interested in only at the superficial surface of the material, which the low loading of 100g was performed. Thus the higher loading parameter might include the bulk material under the surface and the filler component.

Resin-modified glass ionomer cement (Fuji II LC) shown lower surface microhardness after 42d in both deionised water and artificial saliva. The result contrasted to previous studies that the surface hardness of Fuji II LC maintained up to one-year storage in distilled water [35] and artificial saliva [36]. The microhardness of Fuji II LC might be the influenced from calcium, phosphorus, potassium, and magnesium ions from artificial saliva, which these elements were found, on the specimen surface from SEM-EDX, higher in artificial saliva than in deionised water storage. These ions in artificial saliva can diffused or interacted with polyacrylic acid and performed a harder surface [24, 37]. Other ions influencing the microhardness were silicon, aluminium, and magnesium, which the ICP-OES revealed a high releasing at 21 and 42d. Besides, aluminium and silicon are the main components of the reactive ionomer glass fillers, the high releasing of silicon resulted in a weakening of the glass network on the material surface [19] and may soften the surface hardness of the material. Therefore, in this study, the resin component might play a majority influencing the decreasing microhardness of RMGIC in both storage media.

Activa BioActive showed the lowest surface microhardness, and the microhardness was decreasing with time in both storage mediums. The material contained UDMA and Bis (2(methacryloyloxy) Ethyl) phosphate as resin monomers in their component, which the resin polymer also absorb water and soften the surface microhardness. Another reason was the ion exchange between material and immersion mediums, according to the manufacturers' information, the material can release and recharge with calcium, phosphate and fluoride ions. These correspond to this study on SEM-EDX, which calcium, phosphate, fluoride, and aluminium element were detected on the surface at 42d higher in artificial saliva than in deionised water storage. Moreover, the ICP-OES revealed that the calcium ion releasing in deionised water at 21 and 42d was found higher than other material. The high calcium releasing could be relating to the high concentration of calcium and bioactive glass in the material component. Furthermore, the bioactive glass in Activa BioActive may form hydroxylcarbonate apatite formation at the surface of bioactive glass, when the bioactive glass exposes to the simulated body fluid solution [38], which, however, this apatite formation may result different to the present study. Therefore, the significantly decreasing surface hardness of Activa BioActive both in deionised water or in artificial saliva storage could mainly be related to the resin as the elements on the surface and the changing ions were a minor effect.

Nevertheless, in this study potassium, calcium, and magnesium in artificial saliva played an essential role that the higher surface microhardness of material stored in artificial saliva than in deionised water. Potassium is an element in group I in the periodic element table, which the property of group I elements is high reactivity and could easily produce a chemical reaction with the carboxylate group. Calcium ion created chemical interaction with the carboxylic acid in the cement matrix and formed calcium polyacrylate matrix [19], which similar to the chemical interaction between tooth substrate and polyacrylic acid in GIC and RMGIC [10]. Magnesium can also form an interaction with a polyacrylic acid group [25], however, the study on the influence of magnesium on surface hardness needs to be confirmed.

SEM-EDX showed several additional elements apart from the manufacturers' information (Figure 2). Barium (Ba) in Activa BioActive, which incorporates in the filler formulation for achieving material radiopacity [39]. Lanthanum (La) in Equia Forte Fil and Ketac Universal, which lanthanum is a trivalent metal that included in FAS glass fillers [40]. Rhenium (Re) in Beautifil II and Fuji II LC, and tungsten (W) in most materials, which rhenium and tungsten may be used in the conformal coating of filler and subsequently surface modified to provide a chemical bond between the filler and resin matrix [41].

CONCLUSION

This study investigated the surface microhardness of the restorative materials. The surface microhardness of conventional glass ionomer was stable in deionized water at 42d, although the microhardness of GICs gradually increased in artificial saliva at 42 d. Other materials including RMGIC, Giomer, composite, and Activa BioActive, the surface microhardness continually decreased in both media with the time. Phosphorus, calcium, potassium, and magnesium ions from artificial saliva played an essential role in GICs surface microhardness as these elements were found increasing higher on the material surface stored in artificial saliva than deionized water. Therefore, the low ions releasing of calcium, magnesium and phosphorus in deionized water might be role to maintain the surface microhardness of materials.

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FIGURE LEGENDS

Figure 1. The surface microhardness changing with the time in deionised water and artificial saliva. The calculated fitting lines were plotted for each material.

Figure 2. The bar graph presenting the analysis of the elements on the materials surface using SEM-EDX at 1d comparing to at 42d in deionised water and artificial saliva.

Figure 3. The bar graph is representing the concentration of the elements dissolving in deionised water at 1d, 21d and 42d.

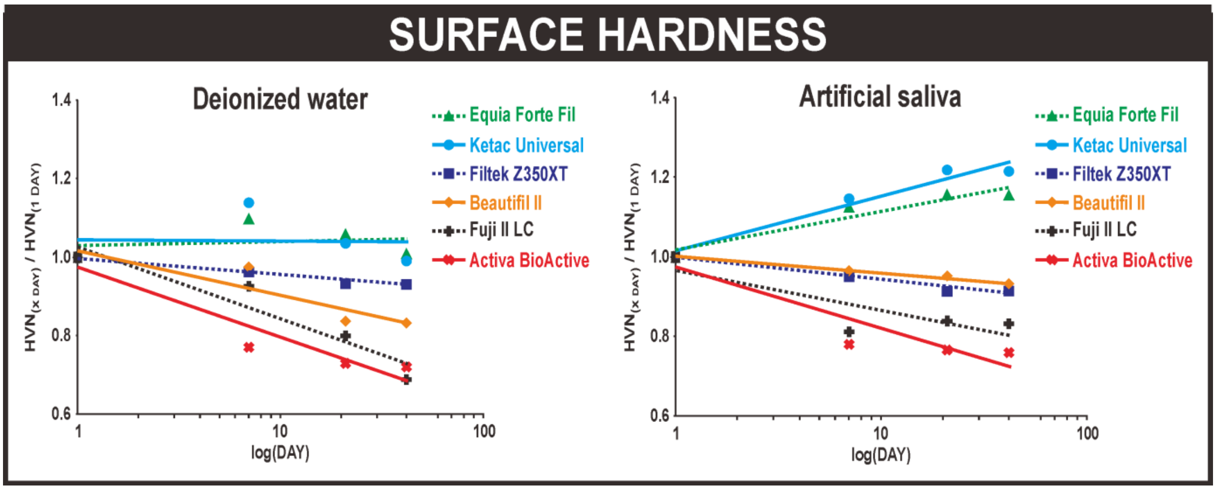

Figure 1: The surface microhardness changing with the time in deionised water and artificial saliva. The calculated fitting lines were plotted for each material.

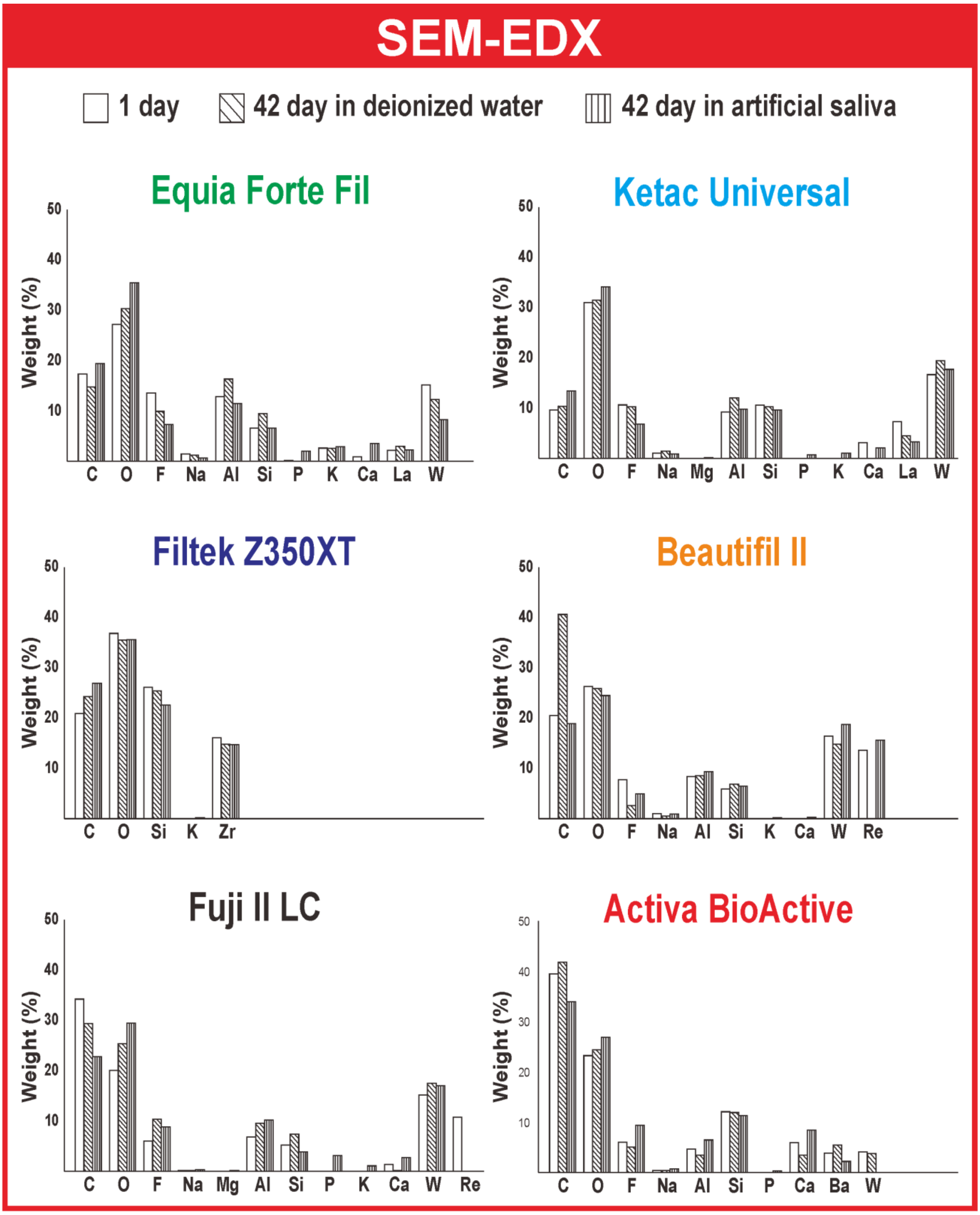

Figure 2. The bar graph presenting the analysis of the elements on the materials surface using SEM-EDX at 1d comparing to at 42d in deionised water and artificial saliva.

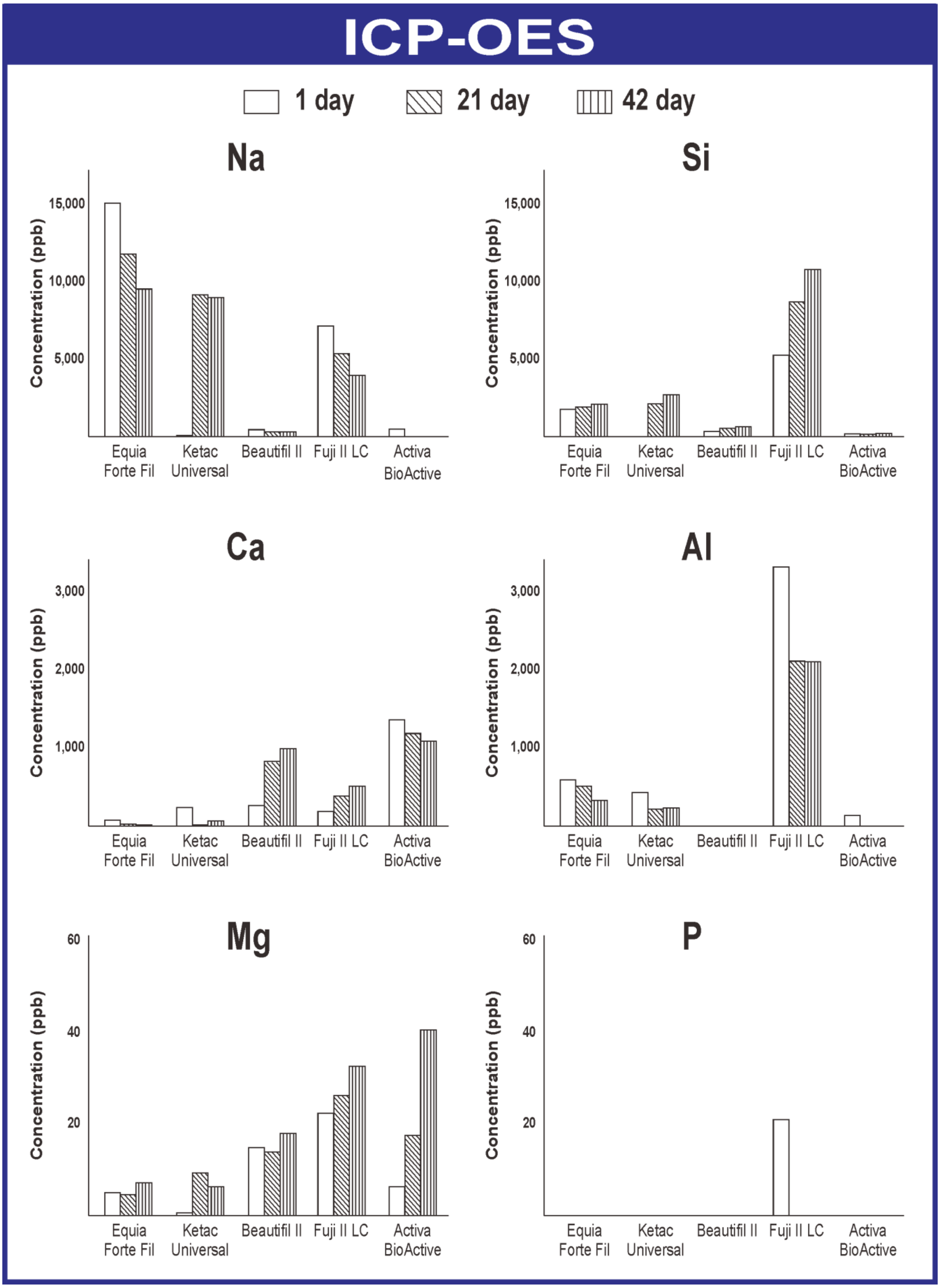

Figure 3. The bar graph is representing the concentration of the elements dissolving in deionised water at 1d, 21d and 42d.

TABLES

Table 1. Composition of the experimental materials.

Materials	Composition[a]
EQUIA® Forte Fil (capsule) (Batch no. 1606221) GC Corporation, Tokyo, Japan	Powder; Ultrafine highly reactive FAS glass, high molecular weight polyacrylic acid powder, Iron(III) oxide(CASRN: 1309-37-1) Liquid; Distilled water, Polybasic carboxylic acid, Tartaric acid(CASRN: 87-69-4)
Ketac™ Universal Aplicap™ (capsule) (Batch no. 637544) 3M, ESPE, St. Paul, MN, USA	Powder; Oxide glass chemicals(CASRN: 65997-17-3) Liquid; Water(CASRN: 7732-18-5), Copolymer of acrylic acid-maleic acid(CASRN: 29132-58-9), Tartaric acid(CASRN: 87-69-4), Benzoic acid(CASRN: 65-85-0)
Filtek™ Z350XT (Batch no. N827976) 3M, ESPE, St. Paul, MN, USA	Silane treated ceramic(CASRN: 444758-98-9), Silane treated silica(CASRN: 248596-91-0), Silane treated zirconia, UDMA, Bis-GMA, TEGDMA, Bis-EMA(6), PEGDMA
Beautifil II (Batch no. 121496) Shofu, The Alpha, Science Park II Singapore	Bis-GMA, TEGDMA, UDMA, Bis-MPEPP, Aluminofluoro-borosilicate glass(CASRN: 65997-18-4), Al_2O_3 (CASRN: 1344-28-1), DL-Camphorquinone(CASRN: 10373-78-1),
GC Fuji II LC ® (capsule) (Batch no. 1508211) GC Corporation, Tokyo, Japan	Powder; (Fluoro) alumino silicate glass (FAS Glass) Liquid; Distilled water(CASRN: 7732-18-5), UDMA, HEMA, 2-hydroxy-1,3 dimethacryloxypropane(CASRN:1830-78-0), Polyarcylic acid(CASRN: 9003-01-04), Tartaric acid(CASRN: 87-69-4), Camphorquinone(CASRN: 465-29-2),
Activa BioActive RESTORATIVE (Batch no. 150212) Pulpdent Corporation, Watertown, MA USA	UDMA, Bis (2-(Methacryloyloxy) Ethyl) phosphate, Barium glass, Ionomer glass, Polyacrylic acid/maleic acid copolymer, Dual-cure chemistry, Amorphous silica, Sodium fluoride

[a] Abbreviations: UDMA: Diurethane dimethacrylate (CASRN: 72869-86-4); Bis-GMA: Bisphenol A diglycidyl ether dimethacrylate (CASRN: 1565-94-2); TEGDMA: Triethylene glycol dimethacrylate (CASRN: 109-16-0); HEMA: 2-Hydroxyethyl methacrylate (CASRN: 868-77-9); Bis-EMA(6): Bisphenol A polyethylene glycol diether dimethacrylate (CASRN: 41637-38-1); PEGDMA: Polyethylene glycol dimethacrylate (CASRN: 25852-47-5).

Table 2. Composition of the artificial saliva.

Composition	Weight/Volume
Sodium chloride (NaCl)	0.798 grams
Potassium chloride (KCl)	1.2 grams
Calcium chloride ($CaCl_2$)	0.147 grams
Potassium dihydrogen phosphate (KH_2PO_4)	0.272 grams
Magnesium chloride hexahydrate ($MgCl_2.6H_2O$)	0.093 grams
Deionised water	990 millilitres

Table. 3 Mean surface Vickers hardness (Hv in kgf/mm^2 ± SD) of materials stored in **deionised water** at 1, 7, 21, and 42 days.

Materials	Time			
	1 day	**7 days**	**21 days**	**42 days**
Equia Forte Fil	79.2 ± 3.4^{Bb}	86.9 ± 6.0^{Ab}	$83.8 \pm 5.6A^{ABa}$	79.6 ± 2.8^{ABa}
Ketac Universal	83.8 ± 2.1^{Bb}	95.4 ± 1.8^{Aa}	86.7 ± 2.5^{Ba}	83.1 ± 3.0^{Ba}
Filtek Z350XT	90.3 ± 1.4^{Aa}	87.2 ± 1.7^{Bb}	84.5 ± 1.4^{Ca}	82.1 ± 1.5^{Ca}
Beautifil II	81.5 ± 1.5^{Ab}	79.6 ± 2.3^{Ac}	68.3 ± 10.5^{Bb}	68.0 ± 6.0^{Bb}
Fuji II LC	66.6 ± 4.1^{Ac}	61.6 ± 1.8^{Ad}	53.3 ± 5.2^{Bc}	46.0 ± 2.0^{Cc}
Activa BioActive	28.4 ± 1.9^{Ad}	22.0 ± 0.6^{Be}	20.8 ± 0.9^{Bd}	20.5 ± 0.3^{Bd}

[1]Different superscript capital or small letter indicates a statistically significant difference in the rows and columns, respectively.

Table. 4 Mean surface Vickers hardness (Hv in kgf/mm^2 ± SD) of materials stored in **artificial saliva** at 1, 7, 21, and 42 days.

Materials	Time			
	1 day	**7 days**	**21 days**	**42 days**
Equia Forte Fil	77.4 ± 3.5^{Bc}	86.9 ± 1.7^{Ab}	89.5 ± 4.3^{Ab}	89.1 ± 2.4^{Ab}
Ketac Universal	85.2 ± 3.2^{Bb}	97.7 ± 6.5^{Aa}	103.6 ± 5.3^{Aa}	103.3 ± 4.2^{Aa}
Filtek Z350XT	90.2 ± 0.9^{Aa}	85.5 ± 2.0^{Bb}	82.3 ± 1.1^{Cc}	83.3 ± 1.7^{BCc}
Beautifil II	81.3 ± 1.6^{Abc}	78.0 ± 1.7^{Bc}	77.4 ± 1.8^{Bc}	75.7 ± 1.5^{Bd}
Fuji II LC	70.6 ± 4.3^{Ad}	57.3 ± 3.1^{Bd}	59.3 ± 3.9^{Bd}	58.6 ± 4.9^{Be}
Activa BioActive	28.4 ± 1.2^{Ae}	22.2 ± 0.4^{Be}	21.8 ± 1.0^{Be}	21.6 ± 1.4^{Bf}

[1]Different superscript capital or small letter indicates a statistically significant difference in the rows and columns, respectively.

ภาคผนวก 4

(Appendix 4)

A CLINICAL STUDY ON FACTORS INFLUENCING SUCCESS AND FAILURE OF NCCLS RESTORATIONS

Wanchanok Saengnil[1], Munlika Anuntasainont[1], Natchalee Srimaneekarn[2], Vesna Miletic[3] and Pong Pongprueksa[1]

Wanchanok Saengnil, DDS, Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, Thailand; 6 Yothi Rd., Ratchathewi, Bangkok 10400, Thailand. E-mail: benzz_15@hotmail.com

Munlika Anuntasainont, DDS, Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, Thailand; 6 Yothi Rd., Ratchathewi, Bangkok 10400, Thailand. E-mail: meuw19th@gmail.com

Natchalee Srimaneekarn, DDS, MSc, PhD, Department of Anatomy, Faculty of Dentistry, Mahidol University, Thailand; 6 Yothi Rd., Ratchathewi, Bangkok 10400, Thailand. E-mail: snatchalee@yahoo.com

Vesna Miletic, BDS, MSc, PhD, DentalNet Research Group, School of Dental Medicine, University of Belgrade, Serbia; Rankeova 4, Beograd, Belgrade 11000, Serbia. E-mail: vesna.miletic@stomf.bg.ac.rs

Pong Pongprueksa, DDS, MSc, PhD, Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, Thailand; 6 Yothi Rd., Ratchathewi, Bangkok 10400, Thailand. E-mail: pong.pon@mahidol.edu

Corresponding author: **Pong Pongprueksa**, Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, Thailand, 6 Yothi Rd., Ratchathewi, Bangkok 10400, Thailand. TEL: +66-96-745-9977, E-mail: pong.pon@mahidol.edu

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ABSTRACT

INTRODUCTION: The adhesively bonded restoration has been a treatment of choice for NCCLs, while several factors relating to the clinical success and failure of restoration. An intrinsic factor that depended on the patient, while an extrinsic factor that depended on dentist.

OBJECTIVES: This retrospective study was to evaluate the factors influencing the clinical success and clinical failure of non-carious cervical lesions (NCCLs) restorations.

METHODS: Patients were routinely treated by undergraduate or postgraduate students and randomly received a clinically recall evaluation. A retrospective study was performed with two experienced calibrated examiners for evaluating NCCLs restorations, which included the key parameters of retention, caries, marginal discoloration, and marginal integrity. The factor related to the restoration was included; gender, age, arch site, tooth position, occlusion, wear, caries risk, operator, adhesive, and composite. The clinical success in the retentive restoration was analyzed using the Kaplan-Meier test. The clinical failure comparison between the key parameters and factors was performed using the Pearson Chi-square or Fisher's exact test with Bonferroni correction.

RESULTS: 460 cervical restorations from 96 patients were evaluated. The clinical retention success was 94.1% for the 2-step etch-and-rinse, 95.7% for the 2-step self-etching adhesive, and 99.5% for the selective enamel etching with phosphoric acid prior 2-step self-etching application at 5 years. The factors influencing clinical failure were depended on the failure parameter, while the adhesive was the factor most influencing the parameters. However, the highest failure parameter was marginal integrity, besides; the marginal integrity failure was 50.7% at the gingival margin and 42.4% at enamel margin.

CONCLUSIONS: The clinical success of NCCLs on survival restoration was not significant depending on the operator and adhesive. However, the main factor influencing the clinical failure of restoration was the adhesive, operator, and the presence of wear facets. Therefore, the marginal integrity was the highest frequently failure parameter.

Clinical relevance: Operator experience and adhesive were not the main factor influencing the clinical success, however, the operator and adhesive factor influenced the clinical failure parameter. Postgraduate student and phosphoric acid etching on the enamel prior 2-step self-etching adhesive performed less clinical failure on NCCLs restoration. Composite was not a factor influencing the clinical success and failure of NCCLs restoration.

INTRODUCTION

An etiology of non-carious cervical lesions (NCCLs) is multifactorial in terms of origins (erosion, abrasion, and abfraction), often initiates by a single factor, but then accumulates the severity through other factors [1]. A recently favorable treatment option for NCCLs includes the placement of an adhesive restoration [2, 3], which the factors are associated with the long-term clinical success and failure of NCCLs restoration including intrinsic factors; patient (behavior) [4], tooth (tooth type, position, occlusion) [5, 6] and extrinsic factors; operator (experience) [7, 8], material (adhesive) [2]. Besides, the extrinsic factor is the most consequential to the clinical success and failure [7, 8].

Moreover, the NCCLs are slowly progressive on the microstructure, leading to a hypermineralized or sclerotic dentin formation to protect the pulpal structure. This microstructure formation presents a higher mineral deposition compared to the sound dentin [9]. Therefore, adhesion to the hypermineralized dentin is less effective compared to the sound dentin as the presence of acid-resistant hypermineralized structure and the obliteration of dentinal tubules prevent acidic penetration into the hybrid layer, thereby reducing the quantity and quality of the hybrid layer and resin tags [10]. Besides, a clinical suggestion for restoration to improve the bonding effectiveness on sclerotic dentin is included a roughened dentin surface with a diamond bur [3] or a phosphoric acid pretreatment on dentin [10]. On the other hand, a recent study recommended that the hypermineralized dentin was unnecessary to remove as this affected the chemical interaction between functional monomers (10-MDP) and the hypermineralized layer of dentin [11].

Regarding the recently adhesive system, a dental adhesive system is classified into two major mode systems: etch-and-rinse mode (E&R) and self-etching mode (SE) [12]. Thus, the best clinical effectiveness of adhesives for the NCCLs restoration was for 2-step self-etching (2-SE) following by 3-step Etch-and-Rinse (3-E&R), 1-step self-etching (1-SE) and 2-step Etch-and-Rinse (2-E&R) [13]. In addition, the use of selective enamel etching with phosphoric acid prior to the application of a self-etching adhesive, so call “selective enamel etching technique”, was recommended for improving the efficiency of self-etching adhesives on enamel margin for the long-term clinical success [2, 13, 14]. Nevertheless, a previous study showed that 2-E&R was a higher technique sensitive than 2-SE adhesive for undergraduate students [7], which in contrast to a previous study that 1-SE was a higher technique sensitive than 3-E&R adhesive for undergraduate student [8].

Therefore, the objectives of this study were to investigate the factors which influence the clinical success and failure of NCCLs restorations in different parameters.

MATERIALS & METHODS

Patients were referred to the Operative Dentistry clinic, Mahidol University, Thailand, for the treatment of NCCLs with composite. The lesions were routinely cleaned with pumice and standardly isolated with cervical retraction cord, cotton rolls, and saliva ejector. The adhesives were applied strictly according to the manufacturer instructions, all these procedures were treated under the supervision of instructors. The patient has commonly received a recall examination every 1 year; therefore, the retrospective clinical randomized study was performed between May 1, 2018, and October 31, 2018. The clinical trial protocol was approved by the Center of Ethical Reinforcement for Human Research of the Mahidol University (COA MU-DT/PY-IRB 2018/025.3004). Two-experienced clinical examiners were calibrated to evaluate NCCLs restoration using the modified USPHS criteria, which included an assessment of retention, caries, marginal discoloration, and marginal integrity (Table 1). The inter-examiner agreement was assessed using Cohen's Kappa test at 0.85, which was performed by the naked eye and a dental explorer. In case of disagreement between the investigators, a consensus was reached by reexamination and discussion.

The inclusion criteria were: (1) NCCLs restoration was involved occlusal enamel margin and gingivally dentin margin of vital teeth, (2) the restoration cavosurface margin was not involved more than 50% of tooth height and (3) the restoration was not involved mesial and distal surfaces. The exclusion criteria were: (1) the patient with uncontrolled systemic disease, (2) the controlled systemic disease was influencing salivary flow rate, (3) the patient with orthodontic appliance treatment, (4) the tooth which was for an abutment for fixed or removable prostheses and (5) the restoration was included base or liner materials on the recorded.

The data was collected as possible prediction risk factors for a longevity restoration as the following: gender, age, arch site (upper or lower), tooth position (Quadrant1,2,3,4), tooth type (anterior, premolar, or molar), occlusion (denture, fixed prostheses, natural teeth, or edentulous), occlusal wear facet (present or absent), caries risk (high, moderate, low), operator (undergraduate or postgraduate student), adhesive (types), and composite (types). Besides, the caries risk assessment was evaluated following the ADA guideline for caries risk assessment [15].

The restoration received a score of Alpha, Bravo, and Charlie for the category of the modified USPHS criteria as the key parameters (restoration loss, partial retention loss, caries, marginal discoloration, and marginal integrity) as shown in Table 1. When the restoration was a category on Bravo and Charlie, the restoration was adjusted as clinical failure. The failure restoration was managed for refurbishment or repair or replacement following the clinical management guidelines [16], and their lifespan was defined as the period from the date of the initial treatment to the date of examination.

The sample size was calculated with one population proportion for comparing with the reference value (two-sided) [17], where a significance level of $\alpha = 0.05$, the desire power of 90% at $\beta = 0.10$, the reference value of $p_0 = 0.90$, and the proportion of $p = 0.94$. Therefore, the minimum sample size (n) was 371 restorations.

The survival rate of the retentive restoration success parameter was analyzed using the Kaplan-Meier test. The statistical comparison between the results of factors and key clinical failure parameters was performed using the Pearson Chi-square or Fisher's Exact test at a significance level of 5% ($p<0.05$). Odd ratio was used to compare to the relationship between each factor and each parameter with Bonferroni correction. Cohen's Kappa statistic was used for testing conformity between two examiners and the reliability of each examiner.

RESULTS

Data was collected on 460 NCCLs restorations from 96 patients with 36.1% of men and 63.9% of women. The average patient age was 60.9 years old (37 - 83 years) and the average longevity restoration period was 38.3 months (1 - 10 years). The restorative tooth was distributed at 53% upper teeth, 47% lower teeth, 28.1% anterior teeth, and 71.9% posterior teeth. The use of adhesive was found at 40% of 2-E&R (Single Bond 2; 3M ESPE, Seefeld, Germany, Optibond Solo Plus; Kerr, Orange, CA, USA and ExciTE F; Ivoclar-Vivadent, Schaan, Liechenstein), 20.2% of 2-SE adhesive (Clearfil SE bond; Kuraray Noritake, Tokyo, Japan) and 39.6% of selective enamel etching (phosphoric acid conditioning at enamel prier the Clearfil SE bond application). The use of composite was found at 70% of Filtek Z350 (3M ESPE, Seefeld, Germany), 26.1% of Estelite Sigma Quick (Tokuyama, Tokyo, Japan), and 3.5% of Filtek Z250 (3M ESPE, Seefeld, Germany). The result is presented in Tables 2 and 3. The Cohen's kappa statistical analysis for standardized two examiners was 0.95 for interexaminer and 0.96 for intraexaminer. Therefore, the clinical failure on restoration loss, partial retention loss, marginal discoloration, and marginal integrity parameters were influenced by various factors, while the caries parameter failure was not influenced by any specific factor.

The clinical success rate on retentive restoration, including Alpha and Bravo (A+B), is presented in Figures 1 and 2, while the clinical failure on restoration loss, indicating Charlie (C), is presented in Table 2. These clinical restoration success (A+B) and restoration loss parameter (C) were directly referring to the effectiveness of adhesive. The clinical failure parameter on caries is presented in Table 2, indicating in Charlie categories. Other clinical failure parameters: partial retention loss, marginal discoloration, and marginal integrity are presented in Tables 2 and 3, including Bravo and Charlie categories (B+C).

Survival restoration

The overall clinical success on survival retentive restoration (A+B) among the different adhesives is presented with Kaplan Meier survival analysis in Figure 1a. The survival analysis of a cumulative clinical survival restoration of adhesive mode systems to the survival time in all operators was plotted. The survival restoration rate was accepted for all adhesives to the ADA guideline, in which the clinical restoration survival rate was higher than 90% at 18 months. The overall survival rate from all adhesives was shown not significantly different at p=0.447. The clinical restoration survival rate on the operators in different adhesives was separately presented, the plotting graph for undergraduate student (Figure 1b) was indicated the higher survival restoration trend for selective enamel etching and 2-SE to 2-E&R, however, the result was not statistically significantly different at p=0.606. Moreover, the plotting graph for the postgraduate student (Figure 1c) was shown not statistically different in all adhesives to the clinical restoration success at p=0.253. Besides, the clinical success for survival retentive restoration between the operators with Kaplan Meier survival analysis is presented in Figure 2, which was not a statistically significant difference between the operators at p=0.428.

Restoration loss

The clinical failure on the restoration loss (C) was related to the presence of wear facets (p=0.03) and the adhesive (p=0.017) as shown in Table 2. For the adhesive factor, the odd ratio statistic in the Table 4 revealed that the higher failure restoration loss was found significantly for the 2-E&R adhesive to the selective etching technique at p=0.004, besides, the odd ratio statistical analysis needs to be used for interpretation at p=0.017, which was derived from taking the p-value at 0.05 dividing by 3; from 3 times calculating of 3 pairs according to the types of usage.

Partial retention loss

The clinical failure on partial retention loss (B+C) was separately evaluated on the failure at the occlusal margin and gingival margin. The occlusal partial retention loss was significantly affected by the operator and adhesive factors at p=0.05 and p=0.003, respectively (Table 2). The undergraduate student was significantly higher partial retention loss failure at the occlusal margin than the postgraduate student. The 2-E&R adhesive was significantly higher occlusal partial retention loss than selective etching technique at p=0.001

(Table 4), regarding the odd ratio statistical analysis. The gingivally partial retention loss was related to the patient gender (p=0.005) and the presence of wear facet (p=0.001) as shown in Table 2.

Marginal discoloration

The clinical failure on marginal discoloration (B+C) was separately observed at the occlusal and gingival margin. The occlusal marginal discoloration was only related to the adhesive factor (p<0.001), in which the adhesive failure at the occlusal marginal discoloration was significantly higher for 2-SE adhesive than selective etching adhesive at p<0.001 (Table 4), regarding the odd ratio statistical analysis. The gingivally marginal discoloration failure was related to gender (p=0.01), wear facets (p=0.001), caries risk (p=0.007), operator (p=0.021) and adhesive (p=0.01) as presented in Table 3. The adhesive failure at gingivally marginal discoloration was significantly higher for 2-E&R and 2-SE compared to selective enamel etching technique at p=0.005 and p=0.009, respectively (Table 4).

Marginal integrity

The clinical failure on marginal integrity (B+C) was separately observed at the occlusal and gingival margin, this marginal integrity failure was the worst failure parameter related to several factors. The occlusal marginal integrity failure was found at 42.4% to the total restoration, while the gingivally marginal integrity failure was found at 50.7%. The occlusal marginal integrity failure was related to the operator (p=0.004) and adhesive (p<0.001), while the gingivally marginal integrity failure was related to gender (p=0.004), occlusion (*p*=0.002), the presence of wear facets (*p*<0.001), caries risk (*p*<0.001), operator (*p*=0.001) and adhesive (*p*=0.016) as presented in Table 3.

DISCUSSION

This retrospective randomized clinical study has revealed the factors influencing the clinical success and failure of the NCCLs restoration, which is performed by undergraduate and postgraduate students. The adhesive was comprised of 2-E&R (Single Bond 2; 3M ESPE, Optibond Solo Plus; Kerr, and ExciTE F; Ivoclar Vivadent), 2-SE adhesive (Clearfil SE bond; Kuraray) and the selective enamel etching was performed with 37% phosphoric acid at enamel margin followed by Clearfil SE bond application to the entire cavity. The composite was included two nanofilled composites (Filtek Z350; 3M ESPE and Estelite Sigma Quick; Tokuyama) and a microhybrid composite (Filtek Z250, 3M ESPE). Therefore, the retrospective clinical success on the survival NCCLs restoration was not influenced either on the operator or adhesive. However, the clinical failure was influenced by several factors: gender, occlusion, the presence of wear facets, caries risk, operator, and adhesive.

This retrospective study revealed the success and failure of NCCLs restorations, which had been treated by several undergraduate and postgraduate students, which represented the actual completion of NCCLs restoration in a general dental treatment. Besides, the several operator treatments are provided valuable information since the skill of dentist is an important factor in the clinical success on restoration [7, 8]. On the contrary, the most prospective studies, which were including only a few operators [2, 7, 8, 14], which might have been represented for the general dentists. Nevertheless, the limitation of the retrospective study is lacking information on the original lesion, while the prospective study is better to control and more information on the lesion, material, and treatment procedure.

The clinical success on the NCCLs restoration in the present study (Figure 1 and 2) was satisfied compared to the American Dental Association (ADA) guideline [18], which the restorative survival retention rate should be more than 95% after 6 months, and 90% after 18 months follow up. However, the result was on the expectation since most of the modern adhesives were accomplished this guideline as presented in the meta-analysis study on the clinical effectiveness of adhesives [13, 19].

The clinical failure in the present study was not influenced by some intrinsic factors: age, tooth position, and an extrinsic factor on the composite to any parameters (Tables 2 and 3). The tooth positions on the arch (upper, lower), position (Q1, Q2, Q3, Q4), and tooth type (anterior, premolar, molar) were not influenced by any failure parameters. It was contrasted to a recent meta-analysis study [5], in which the anterior NCCLs restoration was a higher clinical success than posterior restoration. The observing composite in this study was included in two nanofilled composites at 96.5% and a microhybrid composite at 3.5%, which is a huge difference between composites. However, the composite factor was not influenced by any clinical failure parameters, which is following a previous clinical study that hybrid and micro-filled composites performed with the same clinical success [20]. The composite swelling and the wear resistance can be a cause of marginal discoloration and marginal integrity [21], according to the composite swelling relating to the water sorption and water solubility, which consequently depend on the resin matrix and the monomer polymerization [21-23]. Thus, the physical and mechanical properties of the recent nanocomposite, nanohybrid composite, and microhybrid composite are hardly different [24, 25].

The intrinsic factors influencing the clinical failure included: gender, occlusion, the presence of wear facets and caries risk, while the extrinsic factors included: operator and adhesive. Gender was related to most of the failure parameters (partial retention loss, marginal discoloration, and marginal integrity) specifically at the gingival margin, which might be generally better oral hygiene for women than men.

Occlusion only related to the restoration failure on marginal integrity at the gingival margin. Denture opposing to the NCCLs restoration indicated the most failure incidence among the natural tooth and the edentulous area (Table 4). The denture teeth present more occlusal contact, which exhibits a high occlusal loading and stress to the gingival margin of restoration. The previous study showed the relationship between high occlusal force particularly on the buccal cusp and NCCLs at the buccal surface, besides the occlusal guidance scheme for group function showed a higher incidence of NCCLs compared to canine guidance [26]. This high occlusal force from the denture created higher tensile stress in the cervical tooth area, consequently generated a high gingivally defect on the marginal integrity of restoration [27].

The presence of occlusal wear facet was an intrinsic factor associated with the most clinical failure within the restoration loss, partial retention loss, marginal discoloration, and marginal integrity particularly at the gingival margin. The result corresponded to a previous study that found higher staining at the gingival margin with the wear facet appearance [28]. The reason could be that the presence of occlusal wear facet is related to the etiology progression of NCCLs, which concluding the higher incidence of the lesion progression as the high stress and strain forces are concentrated in the cervical area with the presence of occlusal wear facet [6, 27, 29].

Caries risk assessment was associated with the failure at the gingival margin for marginal discoloration and marginal integrity (Table 3). The high caries risk was a significantly higher failure to the low caries risk on marginal discoloration and marginal integrity at the gingival margin, while moderate caries risk was a significantly higher failure to low caries risk on the marginal integrity at the gingival margin. Dental caries is generally accumulated at the gingival margin, which certainly causes more defects at the gingival margin. Thus, bacterial biofilm at the gingival margin produced acid to destroy a tooth structure and created a marginal gap [30], which consequently associating to create a defect at the gingival margin of restoration.

Operator as an extrinsic factor, including the undergraduate and postgraduate students represented in the different skills of operators. In the present study, the skill of operators was not influenced by the failure of restoration loss and caries parameter. Nevertheless, undergraduate students performed a significantly higher failure on partial retention loss at the occlusal margin, marginal discoloration at the gingival margin, and marginal integrity at both occlusal and gingival margin. Besides, the data revealed that the undergraduate student chose 2-E&R (46.9%), selective etching (30.8%), and 2-SE (22.3%), while postgraduate students preferred selective etching (77%), 2-E&R (11.5%) and 2-SE (11.5%). Thus, 2-E&R adhesive was preferred by the undergraduate student than the postgraduate student, whereas the 2-E&R adhesive was prone to salivary contamination than selective etching adhesives at the gingival margin [31]. Moreover, the postgraduate student is more experience in managing moisture control, restorative material, and polishing skill than undergraduate students. This result was following a previous study that showed a higher success rate of restoration marginal integrity in the experienced dentist compared to dental students [7, 8].

The adhesive was the most consequential factor related to restorative failures excepted for caries in this study. Adhesive comparison is presented in Table 4, representing a non-statistically different clinical failure restoration between 2-E&R and 2-SE adhesive. The result was in contrast to a previous clinical study [32] and a systematic review study [13], which concluded that the 2-SE performed better clinical effectiveness than the 2-E&R adhesive. Therefore, the present study, three 2-E&R adhesives (Single Bond 2, Optibond Solo Plus, and ExciTE F) were involved, whereas these adhesives were reliable products as it was a low annual failure rate in the systematic review study [13].

The selective enamel etching was the most favorable adhesive relative to the significantly lowest clinical failure on various parameters (Table 4). The selective etching was less failure than 2-E&R in most parameters (restoration loss, partial retention loss, marginal discoloration, and marginal integrity), which can be the result of phosphoric acid etching on dentin for E&R adhesive comparing to a less aggressive of acidic functional monomer (10-MDP) for Clearfil SE bond priming on dentin [33]. Therefore, the 10-MDP functional monomer chemically interacts with the calcium of hydroxyapatite on dentin, forming a 10-MDP-Ca salts formation, which low water-soluble structure. This stable structure is expected to contribute to the hybrid layer and adhesive layer, which improving clinical longevity of the adhesively bonded restoration [12]. Moreover, selective enamel etching was less restorative failure than 2-SE adhesive only in terms of the marginal discoloration and marginal integrity particularly at occlusal enamel margin, which could be associated with phosphoric acid etching at the enamel margin. This result was associated to the previous studies, which selective enamel etching and 2-SE adhesive were no significant differences in clinical performance on the short period [14, 34], while the selective enamel etching performed only a minor positive effect on marginal integrity and marginal discoloration on the long-term study [2, 35].

CONCLUSION

The clinical success of NCCLs restoration on the survival restoration was not influenced by the operator and adhesive, however, the adhesive might influence the clinical success on the longevity of the restoration. The factor influencing clinical failure was the presence of wear facets, caries risk, operator, and adhesive factor, while the most influencing factor to the clinical failure was adhesive. Thus, additional etching on the enamel margin prior 2-step self-etching adhesive application resulted in reducing the clinical failure at marginal discoloration and marginal integrity of the restoration. Therefore, the marginal integrity was the most frequent failure parameter for NCCLs restoration.

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FIGURE/TABLE LEGENDS

Figure 1. Kaplan-Meier survival analysis related to the restoration survival rate and the different adhesive systems. (a) Overall survival rate restoration of the different adhesive systems. The red dot line is the ADA guideline for survival restoration after 6 and 18 months. (b) Survival restoration of the different adhesive systems treated by undergraduate students. (c) Survival restoration of the different adhesive systems treated by postgraduate students.

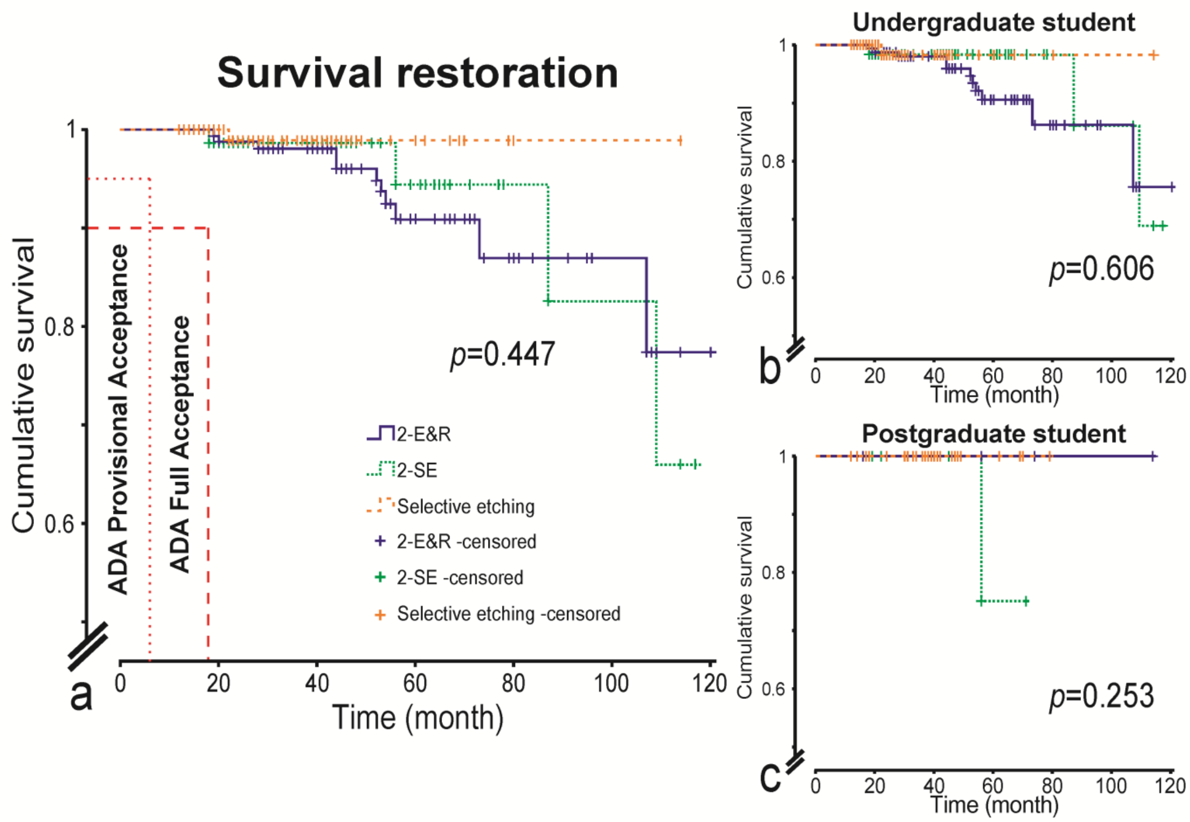
Figure 2. Kaplan-Meier survival analysis related to the restoration survival rate and the different operators.

Table 1. Key parameters determining the overall clinical success rate using modified USPHS criteria**.**

Table2. Distribution of the restorations, restoration loss, partial retention loss and caries failure parameters related to various factors presented in percentage and in number (between brackets).

Table3. Distribution of the marginal discoloration and marginal integrity failure related to various factors presented in percentage and in number (between brackets).

Table4. Odd ratio of the restorations failure parameters on various factors**.**



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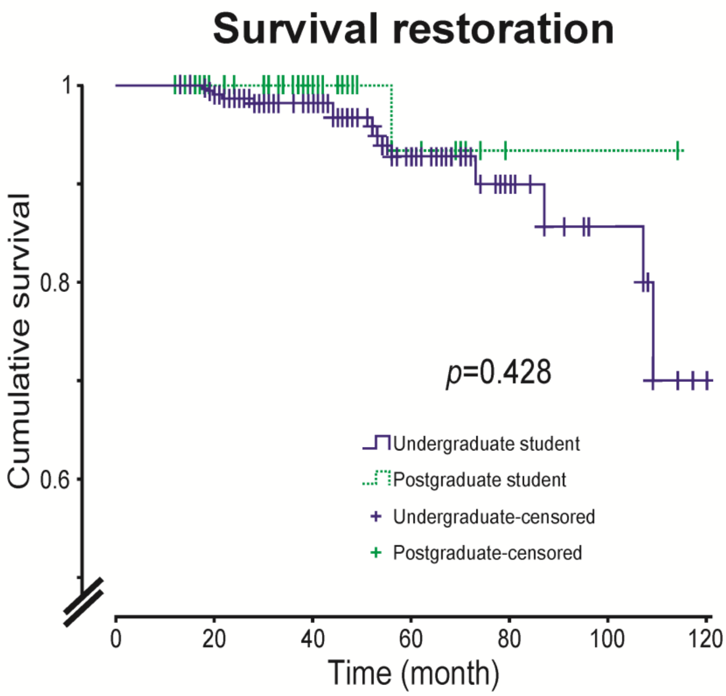

Figure 2. Kaplan-Meier survival analysis related to the restoration survival rate and the different operators.

TABLES

Table 1. Key parameters determining the overall clinical success rate using modified USPHS criteria**.**

Retention	A	Good retention
	B	Partially dislodged restoration
	C	Totally dislodged restoration
Caries	A	No caries present
	C	Caries present
Marginal discoloration	A	No discoloration
	B	Discoloration without axial penetration, can be removed by polishing
	C	Discoloration with penetration in pulpal direction, cannot be removed by polishing
Marginal integrity	A	The restoration appeared to adapt closely to the tooth
	B	Explorer penetrates, edge of the restoration does not adapt closely to the tooth structure, dentin or base are not exposed
	C	Explorer penetrates, crevice in which dentin is exposed

A=Alpha, B=Bravo, C=Charlie

Table2. Distribution of the restorations, restoration loss, partial retention loss and caries failure parameters related to various factors presented in percentage and in number (between brackets).

Factor		Teeth	Restoration loss (C)	*p*-value	Partial retention loss (B+C)				Caries	*p*-value
					Occlusal	*p*-value	Gingival	*p*-value		
Gender	Male	**36.1**(166)	**3.6**(6)	0.905	**9.6**(16)	0.124	**13.9**(23)	0.005*	**0.6**(1)	0.544
	female	**63.9**(294)	**3.4**(10)		**5.8**(17)		**6.1**(18)		**1.0**(3)	
Age	15-59 years	**50.0**(230)	**3.5**(8)	1.0	**7.0**(16)	0.857	**7.8**(18)	0.413	**0.9**(2)	0.688
	≥60 years	**50.0**(230)	**3.5**(8)		**7.4**(17)		**10.0**(23)		**0.9**(2)	
Arch	Upper	**53.0**(244)	**2.0**(5)	0.075	**5.3**(13)	0.103	**7.0**(17)	0.12	**0.8**(2)	0.641
	Lower	**47.0**(216)	**5.1**(11)		**9.3**(20)		**11.1**(24)		**0.9**(2)	
Position	Q1	**28.0**(129)	**2.3**(3)	0.317	**7.0**(9)	0.144	**6.2**(8)	0.156	**1.6**(2)	0.263
	Q2	**25.7**(118)	**1.7**(2)		**3.4**(4)		**8.5**(10)		**0**(0)	
	Q3	**23.5**(108)	**5.6**(6)		**7.4**(8)		**7.4**(8)		**0**(0)	
	Q4	**22.8**(105)	**4.8**(5)		**11.4**(12)		**14.3**(15)		**1.9**(2)	
Tooth type	Anterior	**28.1**(129)	**6.2**(8)	0.107	**8.5**(11)	0.354	**10.9**(14)	0.387	**1.6**(2)	0.49
	Premolar	**54.1**(249)	**2.0**(5)		**5.6**(14)		**7.2**(18)		**0.8**(2)	
	Molar	**17.8**(82)	**3.7**(3)		**9.8**(8)		**11.0**(9)		**0**(0)	
Occlusion	Denture	**12.7**(59)	**3.4**(2)	0.926	**6.8**(4)	0.716	**15.3**(9)	0.174	**1.7**(1)	0.830
	Fixed	**8**(37)	**2.7**(1)		**2.7**(1)		**13.5**(5)		**0**(0)	
	Natural	**76.1**(354)	**3.7**(13)		**7.6**(27)		**7.3**(26)		**0.8**(3)	
	Edentulous	**2.2**(10)	**0**(0)		**10.0**(1)		**10.0**(1)		**0**(0)	
Wear facets	Yes	**61.5**(283)	**4.9**(14)	0.03*	**8.8**(25)	0.081	**12.4**(35)	0.001*	**1.1**(3)	0.501
	No	**38.5**(177)	**1.1**(2)		**4.5**(8)		**3.4**(6)		**0.6**(1)	
Caries risk	High	**7.0**(32)	**6.3**(2)	0.652	**15.6**(5)	0.133	**12.5**(4)	0.117	**0**(0)	0.813
	Moderate	**75.6**(348)	**3.2**(11)		**6.9**(24)		**9.5**(33)		**0.9**(3)	
	Low	**17.4**(80)	**3.8**(3)		**5.0**(4)		**5.0**(4)		**1.3**(1)	
Operator	Undergraduate	**81.1**(378)	**4.0**(15)	0.16	**8.3**(31)	0.05*	**9.9**(37)	0.081	**0.8**(3)	0.569
	Postgraduate	**18.9**(87)	**1.1**(1)		**2.3**(2)		**4.6**(4)		**1.1**(1)	
Adhesive	2 step E&R	**40.2**(185)	**5.9**(11)	0.017*	**11.4**(21)	0.003*	**12.4**(23)	0.087	**1.1**(2)	0.6
	2 step SE	**20.2**(93)	**4.3**(4)		**8.6**(8)		**7.5**(7)		**0**(0)	
	Selective etching	**39.6**(182)	**0.5**(1)		**2.2**(4)		**6.0**(11)		**1.1**(2)	
Composite	Filtek Z350	**70.4**(324)	**2.8**(9)	0.22	**6.2**(20)	0.378	**9.0**(29)	0.419	**0.3**(1)	0.081
	Estelite Sigma Quick	**26.1**(120)	**5.8**(7)		**10.0**(12)		**10.0**(12)		**2.5**(3)	
	Filtek Z250	**3.5**(16)	**0**(0)		**6.3**(1)		**0**(0)		**0**(0)	
Overall	Total	**100**(460)	**3.5**(16)		**7.2**(33)		**8.9**(41)		**0.9**(4)	

*Association between the factor of the restoration and the parameter failures; retention and caries (p-value<0.05, Pearson chi-square or Fisher's exact test)

Table3. Distribution of the marginal discoloration and marginal integrity failure related to various factors presented in percentage and in number (between brackets).

Factor		Marginal discoloration (B+C)				Marginal Integrity (B+C)			
		Occlusal	***p*-value**	**Gingival**	***p*-value**	**Occlusal**	***p*-value**	**Gingival**	***p*-value**
Gender	Male	**15.1**(25)	0.721	**15.1**(25)	0.01*	**47.6**(79)	0.09	**59.6**(99)	0.004*
	Female	**16.3**(48)		**7.5**(22)		**39.5**(116)		**45.6**(134)	
Age	15-59 years	**16.1**(37)	0.898	**9.6**(22)	0.644	**38.7**(89)	0.109	**48.3**(11)	0.305
	≥60 years	**15.7**(36)		**10.9**(25)		**46.1**(106)		**53.0**(122)	
Arch	Upper	**14.3**(35)	0.341	**7.8**(19)	0.067	**43.9**(107)	0.5	**49.6**(121)	0.628
	Lower	**17.6**(38)		**13.0**(28)		**40.7**(88)		**51.9**(112)	
Position	Q1	**15.5**(20)	0.819	**8.5**(11)	0.429	**42.6**(55)	0.817	**53.5**(69)	0.426
	Q2	**13.6**(16)		**7.6**(9)		**45.8**(54)		**44.1**(52)	
	Q3	**16.7**(18)		**12.0**(13)		**39.8**(43)		**52.8**(57)	
	Q4	**18.1**(19)		**13.3**(14)		**41.0**(43)		**52.4**(55)	
Tooth type	Anterior	**16.3**(21)	0.917	**13.2**(17)	0.15	**34.9**(45)	0.091	**55.0**(71)	0.433
	Premolar	**15.3**(38)		**10.4**(26)		**46.6**(116)		**49.8**(124)	
	Molar	**17.1**(14)		**4.9**(4)		**41.5**(34)		**46.3**(38)	
Occlusion	Denture	**22.0**(13)	0.537	**15.3**(9)	0.172	**35.6**(21)	0.46	**71.2**(42)	0.002*
	Fixed	**13.5**(5)		**2.7**(1)		**45.9**(17)		**48.6**(18)	
	Natural	**15.3**(54)		**9.9**(35)		**42.7**(151)		**48.3**(171)	
	Edentulous	**10.0**(1)		**20.0**(2)		**60.0**(6)		**20.0**(2)	
Wear facets	Yes	**18.0**(51)	0.11	**13.8**(39)	0.001*	**42.8**(121)	0.841	**59.4**(168)	<0.001*
	No	**12.4**(22)		**4.5**(8)		**41.8**(74)		**36.7**(65)	
Caries risk	High	**25.0**(8)	0.074	**25.0**(8)	0.007*	**59.4**(19)	0.114	**65.6**(21)	<0.001*
	Moderate	**16.7**(58)		**10.5**(35)		**40.5**(141)		**53.7**(187)	
	Low	**8.8**(7)		**5.0**(4)		**42.4**(35)		**31.3**(25)	
Operator	Undergraduate	**16.9**(63)	0.215	**11.8**(44)	0.021*	**45.6**(170)	0.004*	**54.4**(203)	0.001*
	Postgraduate	**11.5**(10)		**3.4**(3)		**28.7**(25)		**34.5**(30)	
Adhesive	2-step E&R	**16.2**(30)	<0.001*	**13.5**(25)	0.01*	**46.5**(86)	<0.001*	**57.8**(107)	0.016*
	2-step SE	**28.0**(26)		**14.0**(13)		**58.1**(54)		**51.6**(48)	
	Selective etching	**9.3**(17)		**4.9**(9)		**30.2**(55)		**42.9**(78)	
Composite	Filtek Z350	**13.3**(43)	0.059	**9.0**(29)	0.381	**43.2**(140)	0.826	**49.1**(159)	0.384
	Estelite Sigma Quick	**21.7**(26)		**13.3**(16)		**40.0**(48)		**55.8**(67)	
	Filtek Z250	**25.0**(4)		**12.5**(2)		**43.8**(7)		**43.8**(7)	
Overall	Total	**15.9**(73)		**10.2**(47)		**42.4**(195)		**50.7**(233)	

*Association between the factor of the restoration and the parameter failures; marginal discoloration, and marginal integrity (*p*-value<0.05, Pearson chi-square or Fisher's exact test)

Table4. Odd ratio of the restorations failure parameters on various factors**.**

Factor		Restoration loss (C)		Partial retention loss (B+C)				Marginal discoloration (B+C)				Marginal integrity (B+C)			
				Occlusal		Gingival		Occlusal		Gingival		Occlusal		Gingival	
		OR	*p*-value	OR	*p*-value	OR	*p*-value	OR	*p*-value	OR	*p*-value	OR	*p*-value	OR	*p*-value
Occlusion	Denture/ Fixed	0.79	0.671	2.62	0.357	1.15	0.814	1.81	0.298	6.48	0.047	0.65	0.313	2.61	0.026
	Denture/ Natural	1.09	0.636	0.88	0.537	2.27	0.043	1.57	0.194	1.64	0.216	0.74	0.308	2.64	0.001**
	Denture/ Edentulous			0.66	0.555	1.62	0.554	2.54	0.348	0.72	0.502	0.37	0.134	9.89	0.003**
	Natural/ Edentulous			0.74	0.556	0.71	0.542	1.63	0.537	0.44	0.270	0.50	0.220	3.74	0.072
	Natural/ Fixed	0.73	0.611	2.97	0.232	0.51	0.157	1.16	0.773	3.95	0.121	0.88	0.700	0.99	0.968
	Edentulous/ Fixed			4.00	0.384	0.71	0.622	0.71	0.622	9.00	0.110	1.77	0.333	0.26	0.101
Caries risk	High/ Moderate	2.04	0.3	2.50	0.084	1.36	0.38	1.67	0.234	2.98	0.018	2.15	0.039	1.64	0.196
	High/ Low	1.71	0.444	3.52	0.074	2.71	0.161	3.48	0.028	6.33	0.004*	1.88	0.135	4.20	0.001*
	Moderate/ Low	0.84	0.504	1.41	0.536	1.99	0.198	2.09	0.075	2.13	0.156	0.88	0.596	2.56	<0.001*
Adhesive	2-E&R/2-SE	1.41	0.567	1.36	0.479	1.74	0.214	0.50	0.021	0.96	0.915	0.63	0.068	1.29	0.324
	2-E&R/ Selective etching	11.44	0.004*	5.70	0.001*	2.21	0.035	1.88	0.049	3.00	0.005*	2.01	0.001*	1.83	0.004*
	2-SE/ Selective etching	8.14	0.046	4.19	0.018	1.27	0.638	3.77	<0.001*	3.12	0.009*	3.20	<0.001*	1.42	0.168

*,**Association between the factor and the parameter failures with the Odds Ratio(OR) (*p-value<0.017,** p-value<0.008, Pearson chi-square or Fisher's exact test with Bonferroni correction)