

จุฬาลงกรณ์มหาวิทยาลัย

ทุนวิจัย
กองทุนรัชดาภิเษกสมโภช

รายงานผลการวิจัย

การกราฟต์โคพอลิเมอร์น้ำยางธรรมชาติด้วยเอทิลเมทาคริเลต
โดยการฉายรังสีแกมมาเพื่อใช้เป็นวัสดุฐานฟันปลอมอย่างนิ่ม

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โดย

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สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ชื่อโครงการวิจัย: การกราฟต์โคพอลิเมอร์ของน้ำยางธรรมชาติด้วยเอทิลเมทาคริเลตโดยการฉายรังสีแกมมาเพื่อใช้เป็นวัสดุฐานฟันปลอมอย่างนิ่ม

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บทคัดย่อ

ในปัจจุบันได้มีการนำยางธรรมชาติมาใช้ประโยชน์อย่างมากในทางการแพทย์ วัสดุประสังค์ของการวิจัยครั้งนี้ เพื่อพัฒนาวัสดุฐานฟันปลอมอย่างนิ่มจากยางธรรมชาติที่ได้รับการปรับปรุงสมบัติทางกายภาพ ทางกลและทางชีวภาพของน้ำยางให้ดีขึ้น ซึ่งในระยะแรกของการศึกษา ได้ทำการวัลคาไนซ์น้ำยางธรรมชาติโดยใช้รังสีแกมมา จากนั้นทำการกราฟน้ำยางที่ผ่านการวัลคาไนซ์แล้วด้วยสารเอทิลเมทาคริเลตโดยใช้รังสีแกมมาเช่นกัน น้ำยางที่ผ่านการกราฟต์แล้ว (ซึ่งในรายงานนี้เรียกว่ากราฟต์โคพอลิเมอร์) ถูกนำมาทดสอบหาค่าสมบัติทางกายภาพ ทางกล และความเข้ากันได้ทางชีวภาพกับเซลล์จากเหงือกของคน ในการวิจัยนี้ได้เลือกใช้โคชูเปอร์ซอพท์ซึ่งเป็นผลิตภัณฑ์ทางการค้าของวัสดุฐานฟันปลอมอย่างนิ่มเป็นกลุ่มควบคุม ผลการศึกษาลักษณะทางกายภาพและทางกลของกราฟต์โคพอลิเมอร์และโคชูเปอร์ซอพท์ พบว่าค่าความแข็งผิวของวัสดุและค่าการยึดติดกับฐานฟันปลอมของวัสดุทั้งสองมีค่าไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p > 0.05$) ในขณะที่ค่ากำลังการดึงและการฉีกขาดของกราฟต์โคพอลิเมอร์สูงกว่าของกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ผิวหน้าของกราฟต์โคพอลิเมอร์เปียกน้ำได้ดีกว่าโคชูเปอร์ซอพท์ นอกจากนี้ยังพบว่าวัสดุกราฟต์โคพอลิเมอร์มีการดูดซึมน้ำน้อยกว่าโคชูเปอร์ซอพท์ เมื่อแช่วัสดุทั้งสองในน้ำกลั่นเป็นเวลา 10 เดือนจากการศึกษาด้วยวิธีการเพาะเลี้ยงเซลล์ พบว่าเซลล์ไฟโบรบลาสต์จากเหงือกของคนสามารถเจริญเติบโตและยึดเกาะบนผิวหน้าของวัสดุกราฟต์โคพอลิเมอร์ได้ดี แต่ไม่พบปรากฏการณ์ดังกล่าวกับวัสดุโคชูเปอร์ซอพท์ ผลการวิจัยนี้แสดงให้เห็นว่า การกราฟต์น้ำยางที่ผ่านการวัลคาไนซ์ด้วยรังสีด้วยสารเอทิลเมทาคริเลตโดยใช้รังสีแกมมา ช่วยพัฒนาให้ยางพรีวัลคาไนซ์มีสมบัติทางกายภาพและทางกลดีขึ้น และมีความเข้ากันได้ทางชีวภาพกับเซลล์ของคนด้วย ดังนั้นการกราฟต์น้ำยางธรรมชาติด้วยสารเอทิลเมทาคริเลตจึงมีความเหมาะสมและคุ้มค่าที่จะนำไปพัฒนาเป็นวัสดุฐานฟันปลอมอย่างนิ่ม

Project title: Grafting of natural rubber copolymer with ethyl methacrylate by gamma irradiation for using as soft lining denture base material.

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Abstract

Recently, natural rubber has been used in several medical products. The purpose of this study was to develop a soft lining material from natural latex which had been improved its physical, mechanical and biological properties. Initially, natural latex was vulcanized by gamma irradiation. The vulcanized latex was then grafted with ethyl methacrylate using gamma irradiation also. The grafted vulcanized rubber, so-called graft copolymer, was evaluated for its physical and mechanical properties as well as the cellular biocompatibility. Coe Supersoft[®], the commercial soft lining material, was used as a control. The results from physical and mechanical evaluation showed that the surface hardness and the tensile bond strength to the denture base material of both the graft copolymer and control material were not significantly difference ($p > 0.05$) while the tensile strength and tear strength of graft copolymer were significantly higher than control group ($p < 0.05$). In addition, the surface wettability of graft copolymer was better than Coe Supersoft[®] and the water absorption of graft copolymer was lower than the control when both materials were immersed in water for 10 months. The result from *in vitro* study demonstrated that human gingival fibroblasts well proliferated and attached onto the surface of the graft copolymer while the cells co-cultured with the control material could not survive in the parallel experiment. These results suggested that grafting of ethyl methacrylate to the gamma irradiated vulcanized latex could improve the physical and mechanical properties as well as the cellular biocompatibility of the graft copolymer. Therefore, it would be worth to modify natural rubber with ethylmethacrylate grafting to be used as a denture soft lining materials.

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List of Abbreviations

ASTM	American Society for Testing and Materials
°C	Degree Celcius
cm	Centimetre
CO ₂	Carbon dioxide
Co-60	Cobalt sixty
DAP	Diammonium hydrogen phosphate
DMEM	Dulbecco's Modified Eagle Medium
DRC	Dry rubber content
EMA	Ethyl methacrylate
ISO	International Standard Organization
kGy	KiloGray
kV	KiloVoltage
Mg ⁺⁺	Magnesium ion
min	Minute
mm	Millimetre
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MPa	Mega Pascal
<i>n</i> -BA	Normal butyl acrylate
NH ₃	Ammonium
PEMA	Polyethyl methacrylate
phr	Part per hundred rubber
PMMA	Polymethyl methacrylate

List of Abbreviations (continue)

ppm	Part per million
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
T _g	Transitional glass temperature
TMTD	Tetramethyl thiuram disulfide
TSC	Total solid content
UTM	Universal testing machine
VFA	Volatile fatty acid
μl	Microlitre
μm	Micrometre, micron
w/v	Weight/volume
w/w	Weight/weight

สถาบันวิทยบริการ
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Introduction

The success of complete or partial dentures depends on esthetics, comfort, and function. Chronic soreness is a significant problem for denture patients with diabetes or other debilitating diseases and for many geriatric patients. In addition, patients with heavy bruxing or clenching habit may suffer the same consequence. The health of the denture supporting tissue in the patient has an influence on the denture function. The soft denture-bearing mucosa is confined between the hard denture base and the bony support. During function, the damage can be occurred to the supporting tissues resulting in chronic soreness, pathologic changes, and bone loss. To alleviate functional and nonfunctional stresses from the denture bases to the underlying supporting tissues, the soft resilient lining material is designed to place between the hard denture base and the soft mucosa covering the alveolar bone. These properties make soft denture lining materials useful for the treatment of patients with ridge atrophy or resorption, bony undercuts, bruxing tendencies, congenital or acquired oral defects requiring obturation, and patients with xerostomia. Recently, there are several commercial products of soft lining materials available in the market. The commonly used soft lining materials are heat polymerized and autopolymerized acrylic resin, heat polymerized and autopolymerized silicone. Even though these products work efficiently, they still have some disadvantages and limitations. These products cannot remain well serviceable for extended periods of time. They are considered temporary expedients. Their service expectancy does not compare with that of the hard denture base. Failures are associated with poor physical and mechanical properties that foul the lining materials by fungal and bacterial growth and poorly bond to denture base materials. Attempt to find a new soft lining material with the better quality has been

performed to solve these problems. We have found that natural rubber has some qualifications that fit for being a soft lining material. Rubber had been recorded as the earliest soft lining material. It was used for a short period of time since it had high water absorption and became foul and ill fitting. To overcome this problem, appropriate modifications of rubber have to be considered.

The objectives of this study were to develop a soft lining material from natural rubber and to investigate the properties of the newly developed material. In this study, the grafted natural rubber was prepared from the high-ammonium natural latex from *Hevea brasiliensis* tree and then studied for its physical, mechanical, and biological properties. The results from this study will hopefully lead to the accomplishment of a new soft lining material from natural rubber and new knowledges on modifications of natural rubber.



สถาบันวิทยบริการ
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Survey of Related Literature

Definition of soft lining material

Soft lining materials have been defined as soft elastic and resilient materials forming all or parts of the fit surfaces of dentures. The purpose of using the soft lining material is to absorb the stresses produced by masticatory force⁽¹⁻⁴⁾. Therefore, it serves as a cushion between the inner surface of the denture base and the oral tissue when patients cannot tolerate the hard denture base. Soft lining materials are also used to improve the retention for ill-fitting dentures or obturators. Elasticity of the lining material ensures that the material will regain its original shape following deformation, while resilience determines the recovery rate. This has led some to label these materials as “Resilient”, but soft lining material is more correct because of the softness or ease of deformation that particularly separates them from other denture base materials⁽⁴⁾.

History of soft lining material

The earliest soft lining material recorded was the soft rubber and used by Twitchell in 1869. The soft natural rubber known as “Velum” was vulcanized in conjunction with obturators and used as a soft lining for mandibular complete denture. However, this material had high water absorption, and it became foul and ill fitted after a period of usage (reviewed by Braden et al⁽⁴⁾).

One of the first synthetic resins used as a soft lining material was polyvinyl chloride (PVC), in which a plasticizer was necessary. The purpose of plasticizer was to reduce the transition of polymer from liquid to solid below mouth temperature. Thus at mouth temperature, the material was still soft. In 1945, Matthews⁽⁵⁾ used PVC

powder with a liquid di-n-butylphthalate plasticizer for facial prostheses and also soft lining materials for patients with chronic mucosal tenderness. He found that it eliminated the soreness under complete dentures in some selected patients. Lammine and Storer⁽⁶⁾ described the use of PVC with di-butyl phthalate plasticizer and found it to be a very unsatisfactory material. The material hardened in a short period of time (6-12 months) because of the plasticizer leaching out. Dioctyl phthalate was considered a better plasticizer for PVC because the lining remained soft longer⁽⁷⁾.

In late 1940s, there was a report using vinyl chloroacetate with butyl phthalate butyl glycollate plasticizer. This plasticizer improved the adhesion between the lining and the polymethyl methacrylate denture base. It was claimed that there was less leaching out of plasticizer, and therefore the soft lining remained soft for a longer period of time (reviewed by Harris et al⁽⁸⁾). However, Lammine and Storer⁽⁶⁾ reported the unsatisfactory effect of its high water absorption, hardening, and cracking.

In 1961 "Softdent", a hydrophilic gel based on glycol methacrylate ester was developed as a soft lining material. When immersed in water, the material gradually swelled and became soft with final water content of approximately 37 %. Laboratory and clinical studies showed that it was not suitable for using as soft lining material because of continuing changes in its volume. It was finally withdrawn from the market (reviewed by Harris et al⁽⁸⁾).

Silicone rubber materials based on poly(dimethyl siloxane) have been used as soft liners since 1958⁽⁶⁾. Many of the early silicone rubber soft lining materials were processed separately from the denture base and then cemented to it^(9,10). However, the poor adhesion to the denture base resulting from higher water diffusion limited their uses.

Currently available soft lining materials

The currently used soft lining materials on the market are heat polymerized and autopolymerized acrylic resin, heat polymerized and autopolymerized silicone rubbers, and tissue conditioner or treatment liner. In addition, the newly developed soft lining materials, the fluorinated resin and the olefinic material have been mentioned with less report ⁽³⁾. Some examples of those current available soft lining materials with their composition are presented in Table 1- Table 3

Both heat-processed and autopolymerized acrylic resin soft lining materials consist of powder and liquid components. The powder is believed to contain the acrylic polymer or copolymer. The liquid component is the mixture of methylmethacrylate monomer and a plasticizer such as di-n-butylphthalate, which can reduce the modulus of elasticity of the soft material to a satisfactory level ⁽¹¹⁾. The chemical composition of the acrylic resin soft lining shown in Table 1 is similar to that of the acrylic resin denture base material; thus no adhesive is required to bond between the soft lining material and the denture base.

The silicone rubber materials (Table 2) are basically composed of polymers of dimethyl siloxane, a viscous liquid that can be cross-linked to provide good elastic properties. The cross linking agent is normally an alkylsilane, and the reaction is usually catalyzed by an organic metal salt or benzoyl peroxide ⁽¹¹⁾. Molloplast b, the heat polymerized silicone rubber is supplied as one paste system activated by heat; boiling water for 2 hours. The adhesive, γ -methacryloxy propyl trimethoxysilane ⁽¹²⁾, is supplied to aid bonding to the denture base. The silicone autopolymerized, Flexibase, is supplied as a paste and a liquid system. The liquid or the catalyst is a mixture of dibutyl tin dilaurate. Wright ⁽¹²⁾ indicated that the silicone rubbers had no natural adhesion to the polymethyl methacrylate (PMMA) which is the major

denture base material, therefore an adhesive composed of a silicone polymer in a volatile solvent must be used.

Table 1. Composition of two acrylic resin soft lining materials

Material	Polymer	Monomer
Vertex Soft	Poly(ethyl methacrylate)	Acetyl tributyl citrate + methyl methacrylate
Coe Soft	Poly(ethyl methacrylate)	Di-n-butyl phthalate + benzoyl salicylate + ethyl alcohol

Table 2. Composition of two silicone soft lining materials

Material	Polymer	Cross-linking agent	Catalyst	Adhesive
Molloplast-B	Poly(dimethyl siloxane)	Acryloxy alkylsilane	Heat + benzoyl peroxide	Methacryloxy propyl-trimethoxysilane
Flexibase	Poly(dimethyl siloxane)	Triethoxysilanol	Dibutyl tin dilaurate	Silicone polymer in solvent

Table 3. Composition of two treatment liners

Material	Polymer	Cross-linking agent	Catalyst
Viscogel	Poly(ethyl methacrylate)	Ethyl alcohol	Dibutyl phthalate
Coe Comfort	Poly(methyl methacrylate)	Ethyl alcohol	Dibutyl phthalate

Treatment liners usually used as the temporary soft lining materials and tissue conditioners. These materials remain soft for a limited period of days to weeks and can be used when it is necessary to give the oral soft tissue an opportunity to recover before fabricating a new denture or rebasing or relining an existing one. They may also be used as functional impression materials, for immediate denture maintenance, cleft palate, speech aids, and immediate surgical splints⁽¹³⁾. McCarthy and Moser⁽¹⁴⁾ summarized other clinical uses of the treatment liners, and their properties were evaluated. The treatment liners usually consist of two components, powder and liquid. The powder is a higher methacrylate, e.g. polyethylmethacrylate (PEMA) or copolymers, while the liquid is usually a mixture of ethyl alcohol as the solvent and dibutylphthalate as the plasticizer. The solvent swells the copolymer beads and allow the plasticizer to diffuse in and form the gel rapidly. The gel is initially softer with a higher concentration of volatile solvent. However, leaching and evaporation of these components lead to rapid hardening of the material in the mouth.

The plasticizers in the treatment liners can be absorbed by acrylic resin denture base and soften it. The effect is mostly marked in the auto-polymerized acrylic resin of the denture base. The result is decreasing strength of the denture base material by approximately 20%⁽¹⁵⁾. Effects on heat-polymerized acrylic resin are likely to be clinically insignificant.

Ideal Properties of the soft lining materials

For maximum efficacy, soft lining material should display the following properties:

1. They should be easily processed using conventional laboratory equipments.

2. They should exhibit minimal dimensional change during processing and such change should be the same as the denture base materials⁽⁴⁾.
3. Water absorption should be minimal. Bate and Smith⁽¹⁶⁾ commented that high water absorption might lead to swell and stress at the denture base interface and tend to increase distortion and reduce bonding. Ideally, the total water absorption should be close to that of the acrylic resin denture base polymers⁽¹⁷⁾. Braden and Causton⁽¹⁸⁾ had discussed the effects of prolonged immersion of the soft lining material in water. If swelling occurs, the bacteria and nutrient material in the mouth would find their way between the lining and the denture base, and the area becomes unhygienic.
4. The materials should have minimal solubility in saliva. Ideally, the plasticizer should not leach out with time, however, if leaching does occur, it should be minimal.
5. The materials should have the softness property by themselves without using plasticizer such as phthalate ester because of the estrogenic effect⁽¹⁹⁾.
6. They should retain their resilience. The degree of resilience depends on the chemical composition of the material and the thickness of the soft lining. Several authors⁽²⁰⁻²³⁾ suggested that a thickness of 2-3 mm is the most appropriate for soft lining materials.
7. They should bond sufficiently well to PMMA to avoid separation during use. If the bond between the two materials is weak, separation takes place during use and such localized areas of separation rapidly become unhygienic because of the difficulty in cleansing.⁽¹⁻³⁾

8. Adequate tear resistance is practical importance to resist rupture during normal use. The propagation of crack or small tear at the periphery of the soft lining could lead to failure and detachment of the material⁽¹⁻⁴⁾.
9. They should be easily cleaned and not affected by food, drink, or tobacco. It is also important that the resilience and surface texture of the lining are not affected by denture cleansers of all types⁽¹⁻⁴⁾.
10. They should be nontoxic, odorless, and tasteless to encourage long-term wear of the denture by the patients⁽¹⁻⁴⁾.
11. They should be aesthetically acceptable and their color should match of the denture base material⁽¹⁻⁴⁾.

Permanent soft lining materials

It is not possible to estimate accurately the useful functional life of any denture because of the large variation in clinical factors that may necessitate its replacement. It is normally considered, however, that the average conventional complete denture will need replacement approximately once every five years⁽²³⁾. The physical properties of PMMA, the denture base materials, are more than adequate for this life span. It would seem reasonable to expect the soft lining materials to last about this period of time. Gonzalez and Laney⁽²³⁾ proposed that the soft liner that serves for more than two years can be considered as adequate lining material presumably on the basis that replacement every two years is not an excessive liability to the patient and the dentist. Many of the disadvantages of soft lining materials that have identified including the loss of compliance, the poor dimension stability, the failure of bond between the soft liners and denture base materials, the tendency to be torn and abraded, the changes in surface details and the consequences of poor dental hygiene,

especially the colonization by *Candida albicans* ⁽²⁴⁾, might prevent the soft lined denture from fulfilling this requirement.

Clinical failure of soft lining materials

Assuming the provision of a soft lining material meets the patient's expectations, the lined denture will continue to be worn until either the lining or denture fails. Only the former will be considered here and such failure may be attributed to the changes in physical properties of the material i.e. hardening, roughening, cracking or tearing, loss of adhesion to the PMMA denture base, staining, deposition of calculus or oral yeasts. Soft liners have also been shown to be less flexible during and after simulated chewing and clenching test sequences ⁽²⁵⁾ and after thermocycling ⁽⁷⁾.

Loss of compliance in the oral aqueous environment has most often been reported with elasticized acrylic materials ⁽²⁶⁻²⁸⁾ because of the susceptibility of the plasticizer to leaching out of the material. Silicone rubber materials have most often been reported to maintain their softness over a long period of time although these reports are generally based on subjective opinions ⁽²⁹⁻³²⁾. The effect of plasticizer loss may be confused by the absorption of water into the soft lining material which will act as plasticizer itself, thus maintaining or increasing compliance of the material ⁽³³⁾.

The repeated absorption and desorption of water from the surface of the soft liner is one factor in producing the roughening of its surface ^(32,34). Other factors are the constituents of foods and drinks, denture cleansers and brushing. The initial surface roughness may also differ depending on the effects of finishing and polishing the surface. Surface failure can also occur because of poor rupture properties of soft lining materials. Tearing of small pieces of silicone liner from the periphery of the

denture and cracking of the soft acrylic liner related to the crest of the residual ridge have been reported ⁽⁷⁾.

Loss of adhesion of the soft lining material to the PMMA denture base has often been reported as the cause of failure for silicone rubber materials in clinical use^(7, 30). Complete separation does not always occur but local area of separation between the liner and the denture base may become unhygienic because of difficulty in cleansing at the separated surfaces.

Patients rarely complained of staining, color changes or odor from the soft lining when used in the lower complete denture although such changes have often been reported in clinical studies and may lead to a clinical decision of the soft lining replacement. Several reports demonstrated colonization of *Candida albicans* on the surface of the soft lining materials in the clinical situations but there is no evidence that the presence of yeast has any adverse effects on the mandibular oral mucosa ^(29, 30, 35). However, the presence of *Candida albicans* in the mouth in association with the inflammatory changes was observed in denture related stomatitis. Colonization of the yeast is best avoided by the use of appropriate cleansing regimens ⁽³⁶⁾.

Since there were many problems on physical and chemical properties of lining materials, different modifications of these materials have been reported as described below:

Natural rubber/PMMA graft copolymer system

In 1958 Lammine and Storer ⁽⁶⁾ started using rubber as a soft lining material. Later on the Malaysia Rubber Producer Research Association developed a new rubber type lining material comprising a natural rubber/PMMA graft copolymer, cured at 100 °C sulfur/zinc dimethyl dithiocarbamate systems. Adhesion of the new material was

achieved with a toluene solution of the graft polymer, applied to the PMMA denture. This preparation gave excellent mechanical results and showed great promising clinical results. Unfortunately, it had to be abandoned because of the potential dangers of the mucosal reaction to the dithiocarbamate ⁽⁶⁾.

Powdered elastomer soft acrylic systems

The concept that initiated this work was to retain the advantage of soft acrylic systems, and avoid the need for plasticizers. Elastomer in powder form was manufactured by cryogenic grinding of the elastomer and using talc or other silicates as a separating agent. Using the separating agent should be avoided since it results in very high water uptake of the elastomer. Powdered elastomers react easily with the right choice of monomer and the cured products have acceptable strength and adhesion to PMMA ⁽⁴⁾.

Fluoropolymers

The use of visible light cured soft resins prepared by combining fluoroakyl methacrylate monomers with a vinylidene fluoride/ hexafluoropropylene copolymer, or a vinylidene fluoride/tetrafluoroethylene/ hexafluoro propylene copolymer was reported by a group of Japanese researchers (reviewed by Braden et al ⁽⁴⁾). These fluoropolymers were reported to be more wettable than silicones and had low solubility and low residual monomer. Softer resins could be prepared by using lower molecular weight fluoropolymers. Such materials were previously reported ⁽³⁷⁾ but they have not become widely commercially available. There is one heat-cured material of similar chemistry, Kurepeet[®] currently on the market.

The successful of clinical use of soft lining material is very importance. Hence, there are many previous tests on physical and mechanical properties of these materials as be described below.

Bonding to PMMA

The bonding ability between soft lining materials to PMMA material is one of the most important factors for clinical use. The silicone soft lining materials cannot efficiently bond to PMMA. The bonding between silicone and PMMA is deteriorated after immersion in water for a short period of time. Therefore, the adhesive or primer was used to enhance the bonding with the report of a little success ⁽³⁸⁾. To determine the bonding property of the materials, the peel test has to be performed. The bond strength of different soft lining materials to PMMA by the peel test in one study was shown in Table 4 ⁽³⁹⁾.

Table 4. The peel strength (KJ/m²) of some soft lining materials

Material	Dry	7 days in water	90 days in water
Coe Supersoft [®]	13.39	13.69	14.97
Molloplast B	1.80	2.23	1.98
Flexibase	0.63	1.19	0.3

From the table, Coe Supersoft[®] showed the best bonding ability which was not affected by water immersion. The two silicone type materials, Molloplast B and Flexibase, had far lower peel strength and both were reduced after long- term water immersion.

Another method for investigation of the bond strength is the tensile bond strength testing^(40, 41). This method is more effective than the peel test because it can determine both of bond strength and mode of failure.

Tensile properties

Both natural rubber and silicone material are elastomeric materials that are non-linear in tension, except at very near the origin. The ultimate tensile strength and the elongation at break values are important because they refer to the resilience and elasticity of the tested materials. The tensile properties of some soft lining materials were shown in the Table 5⁽⁴²⁾.

Table 5. The tensile properties of some soft lining materials

Material	Tensile strength (MPa)	Elongation at break (%)
Coe Supersoft [®]	2.66	230
Molloplast B	4.28	325
Novus (A polyphosphazine)	3.60	240

Tearing properties

The tear strength is another important property of the soft lining materials^(43, 44). It represents the maximum strength that caused tear in the material. If the soft lining materials have low tear strength, it will be easily torn and cause the soft tissue irritation or food deposition that leading to the bad smell later. The tear strength of several soft lining materials from one study was shown in Table 6⁽⁴²⁾.

From the table, Molloplast B showed the least tear strength that demonstrated the disadvantage of silicone type materials.

Table 6. The tear strength of some soft lining materials

Material	Tear energy (KJ/m ²)
Coe Supersoft	11.5
Molloplast B	1.43
Novus	21

Hardness

Unlike the hardness testing of metals, the hardness testing of soft lining materials requires different equipment since the deformation of soft materials by the indenter can recover. For this phenomena, the shore A hardness tester is used for soft materials. There was a study demonstrated that the hardness of soft acrylic resin type increased with age of the material in the mouth as a result of leaching of the plasticizer⁽⁴⁵⁾ leading to irritation of the oral soft tissue and ill fitting denture..

Water absorption

Water absorption of the soft lining materials needs a great circumspection because the soft materials always contain plasticizers to produce softness. These plasticizers were physically blended with the polymer therefore they were easily leached out when stimulated by mastication or immersion in aqueous environment. In addition, soft lining material can absorb liquids . This is the major pitfall of any elastomer in an aqueous environment The water uptake phenomenon of the soft lining materials was usually lasted for many years, and irrespective of the chemical

structure of the elastomers⁽⁴⁶⁾. The water uptake is governed not only by diffusion, but also by water-soluble impurity within polymers. The explanation of water absorption was shown in Figure 1.

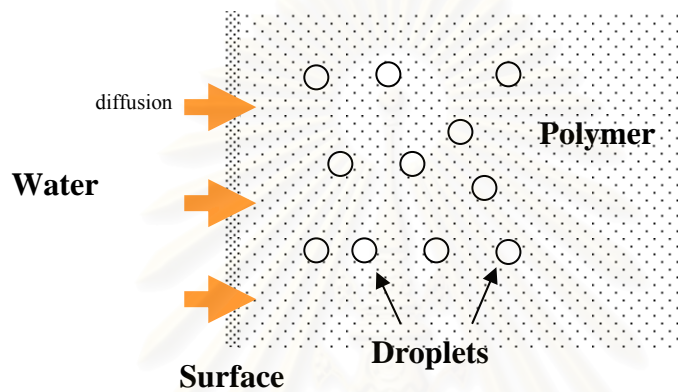


Figure 1. The water uptakes by water-soluble impurities

When the diffusing water reaches the water-soluble impurity site, the solution droplets are formed. The amount of droplets increase as the water absorption increases and the process can continue for a long time. The water diffusion and the droplet formation occur until osmotic pressure and elastic forces balanced. The water uptake of the acrylic soft lining material demonstrated by increasing of the material weight when it was submerged in the water at different time periods in one study⁽⁴⁶⁾ was shown in the Figure 2.

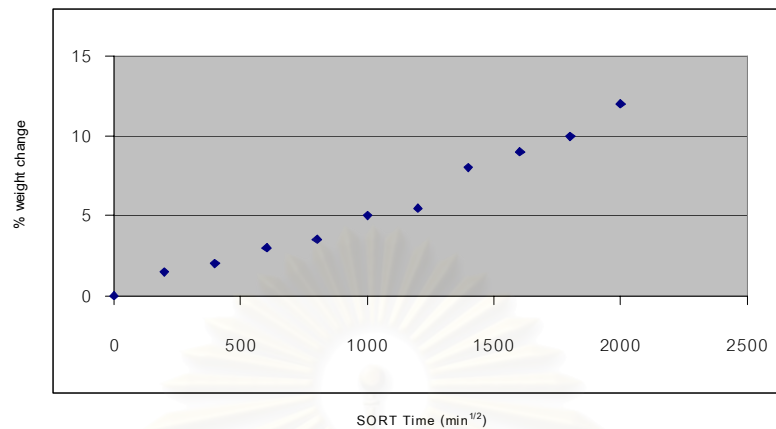


Figure 2. The water uptake of the acrylic soft lining material.

The water uptake is the unwanted property of the soft lining material since it affects the dimensional stability of the material. This problem seems to be serious in producing a satisfactory material and is potentially not easy to solve.

Clinically, the soft lined denture is rarely placed in pure water but it is bathed in saliva in the mouth and is often soaked in solutions of denture cleanser overnight. The effects of immersion some soft lining materials in the artificial saliva have been shown to be different to those found in the distilled water⁽⁴⁷⁾ and the compatibility of soft lining materials and the main types of denture cleansers has also been studied⁽⁴⁸⁾. There was no adverse effects identified in both studies. Recently the new oxidizing denture cleanser without any adverse effect on some soft lining materials has been reported⁽⁴⁹⁾.

Natural Rubber

Natural rubber is the polyisoprene formed through a natural polymerization in the tree. It is obtained from more than 2,000-plant species all over the world. Natural

rubber from *Hevea brasiliensis* holds shares more over than 99% of the market of natural polyisoprenes. Other plants that contain rubber are guayule, balata and gutta percha tree^(50,51). Generally, the term “natural rubber” is used for *Hevea* rubber coming from the rubber tree *Hevea brasiliensis*. The natural rubber from plants is a high-molecular weight hydrocarbon polymer consisting almost entirely of five-carbon isoprene units, C₅H₈. The rubber from *Hevea brasiliensis* and guayule are *cis*-1,4-polyisoprene, while those from balata and gutta percha are *trans*-1,4-polyisoprene⁽⁵²⁾. The molecular structures of *cis* and *trans* polyisoprene are shown in Figure 3 and 4

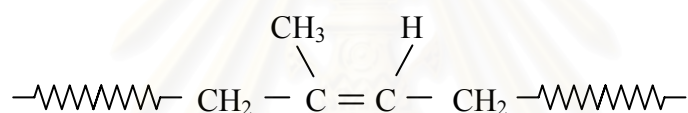


Figure 3. The molecular structure of *cis*-1,4-polyisoprene.

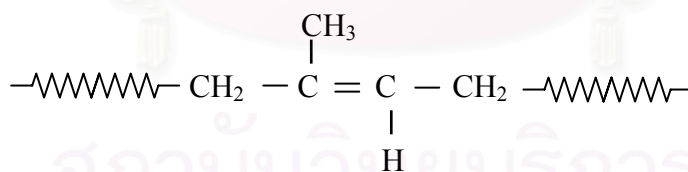


Figure 4. The molecular structure of *trans*-1,4-polyisoprene.

Natural rubber latex

Latex is the form of liquid resin secreted from the inner bark of some trees. Latex is not tree sap. Natural rubber latex is the aqueous emulsion form of rubber extruded from the *Hevea* trees. In the natural rubber latex, the rubber and non rubber

particles dispersed in an aqueous serum phase, as a milk-like liquid. Latex is harvested from the tree by a process call “tapping” and it is collected in a small cup as shown in Figure 5. The freshly collected latex (fresh field latex) has a pH of 6.5-7.0, a density of 0.98 g/cm^3 , and a surface energy of $4.0\text{-}4.5 \text{ }\mu\text{J/ cm}^2$. The total composition of fresh latex, apart from water, is summarized in Table 7 ⁽⁵³⁾. The % content of the components varies according to clonal variations of the rubber clones.

Figure 5. The fresh latex was collected from *Hevea* tree by tapping.



Table 7. The composition of fresh natural rubber latex

Component	Content (% , wt/vol)
Rubber hydrocarbon	25-45
Proteins	1-2
Carbohydrate	1-2
Lipids	0.9-1.7
Organic solutes	0.4-0.5
Inorganic substances	0.4-0.6

After ultracentrifugation, the freshly tapped latex is separated into three principal phases⁽⁵⁴⁾ as shown in Figure 6. The top layer which contains the cream of rubber particles is called the rubber phase. It accounts for 25-45% of the latex by weight. The middle or serum phases occupies approximately 45-65 % and the bottom layer, the bottom phase, contains predominantly the lutoids occupying about 10% by weight .

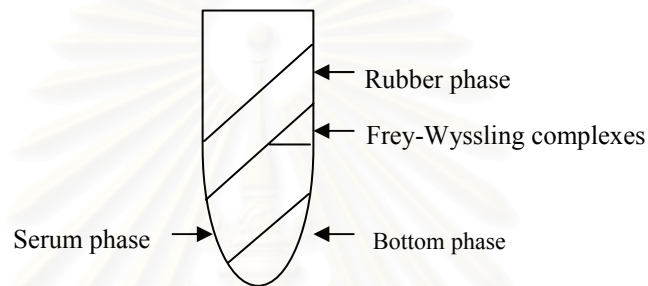


Figure 6. The ultracentrifugation of natural rubber latex.

The rubber phase

This phase contains the rubber particles (Figure 7). These particles are usually spherical in shape with a diameter ranging from 0.01 μm to 5 μm . The hydrophobic rubber molecules, the hydrocarbon, are protected from the hydrophilic medium by a complex film of protein and phospholipid⁽⁵⁵⁾.

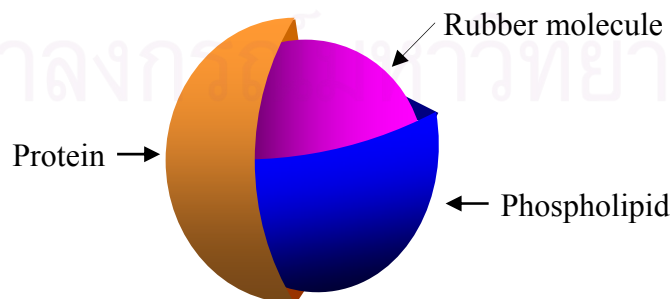


Figure 7. Presumed structure of a rubber particle

The serum phase

The serum phase, also called the clear serum or C-serum or aqueous phase, contains many different chemical species, including carbohydrates proteins, enzymes, and nitrogenous bases. The yellow color of this phase is caused by “Frey Wyssling particles” which are spherical, non-rubber particles. This color is due to the presence of carotenoid pigments⁽⁵³⁾.

The bottom phase

The bottom phase or lutoid phase consists mainly of lutoid particles. These are vacuoles with spherical membrane-bounded bodies. Within the lutoids there is an aqueous environment containing dissolved substances such as acids, minerals, proteins and sugar⁽⁵³⁾.

The preservation of natural rubber latex

Fresh latex coagulates within a few hours after tapping from the tree. It has to be stabilized with ammonia (NH_3) and transported from the plantation to a factory where it undergoes continuous centrifugation to produce concentrated natural rubber latex containing approximately 60% dry rubber content. For a long time preservation of concentrated latex, the NH_3 content is usually raised to 0.6-0.7%. This is referred to high-ammonium preserved concentrated latex. The low-ammonium preserved concentrated latex contains only 0.2-0.3% NH_3 plus tetramethyl thiuram disulfide (TMTD), as a bactericide⁽⁵⁶⁾.

Natural rubber has been widely used as a raw material of several industrial products such as tires, gloves, condoms, cushions, outsole of footwear, etc. Modification of natural rubber is necessary for the improvement of the properties and

quality of rubber to match the purpose of usage. One of the most common modification methods is the grafting.

Graft copolymers of natural rubber

The term “graft copolymer” is defined as a special type of block copolymer in which one or more blocks of homopolymer are grafted as a branches onto a main chain or backbone homopolymer that consists exclusively of units derived from the other monomer ⁽⁵⁷⁾. The polymerization is largely initiated by chemical or irradiation methods .

The graft copolymer of natural rubber is produced by polymerization of a monomer onto an existing polyisoprene chain, which acts as the backbone polymer. The physical properties of graft copolymer are greatly depended on the grafted component, frequency and extent of grafting. The general structure of graft copolymer is indicated in Figure 8.

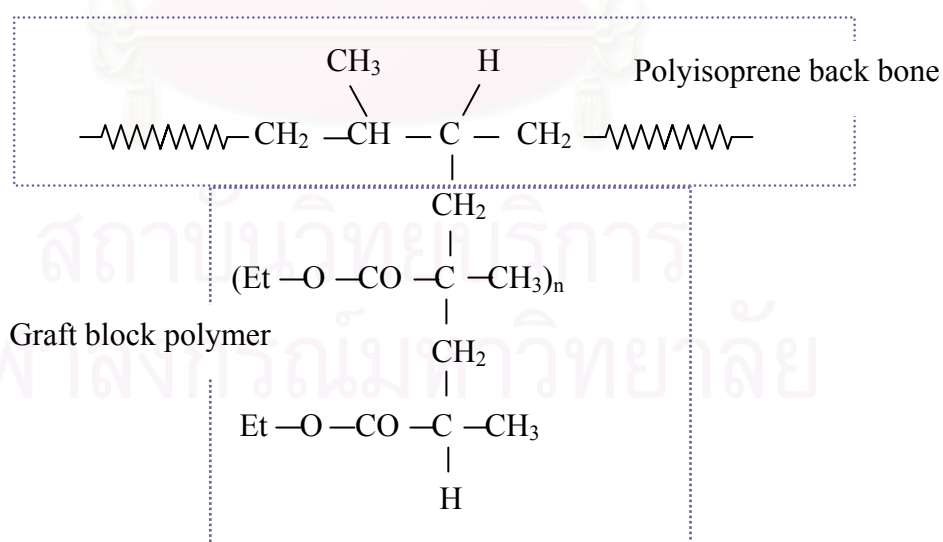


Figure 8. Structure of the graft copolymer. Polyisoprene is the back bone and polyethyl methacrylate is the graft block polymer.

Nowadays, there are still no completely satisfactory soft lining materials. All of the soft lining materials available in the market are synthetic materials and quite expensive. There were many researchers tried to develop the synthetic polymers for using as the soft lining materials but they were not successful in clinical use. There was only one report on using conventional method for grafting natural rubber with PMMA as a soft lining denture base material but this material was abandoned because the reagents used for processing were toxic to the oral mucosa ⁽⁶⁾.

Considering the good physical and mechanical properties of the rubber, it is of interest to modify natural rubber for using as soft lining denture base materials. Presently, the gamma irradiation has been introduced to a lot of medical and industrial productions with many advantages. One of the major advantages is the gamma ray can reduce the usage of toxic reagents usually used in several conventional methods of polymer vulcanization and grafting. In this study, the natural rubber was modified to improve its physical and mechanical properties as well as its cellular biocompatibility by using gamma irradiation in the vulcanization and grafting with ethyl methacrylate processes.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Procedure

Materials:

Biological materials

Fresh field natural latex preserved with .03% (v/v) ammonia (RRIM 600 clone, Pan Asia Biotechnology, Thailand).

Human gingival fibroblast

Chemical for natural rubber grafting and testing

Acetone

Ethyl methacrylate monomer

Lauric Acid

Light petroleum ether

Methyl methacrylate monomer and polymer

Normal butylacrylate

Potassium hydroxide

Sodium sulphite (anhydrous)

Sodium azide

Coffee solution

Tea solution

Capsaicin solution

Chemicals for cell culture and scanning electron microscopy

Dulbecco's Modified Eagle Medium (DMEM) (GIBCO BRL,USA)

DMEM without phenol red (GIBCO BRL, USA)

Antibiotic- antimycotic solution (GIBCO BRL, USA)

L-glutamine (GIBCO BRL,USA)

Fetal bovine serum (GIBCO BRL, USA)

Normal saline solution

Glutaraldehyde

Ethyl alcohol

Osmium tetroxide

Apparatus

Fourier-transform infrared spectroscopy (Perkin Elmer, USA)

Lloyd universal testing machine (Model LR10K, UK)

Durometer (Shore A) (Pacific Transducer, USA)

CO₂ incubator (Shel Lab, USA)

Biohazard Hood (MDH, UK)

Co-60 radiation source (Office of Atom for Peace, Thailand)

Spectrophotometer (Hunter Lab, USA)

Spectrophotometer (Ultrospec 3000, UK)

Inverted phase contrast microscope (Olympus ck2, Japan)

Scanning electron microscope (JEOL, JSM-5410 LV, Japan)

Transmission Electron Microscopy (TEM Model JEM-200CX, Japan)

Contact Angle Measurement (Model CAM-Micro, USA)

Differential Scanning Calorimeter (Model DSC 200, Germany)

Digital balance

Bench-top centrifuge

Hemocytometer

Orbital shaker

Methods

The methods were divided into three parts:

Part I: Preparation and characterization of graft copolymer.

Part II: Investigation of physical and mechanical properties of the graft copolymer

Part III: In vitro study of the cellular response to the graft copolymer.

In this study Coe Supersoft[®], the commercial soft lining material of soft acrylic resin type, was prepared according to the manufacturer recommended procedure and used as the control material.

Part I: Preparation and characterization of graft copolymer.

This part consisted of (1) preparation of high-ammonium concentrated natural rubber latex from fresh field latex, (2) preparation of gamma irradiated prevulcanized natural rubber latex, (3) grafting of ethyl methacrylate monomer onto the prevulcanized natural rubber latex by gamma irradiation, and (4) characterization of the graft copolymer.

(1) Preparation of high-ammonium concentrated natural rubber latex from fresh field latex

Fresh field latex was purchased from Pan Asia Biotechnology (Rayong, Thailand) in the form of milky-like emulsion with ammonia as a preservative (Figure 9). The latex was determined for its dried rubber content (Figure 10 a, b) and total solid content (TSC). Then the water soluble chitosan and ammonium hydroxide were added to the latex for the final concentrations of 0.1% and 0.3% w/w, respectively to preserve and anti-coagulate the latex. The ammoniated latex was again tested for

DRC and TSC as well as volatile fatty acid (VFA) (Figure 11), NH_3 , and magnesium ion (Mg^{++}) contents. Reduction of Mg^{++} content was performed by adding 50 ppm (maximum) of diammonium hydrogen phosphate (DAP) into the latex. To increase mechanical stabilization time, ammonium laurate was added into DAP-ammoniated latex for the final concentration 0.05% w/w. The whole mixture of latex was kept standing over night. The precipitate was discarded and the supernatant latex was determined for DRC, TSC, VFA, NH_3 , and Mg^{++} contents again. Then the soluble alginate and potassium hydroxide were added into the supernatant latex for the final concentration of 0.01 phr and 0.1% w/w, respectively. The latex was adjusted to 25% DRC by 0.3% ammonium hydroxide. After being kept standing for 3 hours the 25% DRC latex was centrifuged at 7,000g (Figure 12). The concentrated latex was finally adjusted to 60% DRC and 0.6% NH_3 . The final preparation of latex was called high-ammonium concentrated latex and was adjusted for TSC, VFA, KOH number, and Mg^{++} content to comply with the ISO specification for high ammonium concentrated latex.

Figure 9. The fresh field latex contained in plastic container was preserved with ammonium hydroxide as a preservative.



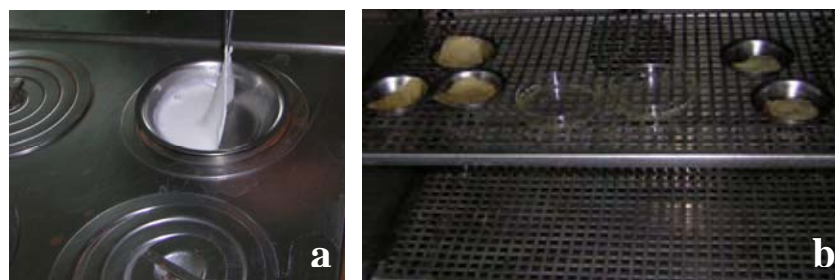


Figure 10. Determination of the dry rubber content.

- a) The natural latex was coagulated and turned into soft rubber by acetic acid and heat on water bath at 100 °C.
- b) The rubber was creped and dried in an oven at 60 °C for 10-12 hours then weighed for DRC calculation.

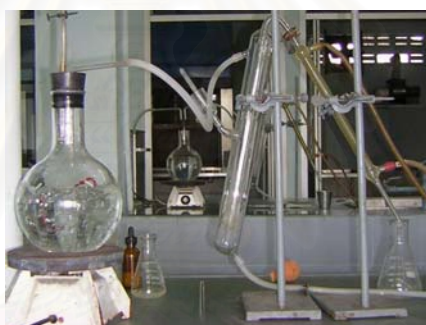


Figure 11. The process of determination of volatile fatty acid by modified Equipments.



Concentrated latex

Figure 12. The latex was centrifuged using a factory scale centrifuge at 7,000g to produce the concentrated latex.

(2) Preparation of gamma irradiated prevulcanized natural rubber latex.

Before vulcanization process high-ammonium concentrated natural rubber latex containing 60% DRC was diluted with 1 % ammonium hydroxide to make up the 50% DRC and stabilized by 0.2 phr potassium hydroxide.

For vulcanization process 5 phr of normal butylacrylate (*n*-BA) was added into 50% DRC latex with slowly continuous stirring (Figure 13) and then irradiated at room temperature by gamma ray from a Co-60 source (Office of Atom for Peace, Bangkok, Thailand) at the irradiation dose of 10, 11, 13, 14, 15, 16,17, and 18 kGy. After irradiation the prevulcanized latex was casted in glass mold, air dried in room temperature for 24 hours and heat dried in hot air oven at 80°C for 48 hours. The dry rubber sheet was cut into a dumbbell shape according to the American Society for Testing and Materials (ASTM) D638. Evaluation of the tensile strength is performed using a universal testing machine (UTM) with the crosshead speed of 50 mm/min at room temperature. The data were evaluated and the appropriated dose of gamma ray that produced the tensile strength of 6 MPa was chosen. In this study, the total dose of gamma ray using for vulcanization and grafting process was chosen at 15 kGy.



Figure 13 The normal butyl acrylate (*n*-BA) was added into 50%DRC latex with slowly continuous stirring.

(3) Grafting of ethyl methacrylate monomer onto the prevulcanized natural rubber latex by gamma irradiation

Different concentrations of ethyl methacrylate (EMA) monomer in the form of emulsion were mixed thoroughly into the prevulcanized latex by mole ratio of prevulcanized latex to EMA equal to 90:10, 80:20, 70:30, 60:40, and 50:50 (Figure 14) and keep the mixture standing for 24 hours. They were then irradiated by gamma ray using a Co-60 source at room temperature and at a total dose of 5 kGy. The irradiated prevulcanized latex/EMA product was called **the graft copolymer** in this study.

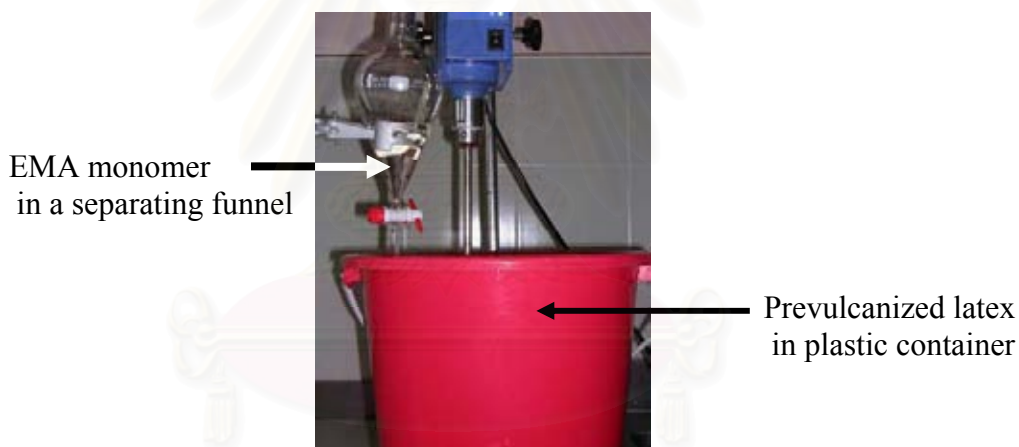


Figure 14 The EMA monomer was added into prevulcanized latex with slowly continuous stirring.

(4) Characterization of the graft copolymer

Determination of the graft copolymer morphology by SEM

The graft copolymer latex was diluted 400 times with distilled water. Then, 2 drops of 2 % aqueous of osmium tetroxide was incubated with 200 cm³ of diluted latex for 1 day to stain the rubber particle. A drop of the stained latex was applied on

a carbon-coated Formvar film deposited on a grid and dried in a dessicator. The morphology of the graft copolymer was examined by Transmission Electron Microscopy (TEM) at 120 kV.

Determination of grafting efficiency

To determine the grafting efficiency, the graft copolymer had to be casted in an open glass tray (170 x 170 x 3 mm³) and dried at room temperature for 48 hours (Figure 15 a). Once the graft copolymer sheet turned clear (Figure 15 b), it was removed from the tray and continuously dried in an oven at 80 °C for 24 hours. After the graft copolymer sheet was dried out, the water-soluble impurities were leached out by distilled water. Finally, the sheet is thoroughly dried again in the oven at 70 °C for 24 hours.

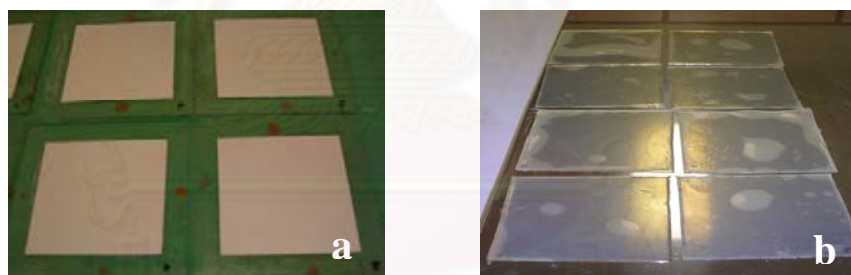


Figure 15 Casting of the graft copolymer sheets.

- a) The graft copolymer latex was casted in open glass trays and dried at room temperature for 48 hours.
- b) The graft copolymer sheets turned clear before moving to hot air oven.

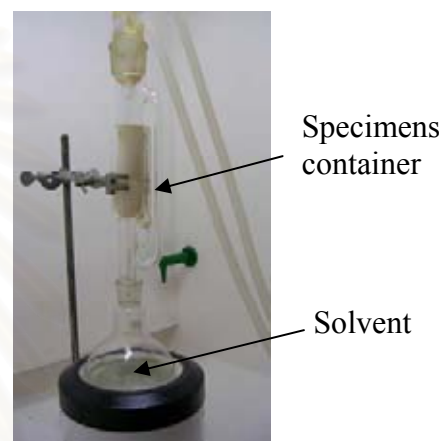
Soxhlet extraction procedures were carried out to assess the amounts of residual ungrafted natural rubber and residual EMA in the final product (Figure 16). The residual ungrafted natural rubber was extracted in the Soxhlet extractor by light petroleum ether for 24 hours and the residual EMA was extracted with acetone for 24

hours. After each extraction, the graft copolymer is weighed for the residual weight.

The grafting efficiency was defined by the formula below.

$$\% \text{ of grafting efficiency} = \frac{\text{Weight of extracted graft copolymer} \times 100}{\text{Total weight of graft polymer}}$$

Figure 16 The residual ungrafted natural rubber and residual EMA were extracted by Soxhlet extraction procedure.



Investigation of functional group in the graft copolymer

This study was performed to investigate the functional groups of EMA that presented in the graft copolymer. After the removals of residual ungrafted natural rubber and the residual EMA by Soxhlet extraction, the graft copolymer was dissolved with methyl ethyl ketone and toluene. The graft copolymer solution was then prepared into a thin film on potassium bromide (Figure 17) and characterized by infrared spectroscopy.

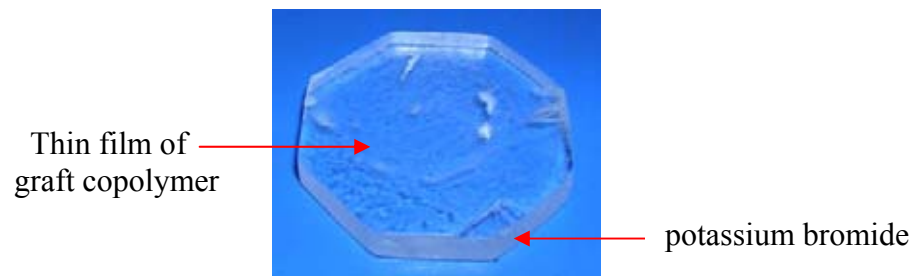


Figure 17. A thin film of graft copolymer was smeared on potassium bromide before characterization by Infrared spectroscopy.

Determination of transitional glass temperature of the graft copolymer.

This study was performed to investigate the transition temperature that the rubber would change from the soft to the hard phase. The graft copolymer and Coe Supersoft[®] specimen were prepared in the circular disk of 5 mm in diameter and 3 mm thick (Figure 18). They were determined for the transitional glass temperature (T_g) by Differential Scanning Calorimeter.

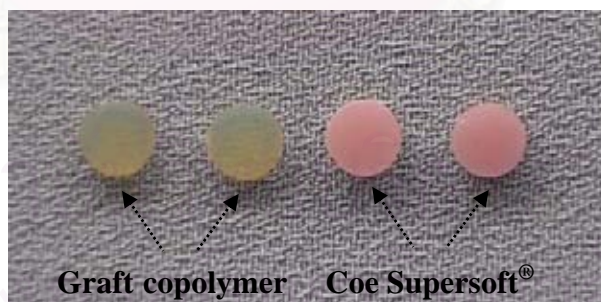


Figure 18. The graft copolymer and Coe Supersoft[®] specimen were prepared in the circular disk for determination of T_g value.

Part II: Investigation of physical and mechanical properties of the graft copolymer

The specimens used in these tests were the graft copolymer prepared in different forms. The specimens of graft copolymer and the Coe Supersoft[®] were used for each testing.

Hardness test

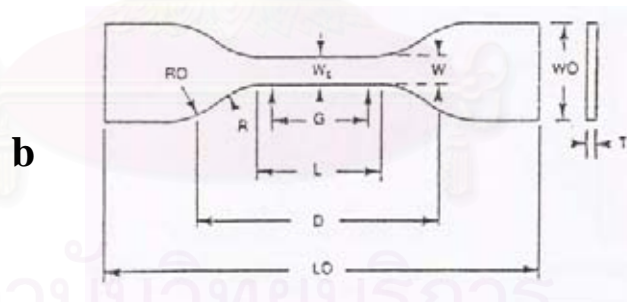
The hardness test was performed according to ASTM D 2240-91 (Standard Test Method for Rubber Property Durometer Hardness) based on the measurement of the penetration of a rigid plunger into the rubber specimen under specified condition. Ten flat and smooth rectangular sheets (70x70x3 mm³) of tested materials were prepared. Then the specimens were immersed in distilled water at 37° C at the different immersion times (30 minute, 1-6 hours, 1-3 weeks, and 1-10 months). The specimen from each immersion time was placed under the plunger of Durometer (Figure 19) then the plunger was pressed with the minor force on the specimen. The scale of the durometer showed the value of the hardness in Shore A value at room temperature. The measurement of the hardness was performed at 5 different positions at least 6 mm apart on the same specimen. The arithmetic mean was calculated from the Shore A values.



Figure 19. The durometer apparatus was used for measuring the surface hardness.

Tensile strength test

Tensile strength was measured according to ASTM D638-01. The flat and smooth rectangular sheet test specimens of the graft copolymer and control group material with 3 mm thick were prepared (Figure 20a). The specimens were cut with one of the steel dies conforming to the dimensions shown in Figure 20b. For the tensile strength test, twenty specimens of each material were measured by the UTM with a crosshead speed of 50 mm/min at room temperature (Figure 21).



$W = 6 \text{ mm}$	$WO = 19 \text{ mm}$	$G = 25 \text{ mm}$	$T = 3 \text{ mm}$
$R = 14 \text{ mm}$	$T = 2 \text{ mm}$	$RO = 25 \text{ mm}$	
$L = 33 \text{ mm}$	$LO = 115 \text{ mm}$	$D = 65 \text{ mm}$	

Figure 20. Preparation of the specimens for tensile strength test.

- The flat and smooth rectangular sheet of tested materials.
- Dumbbell-shaped test piece (type IV) with its dimensions.

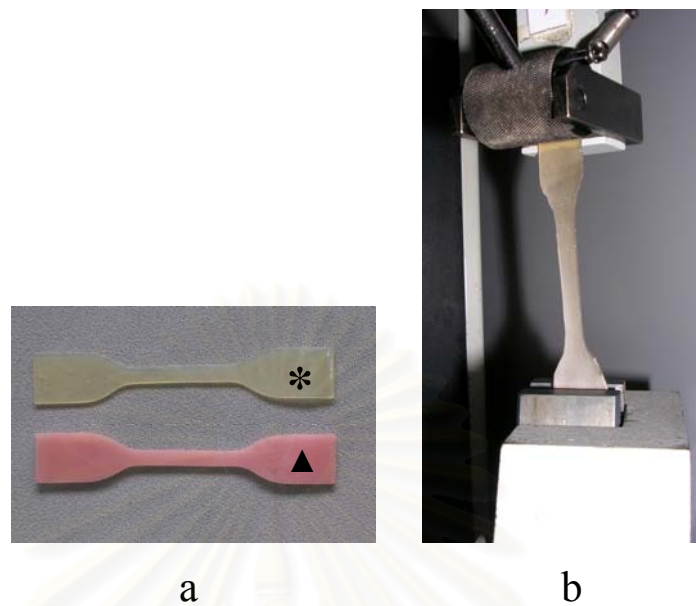


Figure 21. The tensile strength test.

- a) The dumbbell-shaped test piece of graft copolymer () and Coe Supersoft[®] (▲) .
- b) The specimen was fixed to the holder of the UTM for determination of the tensile strength.

Tear strength test

Tear strength was measured according to ASTM D624. The flat and smooth rectangular sheets of the graft copolymer and control material with 3 mm thick were prepared. The specimens were cut with one of the steel dies conforming to the shape of specimens according to Figure 22 a and b. Twenty specimens of each material were measured for the tear strength by the UTM with a crosshead speed of 500 mm/min at room temperature (Figure 22 c).

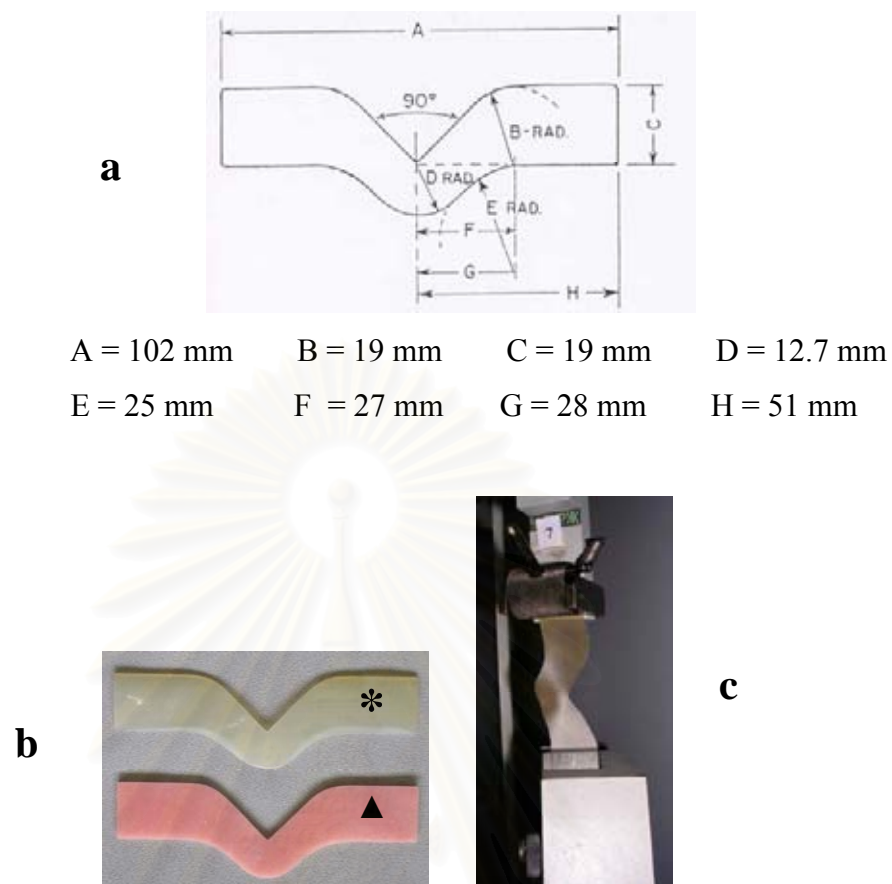


Figure 22. Preparation of the specimens and the tear strength test.

- a) The shape and dimensions of test piece for tear strength testing.
- b) The graft copolymer () and Coe Supersoft[®] (▲).
- c) The test piece was fixed to the holder of the UTM.
for the tear strength measurement.

Tensile bond strength test

The tested specimens were made in the gypsum moulds to perform the rectangular bars consisting of a small part of either the graft copolymer or the Coe Supersoft[®] connected with the heat polymerized denture base materials (10x10x73 mm³/bar) at both ends (Figure 23).

After removing the specimens from the moulds, they were stored in distilled water at 37⁰C and tested at 24 hours. For the tensile bond strength, 15 specimens of each material were measured by UTM at the crosshead speed of 5 mm/min (Figure 24).

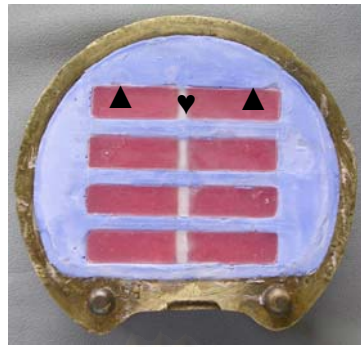


Figure 23 Preparation of the specimens for the tensile bond strength test.

The mould Preparation of the specimens and the tear strength test for the tested specimens. Each specimen was made up of two bars of denture base materials (▲) with the tested material (♥) either the graft copolymer or the Coe Supersoft[®], in the middle.

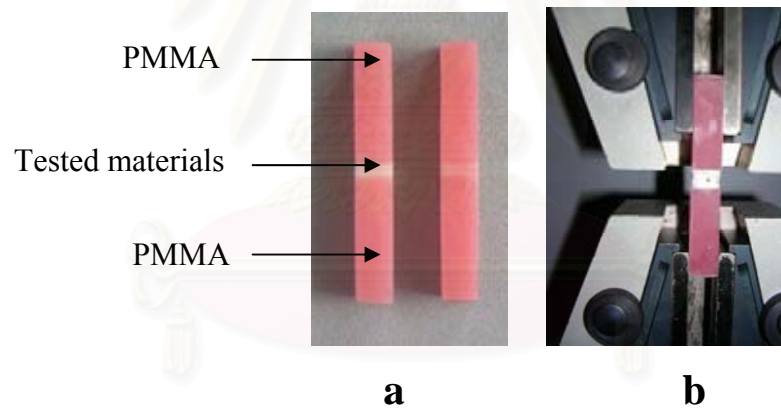


Figure 24 The tensile bond strength test.

a) The tested specimens.

b) The specimen was fixed to the holder and measured the tensile bond strength by the Lloyd UTM.

In order to study the nature of the interface between the PMMA and either the graft copolymer or Coe Supersoft[®], the specimens were prepared for scanning electron microscopic (SEM) examination by cutting the specimens into small pieces. Each piece composed of 2 mm of soft liner bonded to 2 mm of PMMA. Investigation

by SEM was performed and the interfaces between the PMMA and the soft lining materials were recorded.

Water absorption test

The flat and smooth rectangular sheets with 3 mm thick of the graft copolymer and Coe Supersoft[®] was prepared. The specimens were cut with one of the steel dies conforming to the square shape of 20x20x3 mm³ (Figure 25a.). Fifteen specimens of each material were placed in a desiccator at 37 °C with silica gel. Daily weights of the desiccated specimens were obtained until a stable weight reading (m_o) was obtained. The specimens were placed in distilled water and stored at 37 °C in the dark (Figure 25 b.). At each storage interval time: 30 minutes, 1 day-14days, 3 weeks- 4 weeks, 2 months-10 months, the specimens were removed. Excess moisture on the specimen was wiped off quickly and the specimens were weighed (m_t).

The percentage of water absorption (M_T) was determined by the following formula:

$$M_T = \frac{(m_t - m_o) \times 100}{m_o}$$



Figure 25 Preparation of the specimens and water absorption test.

a) The specimens were cut into a square shape.

b) The specimens were immersed in distilled water at 37 °C.

Contact angle test

The flat and smooth rectangular sheet test specimens of the graft copolymer and the control material with 3 mm thick were prepared and cut into 20x40x3 mm³. After preparation, 15 specimens of each material were stored in distilled water at 37°C for 24 hours before testing. The test specimen was placed horizontally on the platform of a contact angle meter (Figure 26). One drop of deionized distilled water was placed carefully from a hypodermic syringe onto the surface of each specimen. The drop was placed near the edge of the specimen to aid the viewing. The contact angle from each drop of water presented on the scale board was measured and calculated. Three measurements were made for each specimen.

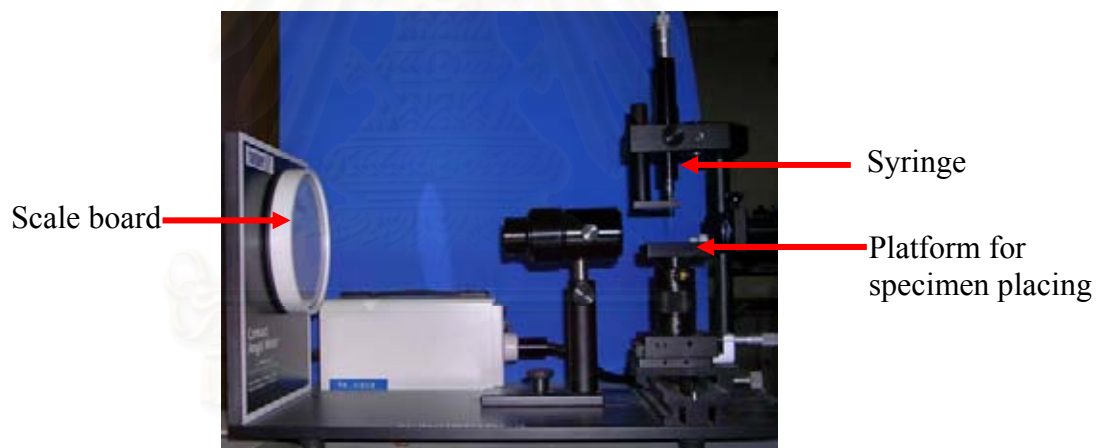


Figure 26. The contact angle meter was used for measuring the surface wettability of the specimens.

Statistical analyses

The data from physical and mechanical properties of graft copolymer and Coe Supersoft[®] used as a control group were statistically analyzed by independent T-test and one sample T-test at 95% confidential level.

Part III : In vitro study of the cellular response to the graft copolymer.

Cell culture

Clones of normal human gingival fibroblasts were prepared from healthy gingival tissues. Briefly, the tissue sample from gingivectomy were cut into small pieces and transferred to 35 mm culture plates (Falcon, Germany) The tissue samples were cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum, 2mM L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin and 5 µg/ml amphotericin B and maintained at 37 °C in humidified atmosphere of 95% air and 5% CO₂. All these ingredients for culture medium were purchased from Gibco BRL (Gibco Laboratories, USA). The medium was replaced every day. The explant was observed under the inverted microscope daily. On day 3 to 10 after tissue explant, a lot of cells migrated out of the tissue. Subculture of the cell clusters was performed in order to reduce the density of cell population and evenly distribute the cells in the new culture plates. This process allowed the cells to receive enough nutrients from the growth medium. In addition, this process could help eliminate other cell types except fibroblasts. Since the fibroblasts adhere on the substrate faster than other cell types do.

The method of subculture was described below:

1. Remove the culture medium and wash twice with simple DMEM to remove the traces of serum that would interfere the action of trypsin.
2. Detach the cells with trypsin-EDTA. This reagent will destroy the protein promoting the adhesion between cell to cell and cell to the substrate. As the result, the cells were freed from the attached surface.
3. Stop the trypsin-EDTA reaction with trypsin inhibitor.

4. The cell suspension was then filtered with lens paper. The filtered cell suspension was centrifuged at 2,000g to pellet the cells and the supernatant was discarded. The pellet was resuspended in the fresh medium.
5. The cells were counted by hemocytometer and replated at the cell density of 2×10^4 per ml in culture medium. The culture plate was kept at 37°C in humidified atmosphere of 95% air, 5% CO₂.
6. After leaving the cells to attach the plate for 1 hour, the old culture medium with unattached cells was replaced with a fresh one. The medium was replaced every 2 days. When the population of cells was high in density, the subculture was repeated as described above. With this method of selective attachment of the cells, clone of fibroblasts can be established approximately at the 5th passage. For this study, cells from the fifth passages were used in the study of the cellular response to graft copolymer and Coe Supersoft[®].

Cytotoxicity test

For cytotoxicity test, the human gingival fibroblasts from the 5th passage were seeded in a 24-well plates at the cell density of 4×10^4 cells/well and incubated at 37°C in humidified atmosphere of 95% air, 5% CO₂ for 24 hours. Cell cultures were immediately exposed to the vulcanized graft copolymer sheets and the Coe Supersoft[®] sheets (3 mm in diameter, 3 mm thick) stabilized in the well. The control group was the wells without any material. After another 24 hours of incubation, the viability of cells was immediately evaluated using the MTT assay. In each test, different formulas of graft copolymer were tested and each formula was test in 3 wells. The experiment was repeated 3 times with cells from different patients. Concentrations corresponding to 50% death of cell were evaluated from the standard curves.

Study the cellular response of fibroblasts to the graft copolymer by scanning electron microscopy

The specimens (20x20x3 mm³) were sterilized by autoclave before being fixed onto the 35 mm culture plates with sticky wax. Then, the cells from the 5th passage were plated into the prepared culture plates at the concentration of 10,000 cells /ml. The culture plates were maintained at 37°C in humidified atmosphere of 95% air, 5% CO₂ for 72 hours. The specimens were investigated with inverted phase contrast microscope everyday. After 72 hours of co-culture, the specimens were prepared for SEM examination. Briefly, The specimens were fixed with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 at 4 °C for 1 hour and 1% osmium tetroxide for 1 hour. After fixation, the specimens were dehydrated with graded ethanol. The specimens were then dried at critical point drying, fixed on the stubs and coated with gold particles before investigation under the SEM.

Results and Discussions

Preparation of high-ammonium concentrated natural latex

In the process of high-ammonium concentrated latex preparation, we added the water soluble chitosan and ammonium hydroxide as antifungal agents ⁽⁵⁸⁾. This was different from the previous studies ^(59, 60) that used only ammonium hydroxide. With this protocol, the latex could be easily centrifuged without coagulation and the concentrated latex was free from TMTD which was usually used as a preservative reagent. It is known that TMTD causes allergic contact dermatitis and when this reagent degrades it forms the carcinogenic nitrosamines ⁽⁶¹⁾. Several countries seriously concerned the contamination of TMTD in the rubber products. Therefore, the concentrated latex prepared by this protocol should be safe enough to be used as the raw material for production of soft lining material.

Determination of gamma irradiation doses that produced high tensile strength

Since the gamma ray was used in vulcanization and grafting of the natural latex, the total dose and the appropriate dose for each use had to be determined. Up to now, there is no specification of the tensile strength for the soft lining materials. In this study, the total irradiation dose was defined as the dose that gave the tensile strength of the final product 6-7 MPa which was the average tensile strength of the rubber gloves and the dental dam sheets. To determine the total irradiation dose, the natural latex was vulcanized by different doses of gamma ray and the tensile strength of rubber sheets casted from different irradiated latex was measured. The data of their tensile strengths were shown in Figure 27 and Table 8.

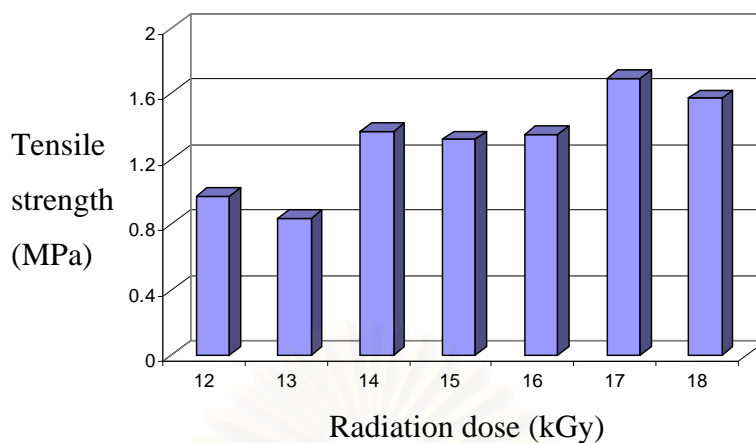


Figure 27. The mean tensile strength of vulcanized rubber at different doses of gamma irradiation

Table 8. The mean and standard deviation of tensile strength of vulcanized rubber at different doses of gamma irradiation

Tensile strength (MPa)	Doses of gamma ray (kGy)						
	12	13	14	15	16	17	18
Mean	0.97	0.84	1.37	1.32	1.35	1.69	1.57
SD.	0.16	0.11	0.21	0.18	0.14	0.24	0.19

The data showed that the tensile strengths of specimen performed from the vulcanized latex were lower than 2 MPa in all radiation doses. The data were statically analyzed with One-Sample Test: test value = 6 at 95% confidence level. It was found that the mean tensile strength of vulcanized rubber by gamma radiation doses from 12 to 18 kGy was significantly lower than the tensile strength of rubber dam sheet or the rubber examination grove ($p < 0.05$).

From these results the experiment was reset with the new criteria that controlled the temperature of the mixing *n*-BA process. The hypothesis for this step

was the lower environmental temperature during *n*-BA mixing process would enhance the tensile strength of vulcanized rubber. This idea came from the high vapor pressure of *n*-BA. Which very low dropping rate of *n*-BA to the latex, *n*-BA might be lost that caused the reduction of cross-linking accelerator. The experiment was set to prove this hypothesis. The cooler bath was used to control the temperature of both latex and *n*-BA (Figure 28).

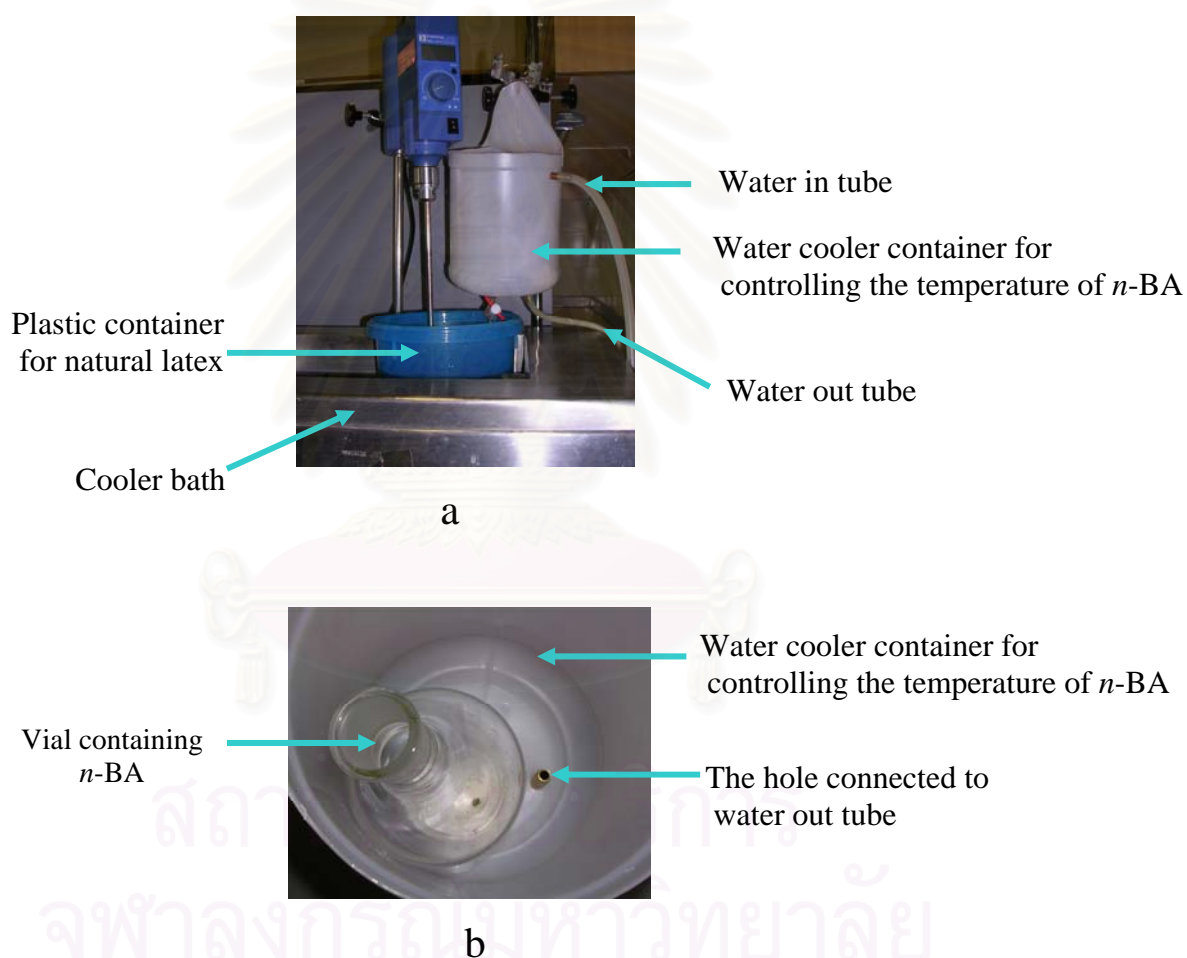


Figure 28. The modified equipments used in controlling the mixing temperature of *n*-BA to the vulcanized rubber.

- a) The water bath was modified for controlling the temperature of latex and *n*-BA.
- b) Shows the inner site of plastic container consists of glass vial for containing *n*-BA.

The temperature was controlled at 10°, 15°, 20°, and 25°C. At each temperature condition, the experiment was repeated as described previously. The specimens were prepared and tested again. The tensile strength from the repeated experiment was shown in Figure 29. It was found that the tensile strength of vulcanized rubber was still lower than 2 MPa. This result may be from the coagulation of rubber particles during storage in the refrigerator before mixing with *n*-BA. Therefore, the above hypothesis that the tensile strength increased if the temperature of environment during *n*-BA mixing decreased was rejected.

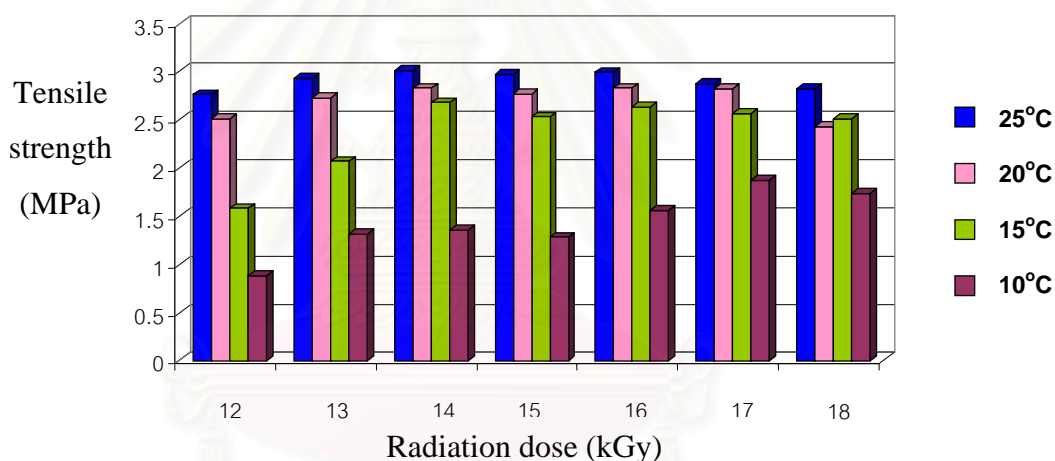


Figure 29. The tensile strength (MPa) of vulcanized rubber at different radiation doses and temperature condition

From the above results, the low temperature might alter the physical properties of concentrated latex. Therefore, the experiment was repeated for the third time at room temperature. The concentrated latex kept in the room temperature was used to evaluate the tensile strength after mixing with *n*-BA at room temperature and irradiated by gamma ray at different doses: 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 kGy. The results from the third trial revealed that the tensile strength of the

vulcanized rubber was higher than 2 MPa (Figure 30). After the data being statically analyzed One-Sample Test : test value = 6 at 95% confidence level, it was found that the mean tensile strength of vulcanized rubber by gamma radiation dose from 15 to 20 kGy was not significantly different from the tensile strength of dental rubber dam sheet ($p>0.05$). Therefore, 15 kGy which was the minimum radiation dose producing the tensile strength equal to rubber dam sheet, was chosen.

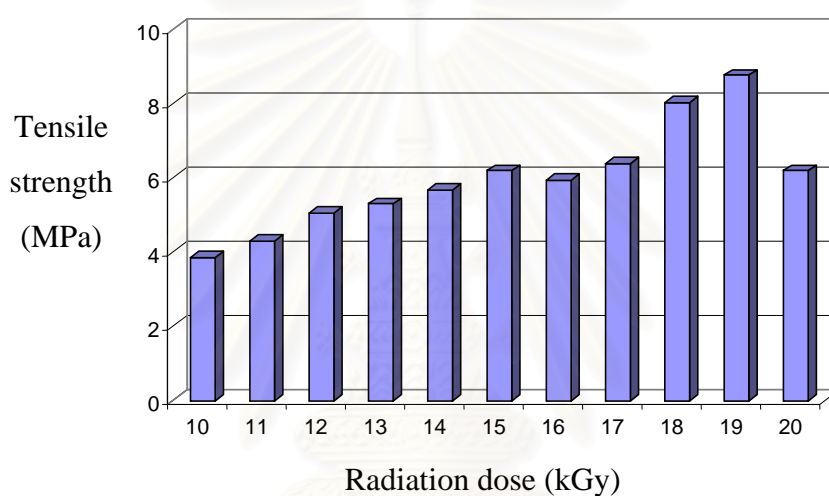


Figure 30. The tensile strength (MPa) of vulcanized rubber irradiated with different gamma radiation doses and prepared at room temperature.

These results suggested that the concentrated latex lost its tensile strength if it was kept at low temperature and it should be stored at room temperature. It also suggested that to get the high tensile strength as 6 MPa, the total irradiation dose should be 15 kGy.

Grafting efficiency

In this study 100 phr of EMA was grafted to 50% DRC vulcanized natural latex. The concentration of EMA and %DRC of vulcanized natural latex used in this

study came from 2 reasons. First in the pilot study, the graft copolymer sheets made from EMA lower than 100 phr was too soft and could not be ground with carbide bur (Figure 31a). In the dental laboratory process or in the dental clinic, the permanent soft lining materials should be easily ground with carbide instrument for boarder adjustment and smoothness (Figure 31b and c). In the case of concentration of EMA higher than 100 phr, it showed the mixture of latex and EMA did not blend homogeneously. The solid granules of polyethyl methacrylate were formed (Figure 32). Secondly, the 30% DRC of pre vulcanized latex was grafted with 100 phr of EMA in another pilot study. The result showed that the graft copolymer prepared in this method had high contraction because of its high water content and low viscosity that caused high shrinkage of dry graft copolymer sheet. This is not suitable for using as the soft ling material. The percentage of grafting efficiency from this method was 84% which was lower than 88% of the other study⁽⁶²⁾. This result suggested that the high DRC (50%) of pre vulcanized latex that had less water content might be necessary for producing free radical for the grafting process⁽⁶¹⁾.

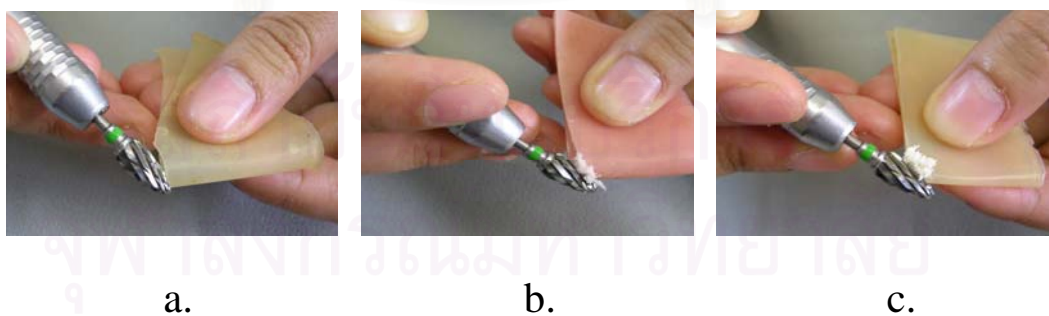


Figure 31. Grinding of specimens prepared from graft copolymer and Coe Supersoft[®]
 a. The graft copolymer sheet of natural rubber and EMA lower than 100 phr can not be ground with carbide bur.
 b and c. The Coe Supersoft[®] sheet and the graft copolymer sheet with 100 phr EMA , respectively, can be ground easily with carbide bur.

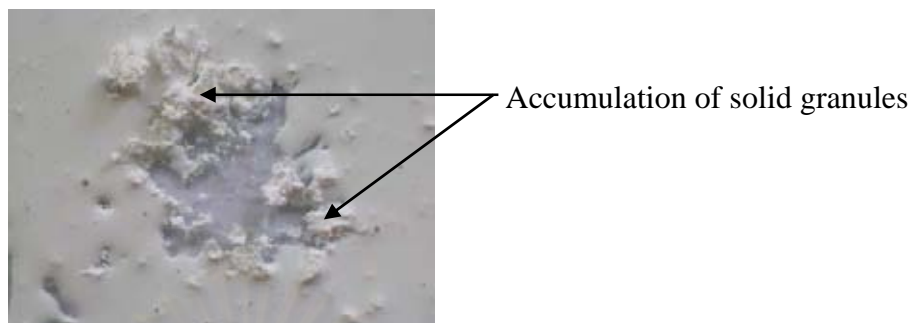


Figure 32. The accumulation of solid granules of polyethyl methacrylate when 110 phr of EMA was used in the grafting process.

Ultrastructure of the graft copolymer

Ultrastructurally, the morphology of natural rubber particles is spherical in shape with different sizes (Figure 33) while the graft copolymer particles demonstrated the natural rubber core with the EMA polymer shells. The EMA polymer was not only coated the surfaces of rubber particles, but also linked these particles together to form polymeric networks (Figure 34). These linkages may lead the graft copolymers to have less porosity than natural rubber resulting in less water absorption of the graft copolymers. Less water absorption is advantageous to soft lining materials since it helps maintain the dimension of the materials as well as decrease water soluble food and microorganism absorption. In addition the linkages may enhance the tensile and tear strength of graft copolymer.

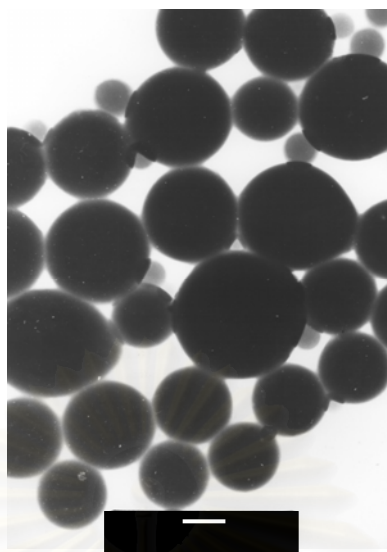


Figure 33. Transmission electron micrograph of prevulcanized latex.

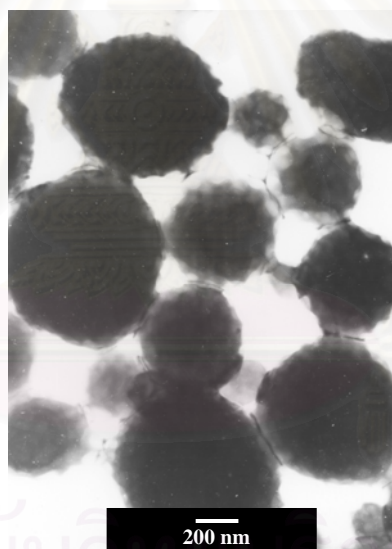


Figure 34. Transmission electron micrograph of graft copolymer by using 50% DRC of prevulcanized latex and 100 phr of EMA monomer.

Characteristics of graft copolymer

To investigate the functional group of EMA in the graft copolymer, the infrared spectroscopy was performed. The graphs of infrared spectrum (Figure 35a, b) showed that the vulcanized rubber had the absorption peak at 1663 cm^{-1} (Figure 35

a) represented the $-C=C-$. On the other hand, it was referred that the unsaturated double bonds were still remained in the vulcanized rubber whereas graft copolymer, the prominent absorption peaks were found at 1732 cm^{-1} ($-C=O$ stretch) and 1140 cm^{-1} ($-C-O-$ stretch) (Figure 35b). These results could be explained that the cross-linking site did not occur at the unsaturated double bond regions⁽⁶¹⁾. Therefore, in the grafting process the unsaturated double bonds were cleaved to form the radical and grafted with polyethyl methacrylate later. In the graft copolymer preparation, the latex was first irradiated for cross-linking and then was irradiated again for grafting. This protocol can enhance the tensile strength and tear strength of final product, graft copolymer, because of cross-linking and entanglement of polymer chains.

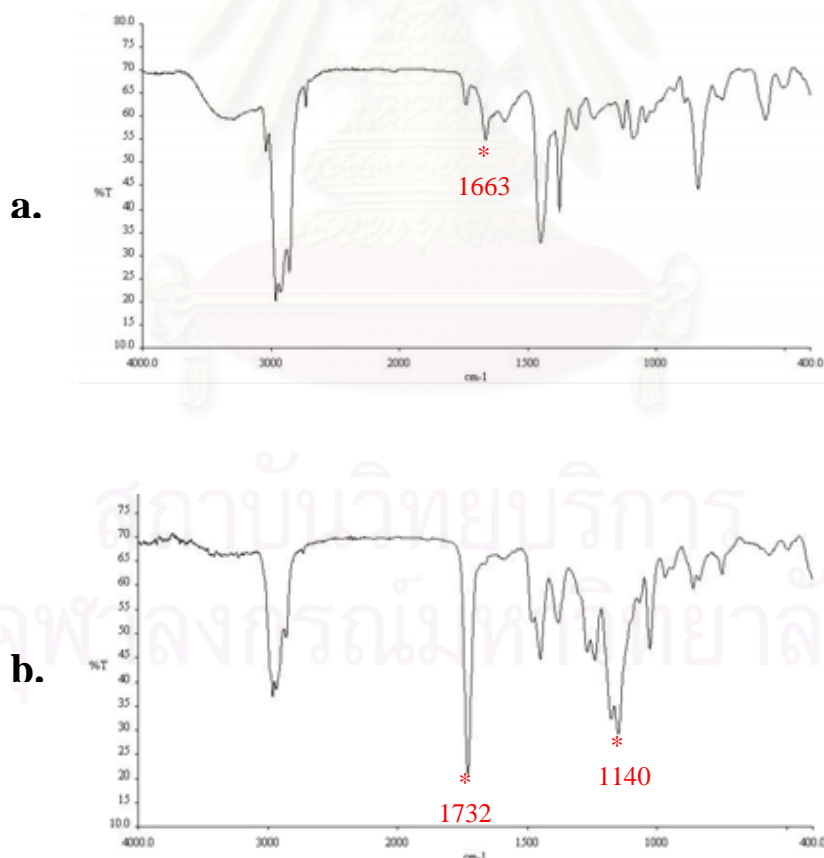


Figure 35. The infrared spectrum of the different preparations of the natural latex.

a) The infrared spectrum of latex vulcanized with 15 kGy irradiation.

b) The graft copolymer prepared by natural latex and 100 phr of EMA.

Transitional glass temperature examination

The results from the Differential Scanning Calorimeter as of the T_g value (Figure 36) showed that the T_g of vulcanized rubber was found at -62.3°C and graft copolymer was at -62.8°C whereas, the T_g of the Coe Supersoft[®] could not found in the range of temperature from -80 to -50°C . The T_g value of pure EMA is 66°C that is much higher than the mouth temperature. Some manufacturers produced the soft acrylic resin by mechanical mixing of PEMA and plasticizer. This product has the lower T_g value. However, it will turn to the hard state after using it for a few months because of the leaching out of plasticizers. Therefore, it is not suitable for this product to be used as a soft lining. For the graft copolymer, PEMA was attached to the polyisoprene chain by chemical bond and its T_g value is very low. The result suggested that the graft copolymer is able to keep the soft state for a long time.

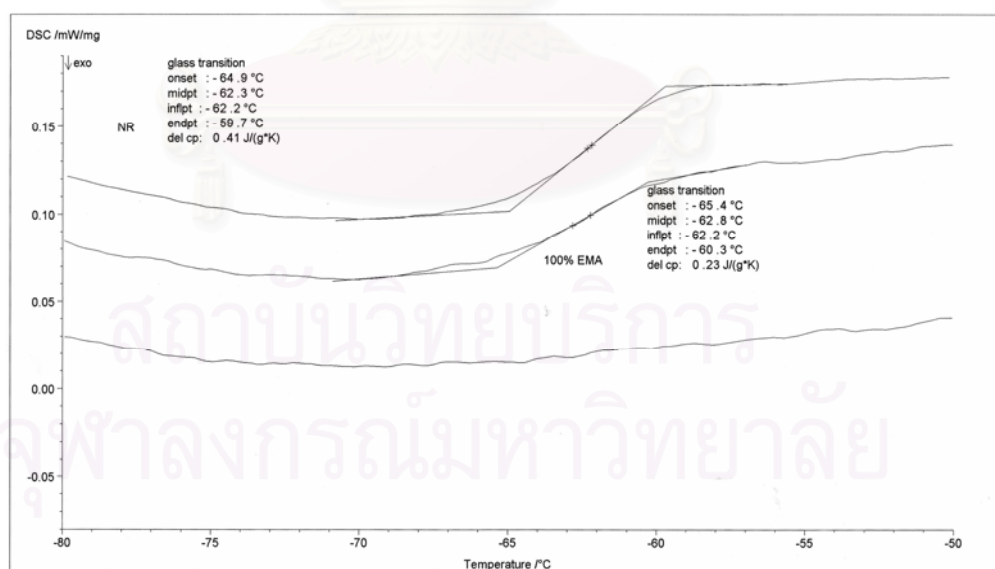


Figure 36. The T_g values of vulcanized rubber (top line), graft copolymer (middle line), and Coe Supersoft[®] (bottom line).

Mechanical properties

Surface hardness

The surface hardness of the graft copolymer was studied by immersion of the graft copolymer as well as the Coe Supersoft[®] in distilled water. The result was shown in Figure 37. From these results, we found that the median value of Shore A of surface hardness was reduced after one day of immersion time in both materials. It may be that water was absorbed into the specimens and acted as a plasticizer⁽⁶³⁾. This level of surface hardness stayed for three months and increased in the 4th month. However, over ten months of immersion in distilled water at 37°C, the surface hardness of both graft copolymer and controlled group were not significant difference. The increasing of surface hardness in the 4th month can be explained as follow. For the Coe Supersoft[®], soft acrylic resin material, is presented in powder and liquid format; comprising a higher methacrylate polymer powder (usually PEMA) with a liquid comprising a higher methacrylate monomer and a plasticizer (commonly a phthalate)⁽¹²⁾. The soft acrylic resin materials undergo two processes when immersed in water⁽⁴⁷⁾. The low molecular weight plasticizer is leached out into the water and at the same time, water is absorbed into the polymer structure. The loss of plasticizer appears to be the most important process as far as the properties are concerned since ageing results not only in a dimensional change but also a loss of softness⁽⁶⁴⁾. Whereas, the graft copolymer that natural particles were grafted with EMA polymer and act as the permanent plasticizing agents but the increasing of surface hardness may be from the degradation of free polyisoprene at the surface. This lead to the remaining PEMA which had hard consistency. If this idea is true, the high percentage of grafting efficiency or the surface coating on the graft copolymer might solve this problem.

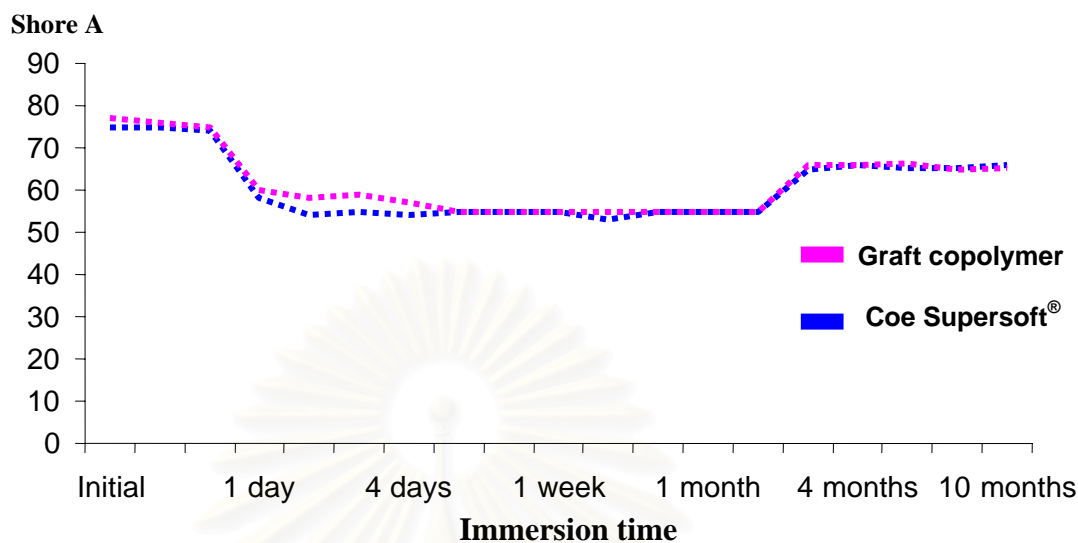


Figure 37. The surface hardness of graft copolymer and Coe Supersoft[®] at different period of immersion time

Tensile properties

The tensile properties of the graft copolymer and Coe Supersoft[®] was shown in Table 9. The data were analyzed by using T-test at 95% confidence level. It was found that the tensile strength, extension at break, and % elongation of graft copolymer were significantly higher than Coe Supersoft[®]. On the contrary, the 100% modulus of graft copolymer was lower than Coe Supersoft[®]. These results suggested that the graft copolymer had higher elasticity than Coe Supersoft[®]. In the previous study we have shown that the tensile strength of the vulcanized rubber with 15 kGy gamma irradiation was 6 MPa. In this study, we found that the prevulcanized latex grafted with 100 phr EMA had twice higher tensile strength than that of the vulcanized rubber. This can be explain that after vulcanization process, the tensile strength increased by the cross-linking between the *cis*-1,4-polyisoprene chains. Then the grafting process caused the entanglement between the polymer chains of

polyethyl methacrylate themselves or between the polyethyl methacrylate and *cis*-1,4-polyisoprene. These cross-links possibly played a role in controlling the tensile strength (Figure 38).

Table 9. The mean and standard deviation of tensile properties of graft copolymer and Coe Supersoft[®].

Materials	100 % modulus (MPa)	Tensile strength (MPa)	Extension at break (mm)	% Elongation
Graft copolymer	1.429 (0.18)	12.92 (2.07)	287.45 (22.35)	856.35 (77.53)
Coe Supersoft [®]	3.43 (0.62)	4.47 (0.77)	72.67 (2.59)	142.3 (8.87)

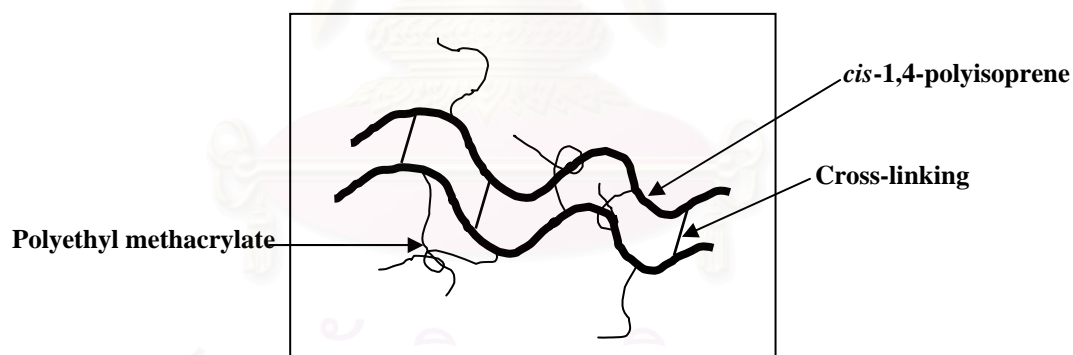


Figure 38. The illustration of cross-linking of *cis*-1,4-polyisoprene and entanglement of polyethyl methacrylate

Tear strength

From the pilot study it was found that the tear strength of the vulcanized rubber irradiated with 15 kGy produced the tear strength of 28.9 N/m. It has been known that vulcanized rubber was high in tensile strength but low in tearing strength.

This property is disadvantageous for all kind of vulcanization method: radiation, peroxide, and sulfur cured. In this study, the vulcanized latex grafted with EMA showed the significantly higher tear strength than Coe Supersoft[®] ($p < 0.05$) (Figure 39). For this result, the high tear strength of the graft copolymer may be from the chain entanglement of polymer chain as described previously. Therefore, to solve the low tear strength of vulcanized rubber, the grafting method should be used. The high tear strength is one of the requirements of the soft lining materials because it provides the good edge of soft lining materials. From the clinical experience, most of the available soft lining materials in the market showed low tear strength that caused the irregular margin and irritated patients' oral tissues.

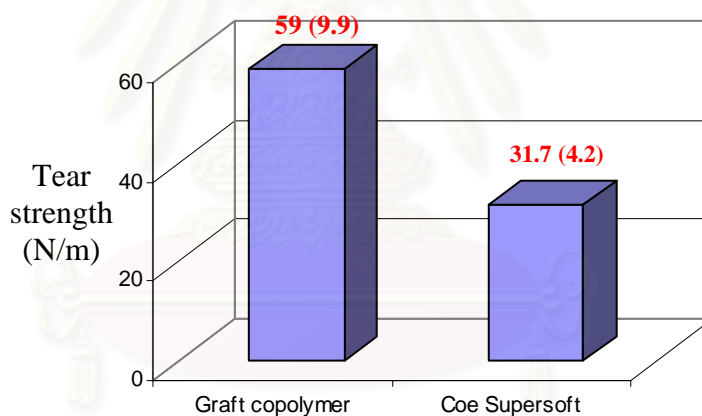


Figure 39. The mean and standard deviation of tear strength of graft copolymer and Coe Supersoft[®]

Tensile bond strength

To study the nature of interface attachment between the graft copolymer and PMMA (denture base material), the investigation at the interface with SEM and the tensile bond strength measurement by Lloyd UTM were performed. It was found from the SEM examination that graft copolymer and Coe Supersoft[®] displayed

differences in the nature of their interfaces with PMMA. Graft copolymer displayed a gap at the interface between itself and PMMA (Figure 40a) suggested the incomplete bonding between these two materials. The Coe Supersoft[®] showed a complete junction with PMMA (Figure 40b). When the specimens were measured the tensile bond strength by UTM, it was found the tensile bond strength between the graft copolymer and PMMA was not significantly difference from the tensile bond strength between Coe Supersoft[®] and PMMA ($p>0.05$). The mean and standard deviation values of tensile bond strength of specimens are shown in Figure 41. The result demonstrated that graft copolymer and Coe Supersoft[®] had the different mode of failure of their bond with PMMA to external damaging loads. Coe Supersoft[®] showed the 8.26 MPa of bond strength and exhibited cohesive failure whereas graft copolymer provided 7.38 MPa of tensile bond strength and demonstrated the adhesive failure or interfacial failure. From the nature of their interface with PMMA, it can be explained that PMMA and Coe Supersoft[®] have the hydrophobic behavior therefore they are compatible and exhibit the complete junction. In the case of graft copolymer when the latex which was in aqueous form was packed next to the dough stage of PMMA, the PMMA surface was contaminated with water from latex resulting in reduction of the complete contact between PMMA and graft copolymer. The incompatible between latex form of graft copolymer and PMMA caused the gap at the junction and enhanced the adhesive failure mode. To solve this problem, elimination of water content in the graft copolymer before packing with the PMMA should be seriously considered.

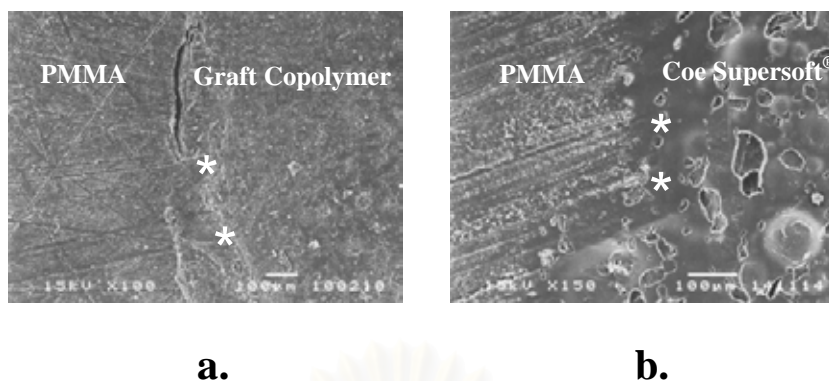


Figure 40. Transmission electron micrographs show the interface (*) between (a) PMMA and graft copolymer and (b) PMM and Coe Supersoft®.

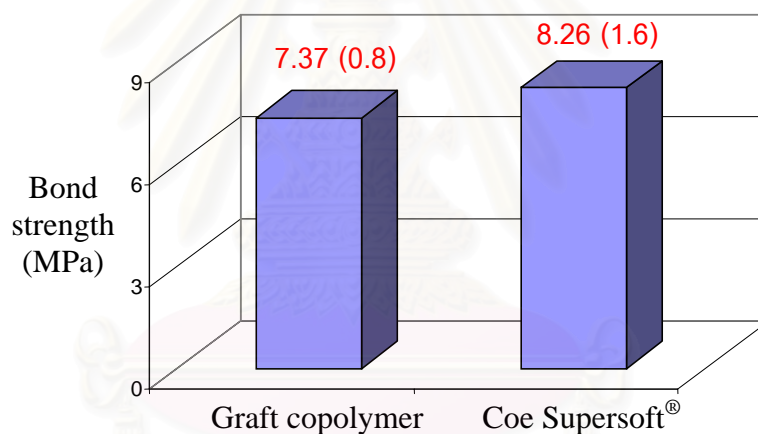


Figure 41. The mean and standard deviation of tensile bond strength of graft copolymer and Coe Supersoft®

Water absorption

In this study, the tested materials were immersed in distilled water up to 10 months. At each immersion time period, the tested materials were weighed and the data was collected as % of water absorption. The mean % of water absorption of graft copolymer, Coe Supersoft® and vulcanized rubber at each time interval was shown in Figure 42. The sorption values (standard deviation) for the materials tested

ranged from a low to high at 10 months: 4.93 %(1.06) for graft copolymer, 6.75 (0.40)% for Coe Supersoft[®], and 42.51 (4.50)% for vulcanized rubber, respectively. The water sorption values of all tested materials increased steadily over the 10 month period of this study. The graft copolymer and Coe Supersoft[®] had much less water sorption than that of natural rubber. One of the ideal properties of soft lining materials is less water absorption. In the past, natural rubber had been used for denture base lining but it absorbed a lot of water. It is of interest that the vulcanized rubber had high water absorption even though the rubber is composed of hydrophobic hydrocarbon molecules. It should have had less water absorption. The increasing water absorption in the vulcanized rubber might be affected by the hydrophilic impurity materials contaminated in the latex ⁽⁶⁵⁾ or the degraded of insoluble protein into soluble protein by irradiation ⁽⁶⁰⁾. In this study the vulcanized rubber was modified by grafting with EMA monomer which helped reduce water absorption from 42.51% to 4.9 %. For the Coe Supersoft[®] water absorption increased steadily that may be caused by leaching out of the plasticizer from the materials and water replacement.

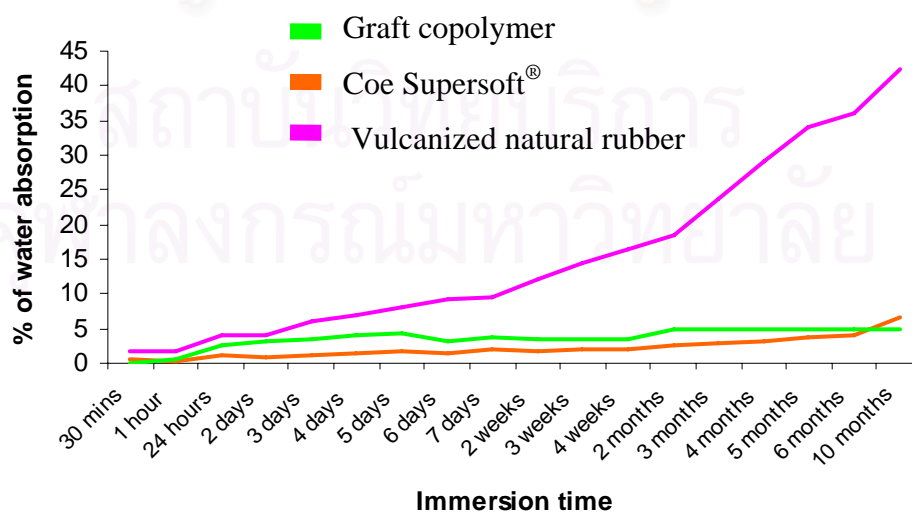


Figure 42. The % of water absorption of three materials: graft copolymer, Coe Supersoft[®], and vulcanized natural rubber.

Contact angle

The mean and standard deviation of contact angles of graft copolymer and Coe Supersoft[®] were 71 degree and 90 degree, respectively. The explanation for the difference of the contact angle of these 2 materials probably is that the graft copolymer had the surface energy higher than Coe Supersoft[®] did or, on the other hand, the surface of graft copolymer material had more hydrophilic property than Coe Supersoft[®] did. The hydrophilic surface of soft lining material will produce the more wettable than the hydrophobic surface. The wettable surface of the soft lining denture base materials is leading to the high capillary reaction and comfortable felling of the patients.

Cytotoxic effects of the graft copolymer and Coe Supersoft[®]

In the co-culture system, the graft copolymer demonstrated a lot of cells around the materials (Figure 43a). These cells attached well on the surface of the graft copolymer (Figure 43b). In contrast, the culture dish containing Coe Supersoft[®] showed a clear band with some cell debris around the specimen (Figure 44a). At the surface of Coe Supersoft[®], there were a few cells found poorly attaching to the material (Figure 44b). These results suggested that the graft copolymer was not only noncytotoxic to the cells, but also offered a hospitable surface for the cells to attach while the Coe Supersoft[®] lack of these properties.

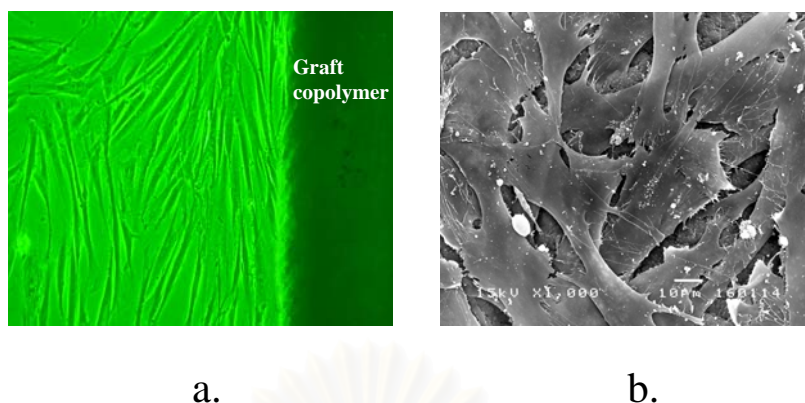


Fig.43. The inverted phase contrast micrograph (a) and scanning electron micrograph (b) of the human gingival fibroblasts co-cultured with the graft copolymer. Note the well proliferated and well attached human gingival fibroblasts around and on the surface of the graft copolymer.

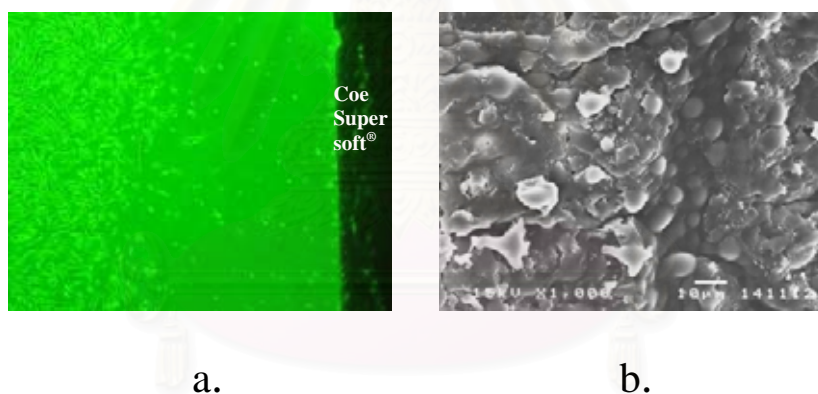


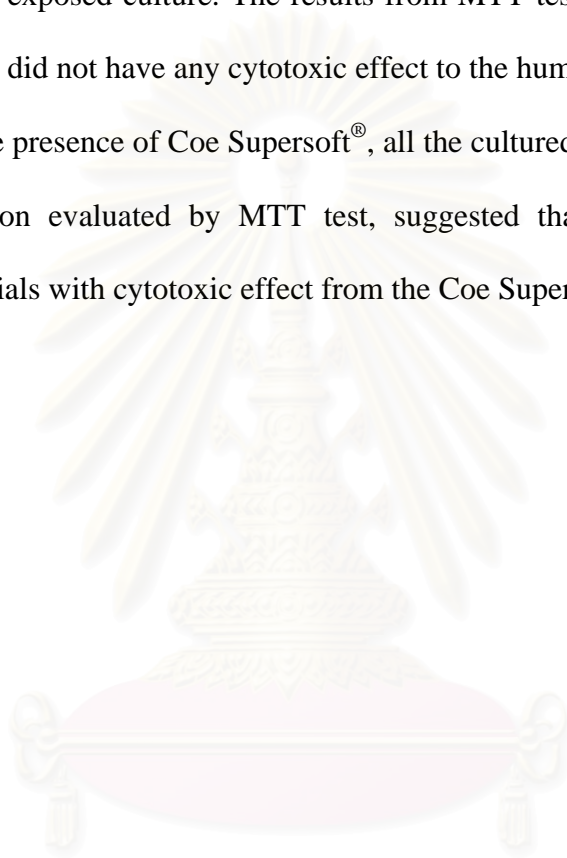
Figure 44. The inverted phase contrast micrograph (a) and scanning electron micrograph (b) of the human gingival fibroblasts co-cultured with the Coe Supersoft®. Note the clear band next to the material and a few poorly attached cells on the material surface.

MTT assay

To test the cytotoxicity of the graft copolymer and Coe Supersoft®, the MTT assay was performed to investigate the cell vitality in the co-culture system. In this study, the mitochondrial activity assessed with the MTT test was inhibited by the

reagents leaching from the Coe Supersoft[®] specimen. The density of cell was reduced to zero in the presence of the Coe Supersoft[®] specimens for 48 hours. Whereas, the graft copolymer specimens did not affect the mitochondrial activity. Therefore, the cell density after incubation with graft copolymer for 48 hours was not different from the cells in non-exposed culture. The results from MTT test also confirmed that the graft copolymer did not have any cytotoxic effect to the human gingival fibroblasts.

In the presence of Coe Supersoft[®], all the cultured cells died after 48 hours of the incubation evaluated by MTT test, suggested that there might be some leachable materials with cytotoxic effect from the Coe Supersoft[®].



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Conclusions

In this study, the graft copolymer was prepared from natural latex by vulcanization with 10 kGy of gamma radiation and graft with ethyl methacrylate using 5 kGy gamma ray. The grafted natural latex, the so-called graft copolymer, was then investigated for its ultrastructure, physical and mechanical properties as well as cellular biocompatibility. The results from all studies showed that

1. The graft copolymer had comparable surface hardness to the Coe Supersoft[®] which was used as the standard group in this study.
2. The graft copolymer had less water absorption than the Coe Supersoft[®]. The least water absorption is an ideal property for the soft lining material.
3. The graft copolymer had higher tensile properties, tensile bond strength and tear strength than Coe Supersoft[®] ($p < 0.05$).
4. The graft copolymer showed incomplete bonding to the denture base material.
5. The graft copolymer showed good cellular biocompatibility to the human gingival fibroblasts.

These results indicated that grafting of ethyl methacrylate to the gamma irradiated vulcanized latex improved the physical and mechanical properties as well as the cellular biocompatibility of the latex. Therefore it would be worth to modify the graft copolymer developed by this protocol for using as denture soft lining materials.

Future Study

1. Investigation of the more appropriate dose for vulcanization and grafting process in the natural latex.

In this study, the total dose of gamma ray used was 15 kGy which gave the tensile strength of 12 MPa in the graft copolymer. For the clinical practice of soft ling denture base material, it may be not necessary to have the high tensile strength as 12 MPa. Therefore, the least irradiation dose that will not alter other properties of graft copolymer must be investigated. Reduction of the irradiation dose means reduction of the hazard, cost and working time.

2. Improvement of the bonding ability of the graft copolymer to the denture base material.

The graft copolymer showed incomplete bonding to the denture base material in this study. This pitfall probably came from high water content in the graft copolymer. To solve this problem, the other form of copolymer with less water content should be developed in the future study.

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