PROPERTIES OF THAI SELF-CURED ORTHODONTIC RESIN

PANADDA NILDAM

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE (ORTHODONTICS)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2006**

Thesis
Entitled

PROPERTIES OF THAI SELF-CURED ORTHODONTIC RESIN

Panadda Nildam

Miss Panadda Nildam
Candidate

Surachai

Assoc. Prof. Surachai Dechkunakorn
B.Sc., D.D.S., Dip.in Orthodontics
Diplomate Thai Board of Orthodontics
Major-Advisor

Niwat

Assoc. Prof. Niwat Anuwongnukroh
D.D.S., M.S.D. (Orthodontics),
Diplomate Thai Board of Orthodontics
Co-Advisor

Dr. Siriporn Tanodekaew
Ph.D. (Polymer Chemistry)
Co-Advisor

Prof. M.R. Jisnuson Svasti, Ph.D.
Dean
Faculty of Graduate Studies

Surachai

Assoc. Prof. Surachai Dechkunakorn,
B.Sc., D.D.S., Dip.in Orthodontics
Diplomate Thai Board of Orthodontics
Chair
Master of Science Programme in
Orthodontics
Faculty of Dentistry

Thesis
Entitled

PROPERTIES OF THAI SELF-CURED ORTHODONTIC RESIN

was submitted to the Faculty of Graduate Studies, Mahidol University
For the degree of Master of Science (Orthodontics)
on
December 21, 2006

Panadda Nildam
....
Miss Panadda Nildam
Candidate

Wanna Suchato
....
Clin. Prof. Wanna Suchato
D.D.S., Training in Orthodontics
Diplomate Thai Board of Orthodontics
Chair

Surachai Dechkunakorn
....
Assoc. Prof. Surachai Dechkunakorn
B.Sc., D.D.S., Dip.in Orthodontics
Diplomate Thai Board of Orthodontics
Member

Siriporn Tanodekaew
....
Dr. Siriporn Tanodekaew
Ph.D.(Polymer)
Member

Niwat Anuwongnukroh
....
Assoc. Prof. Niwat Anuwongnukroh
D.D.S., M.S.D. (Orthodontics),
Diplomate Thai Board of Orthodontics
Member

....
Prof. M.R. Jisnuson Svasti, Ph.D.
Dean
Faculty of Graduate Studies
Mahidol University

Theeralaksna Suddhasthira
....
Asst. Prof. Theeralaksna Suddhasthira,
Ph.D.
Dean
Faculty of Dentistry
Mahidol University

ACKNOWLEDGEMENT

The success of this thesis is attributed to the extensive support and assistance from my major advisor, Associate Professor Surachai Dechkunakorn and my co-advisors, Associate Professor Niwat Anuwongnukroh and Dr. Siriporn Tanodekaew. I would like to express my sincere gratitude and deep appreciation for their valuable advice throughout this research.

I wish to thank Dr. Siriporn Tanodekaew and National Metal and Materials Technology Center (MTEC) for their kindness in synthesis the powder and liquid and examining the particle size, molecular weight and polydispersivity of Thai self-cured orthodontic resin.

I would like to thank Clinical Professor Wanna Suchato for her suggestions and advice as an external examiner.

My special thanks also go to Research Center Unit, Faculty of Dentistry, Mahidol University for providing me some materials and instruments throughtout this study.

Panadda Nildam

PROPERTIES OF THAI SELF-CURED ORTHODONTIC RESIN

PANADDA NILDAM 4837971 DTOD/M

M.Sc.(ORTHODONTICS)

THESIS ADVISORS: SURACHAI DECHKUNAKORN, DIP. IN ORTHODONTICS, NIWAT ANUWONGNUKROH, M.S.D.(ORTHODONTICS), SIRIPORN TANODEKAEW, PH.D.(POLYMER CHEMISTRY)

ABSTRACT

Flexural strength, flexural modulus, surface hardness, water sorption, water solubility, level of residual monomer and biocompatibility are important properties of self-cured orthodontic resins. The purpose of this study is to compare these properties of a Thai self-cured orthodontic resin with the commercial product (Orthocryl®). The tested specimens were classified into 4 groups according to their types and fabrication techniques (Thai-mixing, Thai-infiltration, Orthocryl®-mixing, Orthocryl®-infiltration) and were tested by the methods described by ISO 1567:1999 which is the international standard for dental polymers. Flexural strength, flexural modulus, surface hardness, water sorption, water solubility, residual monomer and biocompatibility of the resins were determined. Kruskal-Wallis, Mann-Whitney and One-Sample T tests ($P<.05$) were used to compare significant differences in the levels of these properties among the four groups of self-cured orthodontic resins. According to ISO1567:1999, the properties of the Thai resin were acceptable. The surface hardness of Orthocryl® was significantly higher than that of the Thai resin. Water sorption and solubility of Orthocryl® were significantly lower than those of the Thai resin. However, there were no significant differences in flexural strength, flexural modulus and level of residual monomer between Orthocryl® and the Thai resin . The specimens prepared by mixing and infiltration technique showed no significant differences in levels of flexural strength, flexural modulus, surface hardness, water sorption, water solubility and residual monomer.

This study suggests that the Thai self-cured orthodontic resin is suitable as an alternative orthodontic denture base. Also, this study will serve as a guidance for further development of Thai self-cured orthodontic resin.

KEY WORDS: SELF-CURED RESIN / ORTHODONTIC RESIN

76 P.

คุณสมบัติของเรซินชนิดบ่มเองของไทยในงานทันตกรรมจัดฟัน (PROPERTIES OF THAI SELF-CURED ORTHODONTIC RESIN)

ปนัดดา นิลดำ 4837971 DTOD/M

วท.ม. (ทันตกรรมจัดฟัน)

คณะกรรมการควบคุมวิทยานิพนธ์: สุรชัย เดชคุณากร, Dip. in Orthodontics,
นิวัต อนุวงศ์นุเคราะห์, M.S.D.(Orthodontics), สิริพร โตนดแก้ว, Ph.D.(Polymer Chemistry)

บทคัดย่อ

ค่าแรงดัดขวาง, ค่าโมดูลัสของแรงดัดขวาง, ความแข็งผิว, การดูดซับน้ำ, การละลายตัวในน้ำ, ปริมาณโมโนเมอร์ที่ตกค้าง, ความเข้ากันได้ทางชีวภาพ เป็นคุณสมบัติที่สำคัญของเรซินชนิดบ่มเองในงานทันตกรรมจัดฟัน วัตถุประสงค์ของการวิจัยนี้เพื่อเปรียบเทียบคุณสมบัติทั้งหมดดังกล่าวของเรซินชนิดบ่มเองของไทยกับเรซินชนิดบ่มเองที่มีขายในท้องตลาดสำหรับการใช้ในงานทันตกรรมจัดฟันยี่ห้อออโธคริล ซึ่งเตรียมโดยวิธีที่แตกต่างกัน 2 วิธี คือ การผสมก่อนเทลงแบบหล่อ และวิธีโรยลงแบบหล่อตามเกณฑ์มาตรฐานที่กำหนดโดยไอเอสโอ (ISO1567:1999) ซึ่งเป็นเกณฑ์มาตรฐานสำหรับโพลีเมอร์ฐานฟันปลอม การเตรียมตัวอย่างทำโดยแบ่งเป็น 4 กลุ่ม คือ เรซินของไทยซึ่งเตรียมโดยวิธีการผสมก่อนเทลงแบบหล่อ, เรซินของไทยซึ่งเตรียมโดยวิธีการโรยลงแบบหล่อ, เรซินชนิดออโธคริลซึ่งเตรียมโดยวิธีการผสมก่อนเทลงแบบหล่อ และ เรซินชนิดออโธคริลซึ่งเตรียมโดยวิธีการโรยลงแบบหล่อ โดยวิธีการเตรียมตัวอย่างเพื่อทดสอบและวิธีทดสอบคุณสมบัติทั้งหมดทำตามเกณฑ์มาตรฐานที่กำหนดโดยไอเอสโอ(ISO1567:1999) จากนั้นนำผลที่ได้มาทดสอบทางสถิติโดยวิธี Kruskal-Wallis, Mann-Whitney และ One-Sample T tests ($P<.05$) เพื่อเปรียบเทียบความแตกต่างอย่างมีนัยสำคัญของคุณสมบัติทั้งหมดดังกล่าวระหว่างเรซินชนิดบ่มเองทั้งหมด 4 กลุ่ม ผลการทดสอบพบว่าคุณสมบัติทั้งหมดของเรซินของไทยผ่านเกณฑ์มาตรฐานที่กำหนดโดยไอเอสโอ(ISO1567:1999) ส่วนความแข็งผิวของออโธคริล มีค่ามากกว่าอย่างมีนัยสำคัญเมื่อเทียบกับเรซินของไทย การดูดซับน้ำและการละลายตัวในน้ำของออโธคริลมีค่าน้อยกว่าอย่างมีนัยสำคัญเมื่อเทียบกับเรซินของไทย สำหรับค่าแรงดัดขวาง,ค่าโมดูลัสของแรงดัดขวางและปริมาณโมโนเมอร์ที่ตกค้างพบว่ามีค่าไม่แตกต่างกันอย่างมีนัยสำคัญระหว่างเรซินทั้งสองชนิด นอกจากนั้นเมื่อเปรียบเทียบวิธีการเตรียมตัวอย่างทั้ง 2 วิธีพบว่า ค่าแรงดัดขวาง, ค่าโมดูลัสของแรงดัดขวาง, ความแข็งผิว, การดูดซับน้ำ, การละลายตัวในน้ำและปริมาณโมโนเมอร์ที่ตกค้าง มีค่าไม่แตกต่างกันอย่างมีนัยสำคัญ

จากผลการศึกษาแสดงให้เห็นว่า สามารถนำเรซินชนิดบ่มเองของไทยมาใช้เป็นวัสดุในการผลิตเครื่องมือทางทันตกรรมจัดฟันชนิดถอดได้ และการวิจัยนี้ยังเป็นแนวทางในการพัฒนาปรับปรุงคุณสมบัติของเรซินชนิดบ่มเองของไทยในงานทันตกรรมจัดฟันต่อไปอีกด้วย

76 หน้า

CONTENTS

LIST OF TABLES

LIST OF TABLES (CONT.)

LIST OF FIGURES

Page

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

CHAPTER 1

INTRODUCTION

Early fabrication of orthodontic removable appliances used vulcanized rubber, which was introduced by Charles Goodyear in 1839. This compound found widespread application as a denture base. Acrylic resins were rapidly developed during the early years of the Second World War when the use of natural rubber for dental vulcanite was prohibited (1). Formerly, orthodontists used heat-polymerized denture base resins for construction of orthodontic removable appliances. Later, autopolymerized or self-cured systems were mainly used due to their favorable rapid and easier fabrication of removable appliances. At present, self-cured acrylic resins are the most commonly preferred materials in fabrication of orthodontic appliances such as retainers and active plates. The "salt and pepper" or infiltration technique allowed the appliances to be constructed more satisfactorily, which eliminated the conventional flasking and packing stages (2).

There are many companies in Thailand marketing self-cured orthodontic acrylic resins, which all are imported from foreign countries. Recently, in co-operation between the National Metal and Materials Technology Center (MTEC) and Faculty of Dentistry Mahidol University (MU), Thai self-cured orthodontic acrylic resins were first produced. Then, assay properties such as flexural strength, flexural modulus, surface hardness, water sorption, water solubility, level of residual monomer and biocompatibility of the recent Thai self-cured orthodontic resins are important for clinical application. The recent study of the properties of Thai resins (3) showed that flexural modulus, surface hardness, water sorption, water solubility except flexural strength were satisfied according to the ISO 1567:1999 requirements (4). However, the properties of Thai resins were inferior to those of the commercial Orthocryl®. The further study on improvement of the properties, especially flexural strength of Thai resins was recommended.

Self-cured resins exhibited higher residual monomer than heat-cured resins (5). Also, self-cured acrylic resins employed for removable orthodontic appliances were reported a greater content of free residual monomer (3, 6). Residual monomer concentration varied with the methods and the conditions of polymerization (5, 7). Residual monomer in denture base polymer was reported in the literatures which had been postulated as etiology of mucosal irritations (8, 9). Therefore, assessment of the level of residual methyl methacrylate monomer and biocompatibility of Thai self-cured resins were crucial for clinical application.

The properties of Thai self-cured orthodontic resins that complied with ISO 1567:1999 would lead to the production of these resins for imported resin replacement which would benefit Thai economy and also lower dental cost.

CHAPTER 2

OBJECTIVES

Objectives of this study

1. To determine and compare flexural strength, flexural modulus, water sorption, water solubility and level of residual methyl methacrylate monomer of Thai self-cured orthodontic resin according to ISO 1567:1999 requirements.

2. To determine and compare biocompatibility of Thai self-cured orthodontic resin according to ISO 7405:1997 requirements.

3. To determine and compare flexural strength, flexural modulus, surface hardness, water sorption, water solubility, level of residual methyl methacrylate monomer and biocompatibility of Thai self-cured orthodontic resin with those of the commercial self-cured orthodontic resin (Orthocryl®).

4. To determine and compare flexural strength, flexural modulus, surface hardness, water sorption, water solubility, level of residual methyl methacrylate monomer and biocompatibility of Thai self-cured orthodontic resin and the commercial self-cured orthodontic resin (Orthocryl®) fabricated by conventional (mixing) and infiltration techniques.

Statements of hypothesis

1. The levels of flexural strength, flexural modulus, water sorption, water solubility and level of residual methyl methacrylate monomer of Thai self-cured orthodontic resin are in limits described by ISO 1567:1999 requirements.

2. The biocompatibility of Thai self-cured orthodontic resin is in limits described by ISO 7405:1997 requirements.

3. There are no statistically significant differences in levels of flexural strength, flexural modulus, surface hardness, water sorption, water solubility, level of residual methyl methacrylate monomer and biocompatibility of Thai self-cured orthodontic acrylic resin and the commercial self-cured orthodontic resin (Orthocryl®).

4. There are no statistically significant differences in levels of flexural strength, flexural modulus, surface hardness, water sorption, water solubility, residual methyl methacrylate monomer and biocompatibility of Thai self-cured orthodontic acrylic resin and the commercial self-cured orthodontic resin (Orthocryl®) fabricated by conventional and infiltration techniques.

The expected benefits of this study

1. This study is a guideline for further development of Thai orthodontic self-cured acrylic resins.

2. If physical and biological properties of Thai self-cured orthodontic resin is found to comply with ISO 1567:1999 requirements, Thai resin can be an alternative for imported orthodontic denture base materials.

3. This study will provide the scientific data for clinicians and technicians to be careful in manipulation and construction of orthodontic appliances that influences the properties of denture bases especially by infiltration technique.

Control of variables

Any identified variables, which may interfere with the experiments have been controlled to ensure the validity of the results. In this study, the powder and liquid portions of the polymers were accurately weighed before mixing to ensure the consistency of powder/liquid ratio. The resins were mixed and cured according to the manufacturer’s instructions. All specimens were accurately measured for dimensional accuracy before tests.

CHAPTER 3

LITERATURE REVIEW

The historical development of orthodontic acrylic resins

Acrylic polymers were first introduced as denture base materials in 1937 (10) and have been used as dental base materials for prosthetic and orthodontic removable appliances. Acrylic resins were rapidly developed during the early years of the Second World War when the use of natural rubber for dental vulcanite was prohibited (1). Formerly, heat-curing denture base resins were used for a construction of orthodontic removable appliances. Later developments of auto-polymerized or “self-cured” resins have produced which facilitated a more rapid and easier fabrication of removable appliances. They have excellent esthetic properties, adequate strength, low water sorption, low water solubility, easier fabrication and repair.

The self-cured denture base is chemically similar to the heat-cured denture base except reducing agent is added to the monomer. The reducing agent is usually a tertiary aromatic amine, though barbituric acid derivatives have been used also. The reducing agent reacts with the benzoyl peroxide at room temperature to produce peroxy radicals, which initiate the polymerization of the monomer in the denture base. There is a wide variation between manufacturers in the molecular weights of the polymers in the powder beads. Some have average molecular weights as low as 190,000. Cross-linker concentrations vary greatly in the monomer liquid, from 0% to 9%, interestingly excess cross-linker is associated with high creep. The size, molecular weight and plasticizer contents are balanced to give a high penetration of monomer into the powder beads without too early increase in viscosity of the mix to allow pouring of the acrylic into the mold. This compromise is difficult to achieve and often results in high free residual monomer content and low cross-linker density. It is the attempt by manufacturers to achieve the best compromise that results in the wide ranges of molecular weights and cross-linker concentrations found in the materials, which inevitably result in large differences in physical and mechanical properties between the various products (11).

Classification of denture base polymers

Denture base polymers covered by the International Organization for Standardization are of the following types and class according to ISO 1567:1999 .

Type 1: Heat- polymerizable polymers
    Class 1: Powder and liquid
    Class 2: Plastic cake
Type 2: Auto-polymerizable polymers
    Class 1: Powder and liquid
    Class 2: Powder and liquid pour-type resins
Type 3: Thermoplastic blank or powder
Type 4: Light- activated materials
Type 5: Microwave cured materials

Acrylic resins

Today most removable dentures are made of acrylic resins. The properties of materials are not ideal but present a compromise between physical properties on the one hand and ease to use and cost on the others (12). There are at least two acrylic resin series that are of dental interest. One series derived from acrylic acid, $CH_2=CHCOOH$, and the other from methacrylic acid, $CH_2=C(CH_3)COOH$.

Although the polyacids are hard and transparent, their polarity related to the carboxyl group causes them to imbibe water. The water tends to separate the chains and cause a general softening and loss of strength (1).

Methyl Methacrylate, MMA

Poly(methyl methacrylate) by itself is not used in dentistry to a great extent in moulding procedures. Rather, the liquid monomer methyl methacrylate is mixed with the polymer which is in the powdered form. The monomer partially dissolves the polymer to form a plastic dough. This dough is packed into the mould and the monomer is polymerized.

Methyl methacrylate is a clear, transparent liquid at room temperature with the following physical properties: melting point of $-48^{0}C$, boiling point of $100.8\ ^{0}C$, density of 0.945 g/ml at 20 ^{0}C, and heat of polymerization of 12.9 kcal/mol. It exhibits a high vapour pressure and excellent organic solvent (1). Although the polymerization of the methyl methacrylate can be initiated by ultraviolet or visible light or heat, it is commonly polymerized in dentistry by the use of a chemical initiator (1).

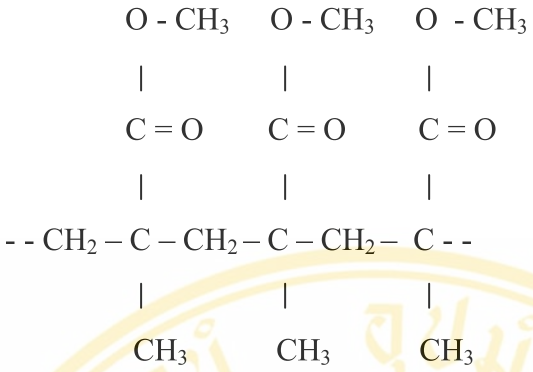
Poly(methyl methacrylate), PMMA (Fig. 3.1)

Poly(methyl methacrylate) is a transparent resin of remarkable clarity. It transmits light into the ultraviolet range to wavelength of 0.25 µm. It is a hard resin with a Knoop hardness number of 18 to 20. Its tensile strength is approximately 59 MPa (8500 psi) and its specific gravity is 1.19. Its modulus of elasticity is approximately 2400 MPa (350,000 psi).

The resin is extremely stable with no discoloration in ultraviolet light. It exhibits remarkable aging property. It is chemically stable to heat. Its softening point is at 125 ^{0}C, which is able to mould as a thermoplastic material (1).

The resin can be colored or tinted to almost any shade and degree of translucence. Its color and optical properties are stable under all normal conditions, and its strength and other physical properties are adequate. The properties of acrylic resins are not ideal, of course, no any properties of other dental materials are ideal. However, it is the combination of desirable characteristics that have made them so acceptable.

The main advantage of poly (methyl methacrylate) is ease of processing. Although it is a thermoplastic resin, it is usually moulded by mixing the methyl methacrylate monomer with the polymer powder. The monomer plasticizes the polymer to doughlike consistency and it can then be easily moulded initially in the mould space. The monomer is subsequently polymerized, and the resulting denture base is composed of a solid, homogeneous resin.



$$--CH_2-\underset{\substack{|\\CH_3}}{\overset{\substack{O-CH_3\\|\\C=O\\|}}{C}}-CH_2-\underset{\substack{|\\CH_3}}{\overset{\substack{O-CH_3\\|\\C=O\\|}}{C}}-CH_2-\underset{\substack{|\\CH_3}}{\overset{\substack{O-CH_3\\|\\C=O\\|}}{C}}--$$

Fig.3.1 Chemical structure of PMMA: Poly (methyl methacrylate)

Polymerization

Polymerization occurs through series of chemical reactions by which the macromolecule, or *polymer,* is formed from large numbers of single molecules known as *monomers* (monomer means one molecule or one *mer*).

The most significant features of polymers are that they consist of very large molecules, that their molecular weight varies over a wide range, and their molecular structure is capable of virtually limitless configurations and conformations (13).

Basically, the polymer is made up of a particular recurring, simple structural unit, which is essentially related to the monomer structure. The structure units are usually connected to each other in the polymer molecule by covalent bonds.

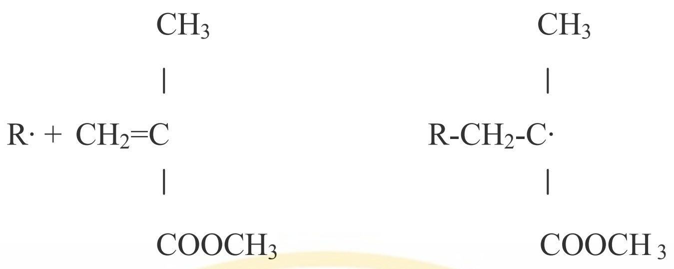
The polymerization can be initiated either by heating the polymer-monomer mixture, by chemical activation at room temperature, or by activating the reaction using microwave energy or visible light (14).

Three stages of addition polymerization of methyl methacrylate (11)

1. Initiation stage

$$C_6H_5COO\text{-}OOCC_6H_5 \xrightarrow[\text{Amines}]{\text{Heat}} C_6H_5COO\cdot + C_6H_5\cdot + CO_2$$

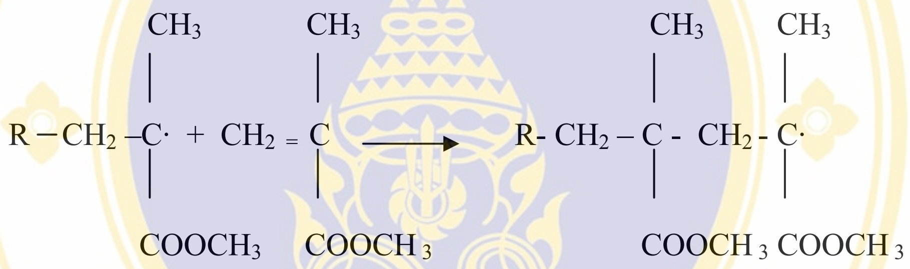
Benzoyl peroxide ⟶ Free radicals (R) + Carbon dioxide



$$R\cdot + CH_2{=}\underset{\displaystyle COOCH_3}{\overset{\displaystyle CH_3}{C}} \qquad R{-}CH_2{-}\underset{\displaystyle COOCH_3}{\overset{\displaystyle CH_3}{C}}\cdot$$

Free radical + Monomer → Free radical
(Activated monomer)

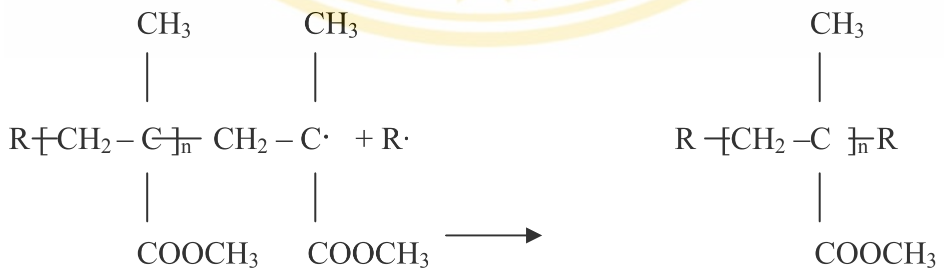
2. Propagation stage



$$R-CH_2-\underset{\displaystyle COOCH_3}{\overset{\displaystyle CH_3}{C}}\cdot + CH_2=\underset{\displaystyle COOCH_3}{\overset{\displaystyle CH_3}{C}} \longrightarrow R-CH_2-\underset{\displaystyle COOCH_3}{\overset{\displaystyle CH_3}{C}}-CH_2-\underset{\displaystyle COOCH_3}{\overset{\displaystyle CH_3}{C}}\cdot$$

Polymer free radical + Monomer → Growing chain

3. Termination stage



$$R\left[CH_2-\underset{\displaystyle COOCH_3}{\overset{\displaystyle CH_3}{C}}\right]_n CH_2-\underset{\displaystyle COOCH_3}{\overset{\displaystyle CH_3}{C}}\cdot + R\cdot \longrightarrow R\left[CH_2-\underset{\displaystyle COOCH_3}{\overset{\displaystyle CH_3}{C}}\right]_n R$$

Free radical polymer + Free radical → Polymer chain

Chemically activated acrylic resins

Chemically activated acrylic resins, first used for dental purposes in Germany during World War II, are known variously as “self-curing”, “cold-cured”, or “autopolymer” resins. Its differences from heat-cured resin are the method of activation of benzoyl peroxide and the degree of polymerization which is not high as heat-cured resins (1).

The chemical-cured dental acrylic system consists of a liquid component and a solid component. A liquid component consists of methyl methacrylate(MMA) monomer, crosslinkers, and activator, typically a tertiary amine such as *N*,*N* dimethyl-*p*-toluidine(DMPT). Pre-polymerized beads of poly(methyl methacrylate)(PMMA) coated with an initiator, e.g., benzoyl peroxide, make up the powder component. When the two components are combined and mixed, MMA dissolves into the PMMA beads, forming a material of doughy consistency. At the same time, *N*,*N* dimethyl-*p*-toluidine (DMPT) comes in contact with benzoyl peroxide, generating benzyl free radicals that go on to initiate the chemical reaction for free radical addition polymerization. Polymerization in the two-part chemical-cured acrylic resin is free radical addition across an aliphatic C=C double bond. As polymerization progresses, the amount of aliphatic C=C double bond decreases. Often, the reaction is not complete, so there will be a finite amount of monomer (MMA) remaining in the chemical- cured acrylic. The polymerization is never as complete as that of the heat-curing type; self-cured resin usually contains 3-5% residual monomer, in comparison with approximate 0.2-0.5 percent free monomer found in resin processed in boiling water. Depending on the proportions of the initiator and activator, the concentration of residual monomer is typically on the order of 3 wt% (15).

The compositions of self-cured resin are the same as those of heat-cured resin, except two differences. Firstly, the powder contains beads of polymer which has lower molecular weight than those of heat-cured resin. Another, the liquid contains chemical activator to initiate the additional polymerization resulting in the greater amount of residual monomer compared to heat-cured resin. This residual monomer creates two major disadvantages. The residual monomer acts as a potential tissue irritant and a plasticizer, which results in decreasing of transverse strength of the cured resin (16).

Self-cured resins have advantages such as excellent esthetic property, easily fabrication and repair. Disadvantages of self-cured resins are lack of color stability, shrinkage, presence of residual monomer, and cracking or crazing. Craze resistance has been improved and the material is closed to 95% polymerization (17). However, the problem of residual monomer has not been totally solved. The color stability of the self-curing resins is inferior to that of the heat-curing type because of the subsequent oxidation of the tertiary amine. The condition can be minimized by the addition of certain stabilizing agents to prevent such oxidation, or the polymerization may be consummated by the use of a more stable activators .

Requirements of auto-polymerizable polymers

Requirements of auto-polymerizable denture base polymers by the International Organization for Standardization according to ISO 1567:1999 (4).

1.Unpolymerized material

1.1 Liquid component consists essentially of monomeric material compatible with the powder and free of deposit that can be observed by visual inspection.

1.2 Solid component shall be free of extraneous material that can be observed by visual inspection.

2. Polymerized material

2.1 Biocompatibility

2.2 Surface characteristics; specimens processed in the manner recommended by the manufacturer should have a smooth, hard and glossy surface.

2.3 Color; a specimen shows no more than a slight difference when compared with the corresponding shade of the shade guide. The colored denture base polymers shall be translucent and evenly pigmented and/or where applicable.

2.4 Color Stability; test specimens shall not show more than a slight change in color.

2.5 Translucency; the shadow of the illuminated opaque disc shall be visible from the opposite side of the test specimen plate.

2.6 Freedom from porosity; specimens strip shall not show voids that can be observed by visual inspection.

2.7 Flexural strength; the flexural strength shall be not less than 60 MPa when tested in water at $(37\pm1)^0$C.

2.8 Flexural modulus; the flexural modulus shall be at least 1500 MPa when tested in water at $(37\pm1)^0$C.

2.9 Residual methyl methacrylate monomer; the upper limit for residual methyl methacrylate is 4.5% mass fraction.

2.10 Sorption; water sorption shall not exceed 32 μg/mm^3.

2.11 Solubility; water solubility shall not exceed 8.0 μg/mm^3.

Requisites for dental resin (14)

Ideal requisites for dental resin are as follows:

- The material should exhibit a translucence or transparency such that it can be made to duplicate esthetically the oral tissues. It should be capable of being tinted or pigmented to this end.
- It should be no change in color or appearance of the material subsequent to its fabrication.
- It should not expand, contract, or warp during processing or during subsequent use by the patient. In other words, it should be dimensionally stable under all conditions of service.
- It should possess adequate strength, resilience, and abrasion resistance.
- It should be impermeable to the oral fluids to the extent that becomes unsanitary or disagreeable in taste or odor.
- It should be completely insoluble in the oral fluids or in any substances taken into the mouth. It should not absorb such fluids.
- The resin should be tasteless, odorless, nontoxic, and nonirritating to the oral tissues.
- It should have a low specific gravity.
- Its softening temperature should be well above the temperature of any hot food or liquid taken into the mouth.
- In case of unavoidable breakage, it should be possible to repair the resin easily and efficiently.

- The fabrication of the resin into a dental appliance should be easily made with simple equipment.

Flexural strength

Flexural strength is an important mechanical property of acrylic denture base materials, since flexural behavior is a guide to service performance. It often describes as the modulus of rupture or transverse strength. Complete denture and removable partial denture bases are subjected to loading, which is flexure in nature, by the mastication during function. Thus, it is important that the materials resist the mastication force without fracture or change in dimension (1, 18) However, the failure rate of acrylic resin dentures as a result of fractures has been reported to be unacceptably high (19).

The flexural strength and modulus (MPa) are calculated from following equations:

$$\text{Flexural strength } (\sigma) = 3FL/\ 2bh^2$$

F = the maximum load (newton)
L = the distance between the supports (millimetre)
b = the width of the specimen (millimetre)
h = the height of the specimen (millimetre)

$$\text{Flexural modulus } (E) = F_1L^3/\ 4bh^3d$$

F_1 = the load (newton) at a convenient point in the straight-line portion of the trace
d = the deflection at load F_1 (millimetre)

This test is a collective measurement of tensile, compressive, and shear stresses. Consequently, the principle stresses on the upper surface are compressive, whereas those on the lower surface are tensile. Shear stress is also produced near the supported ends of the specimen, but it does not play a significant role in the fracture process.

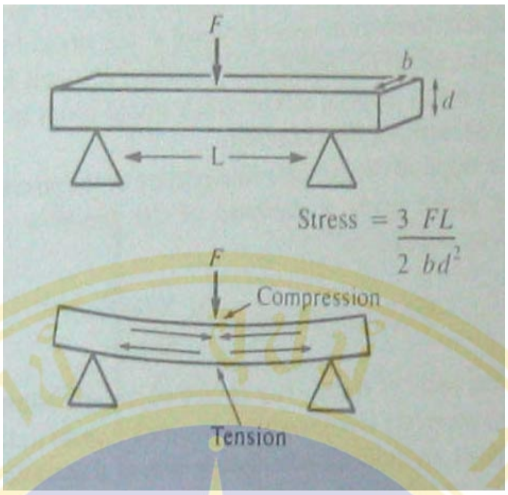


Fig. 3.2 Three Point Bending Test or Transverse Test

Flexural strength was found dependent on:

1. Types of Cross-linking agents

Ruyter et al (20) studied the flexural properties of auto-cured (dough and pour type) denture base resins with different types of cross-linking agents and concluded that the flexural strength of auto-cured denture base using 1,4-BDMA (1,4-butanediol dimethacrylate) as a crosslinking agent was higher than that using EGDMA(Ethylene glycol dimethacrylate). Phillips (14) proposed that cross-linking agent could alter the strength, water sorption and solubility of the cured resin. The exact effect of cross-linking upon the physical properties varied with composition and concentration of the cross-linking agents and the polymer system but excess crosslinking agents caused an increase in viscosity.

2. Molecular Weight

The molecular weight of the polymer powder, size and distribution of the particles might influence their rate of dissolution and swelling in the monomer liquid (20). In particular, a low viscosity at the start of polymerization enhanced the formation of low molecular weight chains in the matrix before the gel stage (21). The strength of polymer increased with an increase of molecular weight (20). The strength became fairly constant at degree of polymerization above 600 and molecular weight of 60,000 for PMMA (22).

3. Residual monomers

Several investigators studied the relationship between residual monomer and physical properties of the self-cured resins (6, 23, 24). They agreed that high residual monomer adversely affected the physical properties of the cured resins. Depending on the type of denture base resin i.e. heat-cured or auto-cured resins and on the polymerization temperature and time, various quantities of residual monomers, predominantly methyl methacrylate (MMA), were left in the cured resins. Dogan et al (25) made conclusion between residual monomer level and mechanical properties that the residual monomer acted as a plasticizer in the polymer matrix and caused the formation of more porosity that also affected the mechanical properties.

4. Polymer : Monomer Ratio

Jerolimov et al (26) studied the effect of powder/liquid ratios on residual monomer levels and flexural properties of heat-cured resins and concluded that flexural properties varied in opposite to amounts of residual monomer. The variations in polymer to monomer ratio could influence the properties in many instances. In general, an increase of polymer/monomer ratio reduced doughing time, the opposite effect was obtained by using an excess monomer. However, the shrinkage and temperature increased during polymerization were directly related to the amount of monomer used. These undesirable effects should be minimized with increased amount of powder.

5. Porosity

Porosity has been attributed to a variety of factors that included the following: air entrapped during mixing, monomer contraction during polymerization, monomeric vaporization associated with exothermic reaction, and the presence of residual monomer (27). Other authors (28) demonstrated that insufficient mixing of monomer and polymer, processing temperatures higher than 74ºC and inadequate compression on the flask caused porosity in denture base resin. Depending on polymerization conditions, up to 11% of porosity has been observed associated with decreased mechanical properties, poor esthetics, potential harboring of organisms and retention of fluid (29). The generation of porosity in poly(methyl methacrylate) (PMMA) denture base resin was a complex phenomenon with many causes.

Winston et al, (30) suggested that the use of a vacuum devices in mixing and pressurizing in curing procedure decreased porosity, increased transverse strength and longevity of auto-polymerizing resin appliances.

6. Curing process

Takahiro et al (31) reported that water temperature during polymerization had affected on flexural strength of auto-cured resin. Polymerization in hot water increased transverse strength and modulus. Polymerization at 60°C and 80°C in water increased transverse strength and modulus two times higher than polymerization at 23°C in air. Dogan et al (25) reported that tensile strength improved with an increase in curing time, which also caused a decrease in residual monomer level.

7. Water sorption

The influence of water sorption on the flexural properties of denture base polymers was recently reported by Takahashi et al (32). They found that denture base polymers of different monomer compositions and activator systems had different resistance to water sorption. Water sorption decreased the flexural strength of denture base polymers. Water acted as a plasticizing effect because of its interaction with polymer structure. It has been reported that water sorption by denture base polymers depended on type of cross-linking agent in the polymer. The cross-linking agent such as EGDMA (Ethylene glycol dimethacrylate) had little effect on the water sorption of denture base polymer (33).

Surface Hardness (18)

Hardness is one of the major important properties. It is defined as the resistance to permanent surface indentation or penetration. The properties that are related to the hardness of materials are strength, proportional limit and ductility to permanent surface indentation. The various hardness tests differ in type and geometry of indenter and load. The indenters are made of steel tungsten carbide or diamond, and shaped as a sphere, cone, or pyramid. Loads typically are in the range from 1 to 3000 kg. The choice of a hardness test depends on the material of interest, the expected hardness range and the desired degree of localization. Common methods used for hardness evaluation include Vickers, Knoop, Brinell and Rockwell. Vickers and Knoop both involve the use of diamond pyramid indentors. The value of hardness, often referred to the hardness number, depends on the method used for its evaluation.

Hardness is, therefore, a measure of the resistance to plastic deformation and is measured as a force per unit area of indentation. Hardness is indicative of the ease of finishing of an appliance and its resistance to in-service scratching. Finishing or polishing is important for esthetic purpose. The general procedure for testing hardness is as follows. A standardized force or weight is applied to the penetrating point through an appropriate mechanism. Such a force application to the indenter produces a symmetrically shaped indentation, which can be measured under a microscope for depth, area, or width of the indentation. The indentation dimensions are then related to tabulated hardness values, with a fixed load applied to the indentation vary inversely with the resistance to penetration of the material tested. Thus, lighter loads are needed for softer materials.

Vickers hardness test using 136-degree diamond pyramid is suitable for testing the surface hardness of dental materials. The Vickers hardness number (usually abbreviated as VHN) is calculated by dividing the load (F) by the surface area of the indentation (ISO 6507-1:1997)

$$\begin{aligned}\text{The Vickers hardness number} &= \frac{\text{Constant} \times \text{test force}}{\text{surface area of indentation}} \\ &= \frac{0.102 \times 2F \sin 136^\circ /2}{d^2} \\ &= 0.1891 \times F/ d^2\end{aligned}$$

The Vickers test is useful in measuring the hardness of small areas and for very hard or brittle materials.

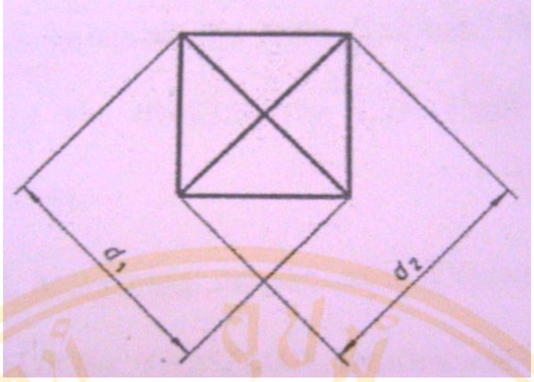


Fig. 3.3 Indentation made by 136-degree diamond pyramidal indenter.

Ruyter and Svendsen (20) showed that physical properties of polymerized resins largely depended on the degree of conversion. The microhardness was a simple and effective way to predict the degree of conversion.

Methacrylate materials that were additionally polymerized with heat increased crosslinking density and tended to demonstrate less wear (34). Polymerization with increased atmospheric pressure and heat increased degree of conversion and minimized porosity (35).

Water Sorption and Solubility

Poly(methyl methacrylate) absorbs water slowly over a period of time. The absorption is undoubtedly due primarily to the polar properties of the resin molecules. However, it has been shown that the mechanism is by diffusion of the water molecules according to the laws of diffusion (1). Water sorption of a material is important property, particularly in relation to the dimensional stability and long-term durability. When the material takes up water, its dimension and structural integrity may be affected. In resin-based materials, expansion as a result of water sorption may be beneficial. It compensates for any initial contraction and could relieve internal stresses caused by the polymerization reaction. However, the absorbed water can also act as a plasticizer, weakening the materials. Water molecule ingress into the vacancies between polymeric chains and push the polymeric chains further apart to cause an expansion (1).

The diffusion coefficient (D) of a typical heat-cured denture acrylic resin is 1.08 X 10^{-12} m^2/sec at 37°C (99°F). When the temperature drops to 23°C (73°F), D is reduced by one-half. For a self-curing acrylic resin, D is 2.34 X 10^{-12} m^2/sec. Diffusion of water is a time-dependent process where water is transported from the environment through the material surface into its body as a function of time (34). Rate of diffusion can be expressed as a diffusion flux (J) which is the mass (dM) diffusing through and perpendicular to a unit cross-sectional area (A) of a solid per unit time.

$$J = \frac{1}{A}\frac{dM}{dt}$$

Water sorption of resins has been shown to cause dimensional changes. In spite of this fact, it has been estimated that for each 1 percent increased in the weight due to the water absorbed, the acrylic resin expanded linearly 0.23 percent. Since the changes that occurred were so small, they could not be clinically detectable. Components of the material may be lost into the water when placed in an aqueous environment. This is referred to leaching. Leaching of components from a material into oral cavity may have an impact on its structural stability and causes undesirable tissue responses. It is therefore desirable that the water sorption and solubility of the materials are as small as possible. The solubility of a material is simply a measurement of the extent to which it will dissolve in a given fluid, for example, water or saliva. When assessing the solubility of materials, it is important to consider the vast range of conditions which may exist in the mouth. The pH of oral fluids may vary from pH 4 to pH 8.5, representing a range from mildly acidic to mildly alkaline.

Standard test of solubility often involve the storage of disc specimens of materials in water for a period of time, the result being quoted as the percentage weight loss of the disc. The most appropriate method for measuring material loss is to estimate volume loss as this can be correlated with material loss from clinical restoration. Judging material loss by weight change is less satisfactory as even though material is lost from the surface the weight of a specimen may increase if water absorption is great enough.

The dynamic of water sorption and solubility is a complex phenomenon; the specimens absorb water and release soluble components during the time of immersion. There is a close relation between water sorption, solubility and dimensional change. If if any resins have a high solubility, they will have high water sorption and large in shape change. The solubility of all resins was a result of leaching out of residual monomer such as methyl methacrylate (36). Then the mechanical properties of denture base materials decreased if the water solubility increased. Jagger (37) reported a correlation between residual monomer and water sorption. The presence of residual monomer resulted in increasing of water absorption and solubility. Braden and Wright (38) reported that the sorption behavior of the acrylic resin materials depended upon the balance between loss of plasticizers by dissolution and absorption of water. When the plasticizers were leached out, the softness was slowly lost and the material became rigid (39). Phillips (14) reported that water uptake by polymer resulted in the hygroscopic expansion due to expansion of the matrix. Lassila and Vallittu (40) stated that water sorption and solubility of polymers depended on the homogeneity of the material.

Residual Methyl Methacrylate Monomer

Self-cured resins appear a level of residual monomer as high as 5%, in comparison with 0.2 - 0.5% for heat-cured resins (41). Residual monomer concentration varied with the methods and the conditions of polymerization (5, 7).

According to ISO 1567:1999, the qualitative and quantitative standard method for the analysis of residual monomers in denture base polymers was gas chromatographic (GC) or high performance liquid chromatographic (HPLC) analyses of methyl methacrylate, extracted with an organic solvent. Gas-liquid chromatography (GLC) has been used with success for residual monomer determinations by many workers (6, 42, 43). However, high performance liquid chromatography (HPLC) has been used previously in dental research and has proven to be a powerful analytical technique. It has been used to analyse dental polymers, including residual monomers, composites or various other dental materials (44).

Fletcher et al (45) determined the level of residual monomer in two types of self-curing acrylic resins by means of gas-liquid chromatography. The results showed that both self-cured resins exhibited higher residual monomer levels than heat-cured acrylics. Stafford and Brooks (2) studied the loss of residual monomer from six orthodontic acrylic resins. Gas chromatography was used for the determination of residual monomer in the cured resins. The results showed that high levels of residual monomer were present in orthodontic resins. There was a rapid loss of monomer in the first 24 hours of soaking of the specimens in the water. Dogan et al (25) studied the effects of varying polymerization times and temperatures on the residual monomer content of denture base materials. The authors showed that increased temperatures and extended polymerization times were accompanied by a decrease in the residual monomer content. Vallittu et al (46) recommended immersing a denture in the water at 37 ^{0} C for one day prior to insert the removable appliances. Shim and Watts (47) examined the residual monomer content after additional immersion cycles of different durations at 100 ^{0} C and demonstrated that the residual monomer content could be reduced considerably.

The residual monomer concentration is the most important parameter in determining variations in mechanical and biological properties of a denture base material. Then, assay the level of residual monomer in self-cured orthodontic acrylic was important to dentists and patients before insertion removable orthodontic appliances.

Residual methyl methacrylate monomer was found to be dependent on:

Effect of polymer to monomer ratio

According to Kedjarune et al (7), the more monomer added to the mixture, the greater the amount of residual monomer and, therefore, the more potential for cytotoxicity. Similarly, Lamb et al (48) investigated the effect of polymer to monomer ratio on residual monomer levels and observed that resins prepared with a high proportion of polymer resulted in significantly lower levels of residual monomer, as compared to those prepared with a lower ratio.

Effect of storage time and water immersion

Sheridan et al (49) reported that the cytotoxic effect of acrylic resins was greater in the first 24 hours after polymerization and decreased with time for all the resins evaluated in their study. Therefore, it is recommended that dentists should soak the denture base in the water at least 24 hours before placing them in the patient's mouth. Jorge et al (50) advocated that the denture base should be immersed in water at 50°C for 60 minutes, to reduce the amount of released monomer and therefore the toxic potential of denture base resins, especially for autopolymerized resins.

Effect of polymerization conditions

Depending on the polymerization temperature and time, various quantities of residual monomer are left in the polymer resulting in different degrees of cytotoxicity. Kedjarune et al (7) observed a reduced amount of residual monomer when the polymerization time was extended, thus resulting in less cytotoxic effects. Lamb et al (48) observed that levels of residual monomers in auto-polymerized resins were higher for specimens polymerized at 22°C, as compared with those polymerized at 55°C. Therefore, it seems reasonable to suggest that the auto-polymerized acrylic resins should be heat-treated to decrease cytotoxic effects. Moreover,Vallittu et al (46) performed a study with 2 auto-polymerized resins in which the reaction was initiated by barbituric acid and 2 heat-polymerized resins activated by benzoyl peroxide. The results showed that auto-polymerized resins exhibited higher contents of residual methyl methacrylate than the heat-polymerized resins.

Effect of polymerization method

The method of polymerization is a decisive feature in the cytotoxicity of denture base acrylic resins. Pettersen et al (51) concluded that the cytotoxic effect was greater in auto-polymerized resins than in heat-polymerized resins. Tsuchiya et al (5) revealed that auto-polymerized resins eluted considerably more substances than did the heat- and microwave-polymerized resins.

Biocompatibility

Biocompatibility is defined as the ability of a material to perform with an appropriate host response in a specific application (52). Biocompatibility tests simulate biological reactions to materials when they are placed on or into tissues of the body. Cytotoxicity as a primary factor of biocompatibility is generally determined by in vitro cell culture. Resins used for the manufacture of denture bases had displayed various degrees of in vitro cytotoxicity and in vivo allergic responses. Various compounds including a residual methyl methacrylate monomer, and additives such as hydroquinone, benzoyl peroxide, and *N*, *N*-dimemethyl-*p*-toluidine were eluted from acrylic polymers. Despite the various methods used to initiate the polymerization of denture base resin, the conversion of monomers to polymers was not complete, and some unreacted monomers MMA were left in the denture base that are leachable into water as well as saliva (7, 53). MMA has been implicated as primary irritant and sensitizer, which can cause allergic eczematous reaction on the oral mucosa as well as skin (54). Residual monomer had the potential to cause irritation, inflammation, and an allergic response of the oral mucosa. Clinical signs and symptoms most frequently reported, included erythema, erosion of oral mucosa, and a burning sensation on the mucosa and tongue (55). Also, there were several reports in the literature regarding levels of residual monomer in denture base polymers which had been postulated that was etiology of mucosal irritations (49, 56). Cytotoxic reactions to acrylic monomers such as methacrylate, the major component of most dental resin liquids, have been reported (57). Tsuchiya et al (58) reported that leachable substances from acrylic resins showed cytotoxic potential in the range of their leaching concentrations. Eluates from various denture base resins had cytotoxic effects on oral epithelial cells (59) and human gingival fibroblasts (GF) (49), as well as inhibiting cell growth, DNA replication, RNA synthesis, and metabolic processes (60). In addition, Cimpan et al (61) demonstrated that resin eluates may enhance cell death by apoptosis and necrosis in human monoblastoid cells in a dose and time dependent fashion. Moreover, Tang et al (56) demonstrated that direct contact of fibroblasts with methacrylate polymers, especially the unpolymerized surface layer, may significantly reduce cell viability than what occurs with the eluates.

Studies have shown that MMA is a cytotoxic agent (7, 58, 62, 63). In addition, MMA was found to induce papilloma and fibroma in terms of sequential histopathological changes on hamster cheek pouches (64). However, the actual mechanism of the cytotoxicity of MMA is not well known. MMA was capable of inhibiting DNA synthesis. Alternation of lipid metabolism (65), cytokine production (66), and inhibition of cell viability via mitochondrial activity (67) may account for the cytotoxicity. In the study of Yanga et al (68), it was found that the concentration level of MMA (10^{-6} (v/v) corresponding to 9.33×10^{-4} μg/ml) could easily reach the effective cytotoxic and cytogenetic level. In the oral cavity, it is possible that the amount of MMA released into oral mucosa may be higher than the concentrations tested in this study that could easily reach the effective toxic level, especially in the area under the denture.

CHAPTER 4

MATERIALS AND METHODS

Materials

1. Two types of self-cured orthodontic resins:
   - Orthocryl$^{®}$ (Dentaurum, Pforzheim, Fr, Germany)
   - Thai resin (MTEC-MU, Thailand)
2. Vasaline
3. Distilled water
4. Diamond discs
5. Silicon carbide grinding papers (600,800,1000,1200 grits)
6. Hydroquinone (HQ)
7. Acetone, HPLC grade
8. Methanol, HPLC grade
9. Methyl methacrylate (MMA), HPLC grade
10. Mouse fibroblasts (L 929) (American Type Culture Collection, Rockville, MD, USA)
11. Completed Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Grand Island, NY, USA)
12. Tissue culture reagents : phosphate-buffered saline (PBS) and trypsin-versin (Amresco, USA).
13. Phenol red (MERCK, Germany)
14. Neutral red (MERCK, Germany)
15. Agar (Wako, Japan)
16. Millipore filter (Sartorius, Germany)
17. Polyvinyl carbonate (Portex, UK)

Apparatus

1. Stainless steel moulds according to ISO 1567:1999
2. Plaster spatula and bowl

3. Racks for holding specimens
4. Desiccator with silica gel
5. Thermometer
6. Pressure pot
7. Rotary pregrinder (Metaserv 2000, 300 cycle/min.)
8. Weighing machine with the accuracy of ± 0.0001 g (Model AB 204, Mettler-Toledo Ltd., Switzerland)
9. Digital Calipers
10. Digital micrometer (Mitutoyo No 293-421 N and Mitutoyo Digimatic Solar No.500-404, Mitutoyo, Japan), capable of measuring to an accurate of ± 0.001 mm
11. Incubator (Model BM 600, Memmert, Schwabarch, FRG, Germany), capable of being maintained at 37±1°C
12. Universal testing machine (Instron model 5566, Instron Corporation, Canton, MA, USA)
13. Microhardness testing machine (Future Tech 700 ex, Future-Tech Corporation, Tokyo, Japan)
14. Volumetric pipettes
15. One-mark volumetric glass flasks
16. Glass centrifuge tubes
17. Culture flasks (Costar Corp, Cambridge, MA, USA)
18. Magnetic stirring apparatus (HB502, Bibby Sterilin, Stone, UK)
19. Centrifuge (Beckman, USA)
20. Inverted microscope QI-16-06 (Nikon, Japan)
21. CO_2 incubator (Thermoforma , USA)
22. Biohazard safety carbinet (Microflow USA)
23. High-performance liquid chromatography (Waters Instruments, Watford, UK) with detector (LDC,4100 UV 210 nm, USA)
24. Digitizing PC program (Un-Scan-It, Silk Scientific, USA)

Fig. 4.1 Orthocryl®, Dentaurum, Pforzheim, Fr, Germany

Fig. 4.2 Thai self-cured orthodontic resin, MTEC-MU

Fig.4.3 Rotary Pregrinder, Metaserv 2000, 300 cycle/min

Fig. 4.4 Weighing machine, Model AB 204, Mettler-Toledo Ltd., Switzerland

Fig. 4.5 Digital Calipers

Fig. 4.6 Digital Micrometer, Mitutoyo No 293-421 N

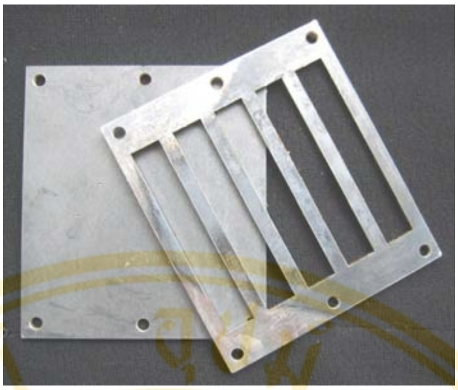


Fig. 4.7 Mould for flexural strength and modulus test : stainless steel mould with 64 mm in length, 10 mm in width, 3.3 mm in height, ISO 1567: 1999

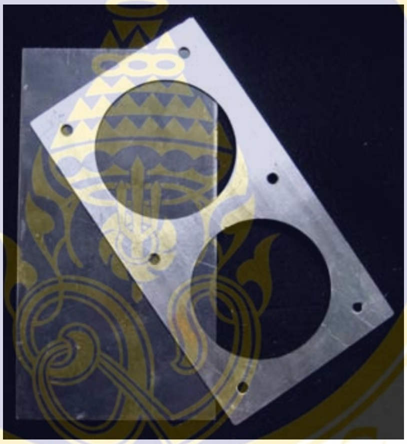


Fig. 4.8 Mould for water sorption and solubility tests:stainless steel mould with 50.0 ± 0.1 mm in diameter and 0.5 ± 0.05 mm in thickness, ISO 1567: 1999

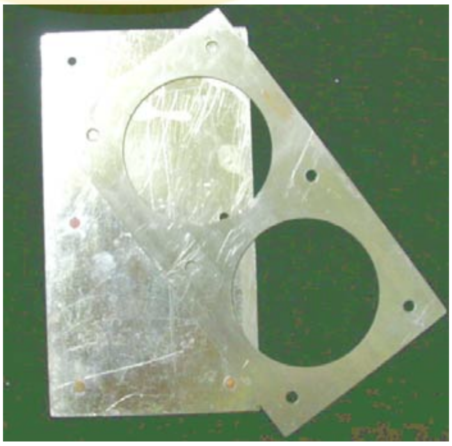


Fig. 4.9 Mould for residual methyl methacrylate monomer test : stainless steel mould with 50.0 mm in diameter and 3.0 ± 0.1 mm in thickness, ISO 1567: 1999

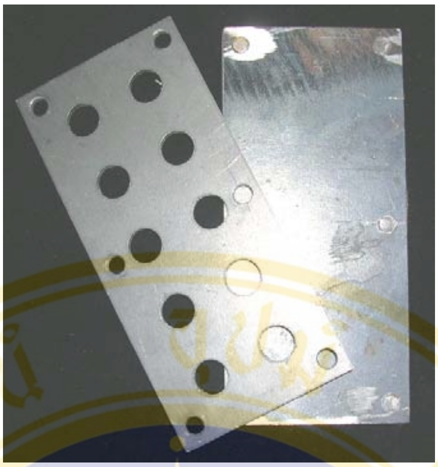


Fig. 4.10 Mould for biocompatibility test : stainless steel mould with 5 mm in diameter and 1 mm in thickness

Preparation of specimens

Specimens were prepared into four groups for two types of self-cured orthodontic resins using stainless steel moulds by conventional (pre-mixing resin before pour into the moulds) and infiltration techniques. Specimens were prepared for each group according to manufacturer's conditions (Table 4.1) and tested by means of ISO 1567 :1999.

Group1: Orthocryl® (Dentaurum, Pforzheim, Fr, Germany), prepared by conventional (mixing) technique, powder : liquid ratio 1.96 : 1 by weight.

Group 2: Orthocryl® (Dentaurum, Pforzheim, Fr, Germany), prepared by infiltration technique, powder : liquid ratio 2.05 : 1 by weight

Group 3: Thai resin, prepared by conventional (mixing) technique, powder : liquid ratio 1.9 : 1 by weight

Group 4: Thai resin , prepared by infiltration technique, powder : liquid ratio 1.7 : 1 by weight

Table 4.1 Polymerization and processing conditions of self-cured orthodontic resins.

Material	P/L ratio (by weight)	Curing process	Manufacturer
Group1:Orthocryl®-mixing	1.96 : 1	35-45 ^{0}C 2.2 bars(30p.s.i.) 25 min	Dentaurum, Pforzheim, Fr, Germany
Group 2:Orthocryl®-infiltration	2.05 : 1		
Group3:Thai-mixing	1.9 : 1	35-45 ^{0}C 2.2 bars(30p.s.i.) 25 min	MTEC-MU
Group 4: Thai-infiltration	1.7 : 1		

Fig. 4.11 Specimen strip for flexural strength and modulus tests (64 ± 0.2 mm × 10± 0.2 mm × 3.3 ± 0.2 mm)

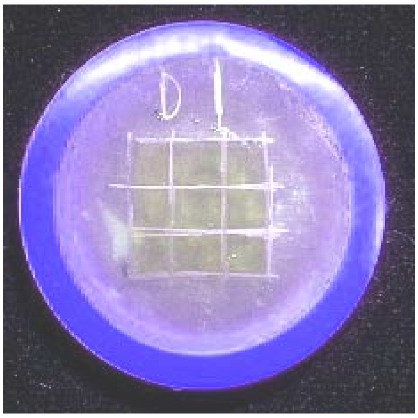


Fig. 4.12 Specimen for surface hardness test (10 ± 0.2 mm × 10.0 ± 0.2 mm × 3.0 ± 0.2 mm)

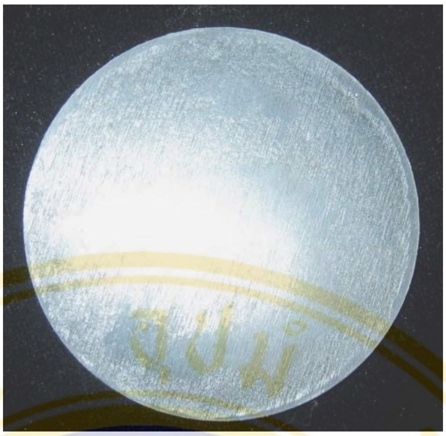


Fig. 4.13 Specimen disc for water sorption and solubility tests

(50.0 ± 0.1 mm in diameter and 0.5 ± 0.05 mm in thickness)

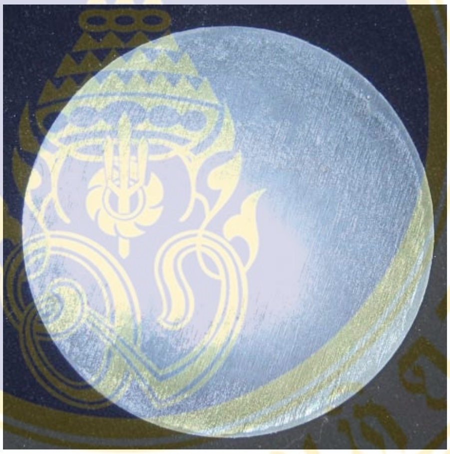


Fig. 4.14 Specimen disc for residual methyl methacrylate test

(50.0 ± 0.1 mm in diameter and 2.0 ± 0.1 mm in thickness)

Part 1: Flexural Strength and Modulus Test

Five strip specimens were prepared. Three measurements of the specimen height were made along the long axis with an accuracy of ± 0.1 mm. The deviation must not more than ± 0.02 mm. Then the specimen was laid on the supports of the flexural test rig. The distance between the center of the supports was 50 ± 0.1 mm. The loading plunger was placed midway between the supports to within 0.1 mm. During testing, the flexural test rig was immersed in the water bath at 37±1°C. The force was increased uniformly on the loading plunger, using a constant crosshead speed 5 ± 1 mm/minute, until the specimen was broken.

Pass/fail determination of flexural strength

According to ISO 1567:1999, flexural strength shall be not less than 60 MPa

- If at least 4 results of 5 specimens > 60 MPa = Pass
- If at least 3 results of 5 specimens < 60 MPa = Fail
- If at least 2 results of 5 specimens < 60 MPa = Repeated test, if at least 5 results of 6 specimens > 60 MPa = Pass

Pass/fail determination of flexural modulus

According to ISO 1567:1999, flexural modulus shall be at least 1500 MPa

- If at least 4 results of 5 specimens > 1500 MPa = Pass
- If at least 3 results of 5 specimens < 1500 MPa = Fail
- If at least 2 results of 5 specimens < 1500 MPa = Repeated test, if at least 5 results of 6 specimens > 1500 MPa = Pass

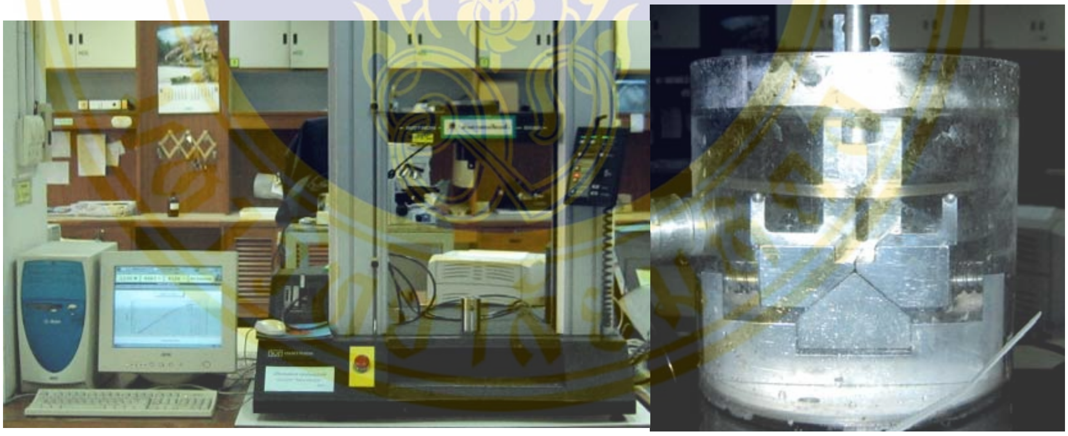


Fig. 4.15 Universal testing machine; Instron model 5566

Part 2: Surface Hardness Test

Specimens were produced and tested by the technique described by ISO 6507-1(E) – Vickers hardness test. The square-shaped specimens (10 ± 0.2 mm × 10.0 ± 0.2 mm × 3.0 ± 0.2 mm) were prepared. The thickness of specimen was an average of six measurements with deviation ≤ 0.02 mm. The specimen was dried and tested within 24 hours by microhardness testing machine with Vickers pyramid diamond indenter and a square base with 136^{o} angle at vertex. The force 50 gram (500 N) was applied in a direction perpendicular to the specimen surface, without shock or vibration for 15 seconds at speed 50 µm/sec. The specimens were indented 9 positions/specimen at room temperature ($23+2^0$C). After unloading, the diagonals of indentation were measured and Vickers hardness number was evaluated using a microscope and computer analysis.

$$\text{The Vickers hardness number} = \frac{\text{Constant} \times \text{test force}}{\text{Surface area of indentation}}$$

$$= \frac{0.102 \times 2F \sin 136° /2}{d^2}$$

$$= 0.1891 \times F/ d^2$$

F = Test force

d = Diameter of specimen

Fig. 4.16 Microhardness Testing Machine; Future Tech 700 ex

PART 3: Water Sorption and Solubility Tests

Conditioned specimens

Specimens were produced and tested according to ISO 1567:1999. The specimens were stored for 23± 1 hours in the rack inside the desiccator which has been placed in the oven at 37±1°C. After that, they were stood at ambient temperature for 60± 10 minutes. Each specimen was weighed using the electric balance to an accuracy of ± 0.2 mg. The desiccator was kept sealed except for the shortest possible period required for removing and replacing specimens. After all the specimens were weighed, silica gel was replaced in desiccator. The cycle described above was repeated until a constant mass, m_1 called the “conditioned mass” was reached which the difference in weight measurement of each specimen was not more than 0.2 mg. At this point, volume V of each specimen was calculated using the mean of three diameter measurements and the mean of five thickness measurements.

Wet specimens

Immerse the conditioned specimens in water at 37±1°C for 7 day ± 2 hours. After that, remove the discs from water, wipe with a clean dry towel until free from visible moisture, wave in the air for 15 ±1 second and weigh within 60 ±1 second after removal from water. Record this mass as m_2.

Reconditioned specimens

After weighing wet specimens, the specimens were reconditioned to the constant mass in the desiccator. Record the mass of the “ reconditioned” specimens as m_3.

Calculation of water sorption and solubility

Water sorption (Wsp)

$$\mathbf{Wsp = \frac{m_2 - m_3}{V} \quad \mu g/mm^3}$$

$\mathbf{m_2}$ = the mass of the specimen after immersion in water (µg)

$\mathbf{m_3}$ = the reconditioned mass of the specimen (µg)

V = the volume of the specimen (mm^3)

Water solubility (Wsl)

$$\mathbf{Wsl = \frac{m_1 - m_3}{V} \quad \mu g/mm^3}$$

$\mathbf{m_1}$ = the “conditioned”mass of the specimen (µg)

$\mathbf{m_3}$ = the reconditioned mass of the specimen (µg)

V = the volume of the specimen (mm^3)

Pass/fail determination of water sorption

According to ISO 1567:1999, water sorption shall not exceed 32 µg/mm^3

- If at least 4 results of 5 specimens ≤ 32 µg/mm^3 = Pass
- If at least 3 results of 5 specimens > 32 µg/mm^3 = Fail
- If at least 3 results of 5 specimens ≤ 32 µg/mm^3 = Repeated test, if at least 5 results of 6 specimens ≤ 32 µg/mm^3 = Pass

Pass/fail determination of water solubility

According to ISO 1567:1999, water solubility shall not exceed 8.0 µg/mm^3

- If at least 4 results of 5 specimens ≤ 8.0 µg/mm^3 = Pass
- If at least 3 results of 5 specimens > 8.0 µg/mm^3 = Fail
- If at least 3 results of 5 specimens ≤ 8.0 µg/mm^3 = Repeated test, if at least 5 results of 6 specimens ≤ 8.0 µg/mm^3 = Pass

Fig. 4.17 Desiccator with specimen discs

PART 4: Residual Methyl Methacrylate Monomer Test

Preparation of test specimen disks

Three specimen disks per group of self-cure resins were prepared from three separate mixes. Specimens were prepared in the form of thin disks with a diameter of 50 mm and a thick of 3.0± 0.1 mm with a flat cover in accordance with ISO 1567: 1999. After 24 hour dark-storage at 23 ^{0} C, the specimen disks were ground equally on both sides using grinding paper to a thickness of 2.0 ± 0.1 mm to remove any un-polymerized or poorly polymerized surface layer. During grinding, the grinding paper and specimen disks were lubricated with running water to avoid frictional heat that could cause loss of monomer or de-polymerization. The ground specimens were stored in the dark laboratory environment of 23 ^{0}C for 24 ± 1 hour prior to extraction of monomer.

Extraction of monomer

Three sample solutions were analysed from each test specimen, i.e. a total of nine sample solutions. Each specimen disk was broken into pieces small enough to pass through the 9 mm neck of a 10 ml volumetric glass flask. Specimen pieces were weighed accurately with a balancing machine. Samples of approximately 650 mg were introduced into separate one-mark 10 ml volumetric glass flasks. Acetone solution was chosen as the extraction solvent and was added until the total volume was 10 ml. Magnetic stirring was used to agitate the sample solutions for 72 ± 2 hour at 23 ^{0}C. A Gilson pipette with separate volumetric tips was used to transfer 2 ml aliquot of each previously prepared sample solution to one-mark 10 ml volumetric flasks. To precipitate the dissolved polymer, methanol solution was added to each sample solution to a total volume of 10 ml. A Gilson pipette with separate volumetric tips was used to transfer 5 ml of the polymer and monomer containing slurry from each of the 10 ml flasks to centrifuge tubes. The slurry was centrifuged at 3000 g_n m/s^2 for 15 minutes.

Use separate volumetric pipettes to transfer a 3 ml aliquot of each centrifuged solution to separate glass tubes. There was remaining polymer in the solution. Then, methanol was added to an aliquot until the total volume was 10 ml. The slurry was centrifuged at 3000 g_n m/s^2 for 15 minutes again. Determine that there is no remaining polymer in the solution. The solution was appeared clear when the beam of light is directed vertically through the test tube containing the solution.

High-performance liquid chromatography (HPLC) test

HPLC operating conditions

Column - 8 mm internal diameter, 100 mm length with octadecyl silanized 10 mm particles

Mobile phase - 67% methanol (HPLC grade), 33% deionized distilled water

Flow rate - 0.8 ml/min

Detecter - UV 205 nm

Temperature - Constant room temperature (23 ^{0}C)

Injection loop - 20 µl

The clear solution from the upper portion of the centrifuged solution was introduced into the injector of the HPLC system. The HPLC was operated for 20 minutes until all components were completely eluted. The HPLC traces were recorded with a standard data-capture system. The peak area of MMA on the HPLC trace was determined by a digitizing PC program. The concentration of MMA monomers (c_{MMA}) in the sample solutions was determined using a linear regression equation obtained from a calibration graph. The following equation was used to calculate total amount of MMA monomer in the sample solution, m_{MMA} (mg):

$$m_{\mathrm{MMA}}\ (\mu\mathrm{g}) = \left[c_{\mathrm{MMA}}\ (\mu\mathrm{g/ml}) \times \frac{10\ (\mathrm{ml})}{2\ \mathrm{ml}} \times \frac{10\ (\mathrm{ml})}{5\ \mathrm{ml}} \times 10\ (\mathrm{ml}) \right]$$

This value was used to calculate the weight percentage of the residual MMA by the following equation:

$$\text{Residual MMA monomer (wt\%)} = \frac{m_{\mathrm{MMA}}\ (\mu\mathrm{g}) \times 100}{m_{\mathrm{SAMPLE}}\ (\mu\mathrm{g})}$$

m_{MMA} = Total quantity of MMA in the sample solution (µg)

m_{SAMPLE} = The mass of sample solution (µg)

Pass/fail determinations of the level of residual methyl methacrylate monomer

According to ISO 1567:1999, the level of residual monomer shall not exceed 4.5% by mass fraction.

- If results obtained for at least 7 of the sample solutions $\leq$ 4.5% by mass fraction = Pass
- If 4 or fewer of the sample solutions $\leq$ 4.5% by mass fraction = Fail
- If only 5 or 6 of the sample solutions $\leq$ 4.5% by mass fraction = Repeated test, if at least 8 of the sample solutions $\leq$ 4.5% by mass fraction = Pass

Fig. 4.18 Sample solutions in 10 ml volumetric glass flasks with magnetic stirring bars

Fig. 4.19 High-performance liquid chromatography, Waters Instruments, Watford, UK

PART 5: Biocompatibility: Agar overlay test

Neutral red is a weakly cationic dye that readily diffuses across plasma and organelle membranes, accumulating in the lysosomes. Loss of membrane integrity induced by tested agents will result in decreased retention of neutral red. Damaged or dead cells thus appear decolorized in comparison to healthy control cells (69).

Sample Preparation

Specimens were prepared and tested by means of ISO 7405: 1999(E) (70). Sample disks of self-cured resins were fabricated in circular discs with a diameter of 5 mm and a thickness of 1 mm, with a flat surface to ensure adequate contact with agar overlay. Three disks of each material were prepared and stored in distilled water at 37 ^{0} C for 48 hours.

Culture medium, reagents and equipment

- Use Dulbecco's Modified Eagle's Medium containing 2.2 g/l sodium bicarbonate, 3.0 g/l HEPES and 50 ml/l new born calf serum as cell culture medium.
- Prepare a double concentration of Eagle's Basal Medium omitting HEPES and reducing sodium bicarbonate to 1 g/l for agar's preparation

The vital stain was prepared by diluting a stock solution of 1% aqueous neutral red solution 1/100 with 0.01 ml/l phosphate-buffered saline solutions immediately before use. Store neutral red solutions protected from the light. Use Petri dishes of diameter 50 mm to 100 mm suitable for tissue culture. For the preparation of the agar, either 3 % agar or 3 % agarose were prepared in a double concentration of the culture medium and then sterilized by autoclaving.

Test procedure

The mouse fibroblast cells were cultured until they reached the end of the log growth phase. Pipette 10 ml of cell suspension (3×10^5 cells/ml) into a sufficient number of Petri dishes and incubate at $(37\pm2)^0$ C in a water-saturated atmosphere with 5%(V/V) carbon dioxide for 24 hours. The sterile agar was heated to 100^0 C in a water bath and allowed it to cool to 48^0 C. One part of agar and one part of double concentration of nutrient medium were mixed and heated to 48 ^{0}C. The liquid nutrient medium was aspirated from each Petri dish and replaced with 10 ml of freshly prepared agar/nutrient medium mixture. Allow the agar nutrient medium to solidify at room temperature (approximately 30 minutes). Then, 10 ml neutral red solution was added and kept dark for 15 to 20 minute. Excess dye was then removed, and the test specimens were placed on the agar surface.

A polyvinyl carbonate disk was used as positive control and millipore filter used as negative control. Keep the specimens as far as possible from each other and from the wall of the Petri dish. Then, the Petri dish was incubated at (37±2) ^{0} C in a water-saturated atmosphere with 5 %(V/V) carbon dioxide for 24 hours. Thereafter, the cultures were examined under an inverted microcope by one examiner experienced in the use of this evaluation technique.

Parameters of assessment

The decolorization zone around the test materials and controls shall be assessed using an inverted microscope with a calibrated screen. Decolorization Index and Lysis Index were determined for each specimen. Each test was repeated twice using the same test specimens. Calculate the median Decolorization Index and Lysis Index for each test material and present the cell response as follows:

Decolorization Index	**Description**
0	No decolorization detectable
1	Decolorization only under the test substance
2	Decolorization zone not greater than 5.0 mm from the test substance
3	Decolorization zone not greater than 10.0 mm from the test substance
4	Decolorization zone greater than 10.0 mm from the test substance
5	The total culture is decolorized

Lysis Index	Description
0	No cell lysis detectable
1	Less than 20% cell lysis
2	20% to 40% cell lysis
3	>40% to <60% cell lysis
4	60% to 80% cell lysis
5	More than 80% cell lysis

Assessment of results

All information gathered in the test shall be taken into account in the test results, particularly any differences in results between the experimental and control groups. The cell response was based on the median Decolorization Index and Lysis Index.

Cell response = Decolorization Index / Lysis Index

Scale	Cell Response	Interpretation
0	0/0	Noncytotoxic
1	1/1	Mildly cytotoxic
2	2/2 to 3/3	Moderate cytotoxic
3	4/4 to 5/5	Severely cytotoxic

Analysis of the test results : Non–Parametric tests

One-Sample T test was used to compare statistically significant differences in levels of flexural strength, flexural modulus, water sorption, water solubility and residual methyl methacrylate monomer, tested according to ISO 1567:1999.

Kruskal-Wallis Test was used to determine statistically significant differences in levels of flexural strength, flexural modulus, surface hardness, water sorption, water solubility, residual methyl methacrylate monomer and biocompatibility among groups of self-cured orthodontic resins.

Mann- Whitney Test was used to compare statistically significant differences in levels of flexural strength, flexural modulus, surface hardness, water sorption, water solubility and residual methyl methacrylate monomer among groups of self-cured orthodontic resins, as well as between conventional and infiltration techniques.
* Statistical analysis was conducted at 0.05 significance level.

CHAPTER 5

RESULTS

The values of flexural strength, flexural modulus, surface hardness, water sorption, water solubility, level of residual monomer and biocompatibility of each orthodontic resin, tested according to ISO 1567:1999 are shown in Tables 4.1 – 4.10, respectively.

Table 5.1 The values of flexural strength and modulus of each orthodontic resin, tested according to ISO 1567:1999

Group	Type	Flexural Strength (MPa)	Flexural Modulus (MPa)
1	Orthocryl®/mixing-1	68.24	2184.86
	Orthocryl®/mixing-2	68.32	1922.43
	Orthocryl®/mixing-3	63.39	2212.67
	Orthocryl®/mixing-4	68.19	2356.58
	Orthocryl®/mixing-5	68.05	2356.58
2	Orthocryl®/infiltration-1	65.56	2211.54
	Orthocryl®/infiltration-2	71.45	2435.79
	Orthocryl®/infiltration-3	72.55	2366.66
	Orthocryl®/infiltration-4	69.43	2129.83
	Orthocryl®/infiltration-5	70.48	2282.36
3	Thai/mixing-1	62.05	1902.20
	Thai/mixing-2	69.61	2254.46
	Thai/mixing-3	64.48	1995.75
	Thai/mixing-4	62.59	2120.92
	Thai/mixing-5	61.80	2006.72
4	Thai/infiltration-1	61.91	2422.45
	Tha/infiltration-2	68.55	2295.61
	Tha/infiltration-3	64.34	2323.00
	Tha/infiltration-4	68.59	2080.33
	Tha/infiltration-5	63.54	2072.40

Table 5.2 Means and standard deviations of flexural strength and flexural modulus of orthodontic resins, tested according to ISO 1567:1999

Group	Mean Flexural	SD	Mean Flexural	SD
1 : Orthocryl®-mixing	67.24	2.15	2206.62	177.66
2: Orthocryl®-infiltration	69.89	2.68	2285.23	121.33
3 : Thai -mixing	64.11	3.25	2056.01	135.38
4 : Thai -infiltration	65.39	3.04	2238.76	155.60

Table 5.3 The values of surface hardness of each orthodontic resin, tested according to ISO 6507-1(E):1997

Group	Type	Surface hardness (VHN)
1	Orthocryl®/mixing-1	16.27
	Orthocryl®/mixing-2	16.69
	Orthocryl®/mixing-3	16.73
	Orthocryl®/mixing-4	16.85
	Orthocryl®/mixing-5	17.17
2	Orthocryl®/infiltration-1	16.16
	Orthocryl®/infiltration-2	17.25
	Orthocryl®/infiltration-3	17.28
	Orthocryl®/infiltration-4	16.52
	Orthocryl®/infiltration-5	16.78
3	Thai/mixing-1	15.24
	Thai/mixing-2	14.93
	Thai/mixing-3	14.56
	Thai/mixing-4	14.55
	Thai/mixing-5	14.61
4	Thai/infiltration-1	15.34
	Tha/infiltration-2	15.01
	Tha/infiltration-3	15.51
	Tha/infiltration-4	15.23
	Tha/infiltration-5	15.19

Table 5.4 Means and standard deviations of surface hardness of orthodontic resins, tested according to ISO 1567:1999

Group	Mean Surface Hardness (VHN)	SD
1 : Orthocryl®-mixing	16.74	0.32
2 : Orthocryl®-infiltration	16.80	0.48
3 : Thai-mixing	14.78	0.30
4 : Thai-infiltration	15.26	0.19

Orthocryl® **Thai resin**

Fig.5.1 Vickers indenter of each type of orthodontic resin from Vickers indenter, Surface hardness test

Table 5.5 The values of water sorption and solubility of each orthodontic resin, tested according to ISO 1567:1999

Group	Type	Water Sorption (µg/mm^3)	Water Solubility (µg/mm^3)
1	Orthocryl®/mixing-1	16.56	0.59
	Orthocryl®/mixing-2	16.62	0.6
	Orthocryl®/mixing-3	17.34	0.69
	Orthocryl®/mixing-4	17.03	0.51
	Orthocryl®/mixing-5	15.96	0.57
2	Orthocryl®/infiltration-1	16.86	0.62
	Orthocryl®/infiltration-2	17.43	0.57
	Orthocryl®/infiltration-3	17.57	0.71
	Orthocryl®/infiltration-4	16.81	0.53
	Orthocryl®/infiltration-5	17.96	0.82
3	Thai/mixing-1	18.41	1.57
	Thai/mixing-2	18.33	1.04
	Thai/mixing-3	17.54	0.89
	Thai/mixing-4	18.35	1.25
	Thai/mixing-5	19.01	1.36
4	Thai/infiltration-1	19.24	1.07
	Tha/infiltration-2	20.03	1.53
	Tha/infiltration-3	19.71	1.45
	Tha/infiltration-4	18.13	0.95
	Tha/infiltration-5	20.85	1.62

Table 5.6 Means and standard deviations of water sorption and solubility of orthodontic resins, tested according to ISO 1567:1999

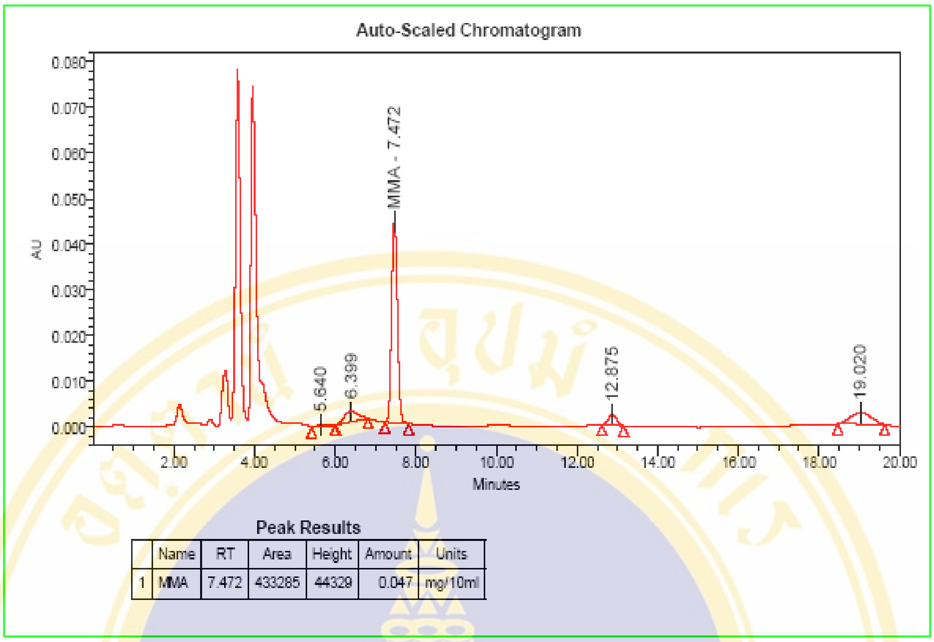
Group	Mean Water Sorption (µg/mm^3)	SD	Mean Water Solubility	SD
1:Orthocryl®-mixing	16.62	0.52	0.59	0.06
2:Orthocryl®-infiltration	16.56	0.49	0.51	0.12
3:Thai-mixing	17.34	0.52	0.69	0.27
4:Thai- infiltration	17.03	1.01	0.60	0.30

Table 5.7 The value of level of residual monomer of each orthodontic resin, tested according to ISO 1567:1999

Group	Type	Level of residual monomer	Group	Type	Level of residual monomer
1	Orthocryl®/mixin	0.63	3	Thai/mixing-	0.47
	Orthocryl®/mixin	1.03		Thai/mixing-	0.25
	Orthocryl®/mixin	0.07		Thai/mixing-	0.24
	Orthocryl®/mixin	0.76		Thai/mixing-	0.78
	Orthocryl®/mixin	1.45		Thai/mixing-	1.04
	Orthocryl®/mixin	0.17		Thai/mixing-	0.20
	Orthocryl®/mixin	0.25		Thai/mixing-	1.52
	Orthocryl®/mixin	1.40		Thai/mixing-	1.53
	Orthocryl®/mixin	0.21		Thai/mixing-	0.29
2	Orthocryl®/infiltr	1.25	4	Tha/infiltratio	1.48
	Orthocryl®/infiltr	1.81		Tha/infiltratio	0.12
	Orthocryl®/infiltr	0.83		Tha/infiltratio	1.89
	Orthocryl®/infiltr	0.72		Tha/infiltratio	1.85
	Orthocryl®/infiltr	1.23		Tha/infiltratio	0.35
	Orthocryl®/infiltr	0.27		Tha/infiltratio	0.50
	Orthocryl®/infiltr	0.30		Tha/infiltratio	0.56
	Orthocryl®/infiltr	1.47		Tha/infiltratio	1.85
	Orthocryl®/infiltr	1.57		Tha/infiltratio	2.21

Table 5.8 Means and standard deviations of level of residual monomer of orthodontic resins, tested according to ISO 1567:1999

Group	Mean Residual Monomer (wt%)	SD
1:Orthocryl®-mixing	0.66	0.53
2:Orthocryl®-infiltration	1.05	0.55
3:Thai-mixing	0.70	0.54
4:Thai-infiltration	1.20	0.81



Peak Results

	Name	RT	Area	Height	Amount	Units
1	MMA	7.472	433285	44329	0.047	mg/10ml

Fig. 5.2 Chromatogram of standard solution (MMA) by HPLC method

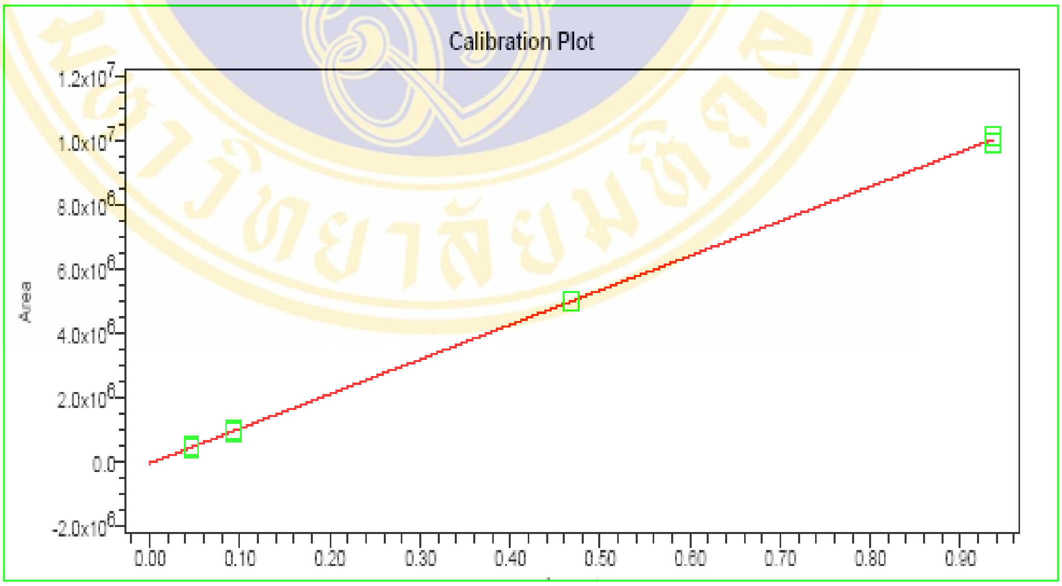
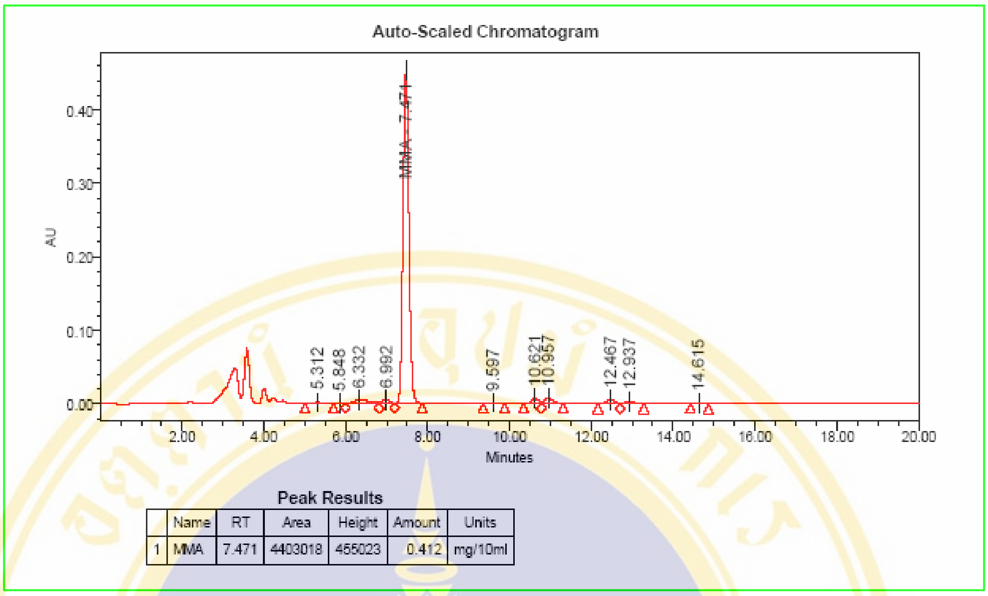


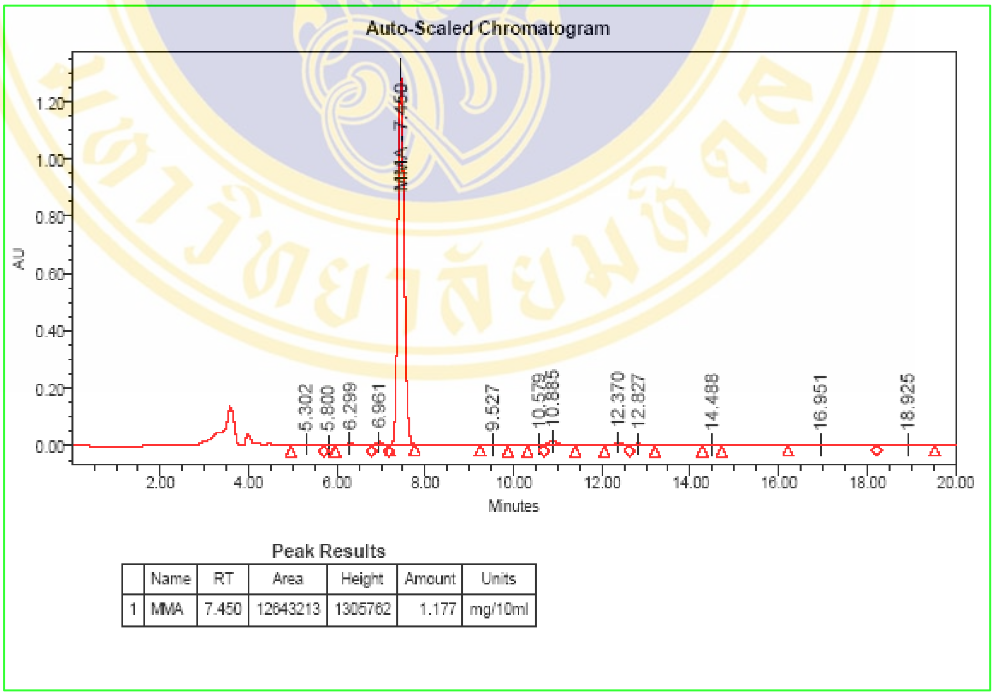
Fig. 5.3 Calibration curve of standard solution (MMA) by HPLC method



Peak Results

	Name	RT	Area	Height	Amount	Units
1	MMA	7.471	4403018	455023	0.412	mg/10ml

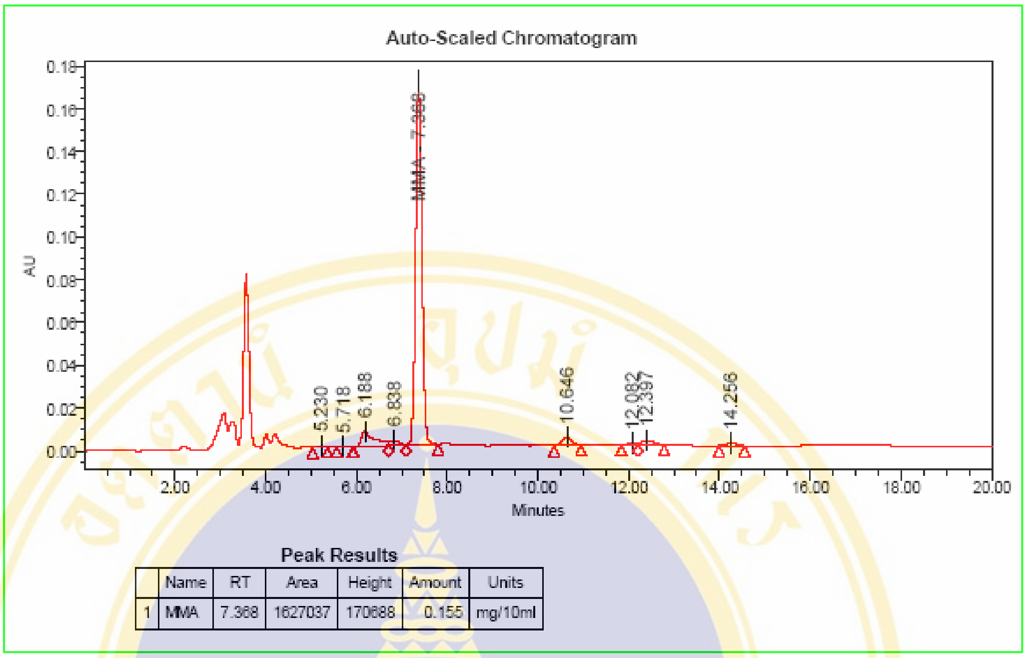
Fig. 5.4 Chromatogram of solution of Orthocryl® prepared by mixing technique, HPLC method



Peak Results

	Name	RT	Area	Height	Amount	Units
1	MMA	7.450	12643213	1305762	1.177	mg/10ml

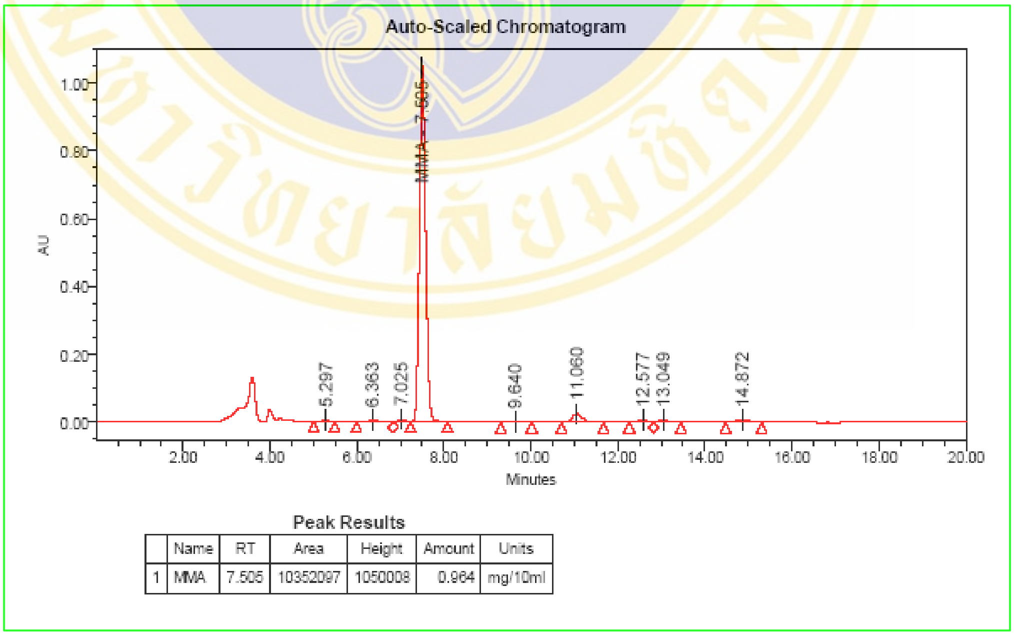
Fig. 5.5 Chromatogram of solution of Orthocryl® prepared by infiltration technique, HPLC method



Peak Results

	Name	RT	Area	Height	Amount	Units
1	MMA	7.368	1627037	170688	0.155	mg/10ml

Fig. 5.6 Chromatogram of solution of Thai resin prepared by mixing technique, HPLC method



Peak Results

	Name	RT	Area	Height	Amount	Units
1	MMA	7.505	10352097	1050008	0.964	mg/10ml

Fig. 5.7 Chromatogram of solution of Thai resin prepared by infiltration technique, HPLC method

Table 5.9 The values of biocompatibility test of orthodontic resins, tested according to ISO 7405:1997(E)

Group	Decolorization zone (mm.)	Decolorization Index	Lysis Index	Cell Response Index	Inter-pretation
1: Orthocyl® -mixing	0	0	0	0/0	Non-cytotoxi
2: Orthocyl® -infiltration	0	0	0	0/0	Non-cytotoxi
3:Thai-mixing	0	0	0	0/0	Non-cytotoxi
4:Thai-infiltration	0	0	0	0/0	Non-cytotoxi

Table 5.10 Means and standard deviations of flexural strength & modulus, surface hardness, water sorption & solubility, level of residual monomer and biocompatibility tests of self-cured resins

Groups / Properties	Orthocryl®-mixing	Orthocryl®-infiltration	Thai -mixing	Thai - infiltration
Mean Flexral Strength (MPa)	67.24±2.15	69.89±2.68	64.11±3.25	65.39±3.04
Mean Flexural Modulus (MPa)	2206.62±177.66	2285.23±121.33	2056.01±135.38	2238.76±155.60
Mean Surace Hardness (VHN)	16.74±0.32	16.80±0.48	14.78±0.30	15.26±0.19
Mean Water Sorption (µg/mm^3)	16.62±0.52	16.56±0.49	17.34±0.52	17.03±1.01
Mean Water Solubility (µg/mm^3)	0.59±0.06	0.51±0.12	0.69±0.27	0.60±0.30
Level of MMA	0.66±0.53	1.05±0.55	0.70±0.54	1.20±0.81
Biocompability	Non-toxicity	Non-toxicity	Non-toxicity	Non-toxicity

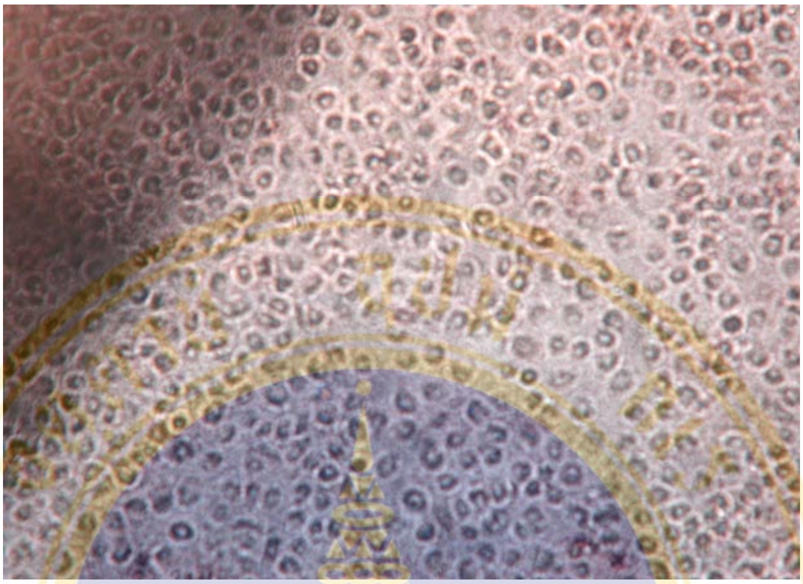


Fig. 5.8 Photograph (100×) of L 929 cell line from positive control :

Polyvinyl carbonate, Agar overlay test

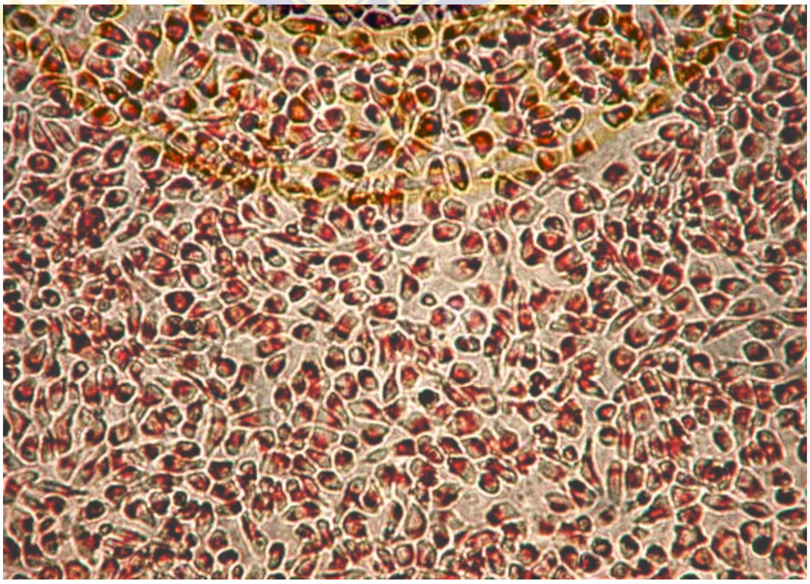


Fig. 5.9 Photograph (100×) of L 929 cell line from negative control :

Millipore filter, Agar overlay test

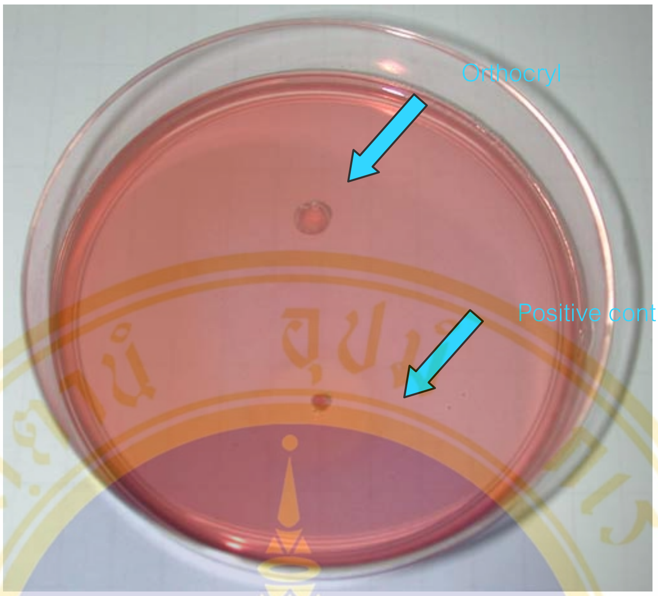


Fig. 5.10 Agar plate showing undecolorization zone of Orthocryl® (mixing technique) compared with decolorization zone of positive control

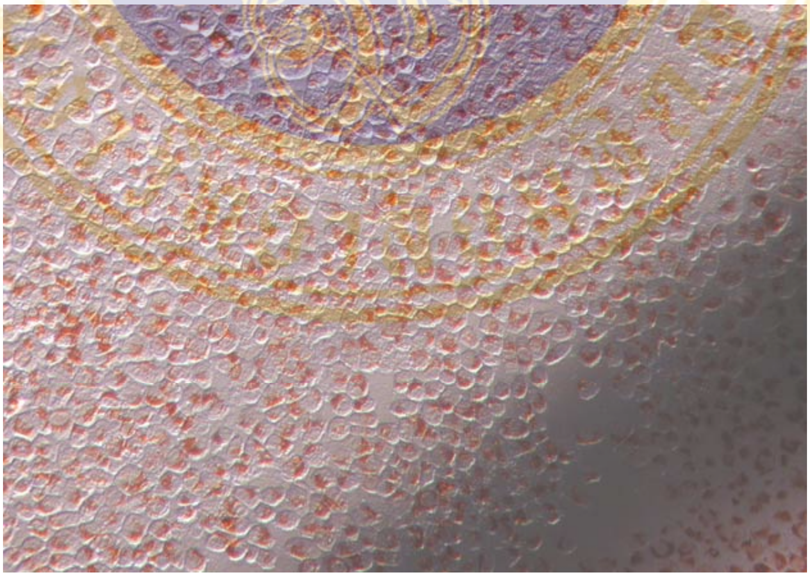


Fig. 5.11 Photograph (100×) of L 929 cell line from Orthocryl® (prepared by mixing technique), Agar overlay test

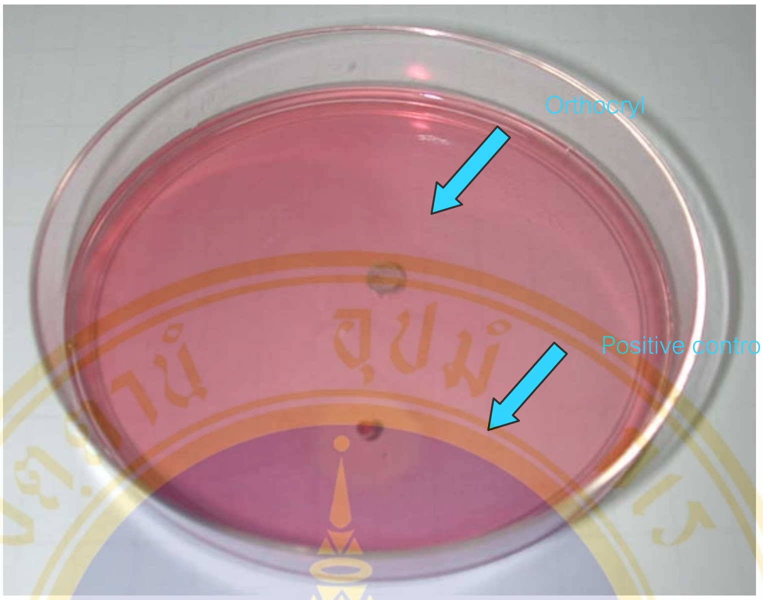


Fig. 5.12 Agar plate showing undecolorization zone of Orthocryl® (infiltration technique) compared with decolorization zone of positive control

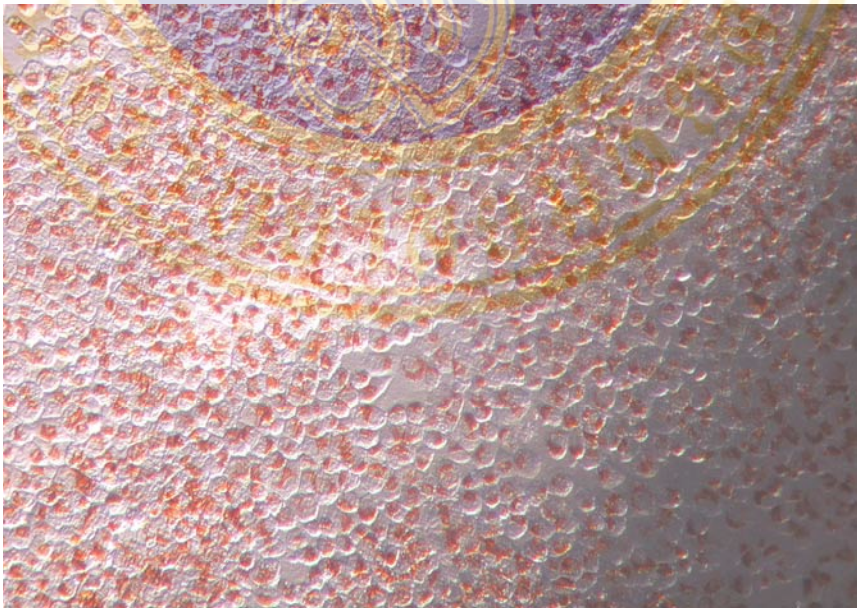


Fig. 5.13 Photograph (100×) of L 929 cell line from Orthocryl® (prepared by infiltration technique), Agar overlay test

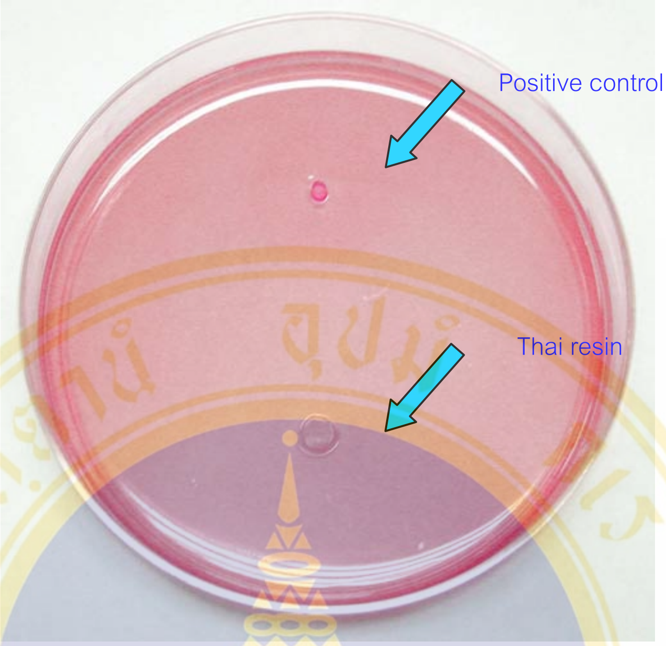


Fig. 5.14 Agar plate showing undecolorization zone of Thai resin (mixing technique) compared with decolorization zone of positive control

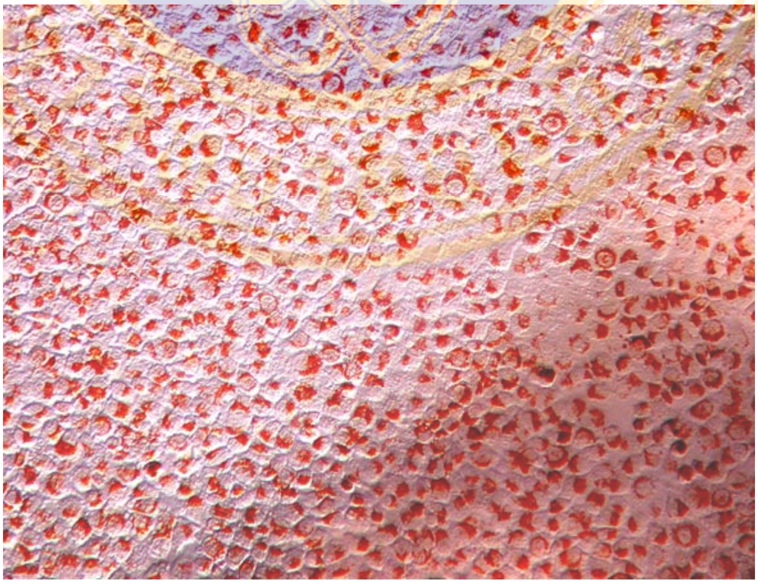


Fig. 5.15 Photograph (100×) of L 929 cell line from Thai resin (prepared by mixing technique), Agar overlay test

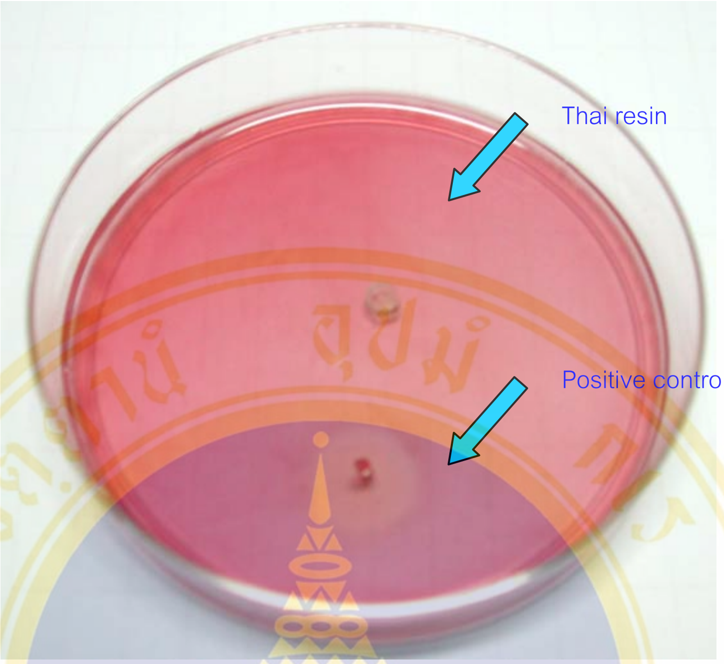


Fig. 5.16 Agar plate showing undecolorization zone of Thai resin (infiltration technique) compared with decolorization zone of positive control

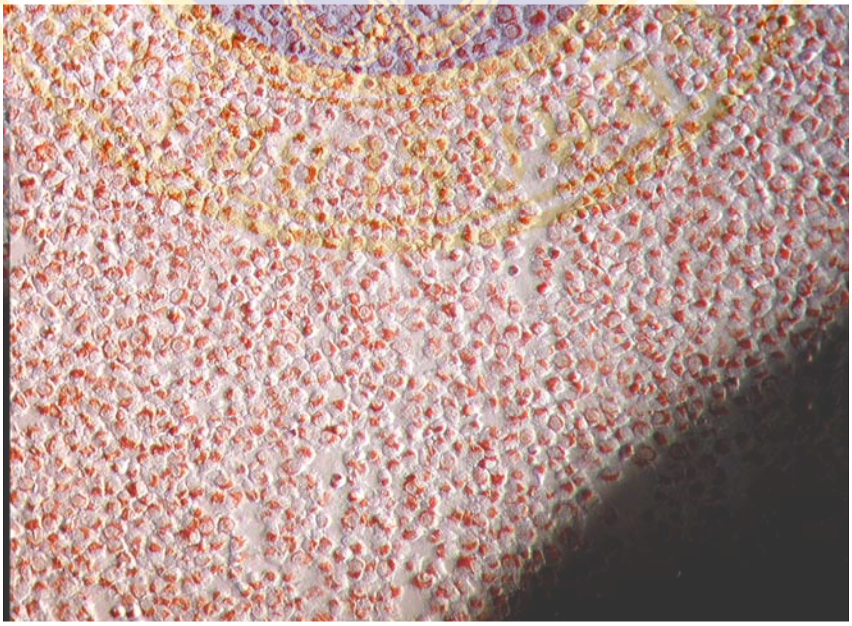


Fig. 5.17 Photographs (100×) of L 929 cell line from Thai resin (prepared by infiltration technique), Agar overlay test

The statistical tests were used to determine and compare significant differences in the levels of flexural strength, flexural modulus, surface hardness, water sorption, water solubility and level of residual monomer of orthodontic resins. The statistical results are shown in Tables 4.11 – 4.14.

Table 5.11 One-Sample T test of flexural strength, flexural modulus, water sorption, water solubility and level of residual monomer of self-cured orthodontic resins

	Flexural Strength	Flexural Modulus	Water Sorption	Water Solubility	Residual monomer
t	8.724	19.152	48.988	81.219	16.959
df	19	19	19	19	35
Sig.(2-tailed)	.000*	.000*	.000*	.000*	.000*

* Significant, P<0.05

Table 5.12 Kruskal-Wallis Test of flexural strength, flexural modulus, surface hardness,water sorption, water solubility and level of residual monomer of self-cured orthodontic resins

	Flexural Strength	Flexural Modulus	Surface Hardness	Water Sorption	Water Solubility	Residual monomer
Chi-Square	7.571	5.553	15.343	15.229	14.620	5.185
df	3	3	3	3	3	3
Asymp. Sig.	.056	.136	.002*	.002*	.002*	.159

* Significant, P<0.05

Table 5.13 Mann- Whitney Test of surface hardness, water sorption and solubility between groups of self-cured orthodontic resins

Asym. Sig.(2-tailed) between group	Surface Hardness	Water Sorption	Water Solubility
Group 1 – Group 2	.754	.076	.402
Group 1 – Group 3	.009*	.009*	.009*
Group 1 – Group 4	.009*	.009*	.009*
Group 2 – Group 3	.009*	.028*	.009*
Group 2 – Group 4	.009*	.028*	.009*
Group 3 – Group 4	.047*	.076	.548

* Significant, P<0.05

Table 5.14 Mann- Whitney Test of of flexural strength, flexural modulus, surface hardness,water sorption, water solubility and level of residual monomer of self-cured orthodontic resin between mixing and infiltration techniques

	Flexural Strength	Flexural Modulus	Surface Hardness	Water Absorption	Water Solubility	Residual monomer
Z	-1.587	-1.739	-.832	-1.436	-.567	-1.426
Asymp.Sig. (2-tailed)	.112	.082	.406	.151	.571	.154
Exact Sig	.123	.089	.436	.165	.579	.156

* Significant, P<0.05

Within the parameters of the present study, designs and material tests, statistical tests were applied to the results for statistical analysis and from that led to the following conclusions:

1. Flexural strength, flexural modulus, water sorption, water solubility, level of residual monomer and biocompatibility of Thai self-cured orthodontic resin were acceptable according to ISO 567:1999.

2. Orthocryl® and Thai self-cured orthodontic resin were non-cytotoxic, as tested by the method of the agar overlay.

3. There were no significant differences in flexural strength, flexural modulus and level of residual monomer between Orthocryl® and Thai self-cured orthodontic resin.

4. Surface hardness of Orthocryl® was statistically significantly higher than that of Thai self-cured orthodontic resin.

5. Water sorption and solubility of Orthocryl® were statistically significantly lower than those of Thai self-cured orthodontic resin.

6. There were no statistically significant differences in levels of flexural strength, flexural modulus, surface hardness, water sorption, water solubility and residual monomer of Orthocryl® and Thai self-cured orthodontic resin fabricated by mixing and infiltration techniques.

CHAPTER 6
DISCUSSION

In the recent study of MU and MTEC in 2005 (3), Thai NO.30 and 32 were tested according to ISO 1567:1999. From the result, it showed that flexural modulus, surface hardness, water sorption , water solubility except flexural strength of those two Thai resins met the ISO requirements, but still inferior to the commercial Orthocryl®. In this study, Thai self-cured orthodontic resin was further developed to improve mechanical, physical and biological properties, and especially its flexural strength. Flexural strength is an important mechanical property of acrylic denture base materials, since flexural behavior is a guide to service performance. Thus, it is important that the materials resist the mastication force without fracture or change in dimension (1, 18). Several factors were reported to affect the flexural strength. These included the average molecular weight which is an indicative of the strength of resin. In general, longer chains with higher molecular weight resulted in increased strength, hardness, stiffness, and resistance to creep of the polymer (11). The resulting increased strength and stiffness contributed the appliances to withstand occlusal stresses during function. Tager (22) reported the strength of polymer increased with increasing molecular weight until a certain molecular weight was attained. Above this molecular weight, there was not a great change in strength with further polymerization. It was shown that the strength became constant at degree of polymerization above 600 and molecular weight of 60,000 of PMMA. Strength and modulus were also found to increase as the cross-linking agent concentration increased. Cross-linking agents, usually less than 15%, have been added to the liquids of denture base resins to improve craze resistance, stiffness, and surface hardness (79, 80).

In previous study of Thai No 30 and 32, the effects of molecular weight and crosslinking agent on flexural strength were investigated. The results showed slightly increasing in flexural strength with increasing crosslinking agent content whereas the use of powder having high molecular weight, although with the plasticizer to promote wetting ability, did not increase flexural strength. The high molecular weight of powder caused an increase in viscosity during mixing. Accordingly, more amount of monomer was required to wet the powder thoroughly. The more monomer added to the mixture, the greater the amount of residual monomer would be remained in the cured resin. It was concluded that this low powder/liquid mixing ratios of Thai no.30 and 32 which resulted in the high remains of residual monomer was the main factor that caused the poor flexural strength of the cured resins.

In this study, to raise the powder/liquid ratio, the particle size and molecular weight of the powder were then reduced from what had been used for Thai NO.30 and 32, in order to allow a homogeneous mixing of resin and a high penetration of monomer into the polymer without too early increased in viscosity. That would lead to the reduction of voids and hence improvement of mechanical properties, water sorption and solubility. The powder characteristics, compositions and powder/ liquid ratio of Thai resin, Thai no.30 and 32 and Orthocryl® are illustrated in Tables 6.1 -6.3.

Table 6.1 The values of particle size, molecular weight and polydispersity of powder components of four types of self-cured orthodontic resins

Type of resins	Particle size (µm)	Molecular weight (Dalton)	Polydispersivity
Orthocryl®	63	494900	3.65
Thai resin	72	656000	3.69
Thai NO. 30	93	769300	4.29
Thai NO. 32	80	785200	3.81

Table 6.2 The composition of powder and liquid components of Thai self-cured orthodontic resins

Type of resins	Powder component			Liquid component		
	PMMA(g)	Benzoyl Peroxide (g)	Dibutylph-thalate(g)	MMA (ml)	Crosslinking agent (ml)	Activator (ml)
Thai resin	100	0.5	0	100	6	1
Thai NO. 30	100	0.5	1-1.5	100	6	1
Thai NO. 32	100	0.5	0	100	6	1

Table 6.3 The mean powder:liquid ratio of Orthocryl® and Thai self-cured orthodontic resins

Group	Mean powder:liquid ratio (by weight)
1: Orthocryl®-mixing	1.96 :1
2: Orthocryl®-infiltration	2.05:1
3: Thai resin-mixing	1.9:1
4: Thai resin-infiltration	1.7 : 1
Thai No. 30	1.2:1
Thai No. 32	1.8:1

As shown in Table 6.1, the powder of Thai resin was smaller in molecular weight and particle size compared to Thai no.30 and 32, but still higher than Orthocryl®. When mixing the powder with the liquid component composed of 6% crosslinking agent (Table 6.2), the smaller particle size and lower molecular weight of the powder led to a better wetting, a smoother mixing and a greater dissolving of the particles. As a result, the powder of Thai resin was able to mix at a higher powder/liquid ratio i.e. 1.9g/1g compared with 1.2g/1g and 1.8g/1g of Thai NO.30 and 32, respectively (Table 6.3).

The increase in powder/liquid ratio showed the considerable improvement in flexural strength of Thai resin as observed from its compliance with the ISO test. Water sorption and solubility, surface hardness and the amount of residual monomer of Thai resin were also improved and satisfied the ISO test by increasing powder/liquid ratio. The statistic results showed insignificant differences in flexural strength, flexural modulus and the level of residual monomer between Orthocryl® and Thai resin. However, surface hardness, water sorption and solubility of Thai resin seemed to be inferior to Orthocryl® which would be expected from the lower powder/liquid ratio of Thai resin. Many studies have reported the correlated effects of powder/liquid ratio and level of residual monomer on mechanical and physical properties, as well as biocompatibility of the cured resin. The different polymer to monomer ratios can result in the acrylic resin mixtures that could be either too stiff or too fluid for clinical use. When the polymerization reaction was activated, the polymers were formed by monomers joined together with high energy link (crossed-links) and this reaction would finish when all monomer reacted. However, some amount of residual monomers varied in the range of 0.54 to 1.08% (71) were remained after polymerization. The more crossed-links formed, the less residual monomer releasing, promoted lower solubility. Jacobsen et al (72) reported that powder/liquid ratio, manipulation, and polymerization were the extremely important influences for the success and longevity of orthodontic appliances. Lamb et al (48) investigated the effect of polymer to monomer ratio on residual monomer levels and observed that resins prepared with a high proportion of polymer resulted in significantly lower level of residual monomer. The presence of high level of residual monomer in the specimen affected the mechanical properties as reported by Wolfaardt et al (73). The decrease in mechanical properties was due to the loss of residual monomer, which leached out into either water or saliva, and to the absorption of water, which led to plasticization of the self-cured resin, making it more flexible and resilient (6). Baker et al (74) showed that the porosities in the structure of chemical-cured PMMA may affect the release of MMA by enhancing the diffusion between PMMA and water. Similarly, high powder/liquid ratio of Thai resin should reduce the amount of entrapped residual monomers which resulted in less porosity in specimen and hence increased flexural strength, flexural modulus, surface hardness, decreased sorption and solubility compared to Thai No 30 and No 32 resins.

In the technique of appliance's fabrication, Sweeney et al and Taylor (28, 75) reported that insufficient mixing of monomer and polymer may cause porosity in denture base resin. Depending on polymerization conditions, up to 11% porosity has been observed associated with the decreased mechanical properties, poor esthetics and the potential harboring of organisms and retention of fluids (29). The present study showed that there are no statistically significant differences in level of flexural strength, flexural modulus, surface hardness, water sorption, water solubility and residual monomer of the cured resins between mixing and infiltration techniques. However, the levels of residual monomer of specimens prepared by infiltration technique were higher than those prepared by mixing technique which was a result of excess monomer and insufficient mixing of monomer and polymer during infiltration. These results caused denture base resin with high porosity and high water sorption and solubility.

Biocompatibility is one of the important properties of self-cured resins. Biocompatibility is defined as the ability of a material to perform with an appropriate host response in a specific application (52). In the evaluation of medical devices, cytocompatibility tests are used both for screening and for the study of toxicity mechanisms (76). Cytotoxicity as a primary factor of biocompatibility is generally determined by in vitro cell culture. In vitro methods permit assessment of various parameters in a simplified system, while minimizing variables and allowing more specific determination of cytotoxic mechanisms. Experimental studies have demonstrated the good correlation between in vitro and in vivo tests, thus confirming the usefulness of in vitro tests as systems to select the materials.

Several clinical reports demonstrated tissue cytotoxicity when tissue was exposed to components released from the curing resins. Self-cured resins appeared to have a level of residual monomer as high as 5%, in comparison with 0.2 - 0.5% for heat-cured resins (41). Various compounds including a residual methyl methacrylate monomer, and additives such as hydroquinone, benzoyl peroxide, and *N*, *N*-dimemethyl-*p*-toluidine were eluted from acrylic polymers. These compounds diffused into saliva and come into contact with the oral mucosa, resulting in 'denture stomatitis' in which redness or burning sensation frequently occurred in the area beneath the dentures. Residual monomer, resulting from incomplete conversion of monomers into polymer, had the potential to cause irritation, inflammation, and an allergic response of the oral mucosa. Tsuchiya et al (58) reported that leachable substances from acrylic resins showed cytotoxic potential in the range of their leaching concentrations. Eluates from various denture base resins caused cytotoxic effects on oral epithelial cells (59) and human gingival fibroblasts (GF) (49), as well as inhibited cell growth, DNA replication, RNA synthesis, and metabolic processes (60). Moreover, Tang et al (56) demonstrated that direct contact of fibroblasts with methacrylate polymers, especially the unpolymerized surface layer, may more significantly reduce cell viability than what occurs with the eluates. Sheridan et al (49) reported that the cytotoxic effect of acrylic resins was greater in the first 24 hours after polymerization and decreased with time for all the resins evaluated in their study. Kedjarune et al (7) observed a reduced amount of residual monomer when the polymerization time was extended, thus resulting in less cytotoxic effects. This present study showed that levels of residual monomer of Thai self-cured resin and Orthocryl® were satisfied the ISO requirements. Also, Thai self-cured resin and Orthocryl® were non-cytotoxic in the agar overlay test with mouse fibroblast cells. However, dentists are recommended to reduce the amount of released monomer by soaking the denture base appliances, especially the ones processed by infiltration which seemed to give higher quantities of leaching residual monomers, in the water at least 24 hours (49) or immersed in water at 50°C for 60 minutes (50) before placing them in the patient's mouth.

From the results of this study, many factors affected the properties of the cured resins including particle size and molecular weight of powder, chemical compositions of the powder, and liquid and powder/liquid ratio. The reduction in particle size and molecular weight of PMMA powder could lead to the improvement of mechanical and physical properties of Thai self-cured orthodontic resin. Flexural strength, flexural modulus, water sorption, water solubility, level of residual monomer and biocompatibility of Thai resin were acceptable according to ISO 1567:1999. Thus, it has a potential to be an alternative for orthodontic denture base materials. However, clinicians should be careful in manipulation of orthodontic appliances since it had great effect on the properties of the cured denture bases, especially if the infiltration technique was used. The residual monomer concentration which affected the variations in mechanical and biological properties of appliances, was also the other important parameter that clinicians should be considered to minimize. Even though Thai self-cured resin was acceptable according to ISO 1567:1999 requirements, its water sorption and solubility, and surface hardness were found to be inferior to Orthocryl®. Then further study on improvement these properties of Thai resin i.e. by the further reduction of particle size and molecular weight of powder was recommended.

CHAPTER 7

CONCLUSIONS

Within the parameters of the present study, designs and material tests, statistical tests were applied to the results for statistical analysis and from that led to the following conclusions:

1. Flexural strength, flexural modulus, water sorption, water solubility, level of residual monomer and biocompatibility of Thai self-cured orthodontic resin were acceptable according to ISO standard.
2. Orthocryl® and Thai self-cured orthodontic resin were non-cytotoxic, according to the agar overlay test.
3. There were no significant differences in flexural strength, flexural modulus and level of residual monomer between Orthocryl® and Thai self-cured orthodontic resin.
4. Surface hardness of Orthocryl® was statistically significantly higher than that of Thai self-cured orthodontic resin.
5. Water sorption and solubility of Orthocryl® were statistically significantly lower than those of Thai self-cured orthodontic resin.
6. There were no statistically significant differences in levels of flexural strength, flexural modulus, surface hardness, water sorption, water solubility and residual monomer of Orthocryl® and Thai self-cured orthodontic resin fabricated by mixing and infiltration techniques.

From the results of this study, many factors affected the properties of the self-cured orthodontic resins including the particle size, molecular weight of powder, chemical compositions of powder and liquid, cross-linking between polymer chains, powder/liquid ratio and processing techniques. The improvement of Thai self-cured orthodontic resin by reducing particle size, molecular weight of PMMA and monomer content could lead to the cured resin with the higher mechanical properties and good biocompatibility. The results showed that flexural strength, flexural modulus, water sorption, water solubility, level of residual monomer and biocompatibility of Thai self-cured orthodontic resin were acceptable according to ISO 1567:1999. Besides, the present study confirmed that clinicians should be careful in fabrication of orthodontic appliances in order to minimize residual MMA remaining in appliances, especially in those prepared by infiltration technique. Even though the properties of Thai self-cured orthodontic resin complied the requirements of ISO, its surface hardness was significantly lower whereas its water sorption and solubility were significantly higher than those of Orthocryl®. The present study suggested that Thai self-cured orthodontic resin can be suitable as an alternative for orthodontic denture base. Improvement the properties of Thai self-cured orthodontic resin by reducing particle size and molecular weight in order to obtain a higher wetting ability and powder/liquid ratio was suggested. The higher powder/liquid ratio of mixing would result in reduction of residual monomer and voids, and hence increased flexural strength, surface hardness, decreased sorption and solubility of Thai self-cured orthodontic resins.

REFERENCES

1. Anusavice KJ. Phillips' Science of dental materials.10th ed. Philadephia: W.B. Saunders Company; 1998.
2. Stafford G, Brooks S. The loss of residual monomer from acrylic orthodontic resins. Dent Mater 1985;1:135-138.
3. Nildam P, Dechkunakorn S, Anuwongnukroh N, Tanodekaew S. Properties of Thai self-cured orthodontic resins. Specialty training program in Orthodontics. Faculty of Dentistry, Mahidol University, 2005.
4. The International Organization for Standardization ISO 1567 Dentistry-Denture base polymers. 3rd ed. Geneva: ISO; 1999.
5. Tsuchiya H, Hoshino Y, Kato H, Takagi N. Flow injection analysis of formaldehyde leached from denture-base acrylic resins. J Dent 1993;21:240-243.
6. Douglas W, Bates J. The determination of residual monomer in polymethyl methacrylate denture base resin. J Mater Sci 1978;13:2600-4.
7. Kedjarune U, Charoenworaluk N, Koontongkaew S. Release of methyl methacrylate from heat-cured and autopolymerized resins : cytotoxicity testing related to residual monomer. Aust Dent J 1999;44:25-30.
8. Basker R, Sturdee D, Davenport J. Patients with burning mouths: a clinical investigation of causative factors. British Dental Journal 1978;145:9-16.
9. Ali A, Reynold A, Wallace D. The burning mouth sensation related to the wearing of acrylic dentures: an investigation. British Dental Journal 1986;161:444-447.
10. Craig R. Denture materials and acrylic base materials. Curr Opin Dent 1991;1:253-43.
11. O' Brien WJ. Dental materials and their selection. 3rd ed. Chicago:Quintessence Publishing Co. Ltd.; 2002.
12. Winkler S. Denture base resins. Dent Clin North Am 1984;28:287-97.

13. Ali A, Reynold A, Wallace D. The burning mouth sensation related to the wearing of acrylic dentures : an investigation. British Dental Journal 1986;161:444-447.

14. Phillips RW. Science of dental materials. 9th ed: Philadephia: W.B. Saunders Company; 1991.

15. Smith D, Bains M. The detection and estimation of residual monomer in poly (methyl methacrylate). J Dent Res 1956;1:16-24.

16. Ferracane JL. Materials in Dentistry: Principles and Applications. Philadephia: J.B. Lippncott Company; 1995.

17. Lewis B, Chestner S. Formaldehyde in dentistry: a review of mutagenic and carcinogenic potential. J Am Dent Assoc 1981;103:429-34.

18. Craig RG. Restorative dental materials. 10th ed. St.Louis: Mosby-Year Book Inc.;1993.

19. Cunningham J. Bond strength of denture teeth to acrylic bases. J Dent 1993;21:274-80.

20. Ruyter E, Svendsen S. Flexural properties of denture base polymers. J Prosthet Dent 1980;43:95-104.

21. Schulz G, Harborth G. Ueber den Mechanismus des explosiven Polymerizations verlaufes des Methacrylsaeuremethylesters. Makromol Chem 1947;1:106.

22. Tager A. Physical Chemistry of Polymers. Moscow: Mir Publishing;1972:221-222.

23. Harrison A, Huggett R, Jagger R. The effect of cross-linking agent on the abrasion resistance and impact strength of an acrylic resin denture base material. J Dent 1977;6:299.

24. Yunus N, Harrison A, Huggett R. Effect of microwave irradiation on flexural strength and residual monomer levels of an acrylic resin repair material. J Oral Rehabil 1994;21:641-8.

25. Dogan A, Bek B, Cevik V, Usanmaz A. The effect of preparation conditions of acrylic denture base materials on the level of residual monomer, mechanical properties and water absorption. J Dent 1995;23:313-8.

26. Jerolimov V, Huggett R, Brooks S, Bates J. The effect of variations in the polymer /monomer mixing ratios on residual monomer levels and flexural properties of denture base materials. Quintessence Dent Technol 1985;9:431-4.

27. Keller J, Lautenschlager E. Porosity reduction and its associated effect on the diametral tensile strength of activated acrylic resins. J Prosthet Dent 1985;53:374-9.

28. Sweeney W, Paffenbarger G, Beal J. Acrylic resins for dentures. J Am Dent Assoc 1942; 29:7-33.

29. Davenport J. The oral distribution of candida in denture stomatitis. Br Dent J 1970; 129:151-6.

30. Winston W, Donovan T, Daftary F, Siu T. The effect of vacuum-mixed autopolymerizing acrylic resins on porosity and transverse strength. J Prosthet Dent 1998;60:517-519.

31. Takahiro O, Mutsuko T, Kiyoshi K. Effect of water temperature during polymerization on strength of autopolymerizing resin. J Prosthet Dent 2002; 84:222-224.

32. Takahashi Y, Chai J, Kawaguchi M. Equilibrium strengths of denture polymers subjected to long-term water immersion. Int J Prosthodont 1999;12:348-52.

33. Arima T, Murata H, Hamada T. The effects of cross-linking agents on the water sorption and solubility characteristics of denture base resin. J Oral Rehabil 1995;23: 476-80.

34. Callister Jr WD. Materials Science and Engineering. 6th ed . New York: John Wiley and Sons;2002.

35. Rawls HR, Esquivel-Upshaw JF. Restorative resins. In: Anusavice KJ, editor. Phillips' science of dental materials. 11th ed. St Louis: Elsevier Science; 2003:399-442.

36. Cucci A, Vergani C, Giampaolo E, Alfonso M. Water sorption, solubility and bond strength of two auto-polymerizing acrylic resins and one heat- polymerizing acrylic resin. J Prosthet Dent 1998;80:434-878.

37. Jagger R. Effect of the curing cycle on some properties of a polymethyl methacrylate denture base material. J Oral Rehabil 1978;5:151-7.

38. Braden M, Wright P. Water sorption and solubility of soft lining materials for acrylic dentures. J Dent Res 1983;62:764-8.

39. Takahashi Y, Chai J, Kawaguchi M. Equilibrium strengths of denture polymers subjected to long-term water immersion. Int J Prosthodont 1999;12:348-52.

40. Lassila L, Vallittu P. Denture base polymer Alldent Sinomer: mechanical properties, water sorption and release of residual compounds. J Oral Rehabil 2001;28:607-13.

41. McCabe JF, Walls AWG. Applied Dental Materials. 8th ed. Blackwell Science Ltd;1998.

42. Smith D. Acrylic denture base. Residual monomer. British Dental Journal 1958;105:86-91.

43. Austin A, Basker R. The level of residual monomer in acrylic denture base materials. British Dental Journal 1980;149:281-286.

44. Ruyter I, Oysaed H. Composites for use in posterior teeth: composition and conversion. J Biomed Mater Res 1987;21(1):11-23.

45. Fletcher A, Purnaveja S, Amin W. The level of residual monomer in self-curing denture-base materials. J Dent Res 1983;62:118-120.

46. Vallittu P, Ruyter I, Buykuilmaz S. Effect of polymerization temperature and time on the residual monomer content of denture base polymers. Eur J Oral Sci 1998;106:588-593.

47. Shim J-S, Watts D. Residual monomer concentrations in denture base acrylic resin after an additional, soft-liner, heat-cure cycle. Dent Mater 1999;15:296-300.

48. Lamb J, Ellis B, Priestley D. The effects of process variables on levels of residual monomer in autopolymerising dental acrylic resin. Journal of Dentistry 1983;11:80-88.

49. Sheridan P, Koka S, Ewoldsen N, Lefebvre C, Lavin M. Cytotoxicity of denture base resins. Int J Prosthodont 1997;10:73-77.

50. Jorge J, Giampaolo E, Vergani C. Cytotoxicity of denture base resins: Effect of water bath and microwave postpolymerization heat treatments. Int J Prosthodont 2004;17:340-4.

51. Pettersen AH-, Wictorin L. The cytotoxic effect of denture base polymers. Acta Odontol Scand 1981;39:101-106.

52. Williams DF. Definitions in Biomaterials. Elsevier, Am- sterdam, 1987.

53. Huang F, Hu C, Chang Y, Chou M. The level of monomer release from acrylic denture base materials into water. Chin Dent J 2000;19:17–20.

54. Fisher A. Allergic sensitization of the skin and oral mucosa to acrylic denture materials. J Am Med Assoc 1954;156:234–42.

55. Weaver R, Goebel W. Reactions to acrylic resin dental prostheses. J Prosthet Dent 1980;43:138-142.

56. Tang A, Liu Y, Bjorkman L. In vitro cytotoxicity of orthodontic bonding resins on human oral fibroblasts. Am J Orthod Dentofac Orthop 1999;116:132-8.

57. Pellen-Mussi P, Fravalo P, Guigand M, Bonnaure-Mallet M. Evaluation of cellular proliferation on collagenous membranes. J Biomed Mater Res 1997;36:331-336.

58. Tsuchiya H, Hoshino Y, Tajima K, Takagi N. Leaching and cytotoxicity of formaldehyde and methyl methacrylate from acrylic resin denture base materials. J Prosthet Dent 1994;71:618-624.

59. Lefebvre C, Schuster G. Biocompatibility of visible light-cured resin systems in prosthodontics. J Prosthet Dent 1994;71:178-185.

60. Schuster G, Lefebvre C, Dirksen T, Knoernschild K, Caughman G. Relationships between denture base resin cytotoxicity and cell lipid metabolism. Int J Prosthodont 1995;8:580-586.

61. Cimpan M, Cressey L, Skaug N, Halstensen A, Lie S, Gjertsen B. Patterns of cell death induced by eluates from denture base acrylic resins in U-937 human monoblastoid cells. Eur J Oral Sci 2000;108:59-69.

62. Yoshii E. Cytotoxic effects of acrylates and methacrylates: relationships of monomer structures and cytotoxicity. J Biomed Mater Res 1997;37:517–24.

63. Taira M, Nakao H, Matsumoto T, Takahashi J. Cytotoxic effect of methyl methacrylate on 4 cultured fibroblasts. Int J Prosthodont 2000;13:311–5.

64. Huang F, Chang Y, Chiang H, Chiang T, Chou M. Studies on methyl methacrylate carcinogenicity in hamster cheek pouch. Chin Dent J 1998;17:165–71.

65. Schuster G, Lefebvre C, Dirksen T, Knoornschild K, Caughman G. Relationship between denture base resin cytotoxicity and cell lipid metabolism. Int J Prosthodont 1995;8:580–6.

66. Liu Y, Loftenius A, Elghazali G, Troye-Blomberg M, Ma S, Ekstrand J. Differential regulation of in vitro cytokine production by human blood cells in response to methyl methacrylate. J Toxicol Environ Health 1999;56:165–80.

67. Huang F, Tai K, Hu C, Chang Y. Cytotoxic effects of denture base materials on a permanent human oral epithelial cell line and on primary human oral fibroblasts in vitro. Int J Prosthodont 2001;14:439–43.

68. Yanga H-W, Choua LS-S, Choub M-Y, Changb Y-C. Assessment of genetic damage by methyl methacrylate employing in vitro mammalian test system. Biomaterials 2003;24:2909–2914.

69. Babich H, Borenfreund E. Applications of the neutral red cytotoxicity assay to in vitro toxicology. Altern Lab Animals 1990;18:129-44.

70. International Organization for Standardization.ISO 7405 Dentistry—Preclinical evaluation of biocompatibility of medical devices used in dentistry—Test methods for dental materials 1997.

71. Harrison A, Huggett R, Jagger R. The effect of a crosslinking agent on the abrasion resistance and impact strengthof an acrylic resin denture base material. J Dent 1994;21(1):103-9.

72. Jacobsen N, Hensten-Pettersen A. Occupatinal health problems and adverse patient reactions in orthodontics. Eur J Orthod 1989;11:254-64.

73. Wolfaardt J, Cleaton-Jones P, Fatti P. The occurrence of porosity in a heat-cured poly(methyl methacrylate) denture base resin. J Prosthet Dent 1986;55:393-400.

74. Baker S, Brooks S, Walker D. The release of residual monomeric methyl methacrylate from acrylic appliances in human mouth: An assay for monomer saliva. J Dent Res 1988;67:1295-1299.

75. Taylor P. Acrylic resins: their manipulation. J Am Dent Assoc 1941;28:373-87.

76. Ciapetti G, Granchi D, Arciola C, Savarino L, Stea S, Montanaro L. Established Cell Lines and Primary Cultures in Testing Medical Devices In Vitro. Laboratory for Biocompatibility Research on Implant Materials 1999; 13:801-810.

BIOGRAPHY

NAME	Panadda Nildam
DATE OF BIRTH	15 May 1973
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	1993-1999 D.D.S. Chulalongkorn University 2002 The Certificate of Prosthodontics Chulalongkorn University 2003-2005 The Certificate of Specialty Training Program in Orthodontics Mahidol University
RESEARCH GRANT	This research is supported in part by Faculty of Graduated Studies, Mahidol University, In the academic year of 2006
POSITION&OFFICE	Private office