

CYTOTOXICITY AND TENSILE STRENGTH OF ACRYLIC DENTURE
SOFT LINER WITH SILVER NANO-PRISMS.

A Thesis
BY
SANTI EURWONGPANICH

Presented in Partial Fulfillment of the Requirement for the
Master of Science Degree in Prosthodontics
at Srinakharinwirot University

May 2018

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Aa Abstract

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Santi Eurwongpanich (2018). Cytotoxicity and Tensile Strength of Acrylic Denture Soft Liner with Silver Nano-Prisms. Master thesis, M.S. (Dentistry). Bangkok: Graduate School, Srinakharinwirot University. Advisor Committee: Asst.Prof.Dr. Napapa Aimjirakul, and Asst.Prof.Dr. Piyanart Ekworapoj

Objective: While the silver compound and its derivatives have long been used as an effective antimicrobial agent, they also have an influence on the cytotoxicity in humans and the mechanical properties. This study evaluates the effects of both cytotoxicity and the mechanical properties of acrylic denture soft liner with silver-nanoprisms (AgNPs).

Methods: Various concentrations (10, 20, 30, 40, 50 and 60 ppm) of AgNPs have been incorporated into the acrylic denture soft liner and divided into seven groups, a control group and experimental G1-G6 groups respectively. The cytotoxicity of all groups was tested via a duplicate MTT assay. Tensile Tear Strength (TTS) and Tensile Bond Strength (TBS) was tested by a universal testing machine (Shimadzu EZ-L, Tokyo, Japan). The results were analyzed using a two-way ANOVA with Tukey HSD test at significant level 0.05.

Results: The cell viability value was lowest in the G7 twenty four hour group (73.25%), but highest in the G1 twenty four hour group (98.32%). The tensile tear strength test were not significantly differences ($P>0.05$). However, the high concentration of AgNPs group (35.00 N/cm²) had significantly higher tensile bond strength than the low concentration of AgNPs group (18.82 N/cm²)

Conclusions: The experimental acrylic soft lining materials with AgNPs was not harmful to fibroblast cells and improved the mechanical properties.

ผลของความเป็นพิษต่อเซลล์และคุณสมบัติความแข็งแรงดึงของ
วัสดุเสริมฐานฟันเทียมแบบนุ่มที่เติมสารละลายซิลเวอร์นาโนพริซึม



บทคัดย่อ
ของ
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เสนอต่อบัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ เพื่อเป็นส่วนหนึ่งของการศึกษา
ตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต สาขาวิชาทันตกรรมคลินิก
พฤษภาคม 2561

สันติ เอื้อวงศ์พานิชย์ (2561). ผลของความเป็นพิษต่อเซลล์และคุณสมบัติความแข็งแรงดึงในวัสดุเสริมฐานฟันเทียมแบบนุ่มที่เติมสารละลายซิลเวอร์นาโนพริซึม. วิทยานิพนธ์ วท.ม. (ทันตกรรมคลินิก). กรุงเทพฯ: บัณฑิตวิทยาลัย มหาวิทยาลัยศรีนครินทรวิโรฒ. อาจารย์ที่ปรึกษาวิทยานิพนธ์: ผู้ช่วยศาสตราจารย์ ดร.ณปภา เอี่ยมจิรกุล, ผู้ช่วยศาสตราจารย์ ดร.ปิยะนารถ เอกวรพจน์

วัตถุประสงค์ สารประกอบซิลเวอร์และอนุพันธ์ของสารเหล่านั้นถูกนำมาใช้เป็นสารต้านจุลชีพที่มีประสิทธิภาพอย่างแพร่หลาย แต่สารเหล่านี้อาจมีผลกระทบต่อความเป็นพิษต่อเซลล์ของมนุษย์ และคุณสมบัติเชิงกลของวัสดุ การศึกษานี้ประเมินผลของความเป็นพิษต่อเซลล์และคุณสมบัติเชิงกลของวัสดุเสริมฐานฟันเทียมแบบนุ่มที่เติมสารละลายซิลเวอร์นาโนพริซึม

วิธีดำเนินการวิจัย ทำการเตรียมสารละลายซิลเวอร์นาโนพริซึมทั้งหมดหกความเข้มข้น (10, 20, 30, 40, 50 และ 60 ppm) ผสมเข้าไปในชั้นทดสอบที่เตรียมจากวัสดุเสริมฐานฟันเทียมแบบนุ่ม โดยแบ่งเป็น 7 กลุ่มคือ กลุ่มควบคุมและกลุ่มทดสอบ G1 ถึง G6 ตามลำดับ ผลของความเป็นพิษต่อเซลล์ถูกประเมินโดยวิธีการ MTT assay แบบทำซ้ำสองครั้ง การทดสอบความแข็งแรงดึงฉีกขาดและการทดสอบความแข็งแรงดึงยึดติดถูกทดสอบโดยเครื่องทดสอบสากล (Shimadzu EZ-L, เมืองโตเกียว, ประเทศญี่ปุ่น) ผลการทดลองถูกวิเคราะห์ข้อมูลทางสถิติโดยใช้การวิเคราะห์ความแปรปรวนสองทาง และการทดสอบทุกที่ที่ระดับนัยสำคัญ 0.05

ผลการศึกษา ความมีชีวิตของเซลล์ลดลงต่ำสุดในกลุ่ม G7 ที่ระยะเวลา 24 ชั่วโมง เป็นร้อยละ 73.25 และความมีชีวิตของเซลล์มากที่สุดในกลุ่ม G1 ที่ระยะเวลา 24 ชั่วโมง เป็นร้อยละ 98.32 ผลของการทดสอบความแข็งแรงดึงฉีกขาดไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($P>0.05$) ในกลุ่มทดสอบระดับความเข้มข้นของสารละลายซิลเวอร์นาโนพริซึมที่สูงมีค่าความแข็งแรงดึงยึดติด (35.00 นิวตันต่อตารางเซนติเมตร) มากกว่าในกลุ่มทดลองที่มีระดับความเข้มข้นของสารละลายซิลเวอร์นาโนพริซึมที่น้อย (18.82 นิวตันต่อตารางเซนติเมตร) อย่างมีนัยสำคัญทางสถิติ ($P<0.05$)

บทสรุป การเติมสารซิลเวอร์นาโนพริซึมในวัสดุเสริมฐานฟันเทียมแบบนุ่มไม่เป็นอันตรายต่อเซลล์ร่างกายในมนุษย์ และยังช่วยปรับปรุงคุณสมบัติเชิงกลของวัสดุด้วย

The thesis titled
"Cytotoxicity and tensile strength of acrylic denture soft liner
with silver nano-prisms"

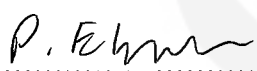
By
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Has been approved by the Graduate school as partial fulfillment of the requirement for the
Master of Science degree in Prosthodontics of Srinakharinwirot University.

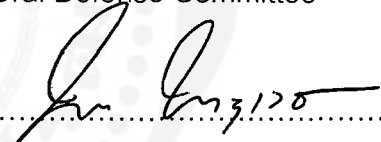

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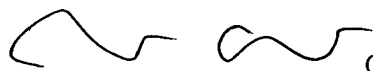

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This research was supported by the grant

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ACKNOWLEDGEMENT

First, I would like to thank Asst.Prof.Dr. Napapa Aimjirakul and Asst.Prof.Dr. Piyanart Ekworapoj, my advisors and co-advisor, for their invaluable, concern and encouragement. Without them, this thesis would never be completed successfully

I am also grateful to Dr. Sanong Ekgasit, Head of Sensor Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, for providing silver nanoprisms.

I am also grateful to Dr. Pornkiatch Cheanjitapirom, Head of Sensor Research Unit, Faculty of Dentistry, Mahidol University, for providing consultation for designed the mold of this study.

I am also grateful to Dr. Numchai Supparekchaisakul, Head of Sensor Research Unit, Faculty of human research behavior, Srinakharinwirot University, for providing consultation for statistics analysis of this study.

I would like to thank all instructors in Department of Stomatology at Faculty of Dentistry, Srinakharinwirot University for supporting and valuable advice

Finally. I am deeply grateful to my family for all love and support.

Santi Eurwongpanich

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CHAPTER 1

INTRODUCTION

1. Background and rational

After long term used of dental prosthesis, the mucosa under the denture base become thin and severe alveolar resorptions. Patients are not tolerate to the hard denture base when they have chewing in the day. Soft lining materials are most commonly used for patients who are not tolerate to the pressures transmitted by prosthesis to enhance the recovery of denture bearing area when it is get trauma to alveolar ridge and get accurate adaptation to the edentulous ridge.¹

Candida albicans which is considered to be the most important etiological factor in denture stomatitis, can be found on soft liner surface.^{2,3} Moreover, in vitro experiments showed that none of the materials had an inhibitory effect on growth of *Candida albicans* which could penetrate to the inner portions of the soft liner.^{4,5}

There are two methods (mechanical and chemical method) for a daily cleaning the denture. Mechanical method which use a toothbrush will cause the damage for the soft liner. The method should be used to clean, is chemicals method. However, the chemical cleanser can alter some denture properties.⁶

Tissue conditioners act as drug delivery agents to bring antiseptic agent to cure denture stomatitis patients. Antifungal drug such as nystatin was commonly used for the treatment of the denture stomatitis.⁷ However, the antifungal drug is board spectrum target in humans when doctors use for treating patients. Amphotericin B and nystatin were the good antifungal drug, but they were not effective when they were contained in the viscogel soft liner.⁸

Recently, there are new antifungal agents such as metallic oxide powders⁹, natural and herbal oils¹⁰, and silver nanoparticles (AgNPs)^{11, 21-23, 38, 40-42} into tissue conditioners have been investigated. Silver nanoparticles have been widely used in clothing, electronics, bio-sensing, the food industry, paints, sunscreens, cosmetics and medical devices. Not only silver nanoparticles have an ability to disturb metabolism activity of microorganisms but is

also nontoxic to humans. Moreover, silver nanoparticles are used in very low concentration for against microorganisms.^{12,21,22}

The purpose of this study was to study cytotoxic effects of silver-nanoprisms containing on acrylic soft lining materials, and evaluate the mechanical properties on tensile bond strength and tensile tear strength of silver-nanoprisms containing on acrylic soft lining material.

2. Purpose of this study

1. To study cytotoxic effect of acrylic soft lining material containing on silver nano-prisms agent.
2. To evaluate the mechanical properties on tensile tear strength and tensile bond strength of acrylic soft lining material containing on silver nano-prisms agent.

3. Limitation of this study

This study is based on experimental research.

4. Definition of terms

Silver nanoprisms

Silver nanoprisms in colloidal substances of between 0.6 nm to 2.5 μm . in size of the particles.

Tensile bond strength

A measurement of the capacity of material to withstand loads tending to elongation, as opposed to compressive strength, which withstands loads tending to reduce size. Tensile bond strength may be involves determining the stress required to rupture a bond formed by an adhesive between two **solid surfaces**.⁴³

Tensile tear strength

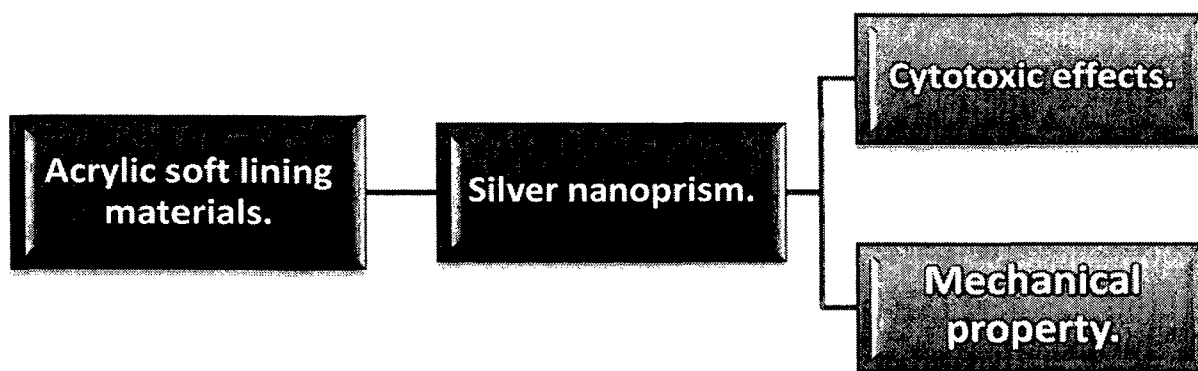
A measurement of the capacity of material to withstand loads tending to a measure of how does can withstand loads tending to the effects of tearing inside them, whereas a normal tensile tear test is done for a non-defective piece of material.⁴⁴

Cytotoxicity test

When cells attach toxic, they was exposed to a cytotoxic compound and respond in the many ways. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. Cytotoxicity is important for testing of new materials used in Humans. Cytotoxicity tests provide an investigation of toxicity in a simplified system that reduces the effects of toxic variables factor. To evaluate potential of such as materials to cause irritation, the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay is applicable. MTT offers a high degree of sensitivity, while providing a considerable savings in time and labor.²³

5. Conceptual framework

Although acrylic soft lining materials are modified by silver nano-prisms have the antimicrobial effect to against fungi and bacteria species. However, the soft lining materials containing silver nano-prisms maybe affect to human toxicity and mechanical property of them such as tensile tear strength and tensile bond strength.



6. Variable for this study

Independent variable

- The concentration of silver nanoprism into acrylic soft lining materials.

Dependent variable

- The level of cytotoxicity indicating as the percentage of cell availability.
- Mechanical properties : Tensile bond strength
: Tensile tear strength

7. Hypothesis

1. Cytotoxic effect

H_0 : There have no significant differences on cytotoxic effect between the acrylic soft lining material from the one with silver nanoprism.

H_1 : There have significant differences on cytotoxic effect between the acrylic soft lining material from the one with silver nanoprism.

2. Tensile tear strength.

H_0 : There have no significant differences on tensile tear strength between acrylic soft lining material from the one with silver nanoprism.

H_1 : There have significant differences on tensile tear strength between acrylic soft lining material from the one with silver nanoprism.

3. Tensile bond strength.

H_0 : There have no significant differences on tensile bond strength between acrylic soft lining material from the one with silver nanoprism.

H_1 : There have significant differences on tensile bond strength between acrylic soft lining material from the one with silver nanoprism.



CHAPTER 2

LITERATURE REVIEW

1. Introduction

It was found that alveolar bone loss under tissue surface of denture occurs in elderly patients who wear the dentures for a long time. There will be a loose denture and loss of stability. The injured tissue beneath the denture base or abused tissue will happen. In these cases, soft lining materials are applied to the fitting surface of the denture beneath abuse tissue before doctors make a new complete denture. Soft lining materials used for relined denture to enhance the recovery of denture bearing area from trauma, damage/ ridge resorption, and get accurate adaptation to the edentulous ridge.¹³

2. Background knowledge

2.1 Type of soft lining materials

Soft lining materials can be divided into two main categories that are (1) Temporary soft lining materials and (2) Permanent soft lining materials.

According to composition and processing method, permanent soft lining materials may be divided into four groups as (1) heat polymerized silicone, (2) autopolymerizing silicone, (3) heat polymerized acrylic resin and (4) autopolymerizing acrylic resin.

The category of soft lining materials and manufacturers of production are shown in figure 1 and table 1.^{13,18}

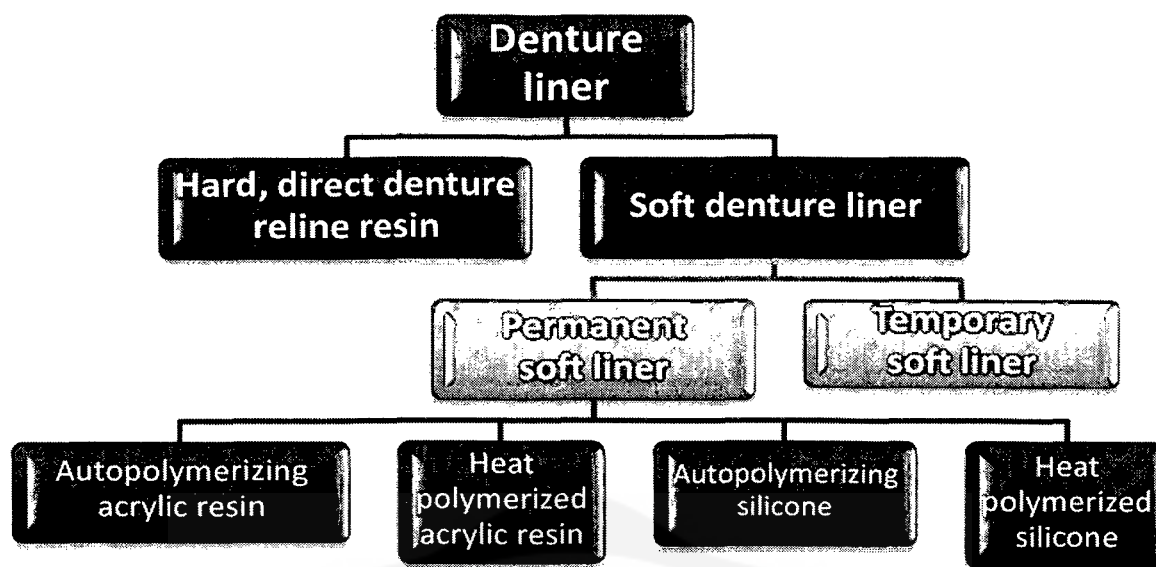


Figure 1 classification of denture soft liner.

Table 1 Soft lining materials¹³⁻¹⁶

Categories /Processing method	Brand name	Manufacturer
Temporary soft lining materials (tissue conditioners)		
Autopolymerizing acrylic resin	Visco-Gel [®]	DeTrey Division,Densply,England
	GC soft [®]	GC dental Industrial Corp,Japan
	Coe comfort [®]	Coe Laboratories Inc,Chicago,Illinois
	Coe soft [®]	Coe Laboratories Inc,Chicago,Illinois
	S-R Ivoseal [®]	Ivoclar AG,Lichtenstein
	Fit [®]	Kerr/Sybron, Michigan, USA
Permanent soft lining materials		
Autopolymerizing acrylic resin	Soften [®]	Kamemizu Chem,Japan
Heat polymerized acrylic resin	Coe supersoft [®]	Coe Laboratories Inc,Chicago,Illinois
Autopolymerizing silicone	GC Denture relining [®]	GC dental Industrial Corp,Japan
Heat polymerized silicone	Molloplast-B [®]	Detax Karl Huber GmbH,Germany

2.2 Composition of soft lining materials

According to composition and processing method, soft lining materials may be divided into two groups that are 1) Acrylic resin soft lining materials and 2) Silicone soft lining materials. The acrylic resin temporary soft lining materials are comprised of non cross-linked amorphous polymers contain, that don't have initiator and monomer. Both the silicone and acrylic permanent materials are comprised of cross-linked amorphous polymers.¹⁴

Composition of acrylic resin soft lining materials^{13,16}

polymer powder : poly (ethyl methacrylate)

monomer : Methyl methacrylate, N-butyl methacrylate, 2-Ethoxyethyl methacrylate,
Lauryl methacrylate, Ethyl methacrylate

plasticizer: Butyl benzyl phthalate(BBP), Butyl phthalyl butyl glycolate(BPBG),
Dibutyl phthalate(DBP), Dibutyl sebacate(DBS), Ethyl alcohol, Benzyl
benzoate(BB), Benzyl salicylate(BS)

Composition of silicone soft lining materials^{13,15}

Polymer : α - ω -Dihydroxy end blocked poly(Dimethyl siloxane)

Cross-linking agent : Triethoxy silanol, Ethyl poly silicate, Tetraethoxy silane,
Methyltriacetoxysilane, Acryloxyalkyl silane

Catalyst : Dibutyltin dilaurate, Stannous octoate, Moisture, heat and benzoyl
peroxide

Table 2 The plasticizer in origin mixed polymer gel of soft liner(%wt).¹⁶

Material	P/L by Wt	(wt%) plasticizer						
		BB	BS	BBP	BPBG	DBP	DBS	EtOH
Coe-comfort (cc)	0.90	46.0 (87.3)	-	-	-	2.4 (4.5)	-	4.3 (8.2)
Coe Soft (CS)	1.34	-	15.0 (35.1)	-	-	21.2 (49.7)	-	6.5 (15.2)
Fitt (FI)	1.36	-	-	-	36.0 (80.9)	34.0 (80.4)	-	8.3 (19.6)
G-C Soft Liner (GC)	1.25	-	-	39.6 (87.6)	-	1.9 (4.3)	-	6.6 (14.8)
Hydrocast (HC)	1.21	-	-	-	-	-	-	5.6 (12.4)
S-R-Ivoseal (SR)	1.50	-	-	-	-	-	20.8 (51.9)	19.2 (48.1)
Veltec (VE)a	1.76	-	-	-	25.9 (71.7)	4.6 (12.6)	-	5.7 (15.7)
Veltec(VE)b	1.76	-	-	-	-	32.6 (90.0)	-	3.6 (10.0)
Visco-gel (VG)	1.25	-	-	-	38.6 (86.9)	3.6 (90.0)	-	2.2 (4.9)

BB = Benzyl benzoate, BS = Benzyl salicylate BBP, = Butyl benzyl phthalate,

DBP = Dibutyl phthalate, DBS = Dibutyl sebacate, Ethyl alcohol,

BPBG = Butyl phthalyl butyl glycolate

The results showed that silver nanoprisms or amphotericin B-treated cells both accumulated more intracellular glucose and trehalose than the compound-untreated cells. The extracellular glucose and trehalose, induced by amphotericin B, were measured as being 27.4 μg per fungal dry weight of 1 mg. The extracellular glucose and trehalose amounts, however, induced by nano-Ag were measured as being 30.3 μg per fungal dry weight of 1 mg. This rate was significantly higher than that induced in the compound-untreated cells.³²

Panacek (2009) found the use of silver nanoprisms inhibit the growth of the yeasts at very low concentrations that are comparable to those of common antifungals. Furthermore, the silver nanoprisms exhibit no cytotoxic effects on human fibroblasts at low concentrations.²²

Chladek (2012) found when they observed tendency of AgNP to form large aggregations of more than several hundred nanometer in the material, particularly noticeable at concentrations of AgNP exceeding 80 ppm, can result in a decrease in antimicrobial effectiveness of the nano-particle introduced into the material by the reduction of effective surface of AgNP which can contact with microorganisms.²³

Kim (2009) study in the antifungal effects of silver nano-particles and their mode of action on *Candida albican*. They found the antifungal activity of their to *Candida albican* similar to the antifungal activity in the MIC values of amphotericin B, which was used as a positive control. However amphotericin B shows a slightly higher hemolytic activity than silver nano-particles that could be fatal in patients who are treated via this agent for fungal infections.³¹

Ghaffari(2014) In vitro: AgNPs in two concentration groups at 0.2 and 2 wt% were mixed with heat-curing acrylic resin results for compressive strengths in various groups showed that acrylic resin at 0.2% and 2% AgNPs concentrations had a significantly higher compressive strength compared with the control group ($P < 0.05$), but the strength difference between the groups containing 0.2% and 2% AgNPs was not significant ($P > 0.05$). There were no significant differences between the test groups ($P > 0.05$).²⁶

Issa (2015) In vitro: found a highly Silver nanoparticles were incorporated into soft denture liner in different percentages (0.05%, 0.1% and 0.2% by weight) found

2.3 Properties of soft lining materials

Acrylic resin soft liner materials can be classified into two main types such as temporary materials (tissue conditioners) and permanent materials. Temporary materials remain soft for a limited period (days to weeks) and used when require to recover soft tissue before make a new denture, rebasing or relining denture, they may used for functional impression of immediate denture, immediate surgical splint and cleft palate speech aids.^{1,19} Plasticizer reduce the transition of the polymer from liquid to solid below mouth temperature and reduce the modulus of elasticity of soft lining materials.^{13,16}

However, leaching and evaporation of a plasticizer leads to hardening of the material and water are absorbed into materials cause to dimensional and mechanical changes. Thus, these material cannot considered for the permanent soft lining materials.

Wright (1981) reported that none of soft liner materials had any effects on the growth of *Canida albicans*, and found the plasticizers are leached out of the materials into aqueous environment such as the mouth and consider has been expressed to their possible toxics effects when ingested in humans. However, they have not ever been shown to have any adverse effects in human begins. Although the acryloxyalkyl silane additive used in Molloplast-B silicone soft lining materials had mild inhibitory effect on *Canida albicans*, this is unlikely to be clinically significant.¹⁵

In the past, this silicone soft lining material was provided better adhesion to the resin denture base and was less prone to growth of *Candida albicans* than other materials. However, the main limitation associated with this material was a lower resistance to tearing than other soft liners. The resilience of silicones was not based on plasticizers but that was on an intrinsic property of the polymer to retain resilience throughout the working life of the material.¹⁶

Nowadays, there have some clinical limitation in acrylic soft lining material properties such as resilience, tear resistance, viscoelasticity, biocompatibility, lack of odor and taste, adhesive bond strength, low solubility in saliva, low adsorption in saliva, ease of

adjustability, dimensional stability, color stability, resistance to abrasion, and easy of cleaning.¹⁷

2.4 Indication of soft lining materials

The clinical indication for denture wearers who complain of masticatory pain, have a thin and non-resilient oral mucosa, poor alveolar ridge (atrophic or knife edge). When chewing or biting forces are transmitted to the basal seat mucosa through a hard denture base, this oral mucosa will be injured, which will cause the sore spots, masticatory pain, further resorption of alveolar bone.¹⁸

According to extensive bone resorption, the mental nerve lies on the surface of the edentulous ridge. Soft lining material is indicated to reduce pressure on the mental nerve, which otherwise becomes trapped between the hard denture base and the underlying bone¹³ and can be used for relief of maxillary tori or other heavy bony prominences, prosthetic restorations for congenital or acquired oral defects, and stabilizing registration bases for jaw relation records.¹⁹

2.5 Problem of soft lining materials

When the denture is used by the patient for long periods, the plasticizer will be leached out and caused by the lining denture to harden and limit its usefulness. Thermal effects from the ingestion of hot and cold food and drinks may also have a deleterious effect. The porosity of soft linings allows water absorption and the diffusion of nutrient materials, which can easily become colonized by candidal organisms.¹³

Another problem of soft lining materials, including a failure of adhesive bonding between soft lining materials and the resin acrylic denture base especially the silicone type. Reduction of the denture base strength since the denture base material must be reduced at least 2-3 mm. and replacement with soft lining materials. Moreover, the dimension of materials was unstable because of water absorption to soft lining materials. There was caused by colonization of many organisms.¹⁹

Graham *et al.*(1991) studies in twenty eight patients in two materials found the resilient denture lining surface supported the presence and growth of oral fungi and there have not significant difference in two resilient denture liner test.⁵

2.6 Silver nanoparticle

Silver is a white and shiny metallic element and have atomic number as 47 and atomic mass as 107.87. It's the chemical symbol "Ag" in the periodic chart. Pure silver is ideally ductile, malleable, the highest electrical and thermal conductivity as well as the lowest contact resistance of any metal.

Silver nanoparticles have been widely used in clothing, electronics, bio-sensing, the food industry, paints, sunscreens, cosmetics and medical devices, all of which increase human exposure and thus the potential risk related to their short- and long-term toxicity. Many studies indicate that AgNPs are toxic to human health. Interestingly, the majority of these studies focus on the interaction of the silver nanoparticle with single cells, indicating that AgNPs have the potential to induce the genes associated with cell cycle progression, DNA damage and mitochondrial associated apoptosis.²¹

However, the mechanism of actions of silver nanoparticles is still not well understood. The action of silver nanoparticles is broadly similar to that of silver-ion. Silver nanoparticles provide the bactericidal effect by interactions with the peptidoglycan cell wall and the plasma membrane. Silver nanoparticles prevent bacterial DNA replication by interacting with the exposed sulfhydryl groups in bacterial proteins, especially with the enzymes involved in vital cellular processes such as the electron transport chain. Silver nanoparticles inhibit a respiratory enzymes, facilitating the generation of reactive oxygen species and consequently damaging the cell. In additions, the antimicrobial activity of silver nanoparticles depended on their sizes. The small size of these particles makes penetration through cell membranes easier, thus affecting intracellular processes resulting in higher antimicrobial activity.²⁰

The mechanism of action of AgNPs involves 2 phases: cell death induction and Reactive Oxygen Species (ROS) induction. Both AgNPs and Ag⁺ contribute to the killing effect, with the much larger proportion of AgNPs present. The first phase involves cell death induction, where the cells were very abruptly killed by AgNPs within 5-30 min. During the

induction, where the cells were very abruptly killed by AgNPs within 5-30 min. During the killing mechanism, the AgNPs release a small fraction of Ag^+ . Both forms can bind with electrostatic force to the negatively charged phospholipids of the outer membrane, leading to surface charge neutralization and also cell membrane disruption. The second phase takes place after the first phase, with ROS induction found 1 ± 4 hr. after the AgNP treatment. These ROS were slowly accumulated within the first hour and then prominently increased within 4 hr.³¹

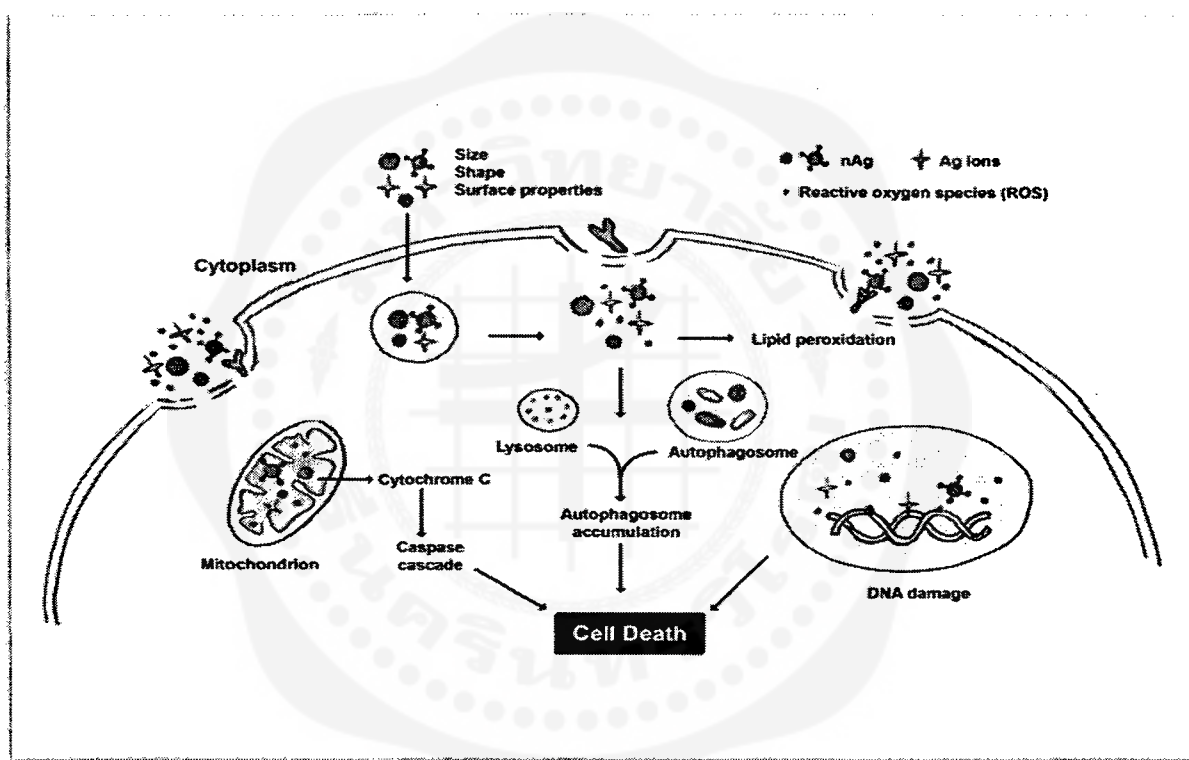


Figure 2 The mechanism of actions of silver nanoparticles into microorganisms.

Soure: The schematics figure is modified from Simeonidis, *et al* (2016).²⁹

Antifungal activity of silver nanoprisms showed antifungal activity against fungal strains tested. Silver nanoprisms exhibited a MIC value of 2 $\mu\text{g/ml}$, and the MIC values of this compound were approximately the same level as those of amphotericin B, showing MIC values of 2.5–5 $\mu\text{g/ml}$ toward the fungal strains tested.³²

significant decrease in colony forming units of *C. albicans* in comparison to control group. There was no inhibition zone around any test specimen of any test group. There was no silver detected to be released. The addition of AgNPs resulted in a highly significant decrease in water sorption, while only 0.2% group showed highly significant decrease in solubility. Non significant differences in shear bond strength were found. A highly significant increase in light absorption percentage was observed in all experimental groups.²⁷

Casemiro (2008) In vitro: experiment of antimicrobial activity in acrylic resins with incorporated silver–zinc zeolite (0% , 2.5%, 5.0%, 7.5% and 10%) found the addition of 2.5% of zeolite was enough to assign antimicrobial activity against *Candida albican*.²⁸

2.7 Cytotoxicity

Cytotoxicity tests are the essential screening step for testing of new materials used in humans. They provide an investigation of toxicity in a simplified system that reduces the effects of toxic variables factor. To evaluate potential of such materials to cause irritation, the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay is applicable. MTT offers a high degree of sensitivity, while providing a considerable savings in time and labor by eliminating the need to solubilize the formazan product prior to absorbance measurements thus making it quick, easy and safe to perform.²³

The MTT assay use of MTT reduction as a direct assay for mitochondrial activity. It's mainly reduced in the cytoplasm by NADH and dehydrogenases associated to the endoplasmic reticulum(ER), endosome/lysosome vesicles, and plasma membrane. It has been claimed that the MTT formazan gives rise to extracellular deposits by exocytosis. The view that MTT formazan is eliminated by exocytosis. Measurement of the brightness of formazan crystals under polarized light was proposed for assessing drug-induced cytotoxicity in tumors. The viability of bacterial cultures was also evaluated by resonance light scattering of formazan crystals using a fluorescence spectrophotometer.²⁴

The quantity of formazan is measured by recording changes in absorbance at 570 nm using a plate reading spectrophotometer. A reference wavelength of 630 nm is sometimes used, but not necessary for most assay conditions. Viable cells with active metabolism convert MTT into a purple colored formazan product with an absorbance

maximum near 570 nm. (Figure2). When cells die, they lose the ability to convert MTT into formazan, thus color formation serves as a useful and convenient marker of only the viable cells.²⁵

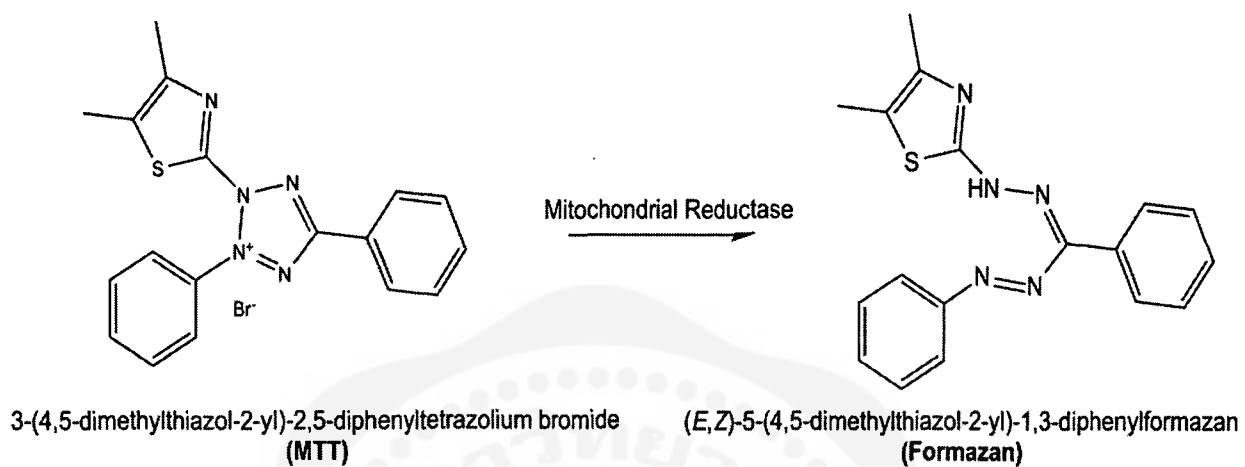


Figure 3 Structures of MTT and colored formazan product²⁵

CHAPTER 3

RESEARCH METHODOLOGY

Materials and methods

Materials

The materials used in this experiment are

1. Acrylic soft liner materials : Visco-gel Dentsply, Germany shows in table 3 and figure 4.
2. Antimicrobial agent : Silver nanoprism (AgNPs) from Prime NANO technology shows in figure 5.
3. The acrylic heat-cured resin material: Vertex, Netherlands shows in figure 6.
4. Stainless steel mold : disc-shaped for cytotoxic test, trouser-leg designed for tensile tear strength test, bar-shaped for tensile bond strength test shows in figure. 9,13, 17.

Table 3 Details of materials used in the experiment : Visco-gel

Brand of material	Visco-gel
Manufacturer	Dentsply, Germany
Type	Temporary soft lining materials
Compositon	Powder: Polyethyl methacrylate Liquid: Phthalyl butyl glycolate, Ethanol
Method to application	Powder/liquid : 3 g / 2 ml

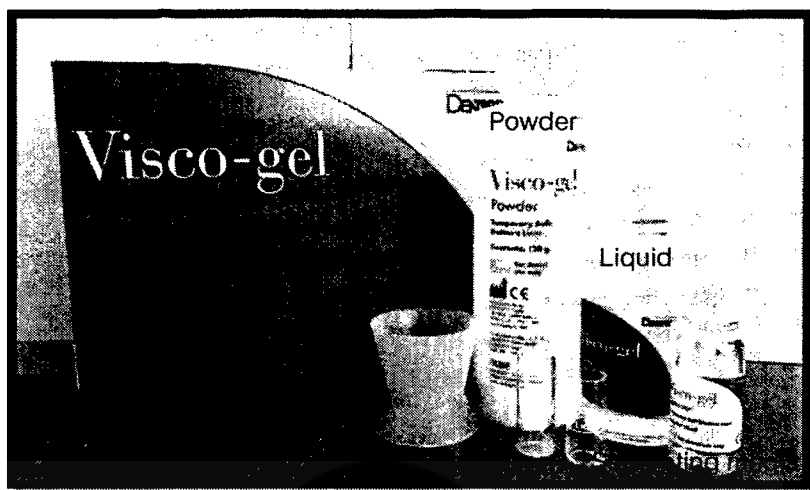


Figure 4. Acrylic soft liner materials (Visco-gel, Dentsply, Germany)



Figure 5 Silver nanoparticles from Prime NANO technology (Prime Nanotechnology Co. Ltd., Chulalongkorn University, Bangkok, Thailand).

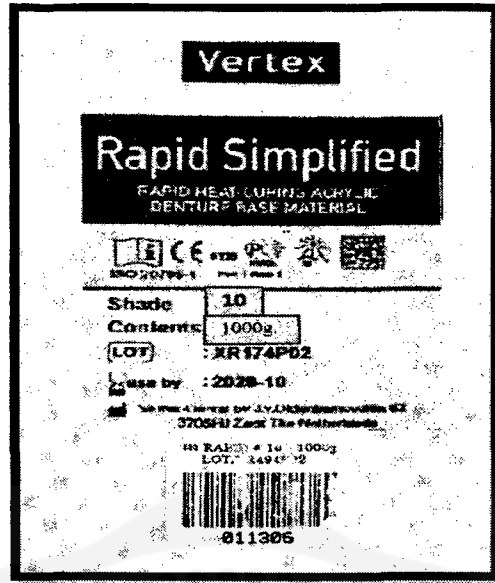


Figure 6 Acrylic heat-cured resin material as denture base materials in study. (Vertex, Switzerland)

Methods

Visco-gel (acrylic soft lining materials) was prepared by mixing with powder/liquid ratio as 3g/ 2ml according to the manufacturers' instructions, then they add the silver nanoparticles into sixth groups following table 4 and figure 7,8

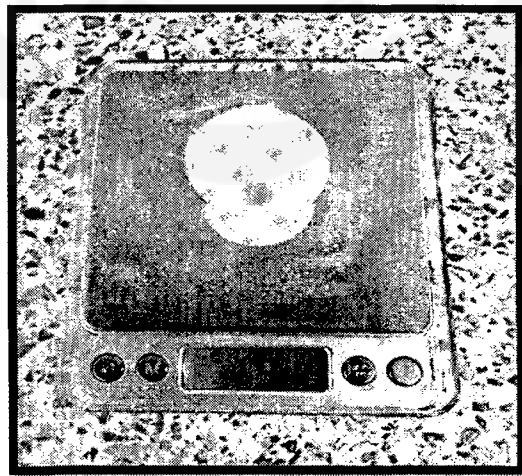


Figure 7 The measurement power of soft lining materials by digital scales. (FX-I series, Tokyo, Japan)

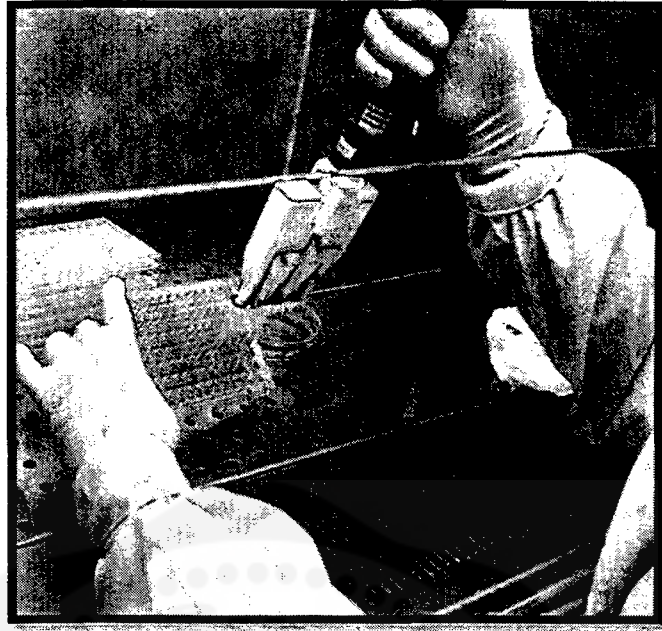


Figure 8 The AgNPs were diluted into the liquid portion of soft lining materials by micropipette.

Table 4 The classification of experimental groups.

Sample group	Silver nanoprisms concentration (ppm)	Method to application Powder(g) : Liquid(ml)	Silver nanoprism Volume (ml)
1	0	3 : 2.000	0
2	10	3 : 2.002	0.002
3	20	3 : 2.003	0.003
4	30	3 : 2.004	0.004
5	40	3 : 2.005	0.005
6	50	3 : 2.006	0.006
7	60	3 : 2.007	0.007

Cytotoxic effects test

The study of cytotoxic that effects from silver nanoparticles containing the acrylic soft lining material, was detected by MTT assay. The specimens are divided into seven groups that are a control group and six treatment groups. These six treatment groups have varied concentration of silver nanoparticle (10, 20, 30, 40, 50 and 60 ppm) in acrylic soft lining materials. Each disc-shaped specimen 10 mm. in diameter and 1mm. thick is prepared in the stainless steel mold (figure 9). Specimens are grouped in accordance to incubation period as 0, 24, 48 and 72 hours (six specimens in a concentration), and are stored at 37°C in an atm of 5% CO₂.

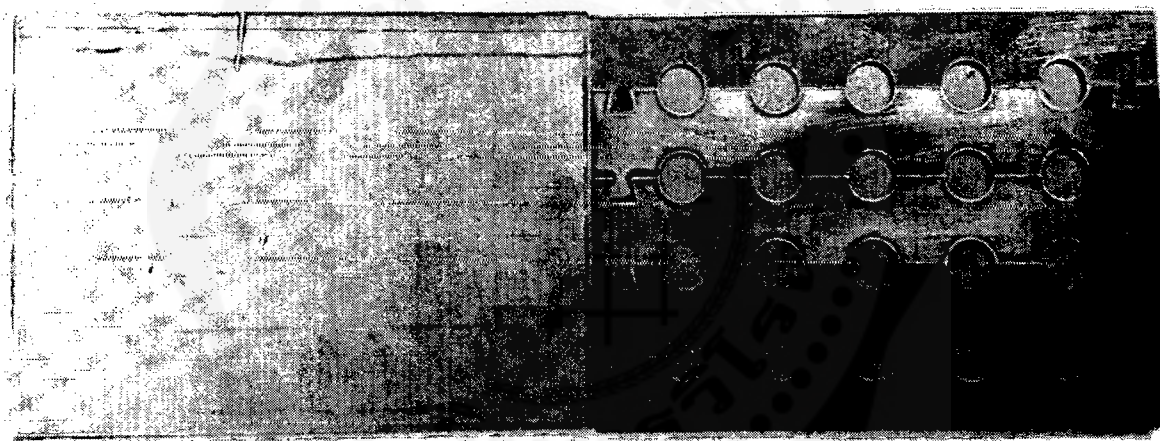


Figure 9 Stainless steel mold for cytotoxic test specimen fabrication.

1.1 The cell line L929 mouse fibroblast cell in 1×10^5 cell/cm³ (ATCC 2869501, obtained from passage 106, ATCC®, USA) were grown as a monolayer culture in cell culture dish at 37°C in an atm of 5% CO₂. The culture medium used supplement with 10% fetal bovine serum (FBS) and 1% antibiotic. Then they were incubated for 24 hours at 37°C. The cell culture medium was removed and the fresh medium containing elutes of experimental materials was added to each wellplate.

1.2 Soft liner were mixed according to the manufacturers' instructions, then they were allowed to set at 37°C for 30 minutes. After removal from molds was stored in 2 ml of eagle's minimum essential medium (E-MEM) without FBS or antibiotics for 1 hour at 37°C. The materials were handled aseptic technique throughout the procedures (figure 10).

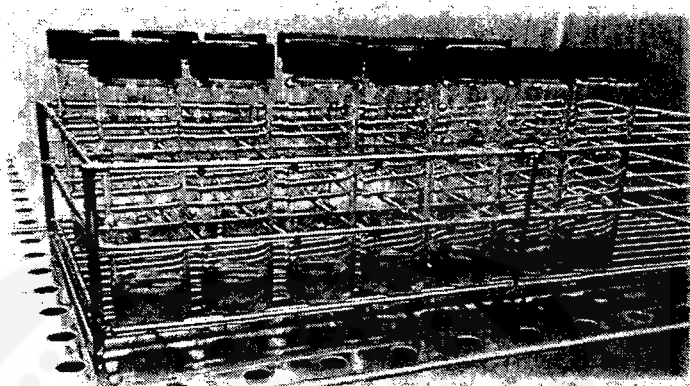


Figure 10 Samples were stored in 2 ml of E-MEM without FBS or antibiotics.

1.3 The sample was applied to center of culture dish, were incubated for 24 hours at 37°C. Then were add 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), and were incubated for 4 hours at 37°C.

1.4 After MTT-formazan was extracted with 0.5 mL of isopropanol for 2 hours at 37°C and 5% CO₂ (figure 11).

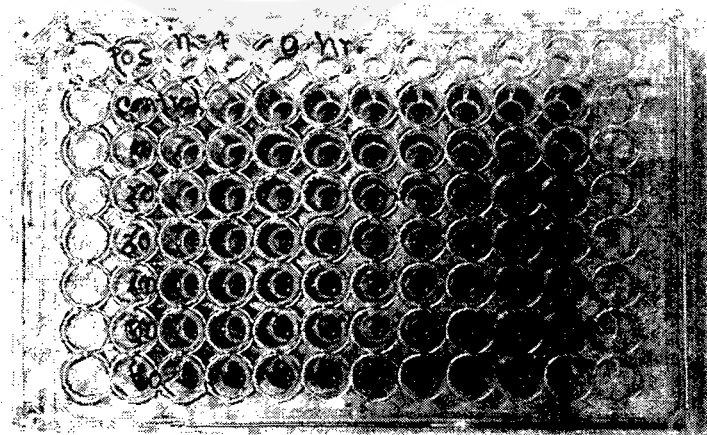


Figure 11 The 96 well plate after MTT-formazan was extracted with 0.5 mL of isopropanol.

1.5 The wavelength used to measure the optical density (OD) of the absorbance of the formazan product was 570 nm. Cell viability and cell cytotoxicity were calculated in percentage of control groups according to the following modified formula:³⁶

$$\text{Cell cytotoxicity (\%)} = 100 - \text{Cell viability (\%)}$$

$$= \left(\frac{\text{OD of the test group}}{\text{OD of the control group}} \right) \times 100$$

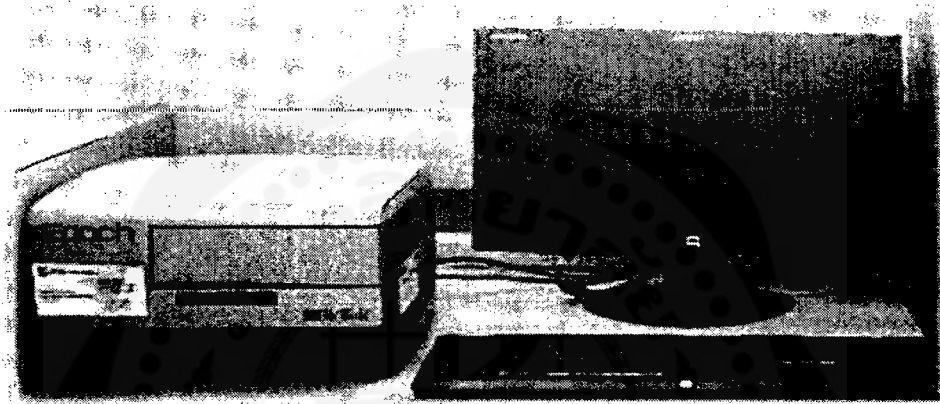


Figure 12 The wavelength absorbance reader (Epoch Spectrophotometer, Vermont, USA)

Tensile tear strength test

2.1 Ten trouser-leg designed specimens of each group were made of the dimensions 50 mm long, 10 mm wide, and 1mm thick (Figure 13) with stainless steel mold.

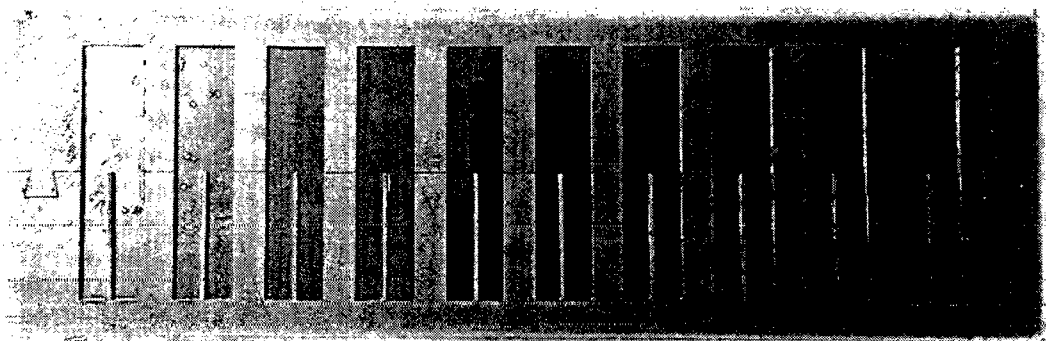


Figure 13 Stainless steel mold for tear strength test specimen fabrication.

2.2 Soft liner were mixed according to the manufacturer's instructions and were allowed to set at 37°C for 30 minutes (Figure 14), then they were cut vertically (25mm long) with blade number 15 to form the trouser-leg design.

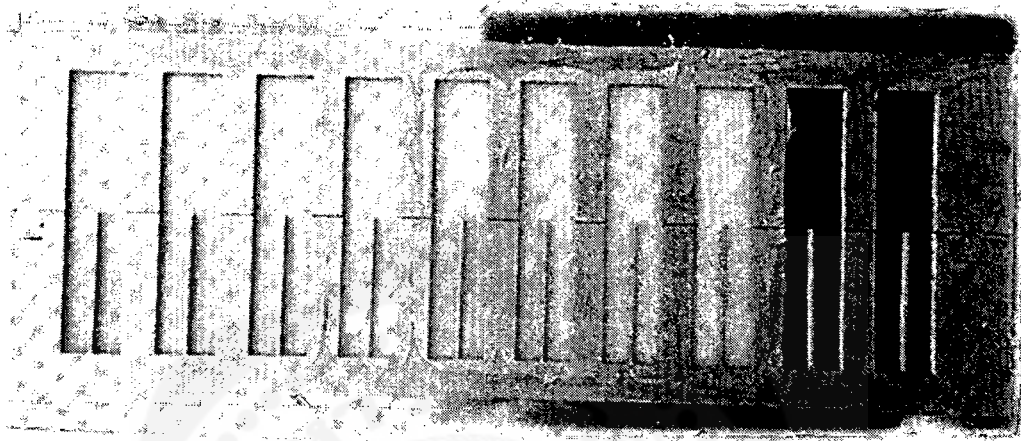


Figure 14 Soft lining material were setting in stainless steel mold.

2.3 The specimens were divided into two main groups, non-thermocycling and 1,000-cycles of thermocycling groups (70 specimens per group). Each the main group were divided into seven sub-groups: control, 10, 20, 30, 40, 50 and 60 ppm (10 specimens per group). The thermocycling groups were set up to a dwelling time of 30 seconds and a resting time of 6 seconds with temperatures of 5°C and 55°C.

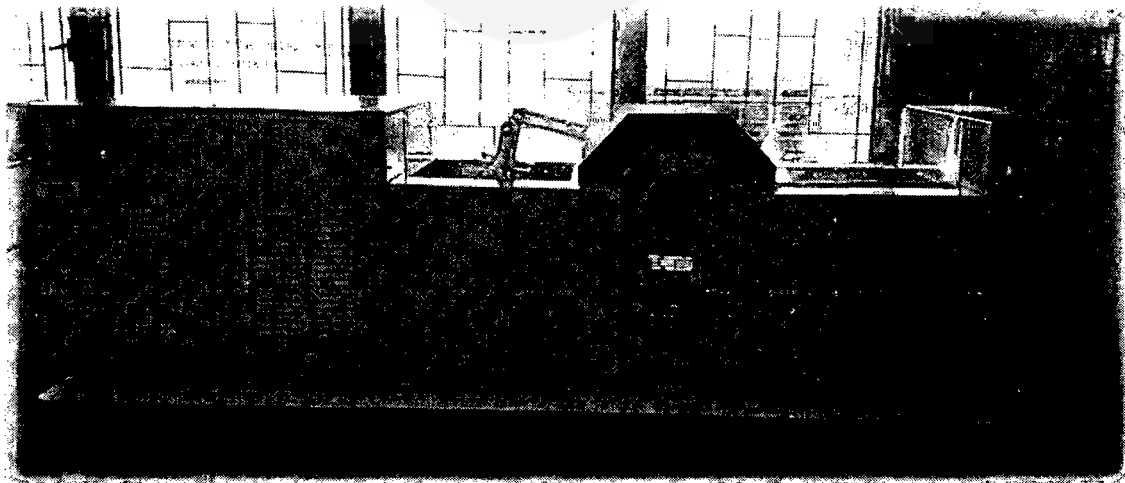




Figure 15 Thermocycling test machine (TC301, Medical&Environmental Equipment Research Laboratory, Bangkok, Thailand)

2.4 Then the specimens were tested on a universal testing machine at a cross-head speed of 50 mm/min, load cell 500 Newtons show in figure16.

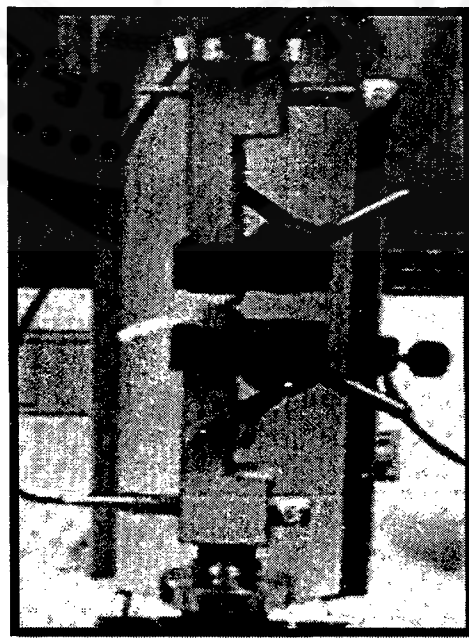


Figure16 Universal testing machine (Shimadzu EZ-L, Tokyo, Japan)

Tensile bond strength test

3.1 Ten stainless bars and spacer designed specimens of each group were made of the dimensions 40 mm long, 10 mm wide, and 3mm thick and 1 aluminum spacer bar (100 mm long; 3 mm wide; and 3 mm thick) with stainless steel mold. (show in fig. 17)

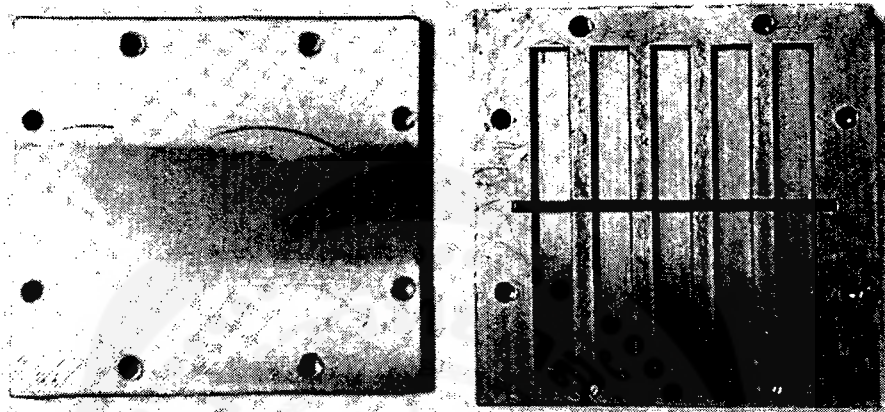


Figure17 Stainless bars and spacer designed for tensile strength test specimen fabrication.

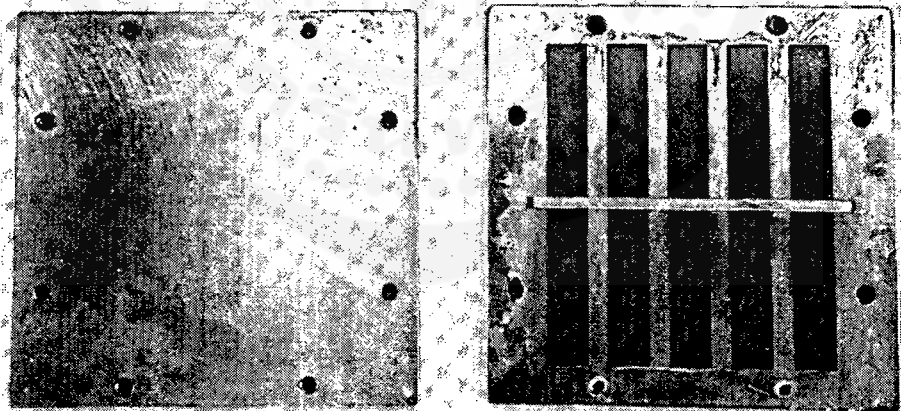


Figure18 The heat-cured acrylic resin as denture base as fabrication in stainless mold.

3.2 Acrylic resin was mixed according to the manufacturer's instructions, pressed into the mold and processed in a water bath at 75 °C for 9 hours. After polymerization, the stainless spacer and acrylic specimens were removed and the acrylic resin bars were trimmed. The surface to be bonded was smoothed using 240-grit silicone carbide paper and cleaned. The soft liner was packed into the space remaining after the spacer bar removal, the mold was reassembled and the liner was processed according to the manufacturers' directions. (show in fig. 19).

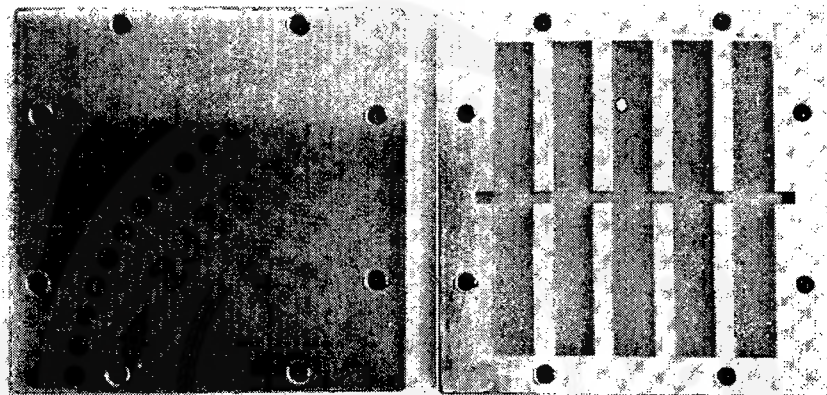


Figure19 Soft liner was packed into the space bar in stainless mold .

3.3 The specimens were divided into two main groups (70 specimens per group); non-thermocycling and 1000-cycles of thermocycling groups. The main group were divided into seven sub-groups (10 specimens per group); control, 10, 20, 30, 40, 50 and 60 ppm. The thermocycling groups were set up to a dwelling time of 30 seconds and a resting time of 6 seconds with temperatures of 5°C and 55°C.

3.4 Then the specimens were tested in a universal testing machine (fig. 20) at a cross-head speed of 10 mm/min, load cell 500 N until the specimens start fracture.

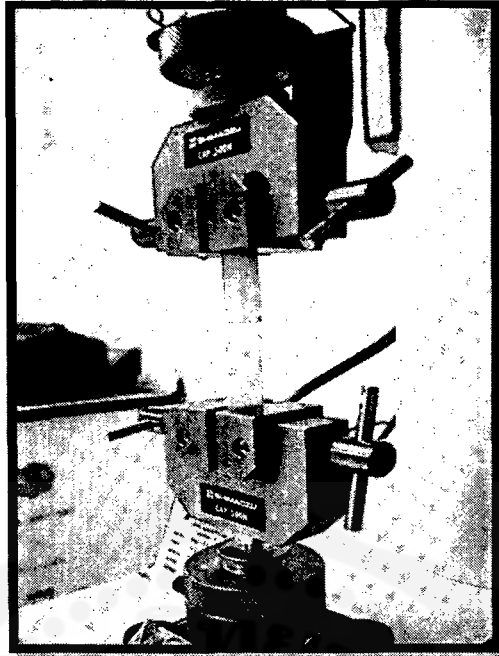


Figure20 Sample place in Universal testing machine for tensile bond strength.

Data of this study were analyzed by two-way anova analysis of variance for group comparison, and one-way anova analysis of variance for individual group comparisons by using SPSS version 20.0 (IBM SPSS Statistics for Windows, Version 20.0, NY, USA). The Kolmogorov–Smirnov test can be modified to serve as a goodness of fit test for comparison with a standard normal distribution, before there were tested by anova analysis of variance. Then means and standard deviations were recorded for both tests and significant differences of the results and between groups were analyzed using Tukey HSD test ($P < .05$).

CHAPTER 4

RESULT

Cytotoxicity effects test

The cytotoxicity effects test of silver nano-prisms in acrylic soft liner was detected by the percentage of optical density (OD) in MTT assay after four periods (0hr, 24hr, 48hr and 72hr) and six concentrations (10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm) of Silver nanoprism (AgNPs). The mean average of cytotoxicity effects test was shown in table 5 and this information is shown in fig. 21.

The results showed that the differences were significant ($P<0.05$). The cell viability value is least in 24 hours group and 60 ppm concentration of AgNPs, but highest in 72 hours group and 10 ppm concentration of AgNPs. Although the group of 24 hours and 60 ppm concentration of AgNPs is least values, the cell viability scored is less than 30 percent cell cytotoxic (that was mean non-cytotoxic scored ranges). In the same concentration of this study the cell viability value of materials is decrease in 24 hours but increase in 48 hours and 72 hours in most concentration of AgNPs.

Table 5 Percentage of optical density (OD) in MTT assay.

Concentration of Silver nanoprism (ppm)	Percentage of OD ($\bar{x}$ (sd))			
	0 hr	after 24 hr	after 48 hr	after 72 hr
10ppm	98.30 (9.4)	95.77 (5.8)	98.32 (4.1)	98.10 (11.4)
20ppm	94.51 (11.1)	95.41(7.2)	97.35 (6.7)	95.29 (8.1)
30ppm	95.10 (4.5)	90.53 (8.5)	96.65 (4.6)	94.11 (4.0)
40ppm	94.00 (6.1)	81.62 (9.6)	95.58 (8.7)	93.57 (7.0)
50ppm	90.58 (9.4)	74.25 (11.1)	93.74 (4.8)	91.63 (9.4)
60ppm	88.05 (8.8)	73.25 (8.8)	91.53 (5.2)	90.53 (3.3)

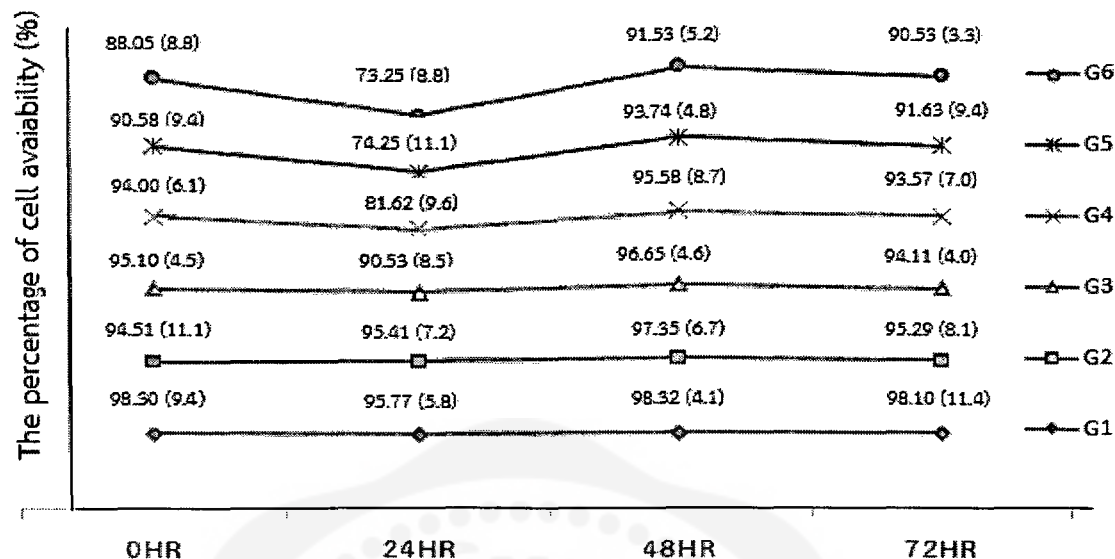


Figure 21 Percentage of OD in MTT assay at 0 hr, 24hr, 48hr, and 72hr incubation period.

Tensile tear strength test

The tensile tear strength test of silver nano-prisms in acrylic soft liner was detected by mean average of tensile tear strength values in two conditions (Non-soak in thermocycling and soak in thermocycling) and six concentrations (10, 20, 30, 40, 50 and 60 ppm) of AgNPs. The mean average of tensile tear strength values is shown in table 6, and this information is shown graphically in figure 22.

The results showed that the differences were non significant ($P < 0.05$). The mean average of tensile tear strength values in high concentration of AgNPs group have a higher trend of tensile tear strength values than low concentration of AgNPs group. Moreover, the samples which soak in thermocycling, have a higher trend of tensile tear strength values than samples which non-soak in thermocycling.

Table 6 Mean values average and standard deviation of tensile tear strength test.

Concentration of Silver nanoprism (ppm)	Maximum force (N/cm ²) ($\bar{x}$ (sd))	
	Non thermocycling	Thermocycling
Control	1.49 (0.13)	2.02 (0.17)
10ppm	1.55 (0.10)	2.04 (0.18)
20ppm	1.55 (0.27)	2.06 (0.27)
30ppm	1.56 (0.17)	2.05 (0.29)
40ppm	1.69 (0.14)	2.03 (0.20)
50ppm	1.71 (0.09)	2.12 (0.19)
60ppm	1.74 (0.14)	2.03 (0.22)

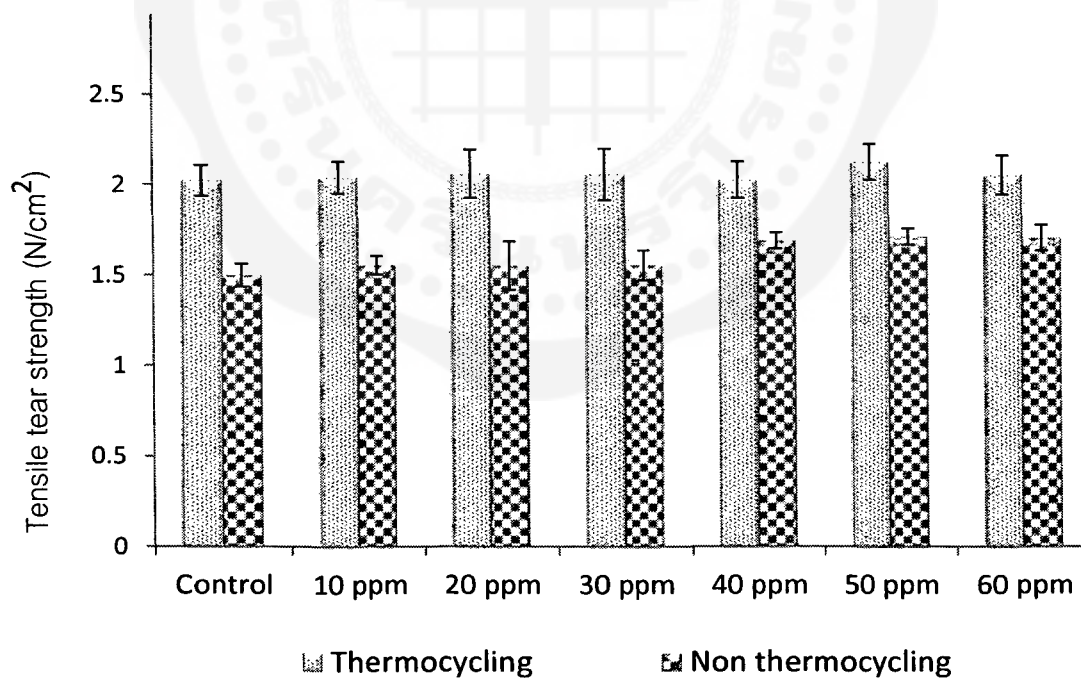


Figure 22 Percentage of mean values average in tensile tear strength test

Tensile bond strength test

The tensile bond strength test of silver nano-prisms in acrylic soft liner was detected by mean of tensile bond strength values average in two conditions (non-thermocycling groups and thermocycling groups) and six concentration (10, 20, 30, 40, 50 and 60 ppm) of AgNPs. The mean average of tensile bond strength values is shown in table 7, and this information is shown graphically in figure 23.

The results showed that the differences were significant ($P<0.05$). The mean average of tensile bond strength values in a high concentration of AgNPs group have a higher trend of tensile bond strength values than low concentration of AgNPs group. Moreover, samples which soak in thermocycling, have a higher trend of tensile bond strength values than samples which non-thermocycling.

Table 7 Mean values average and standard deviation of tensile bond strength test.

Concentration of Silver nanoprism (ppm)	Maximum force (N/cm ²) ($\bar{x}$ (sd))	
	Non thermocycling	Thermocycling
Control	16.12 (1.49)	18.82 (2.20)
10ppm	25.79 (2.78)	23.80 (3.39)
20ppm	27.67 (2.69)	27.84 (3.62)
30ppm	29.11 (2.88)	28.31 (2.83)
40ppm	29.86 (2.89)	28.73 (3.61)
50ppm	33.37 (3.45)	28.68 (3.76)
60ppm	32.54 (1.14)	35.00 (2.19)

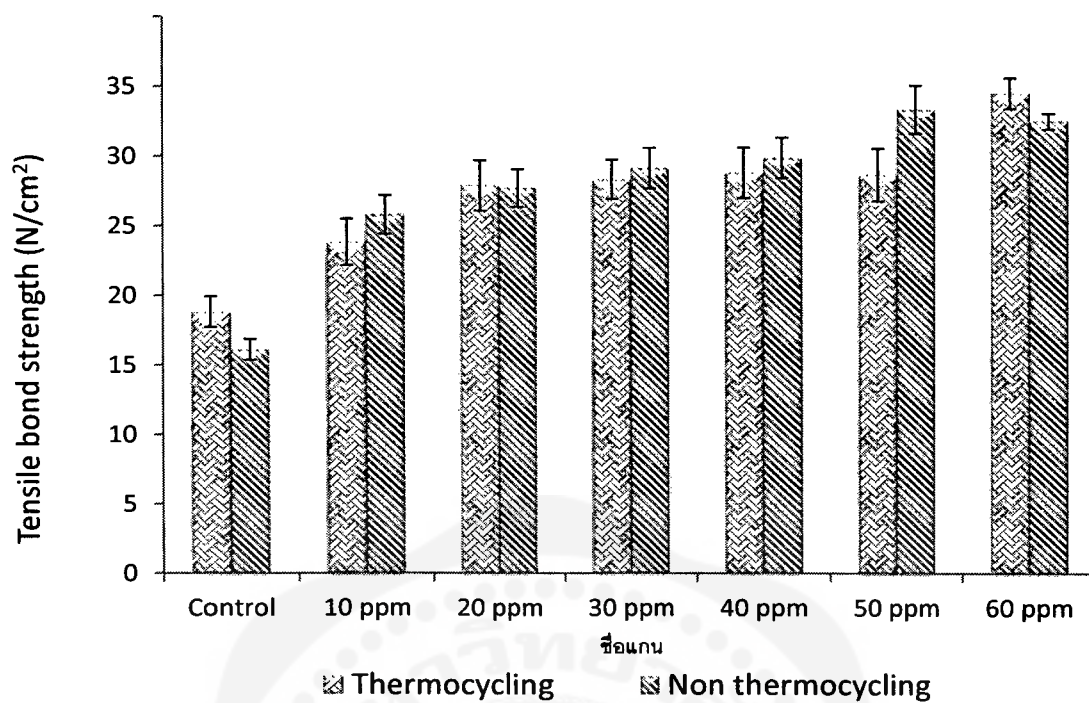


Figure 23 Percentage of mean values average in tensile bond strength test

Failure mode after debonding

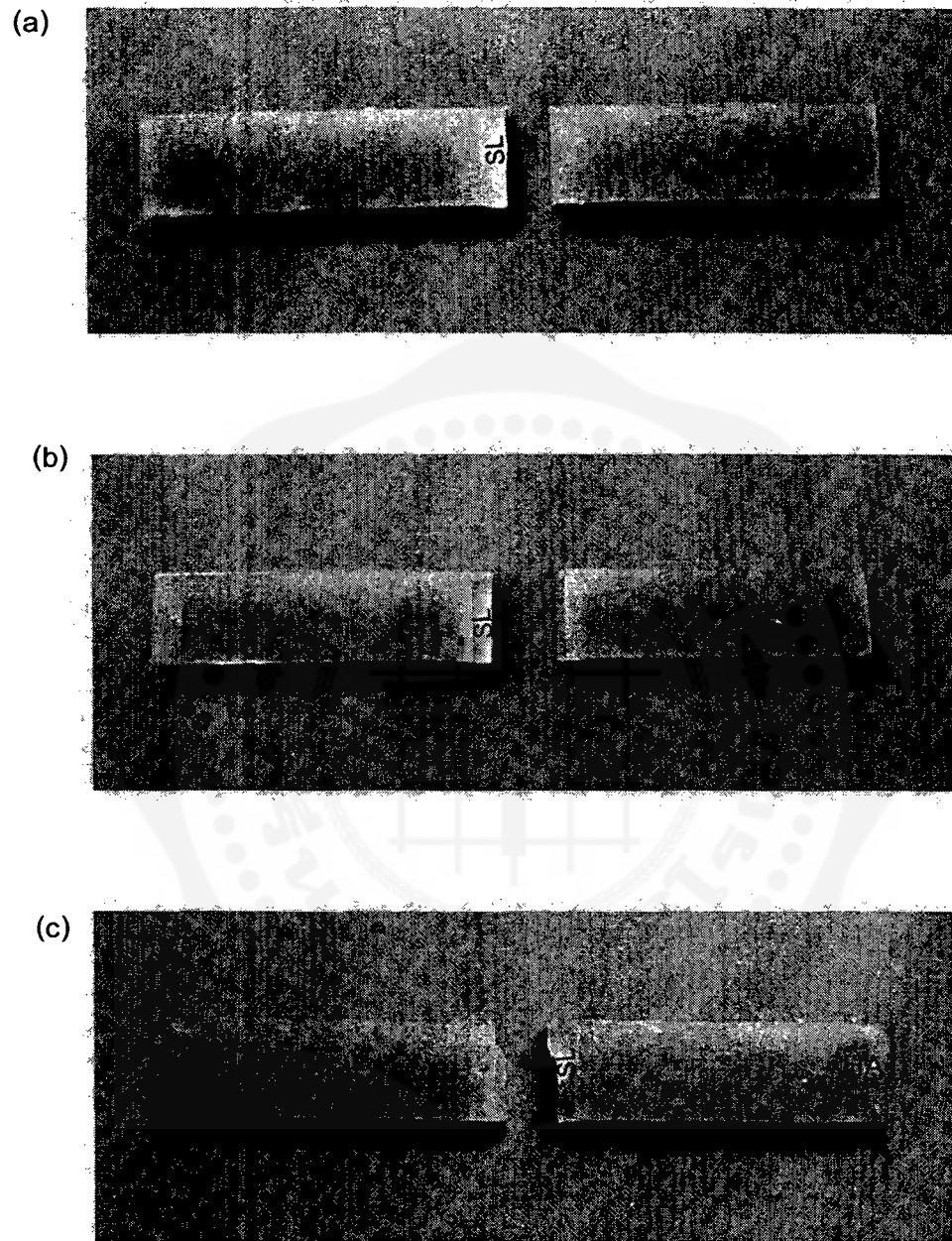
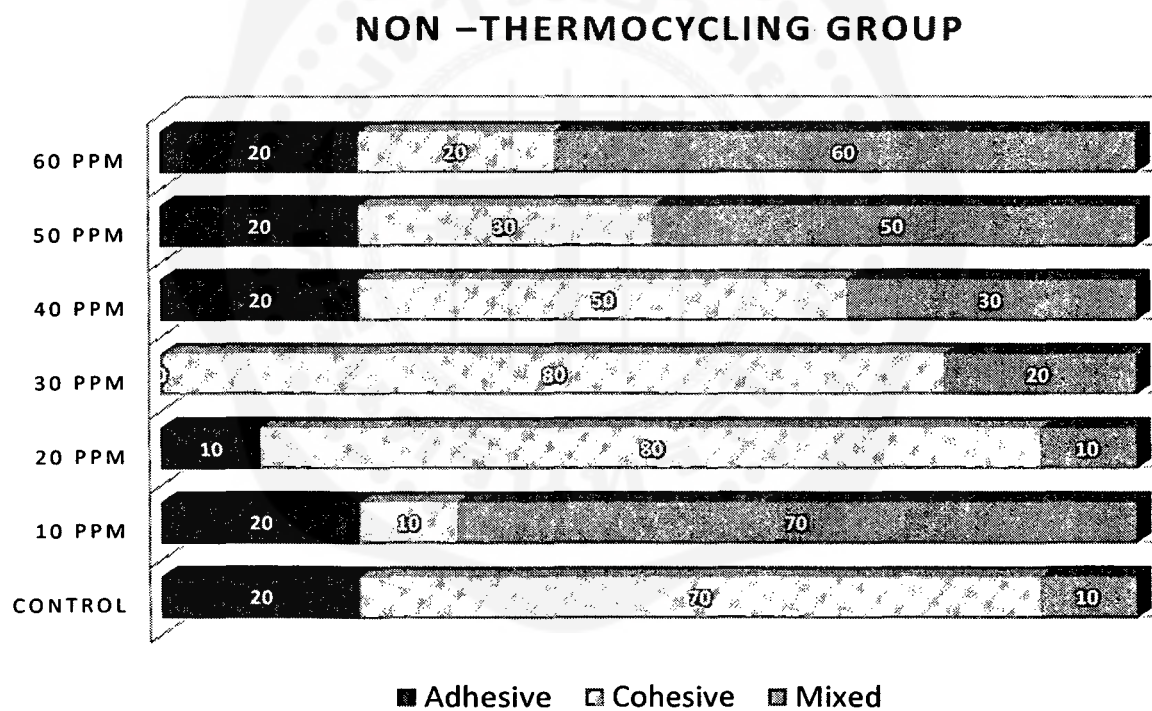


Figure 24 Failure mode after debonding a) adhesive failure mode, b) cohesive failure mode and c) mixed failure mode (HA= Heat-cured acrylic resin, SL= Soft lining material)

After the changes in the mechanical properties about tensile bond strength found that three failure modes after debonding in this study such as a) adhesive failure mode, b) cohesive failure mode and c) mixed failure mode.

Adhesive failure mode is the fracture in the bonding between acrylic denture base and soft lining materials. Cohesive failure mode is the fracture in the inner body of soft lining materials. Mixed failure mode is the fracture in the bonding between acrylic denture base and soft lining materials, and is also the fracture in the inner body of soft lining materials shown in figure 24 and 25.



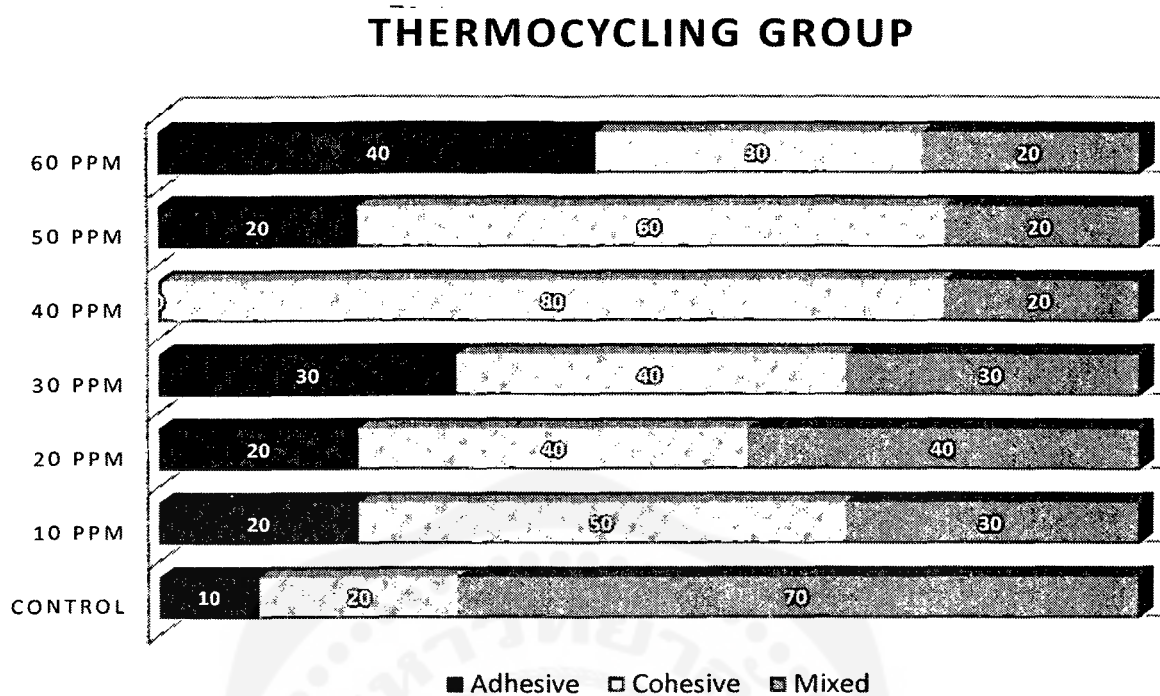
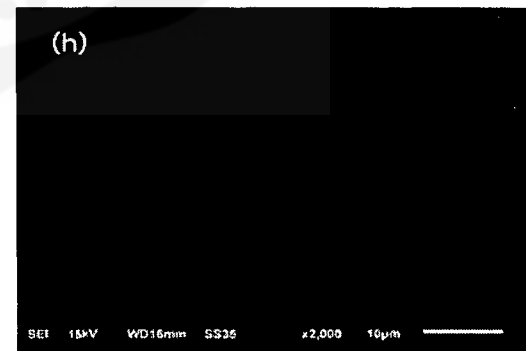
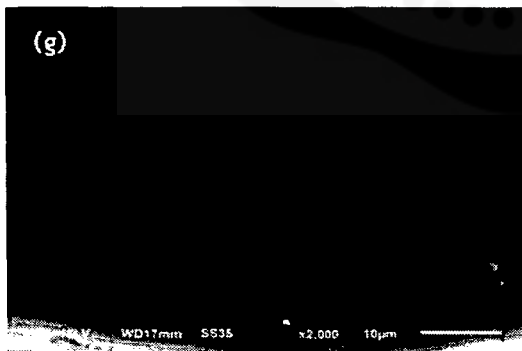
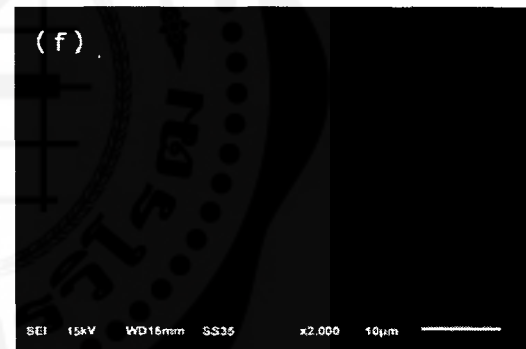
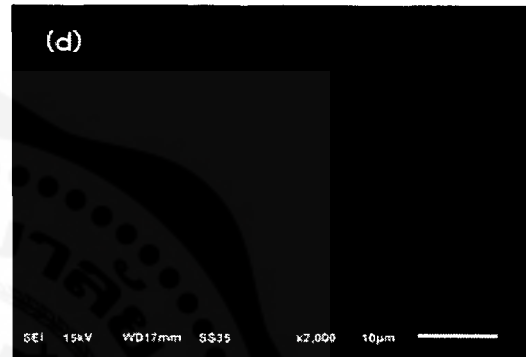
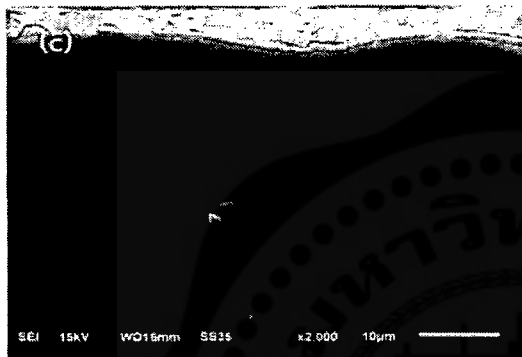
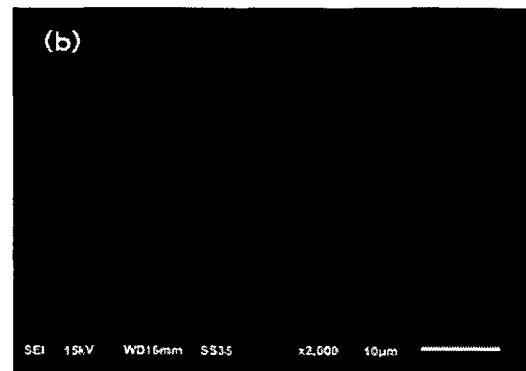
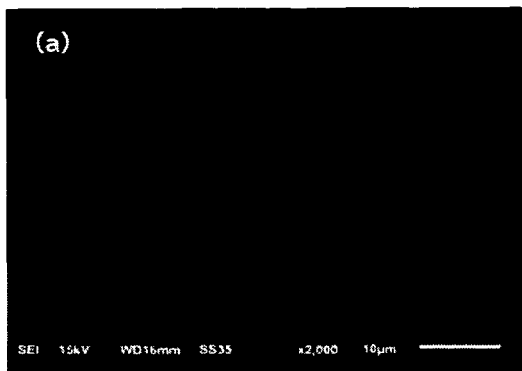


Figure 25 Percentage of failure mode after debonding in non-thermocycling groups and thermocycling groups.

The Scanning Electron Microscopy (SEM) image shows that the control samples don't have any particles of the silver nano-prisms on both specimens (center and border of soft lining materials) shown in figure (a) and (b). The other sample groups have some particles of silver nano-prisms. The most density of the silver nano-prisms is 60 ppm groups show in figure 25 (m) and the least density of the silver nano-prisms is 10 ppm groups show in figure 25 (c).



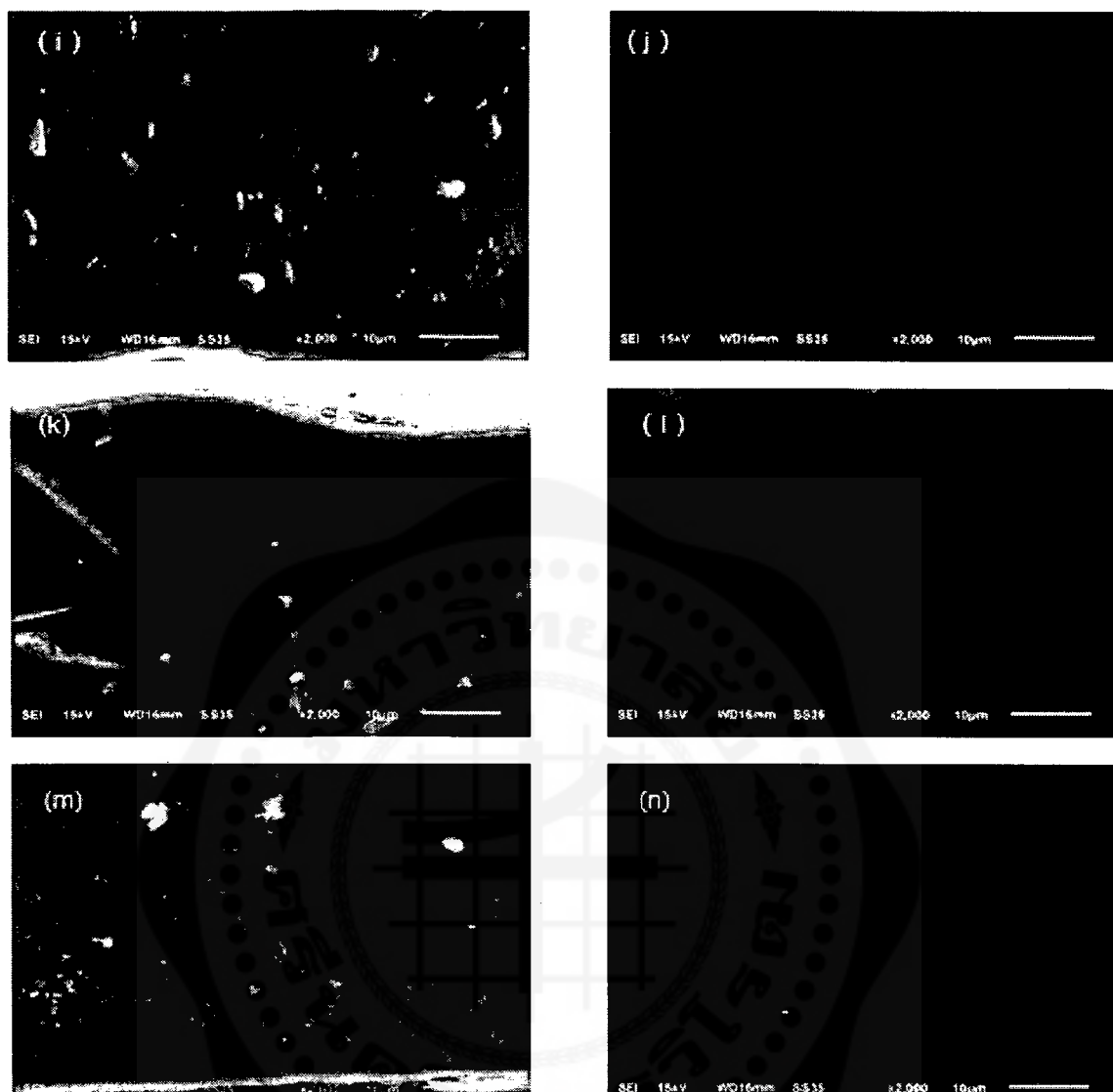


Figure 26. Scanning electron microscopy image (*2000 magnification) of soft lining materials specimens. (a) and (b) are control group specimens. (left side is border of specimens, right side is inner of specimens). (c) to (n) are treatment group with AgNPs in 10, 20, 30, 40, 50 and 60 ppm concentration. (left side is border of specimens, right side is inner of specimens). The red arrows showing AgNPs dispersed in denture soft lining materials.

CHAPTER 5

DISCUSSION

Landayan, *et al* (2014)²⁰ found that four acrylic soft lining materials (Coe-Comfort[®] GC America, USA; Coe-Soft[®] GC America, USA; Visco-gel[®] Dentsply, USA; and Sofreliner Tough M[®], Tokuyama Dental Corporation, Japan). The Visco-Gel[®] was lowest cytotoxicity effects which available sold in thailand, so it was selected for this study to compare cytotoxicity effects of each silver nanoprisms concentration that contained in soft lining materials and use in the denture prosthesis.

Parnklang *et al.* (2013) found that silver nanoprisms which was made from the rapid thermochemical synthesis of AgNPrs employing hydrogen peroxide (H_2O_2), transform in the shape of Silver nanospheres (AgNSs) to Silver nanoprisms (AgNPrs). This synthesized of AgNPrs are stable for prolonged storage of at least 3-6 months in liquids form, and could to manipulate metallic crystals at the nanoscale. The obtained Silver nanoprisms shape exhibited mixed geometries such as hexagonal, triangular, rounded-tip triangular prisms, and circular disks with average bisector lengths of 30 to 120 nm and the thickness of 10 to 20 nm.³⁵ Liquids form of Silver nanoprisms is selected for this study because this liquids form can easily dispersed more than powder form in liquid/ powder composition of soft lining materials. Liquids form of Silver nanoprisms is also stable for prolonged storage of at least 3-6 months.

The highest percentage of cell cytotoxicity effects test was founded in the 24 hours group of all concentrations, and founded decreasing in the 48 and 72 hours groups later. This results have a trend as Landayan JIA, *et al* (2014).²⁰ Likewise, Jones *et al*¹⁶ (1988) study in plasticizer composition and leachability in dental soft polymers found that ethyl alcohol is completely lost within 24 hours from polymer gel materials stored in water at 37°C, and the plasticizer would loss in ranged from 0.30 mg/g to 8.70 mg/g within 14 days.

Therefore, the percentage of cell cytotoxicity effects test in 24 hours group is higher than the others.

In 2011 Chladek, *et al.*⁴¹ studied in antifungal activity of denture soft lining material modified by silver nanoparticles in sixth concentration (10, 20, 40, 80, 120 and 200 ppm) found that the average antifungal efficacy value (AFE) for samples with 40 ppm contained in AgNPs was 31.5%. An additional concentration have increased in the AgNPs concentration resulted in a less dynamic, but there are still visible AFE values increasion.⁴¹ Consequently, the appropriate concentration was the 40 ppm concentration groups.

The Scanning Electron Microscopy (SEM) image shows the diffusion of silver nano-prisms in soft lining materials. This study found that silver nano-prisms more disperse in the border of materials than the other area of materials (show in figure 26). Soft lining materials contain silver nano-prisms which they are the metal-ion had polymerization. The silver nano-prisms had extruded by the polymerize force of materials into the border of materials. When patients use the dentures with soft lining materials to improve denture stomatitis, it is necessary to contact the silver nano-prisms ions in tissue surface of denture for antimicrobial effect.

However, after changes in the mechanical properties in tensile bond strength test, found that the percentage of adhesive failure mode after debonding in the thermocycling group is higher than the non-thermocycling group. In additions, the percentage of cohesive failure mode after debonding in the non-thermocycling group is higher than the thermocycling group. Therefore, the results of this study imply to thermocycling group had high affected to the bonding between denture soft liner and acrylic resin materials more than non-thermocycling group. However, the mean average of tensile bond strength values shown that the differences were not significant as the result of denure soft liner without AgNPs and bond to the denture base of acrylic resin.

Elias, *et al.* (2007)³³ studied in effect of thermocycling on the tensile and shear bond strengths of three soft liners to denture base resin, found that the mean average of tensile

and shear bond strength values of the non-thermocycling group is higher than the thermocycling group.

Because of their study test in silicone soft liner materials, they was lower in the bond strength than the other groups of soft lining materials.

Furthermore, this study found that different concentrations of AgNPs groups were significant. In high concentration of AgNPs have increased the average maximum forces of the tensile bond strength values imply to more strongly bond for the denture base acrylic resin. Owing to the high concentration of AgNPs groups have decreased sorption and solubility in denture soft liner. Likewise, Issa, *et al.* (2015)²⁷ reported that the water sorption and solubility decrease through water gain and loss of soluble component in high concentration of AgNPs.

Nevertheless, this finding does not agree with Chladek, *et al.* (2013)⁴² that studied about an effect of silver nanoparticle incorporation into soft liner material on sorption and solubility. They found that when increasing the silver nano-prisms concentration, as a result, hardness decreased, sorption and solubility increased, and bond strength decreased. Disagreement may be because of differences in soft liner materials type, Chladek, *et al.* (2013)⁴² used silicone-based liner. The silicone-based lining materials generally exhibit much lower sorption and solubility than acrylic-based lining materials because the silicone-based liner have the high contact angle of materials.

Issa, *et al.* (2015)²⁷ studied in evaluating the effect of silver nanoparticles incorporation on antifungal activity and some properties of soft denture lining material. They reported that the bond strength value is not significantly change. However, they found that the mean average of the bond strength values in a high concentration of AgNPs groups have a higher trend of the bond strength values than low concentration of AgNPs groups.²⁷ The result could be explained by the compatibility and high degree of similarity in chemical structure between polymethyl methacrylate denture base material and polyethyl

methacrylate soft liner which would result in a chemical bonding between the two materials. In addition, the flowability of AgNPs in the soft lining material which allows the material to readily adapt to the bonding surfaces and creates an union together.

Meanwhile Chladek *et al.* (2013) they study in sorption, solubility, bond strength and hardness of denture soft lining incorporated with silver nanoparticles in sixth concentration (10, 20, 40, 80, 120 and 200 ppm). They founded that the concentration of silver nanoparticles more than 80 ppm incorporation into soft lining material have decreased the mean bond strength values and 10 ppm to 40 ppm was appropriate concentration.⁴² Agree with this study that silver nanoparticles concentrations between 10 ppm to 60 ppm was selected groups.

Furthermore, this study found that after change the mechanical properties in tensile tear strength test, the mean tensile tear strength values of the thermocycling group is higher than non- thermocycling group because denture soft lining materials was leaching of plasticizer in thermocycling test.

Likewise, in vitro study of Landayan, *et al* (2014).²⁰ They studied on effect of aging on tear strength and cytotoxicity of soft denture lining materials; in vitro. The study do in four commonly used soft denture lining materials (Coe-Comfort[®] GC America, USA; Coe-Soft[®] GC America, USA; Visco-gel[®] Dentsply, USA; and Sofreliner Tough M[®], Tokuyama Dental Corporation, Japan). They found that the vicogel soft denture lining materials in 3000 cycles thermocycling group have the tear strength value more higher than the vicogel soft denture lining materials in 1000 cycle group.²⁰ This result can be supported by the study of Landayan, *et al.* (2014). Consequently AgNPs have not influence on mechanical property of the soft lining materials, when it add on the low concentration.

Although this study found that (after change the mechanical properties in tensile tear strength test) the softness of soft lining materials had decreased when the concentration of AgNP had increased, but the toughness which can be defined form area under the stress–

AgNP had increased, but the toughness values which can be defined from area under the stress–strain curve had slightly increased when the concentration of AgNP had increased.

Therefore, the addition mild concentration of silver nano-prisms incorporation on soft denture lining material could improved the mechanical properties of soft denture lining material.

Color change in study showed that when silver nano-prisms (AgNPs) concentration were increased (from 10 ppm to 60 ppm), the color of the samples does not becomes darker. There have less changed in the color of soft lining materials. Also this finding does not agree with Chladek, *et al.*⁴² They were found that when the AgNPs concentration increased (from 10 ppm to 200 ppm), the color of the samples became darker. They explained by the presence AgNPs in the polymer matrix, as the silver nanoparticles have extraordinary efficient ability to absorb and scatter light due to their optical properties, and a single silver nanoparticle can interact with the light more efficiently than any known organic or inorganic colored particle with same dimensions.

Conclusions

1. The cytotoxicity test found increase the optical density values in 24 hour group in all concentration of AgNPs and decrease in 48 hours and 72 hours in all concentration later. However, the optical density values of all groups less than 30 percent was fall in non-cytotoxic range.

2. The tensile tear strength test showed that the differences were non-significant ($P < 0.05$) in difference concentration. However, the thermocycling groups were showed significant higher than the non-thermocycling groups.

3. The tensile bond strength test showed that the differences were significant ($P < 0.05$) in difference concentration, the high concentration were increase the values in test. However the thermocycling groups were not showed significant than the non-thermocycling groups.

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APPENDIX



Table 8 The optical density values of cytotoxic test at 0 hr. plate1

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
CTL1	control	B2	0.602	0.609	0.026	4.352
		B3	0.62			
		B4	0.598			
		B6	0.601			
		B7	0.608			
		B8	0.606			
		B9	0.617			
		B11	0.547			
Pos		A2	0.057	0.071	0.015	20.841
		A3	0.056			
		A4	0.058			
		A5	0.057			
		A6	0.061			
		A9	0.079			
		A10	0.088			
		A11	0.096			
SPL1	10	C3	0.568	0.595	0.065	10.536
		C4	0.586			
		C5	0.551			
		C6	0.556			
		C7	0.63			
		C8	0.678			
		C9	0.626			
		C11	0.571			
SPL2	20	D3	0.607	0.591	0.053	9.002
		D4	0.587			
		D5	0.537			
		D6	0.563			
		D7	0.692			
		D9	0.559			
		D10	0.629			
		D11	0.561			

Table 8 (continued)

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
SPL3	30	E2	0.58	0.589	0.039	6.703
		E3	0.605			
		E4	0.588			
		E5	0.579			
		E6	0.64			
		E7	0.574			
		E10	0.605			
		E11	0.546			
SPL4	40	F2	0.605	0.588	0.031	5.162
		F4	0.601			
		F5	0.566			
		F6	0.616			
		F7	0.564			
		F8	0.617			
		F10	0.58			
		F11	0.562			
SPL5	50	G2	0.605	0.568	0.067	11.357
		G4	0.591			
		G6	0.607			
		G7	0.452			
		G8	0.528			
		G9	0.533			
		G10	0.634			
		G11	0.603			
SPL6	60	H4	0.562	0.545	0.06	10.922
		H5	0.548			
		H6	0.525			
		H7	0.479			
		H8	0.564			
		H9	0.645			
		H10	0.534			
		H11	0.488			

Table 9 The optical density values of cytotoxic test at 0 hr. plate2

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
CTL1	control	B3	0.768	0.663	0.043	7.15
		B4	0.739			
		B5	0.654			
		B6	0.532			
		B8	0.676			
		B9	0.659			
		B10	0.665			
		B11	0.611			
Neg		A2	0.545	0.303	0.26	85.974
		A3	0.54			
		A4	0.543			
		A5	0.549			
		A6	0.572			
		A9	0.047			
		A10	0.052			
		A11	0.066			
SPL1	10	C3	0.818	0.655	0.067	10.956
		C4	0.747			
		C5	0.633			
		C6	0.662			
		C7	0.699			
		C8	0.74			
		C9	0.559			
		C10	0.536			
SPL2	20	D2	0.75	0.638	0.087	14.837
		D3	0.721			
		D4	0.73			
		D5	0.678			
		D6	0.59			
		D8	0.585			
		D9	0.536			
		D10	0.52			

Table 9 (continued)

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
SPL3	30	E2	0.602	0.619	0.09	13.026
		E4	0.641			
		E5	0.617			
		E6	0.618			
		E7	0.673			
		E8	0.603			
		E9	0.615			
		E10	0.586			
SPL4	40	F3	0.681	0.605	0.094	13.667
		F4	0.603			
		F5	0.582			
		F6	0.579			
		F7	0.601			
		F8	0.601			
		F9	0.537			
		F10	0.661			
SPL5	50	G3	0.67	0.582	0.101	15.858
		G4	0.624			
		G5	0.545			
		G6	0.617			
		G7	0.698			
		G9	0.519			
		G10	0.476			
		G11	0.507			
SPL6	60	H2	0.625	0.576	0.097	15.775
		H4	0.673			
		H5	0.618			
		H6	0.516			
		H7	0.609			
		H8	0.516			
		H10	0.55			
		H11	0.505			

Table 10 The optical density values of cytotoxic test at 24 hr. plate1.

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
CTL1	control	B2	0.08	0.084	0.005	7.405
		B3	0.099			
		B4	0.069			
		B5	0.078			
		B6	0.084			
		B8	0.083			
		B9	0.09			
		B11	0.096			
SPL1	10	C2	0.081	0.076	0.006	8.174
		C3	0.085			
		C4	0.086			
		C5	0.075			
		C6	0.077			
		C7	0.078			
		C8	0.083			
		C10	0.076			
SPL2	20	D2	0.085	0.79	0.006	7.405
		D3	0.083			
		D4	0.079			
		D5	0.068			
		D6	0.079			
		D7	0.082			
		D10	0.079			
		D11	0.078			
SPL3	30	E2	0.071	0.071	0.022	22.776
		E3	0.072			
		E5	0.065			
		E6	0.073			
		E7	0.073			
		E8	0.077			
		E9	0.069			
		E10	0.074			

Table 10(continued)

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
SPL4	40	F2	0.059	0.063	0.01	11.94
		F3	0.061			
		F4	0.061			
		F6	0.071			
		F7	0.061			
		F8	0.059			
		F9	0.063			
		F11	0.066			
SPL5	50	G2	0.057	0.06	0.009	14.779
		G3	0.066			
		G4	0.061			
		G5	0.054			
		G6	0.065			
		G7	0.064			
		G9	0.064			
		G10	0.056			
SPL6	60	H2	0.061	0.06	0.004	5.965
		H3	0.067			
		H4	0.059			
		H5	0.058			
		H6	0.06			
		H7	0.061			
		H9	0.061			
		H10	0.055			

Table 11 The optical density values of cytotoxic test at 24 hr. plate2

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
CTL1	control	B2	0.082	0.0798	0.006	10.013
		B3	0.08			
		B4	0.083			
		B5	0.083			
		B6	0.076			
		B7	0.078			
		B9	0.082			
		B11	0.074			
SPL1	10	C2	0.077	0.0775	0.005	6.982
		C3	0.068			
		C4	0.074			
		C5	0.083			
		C6	0.08			
		C7	0.084			
		C9	0.079			
		C11	0.075			
SPL2	20	D2	0.08	0.0779	0.011	13.185
		D4	0.077			
		D5	0.085			
		D6	0.075			
		D8	0.088			
		D9	0.077			
		D10	0.073			
		D11	0.068			
SPL3	30	E2	0.073	0.077	0.008	10.234
		E3	0.088			
		E4	0.082			
		E6	0.074			
		E7	0.078			
		E9	0.078			
		E10	0.073			
		B2	0.082	0.0798	0.006	10.013

Table 11 (continued)

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
SPL4	40	F2	0.067	0.0714	0.018	19.798
		F3	0.076			
		F4	0.072			
		F5	0.068			
		F7	0.073			
		F8	0.064			
		F9	0.075			
		F10	0.076			
SPL5	50	G2	0.055	0.0613	0.006	9.042
		G3	0.057			
		G5	0.058			
		G6	0.062			
		G7	0.069			
		G8	0.061			
		G9	0.063			
		G10	0.065			
SPL6	60	H2	0.055	0.0603	0.004	6.223
		H3	0.063			
		H5	0.061			
		H6	0.061			
		H7	0.062			
		H8	0.061			
		H9	0.06			
		H10	0.059			

Table 12 The optical density values of cytotoxic test at 48 hr. plate1

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
CTL1	control	B2	0.069	0.075	0.003	4.672
		B3	0.075			
		B6	0.073			
		B7	0.069			
		B8	0.065			
		B9	0.066			
		B10	0.068			
		B11	0.075			
SPL1	10	A2	0.224	0.468	0.239	51.125
		A3	0.27			
		A4	0.238			
		A5	0.231			
		A6	0.251			
		A7	0.639			
		A8	0.66			
		A11	0.743			
SPL2	20	C2	0.066	0.068	0.006	8.765
		C3	0.066			
		C4	0.065			
		C6	0.071			
		C7	0.072			
		C8	0.07			
		C10	0.065			
		C11	0.068			
SPL3	30	D2	0.065	0.067	0.004	5.711
		D3	0.065			
		D4	0.071			
		D6	0.063			
		D7	0.072			
		D8	0.064			
		D9	0.064			
		D11	0.069			

Table 12 (continued)

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
SPL4	40	E2	0.071	0.067	0.004	5.406
		E3	0.067			
		E4	0.065			
		E5	0.066			
		E6	0.067			
		E7	0.066			
		E8	0.07			
		E9	0.068			
SPL5	50	F2	0.066	0.068	0.003	3.85
		F3	0.065			
		F4	0.066			
		F5	0.063			
		F6	0.066			
		F7	0.069			
		F8	0.065			
		F11	0.07			
SPL6	60	G2	0.068	0.066	0.004	6.126
		G3	0.064			
		G4	0.061			
		G5	0.065			
		G6	0.066			
		G7	0.072			
		G9	0.066			
		G10	0.069			

Table 13 The optical density values of cytotoxic test at 48 hr. plate2

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
CTL1	control	B2	0.07	0.0713	0.004	5.104
		B4	0.072			
		B6	0.073			
		B7	0.07			
		B8	0.074			
		B9	0.074			
		B10	0.068			
		B11	0.07			
SPL1	10	A2	0.068	0.07	0.005	7.003
		A3	0.067			
		A4	0.071			
		A5	0.067			
		A6	0.074			
		A7	0.069			
		A10	0.071			
		A11	0.081			
SPL2	20	C2	0.068	0.0711	0.004	5.383
		C4	0.072			
		C6	0.07			
		C7	0.068			
		C8	0.069			
		C9	0.073			
		C10	0.076			
		C11	0.073			
SPL3	30	D2	0.066	0.0698	0.006	8.623
		D5	0.083			
		D6	0.069			
		D7	0.071			
		D8	0.064			
		D9	0.072			
		D10	0.072			
		D11	0.072			

Table 13 (continued)

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
SPL4	40	E2	0.067	0.0691	0.004	6.389
		E3	0.065			
		E4	0.076			
		E7	0.063			
		E8	0.068			
		E9	0.069			
		E10	0.073			
		E11	0.072			
SPL5	50	F2	0.066	0.0688	0.004	5.259
		F3	0.064			
		F4	0.073			
		F5	0.07			
		F7	0.073			
		F8	0.066			
		F9	0.068			
		F10	0.071			
SPL6	60	G2	0.067	0.0661	0.004	5.967
		G3	0.065			
		G4	0.07			
		G5	0.068			
		G7	0.069			
		G8	0.06			
		G9	0.062			
		G10	0.068			

Table 14 The optical density values of cytotoxic test at 72 hr. plate1

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
CTL1	control	B2	0.056	0.059	0.005	9.023
		B3	0.056			
		B4	0.062			
		B5	0.06			
		B7	0.065			
		B8	0.065			
		B9	0.061			
		B10	0.052			
Pos		A2	0.062	0.064	0.009	13.468
		A3	0.059			
		A4	0.059			
		A7	0.062			
		A8	0.059			
		A9	0.055			
		A10	0.06			
		A11	0.083			
SPL1	10	C2	0.06	0.057	0.01	15.616
		C3	0.055			
		C4	0.059			
		C6	0.065			
		C7	0.057			
		C8	0.052			
		C9	0.057			
		C10	0.055			
SPL2	20	D2	0.056	0.056	0.008	13.702
		D4	0.059			
		D5	0.064			
		D6	0.063			
		D7	0.051			
		D8	0.05			
		D9	0.052			
		D10	0.054			

Table 14(continued)

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
SPL3	30	E2	0.055	0.056	0.004	7.574
		E3	0.051			
		E5	0.057			
		E6	0.063			
		E7	0.052			
		E8	0.053			
		E9	0.056			
		E10	0.056			
SPL4	40	F2	0.052	0.055	0.027	41.528
		F4	0.049			
		F5	0.054			
		F7	0.055			
		F8	0.051			
		F9	0.06			
		F10	0.055			
		F11	0.065			
SPL5	50	G2	0.055	0.054	0.006	10.412
		G3	0.053			
		G4	0.048			
		G5	0.048			
		G6	0.053			
		G8	0.063			
		G9	0.058			
		G10	0.054			
SPL6	60	H2	0.056	0.053	0.011	18.2
		H4	0.051			
		H5	0.056			
		H7	0.052			
		H8	0.055			
		H9	0.051			
		H10	0.055			
		H11	0.055			

Table 15 The optical density values of cytotoxic test at 72 hr. plate2

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
CTL1	control	B2	0.049	0.055	0.008	13.674
		B5	0.051			
		B6	0.051			
		B7	0.067			
		B8	0.051			
		B9	0.052			
		B10	0.061			
		B11	0.069			
Pos		A2	0.652	0.621	0.059	9.558
		A3	0.625			
		A4	0.581			
		A5	0.631			
		A8	0.627			
		A9	0.509			
		A10	0.675			
		A11	0.551			
SPL1	10	C2	0.078	0.059	0.01	17.425
		C4	0.048			
		C5	0.049			
		C6	0.051			
		C7	0.053			
		C9	0.064			
		C10	0.06			
		C11	0.049			
SPL2	20	D2	0.051	0.057	0.012	21.685
		D3	0.064			
		D4	0.051			
		D5	0.054			
		D6	0.054			
		D7	0.057			
		D8	0.053			
		D9	0.053			

Table 15 (continued)

Well ID	Conc.(ppm)	Well	570(nm)	Mean	Std. Dev	CV (%)
SPL3	30	E2	0.052	0.051	0.002	4.075
		E3	0.051			
		E4	0.05			
		E6	0.057			
		E7	0.057			
		E9	0.053			
		E10	0.058			
		E11	0.054			
SPL4	40	F2	0.054	0.065	0.036	55.067
		F3	0.058			
		F4	0.05			
		F5	0.051			
		F6	0.051			
		F7	0.055			
		F8	0.053			
		F11	0.057			
SPL5	50	G2	0.05	0.068	0.029	42.999
		G3	0.052			
		G5	0.055			
		G6	0.059			
		G7	0.056			
		G8	0.051			
		G9	0.049			
		G10	0.048			
SPL6	60	H2	0.05	0.056	0.006	10.756
		H3	0.05			
		H6	0.054			
		H7	0.054			
		H8	0.051			
		H9	0.049			
		H10	0.051			
		H11	0.052			

Table 16 The tensile tear strength values in the non thermocycling groups.

Parameter		Max_Force
Unit	Groups(ppm)	(N/cm ²)
1 _ 1	control	1.475
1 _ 2	control	1.350
1 _ 3	control	1.350
1 _ 4	control	1.425
1 _ 5	control	1.450
1 _ 6	control	1.425
1 _ 7	control	1.500
1 _ 8	control	1.750
1 _ 9	control	1.650
1 _ 10	control	1.650
Average		1.4975
Standard Deviation		0.12608
2 _ 1	10	1.575
2 _ 2	10	1.475
2 _ 3	10	1.475
2 _ 4	10	1.400
2 _ 5	10	1.525
2 _ 6	10	1.650
2 _ 7	10	1.725
2 _ 8	10	1.425
2 _ 9	10	1.575
2 _ 10	10	1.650
Average		1.5525
Standard Deviation		0.101

Table 16 (continued)

Parameter		Max_Force (N/cm ²)
Unit	Groups(ppm)	
3 _ 1	20	1.450
3 _ 2	20	1.400
3 _ 3	20	1.800
3 _ 4	20	1.400
3 _ 5	20	1.500
3 _ 6	20	1.500
3 _ 7	20	1.725
3 _ 8	20	1.600
3 _ 9	20	1.525
3 _ 10	20	1.600
Average		1.550
Standard Deviation		0.1328
4 _ 1	30	1.350
4 _ 2	30	1.550
4 _ 3	30	1.750
4 _ 4	30	1.450
4 _ 5	30	1.350
4 _ 6	30	1.750
4 _ 7	30	1.750
4 _ 8	30	1.575
4 _ 9	30	1.425
4 _ 10	30	1.400
Average		1.535
Standard Deviation		0.166

Table 16 (continued)

Parameter		Max_Force
Unit	Groups(ppm)	(N/cm ²)
5 _ 1	40	1.725
5 _ 2	40	1.750
5 _ 3	40	1.525
5 _ 4	40	1.530
5 _ 5	40	1.750
5 _ 6	40	1.600
5 _ 7	40	1.750
5 _ 8	40	1.750
5 _ 9	40	1.600
5 _ 10	40	1.750
Average		1.690
Standard Deviation		0.0974
6 _ 1	50	1.750
6 _ 2	50	1.750
6 _ 3	50	1.750
6 _ 4	50	1.650
6 _ 5	50	1.725
6 _ 6	50	1.725
6 _ 7	50	1.750
6 _ 8	50	1.525
6 _ 9	50	1.875
6 _ 10	50	1.600
Average		1.710
Standard Deviation		0.096

Table 16 (continued)

Parameter		Max_Force
Unit	Groups(ppm)	(N/cm ²)
7 _ 1	60	1.825
7 _ 2	60	1.925
7 _ 3	60	1.525
7 _ 4	60	1.875
7 _ 5	60	1.750
7 _ 6	60	1.535
7 _ 7	60	1.600
7 _ 8	60	1.750
7 _ 9	60	1.525
7 _ 10	60	1.600
Average		1.704
Standard Deviation		0.1423

Table 17 The tensile tear strength values in the thermocycling groups.

Parameter		Max_Force (N/cm ²)
Unit	Groups(ppm)	
1 _ 1	control	1.8500
1 _ 2	control	2.3250
1 _ 3	control	2.1000
1 _ 4	control	1.9750
1 _ 5	control	1.9250
1 _ 6	control	2.150
1 _ 7	control	1.800
1 _ 8	control	2.150
1 _ 9	control	1.850
1 _ 10	control	2.100
Average		2.023
Standard Deviation		0.1689
2 _ 1	10	1.975
2 _ 2	10	1.825
2 _ 3	10	2.025
2 _ 4	10	2.175
2 _ 5	10	2.325
2 _ 6	10	2.200
2 _ 7	10	1.850
2 _ 8	10	2.200
2 _ 9	10	2.150
2 _ 10	10	2.200
Average		2.0375
Standard Deviation		0.1769

Table 17 (continued)

Parameter		Max_Force
Unit	Groups(ppm)	(N/cm ²)
3 _ 1	20	2.250
3 _ 2	20	2.175
3 _ 3	20	1.725
3 _ 4	20	1.775
3 _ 5	20	1.875
3 _ 6	20	2.150
3 _ 7	20	1.825
3 _ 8	20	1.975
3 _ 9	20	1.875
3 _ 10	20	2.575
Average		2.0575
Standard Deviation		0.2685
4 _ 1	30	1.9500
4 _ 2	30	1.9750
4 _ 3	30	2.0500
4 _ 4	30	1.8250
4 _ 5	30	2.2000
4 _ 6	30	2.500
4 _ 7	30	2.250
4 _ 8	30	1.475
4 _ 9	30	2.000
4 _ 10	30	2.325
Average		2.055
Standard Deviation		0.286

Table 17 (continued)

Parameter		Max_Force (N/cm ²)
Unit	Groups(ppm)	
5 _ 1	40	1.825
5 _ 2	40	2.050
5 _ 3	40	2.250
5 _ 4	40	1.750
5 _ 5	40	1.850
5 _ 6	40	2.300
5 _ 7	40	2.200
5 _ 8	40	2.050
5 _ 9	40	1.825
5 _ 10	40	1.800
Average		2.025
Standard Deviation		0.200
6 _ 1	50	1.8000
6 _ 2	50	1.8000
6 _ 3	50	2.2000
6 _ 4	50	2.1000
6 _ 5	50	2.2750
6 _ 6	50	2.075
6 _ 7	50	2.375
6 _ 8	50	2.275
6 _ 9	50	2.275
6 _ 10	50	2.050
Average		2.123
Standard Deviation		0.198

Table 17 (continued)

Parameter		Max_Force (N/cm ²)
Unit	Groups(ppm)	
7 _ 1	60	2.050
7 _ 2	60	2.275
7 _ 3	60	2.425
7 _ 4	60	1.825
7 _ 5	60	2.275
7 _ 6	60	1.925
7 _ 7	60	2.100
7 _ 8	60	1.950
7 _ 9	60	1.975
7 _ 10	60	1.725
Average		2.053
Standard Deviation		0.219

Table 18 The tensile bond strength values in the non-thermocycling group.

Parameter		Max_Force (N/cm ²)
Unit	Groups(ppm)	
1 _ 1	control	15.675
1 _ 2	control	14.350
1 _ 3	control	14.475
1 _ 4	control	14.250
1 _ 5	control	18.625
1 _ 6	control	17.825
1 _ 7	control	16.675
1 _ 8	control	16.200
1 _ 9	control	16.000
1 _ 10	control	17.100
Average		16.118
Standard Deviation		1.490
2 _ 1	10	24.150
2 _ 2	10	26.650
2 _ 3	10	30.775
2 _ 4	10	28.050
2 _ 5	10	22.925
2 _ 6	10	24.025
2 _ 7	10	29.325
2 _ 8	10	25.075
2 _ 9	10	23.325
2 _ 10	10	23.950
Average		25.785
Standard Deviation		2.775

Table 18 (continued)

Parameter		Max_Force
Unit	Groups(ppm)	(N/cm ²)
3 _ 1	20	24.200
3 _ 2	20	29.450
3 _ 3	20	28.675
3 _ 4	20	27.325
3 _ 5	20	24.900
3 _ 6	20	30.850
3 _ 7	20	28.675
3 _ 8	20	22.400
3 _ 9	20	30.750
3 _ 10	20	29.475
Average		27.670
Standard Deviation		2.899
4 _ 1	30	26.775
4 _ 2	30	25.200
4 _ 3	30	31.825
4 _ 4	30	29.425
4 _ 5	30	31.625
4 _ 6	30	26.150
4 _ 7	30	25.900
4 _ 8	30	31.575
4 _ 9	30	30.975
4 _ 10	30	31.650
Average		29.110
Standard Deviation		2.781

Table 18 (continued)

Parameter		Max_Force
Unit	Groups(ppm)	(N/cm ²)
5 _ 1	40	30.650
5 _ 2	40	26.350
5 _ 3	40	31.975
5 _ 4	40	30.650
5 _ 5	40	27.200
5 _ 6	40	26.950
5 _ 7	40	34.750
5 _ 8	40	30.400
5 _ 9	40	26.925
5 _ 10	40	32.775
Average		29.863
Standard Deviation		2.884
6 _ 1	50	32.325
6 _ 2	50	32.025
6 _ 3	50	33.600
6 _ 4	50	30.125
6 _ 5	50	38.775
6 _ 6	50	33.375
6 _ 7	50	27.750
6 _ 8	50	37.575
6 _ 9	50	31.375
6 _ 10	50	36.725
Average		33.365
Standard Deviation		3.451

Table 18 (continued)

Parameter		Max_Force
Unit	Groups(ppm)	(N/cm ²)
7 _ 1	60	33.900
7 _ 2	60	33.825
7 _ 3	60	34.525
7 _ 4	60	36.675
7 _ 5	60	32.625
7 _ 6	60	31.350
7 _ 7	60	37.075
7 _ 8	60	31.875
7 _ 9	60	37.400
7 _ 10	60	36.100
Average		34.535
Standard Deviation		2.198

Table 19 The tensile bond strength values in the thermocycling group.

Parameter		Max_Force (N/cm ²)
Unit	Groups(ppm)	
1 _ 1	control	17.475
1 _ 2	control	15.625
1 _ 3	control	20.900
1 _ 4	control	21.950
1 _ 5	control	17.925
1 _ 6	control	21.375
1 _ 7	control	19.850
1 _ 8	control	15.825
1 _ 9	control	18.450
1 _ 10	control	18.823
Average		18.823
Standard Deviation		2.202
2 _ 1	10	24.950
2 _ 2	10	19.950
2 _ 3	10	26.400
2 _ 4	10	23.450
2 _ 5	10	20.600
2 _ 6	10	21.000
2 _ 7	10	29.500
2 _ 8	10	21.000
2 _ 9	10	23.050
2 _ 10	10	28.075
Average		23.798
Standard Deviation		3.338

Table 19 (continued)

Parameter		Max_Force
Unit	Groups(ppm)	(N/cm ²)
3 _ 1	20	30.900
3 _ 2	20	26.575
3 _ 3	20	28.575
3 _ 4	20	28.675
3 _ 5	20	22.575
3 _ 6	20	30.700
3 _ 7	20	24.825
3 _ 8	20	33.900
3 _ 9	20	23.025
3 _ 10	20	27.570
Average		27.833
Standard Deviation		3.615
4 _ 1	30	29.300
4 _ 2	30	25.300
4 _ 3	30	26.600
4 _ 4	30	26.725
4 _ 5	30	27.975
4 _ 6	30	23.200
4 _ 7	30	27.125
4 _ 8	30	30.450
4 _ 9	30	23.200
4 _ 10	30	31.900
Average		27.200
Standard Deviation		2.831

Table 19 (continued)

Parameter		Max_Force
Unit	Groups(ppm)	(N/cm ²)
5 _ 1	40	25.200
5 _ 2	40	25.025
5 _ 3	40	27.350
5 _ 4	40	31.775
5 _ 5	40	24.475
5 _ 6	40	32.425
5 _ 7	40	24.200
5 _ 8	40	32.425
5 _ 9	40	31.025
5 _ 10	40	32.725
Average		
Standard Deviation		3.606
6 _ 1	50	23.100
6 _ 2	50	29.075
6 _ 3	50	32.600
6 _ 4	50	33.325
6 _ 5	50	25.650
6 _ 6	50	33.450
6 _ 7	50	30.075
6 _ 8	50	29.150
6 _ 9	50	25.300
6 _ 10	50	25.000
Average		28.673
Standard Deviation		3.761

Table 19 (continued)

Parameter		Max_Force
Unit	Groups(ppm)	(N/cm ²)
7 _ 1	60	32.675
7 _ 2	60	28.750
7 _ 3	60	33.350
7 _ 4	60	38.725
7 _ 5	60	39.525
7 _ 6	60	28.525
7 _ 7	60	29.100
7 _ 8	60	31.725
7 _ 9	60	32.650
7 _ 10	60	28.75
Average		32.890
Standard Deviation		3.819

STATISTICS ANALYSIS

1.Cytotoxic test

Tests of Between-Subjects Effects

Dependent Variable: ODper

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	20125.885 ^a	27	745.403	17.161	.000	.525
Intercept	3877601.403	1	3877601.403	89274.019	.000	.995
con	10109.475	6	1684.912	38.792	.000	.357
time	5346.617	3	1782.206	41.032	.000	.227
con * time	4669.793	18	259.433	5.973	.000	.204
Error	18242.627	420	43.435			
Total	3915969.915	448				
Corrected Total	38368.512	447				

a. R Squared = .525 (Adjusted R Squared = .494)

Dependent Variable: ODper

			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
	(I) con	(J) con				Lower Bound	Upper Bound
Tukey HSD	control	10ppm	2.9776	1.16505	.143	-.4741	6.4293
		20ppm	4.3493*	1.16505	.004	.8977	7.8010
		30ppm	5.8965*	1.16505	.000	2.4448	9.3482
		40ppm	8.9428*	1.16505	.000	5.4911	12.3945
		50ppm	12.4426*	1.16505	.000	8.9909	15.8943
		60ppm	14.1518*	1.16505	.000	10.7002	17.6035
	10ppm	control	-2.9776	1.16505	.143	-6.4293	.4741
		20ppm	1.3717	1.16505	.902	-2.0800	4.8234
		30ppm	2.9189	1.16505	.160	-.5328	6.3705
		40ppm	5.9652*	1.16505	.000	2.5135	9.4169
		50ppm	9.4650*	1.16505	.000	6.0133	12.9167
		60ppm	11.1742*	1.16505	.000	7.7225	14.6259

20ppm	control	-4.3493*	1.16505	.004	-7.8010	-.8977
	10ppm	-1.3717	1.16505	.902	-4.8234	2.0800
	30ppm	1.5471	1.16505	.839	-1.9045	4.9988
	40ppm	4.5935*	1.16505	.002	1.1418	8.0451
	50ppm	8.0933*	1.16505	.000	4.6416	11.5450
	60ppm	9.8025*	1.16505	.000	6.3508	13.2542
30ppm	control	-5.8965*	1.16505	.000	-9.3482	-2.4448
	10ppm	-2.9189	1.16505	.160	-6.3705	.5328
	20ppm	-1.5471	1.16505	.839	-4.9988	1.9045
	40ppm	3.0463	1.16505	.124	-.4054	6.4980
	50ppm	6.5461*	1.16505	.000	3.0944	9.9978
	60ppm	8.2554*	1.16505	.000	4.8037	11.7071
40ppm	control	-8.9428*	1.16505	.000	-12.3945	-5.4911
	10ppm	-5.9652*	1.16505	.000	-9.4169	-2.5135
	20ppm	-4.5935*	1.16505	.002	-8.0451	-1.1418
	30ppm	-3.0463	1.16505	.124	-6.4980	.4054
	50ppm	3.4998*	1.16505	.044	.0481	6.9515
	60ppm	5.2091*	1.16505	.000	1.7574	8.6607
50ppm	control	-12.4426*	1.16505	.000	-15.8943	-8.9909
	10ppm	-9.4650*	1.16505	.000	-12.9167	-6.0133
	20ppm	-8.0933*	1.16505	.000	-11.5450	-4.6416
	30ppm	-6.5461*	1.16505	.000	-9.9978	-3.0944
	40ppm	-3.4998*	1.16505	.044	-6.9515	-.0481
	60ppm	1.7092	1.16505	.764	-1.7424	5.1609
60ppm	control	-14.1518*	1.16505	.000	-17.6035	-10.7002
	10ppm	-11.1742*	1.16505	.000	-14.6259	-7.7225
	20ppm	-9.8025*	1.16505	.000	-13.2542	-6.3508
	30ppm	-8.2554*	1.16505	.000	-11.7071	-4.8037
	40ppm	-5.2091*	1.16505	.000	-8.6607	-1.7574
	50ppm	-1.7092	1.16505	.764	-5.1609	1.7424

Based on observed means.

The error term is Mean Square(Error) = 43.435.

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Dependent Variable: ODper

			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
	(I) time	(J) time				Lower Bound	Upper Bound
Tukey HSD	0hr	24hr	7.1858*	.88069	.000	4.9142	9.4575
		48hr	-1.8018	.88069	.173	-4.0735	.4698
		72hr	-.0373	.88069	1.000	-2.3089	2.2343
	24hr	0hr	-7.1858*	.88069	.000	-9.4575	-4.9142
		48hr	-8.9877*	.88069	.000	-11.2593	-6.7161
		72hr	-7.2232*	.88069	.000	-9.4948	-4.9516
	48hr	0hr	1.8018	.88069	.173	-.4698	4.0735
		24hr	8.9877*	.88069	.000	6.7161	11.2593
		72hr	1.7645	.88069	.188	-.5071	4.0361
	72hr	0hr	.0373	.88069	1.000	-2.2343	2.3089
		24hr	7.2232*	.88069	.000	4.9516	9.4948
		48hr	-1.7645	.88069	.188	-4.0361	.5071

Based on observed means.

The error term is Mean Square(Error) = 43.435.

*. The mean difference is significant at the .05 level.

2. Tensile tear strength test statistics.

Tests of Normality

	Treatment	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Maxforce	control	.152	20	.200*	.933	20	.175
	10 ppm	.144	20	.200*	.933	20	.175
	20 ppm	.130	20	.200*	.925	20	.125
	30 ppm	.152	20	.200*	.920	20	.098
	40 ppm	.212	20	.019	.902	20	.045
	50 ppm	.222	20	.011	.905	20	.050
	60 ppm	.101	20	.200*	.953	20	.410

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Tests of Between-Subjects Effects

Dependent Variable: Maxforce

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	7.522 ^a	13	.579	18.191	.000	.652
Intercept	468.626	1	468.626	14732.635	.000	.992
Thermocycling	7.002	1	7.002	220.136	.000	.636
Treatment	.351	6	.058	1.838	.097	.080
Thermocycling * Treatment	.169	6	.028	.886	.507	.040
Error	4.008	126	.032			
Total	480.157	140				
Corrected Total	11.530	139				

a. R Squared = .652 (Adjusted R Squared = .617)

Multiple Comparisons Dependent Variable: Maxforce

Tukey HSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	10 ppm	-.03500	.056399	.996	-.20400	.13400
	20 ppm	-.04375	.056399	.987	-.21275	.12525
	30 ppm	-.04500	.056399	.985	-.21400	.12400
	40 ppm	-.08900	.056399	.697	-.25800	.08000
	50 ppm	-.15625	.056399	.090	-.32525	.01275
	60 ppm	-.11800	.056399	.364	-.28700	.05100
10 ppm	control	.03500	.056399	.996	-.13400	.20400
	20 ppm	-.00875	.056399	1.000	-.17775	.16025
	30 ppm	-.01000	.056399	1.000	-.17900	.15900
	40 ppm	-.05400	.056399	.962	-.22300	.11500
	50 ppm	-.12125	.056399	.330	-.29025	.04775
	60 ppm	-.08300	.056399	.761	-.25200	.08600
20 ppm	control	.04375	.056399	.987	-.12525	.21275
	10 ppm	.00875	.056399	1.000	-.16025	.17775
	30 ppm	-.00125	.056399	1.000	-.17025	.16775
	40 ppm	-.04525	.056399	.984	-.21425	.12375
	50 ppm	-.11250	.056399	.423	-.28150	.05650
	60 ppm	-.07425	.056399	.843	-.24325	.09475
30 ppm	control	.04500	.056399	.985	-.12400	.21400
	10 ppm	.01000	.056399	1.000	-.15900	.17900
	20 ppm	.00125	.056399	1.000	-.16775	.17025
	40 ppm	-.04400	.056399	.986	-.21300	.12500
	50 ppm	-.11125	.056399	.437	-.28025	.05775
	60 ppm	-.07300	.056399	.853	-.24200	.09600
40 ppm	control	.08900	.056399	.697	-.08000	.25800
	10 ppm	.05400	.056399	.962	-.11500	.22300
	20 ppm	.04525	.056399	.984	-.12375	.21425
	30 ppm	.04400	.056399	.986	-.12500	.21300
	50 ppm	-.06725	.056399	.896	-.23625	.10175
	60 ppm	-.02900	.056399	.999	-.19800	.14000

50 ppm	control	.15625	.056399	.090	-.01275	.32525
	10 ppm	.12125	.056399	.330	-.04775	.29025
	20 ppm	.11250	.056399	.423	-.05650	.28150
	30 ppm	.11125	.056399	.437	-.05775	.28025
	40 ppm	.06725	.056399	.896	-.10175	.23625
	60 ppm	.03825	.056399	.994	-.13075	.20725
60 ppm	control	.11800	.056399	.364	-.05100	.28700
	10 ppm	.08300	.056399	.761	-.08600	.25200
	20 ppm	.07425	.056399	.843	-.09475	.24325
	30 ppm	.07300	.056399	.853	-.09600	.24200
	40 ppm	.02900	.056399	.999	-.14000	.19800
	50 ppm	-.03825	.056399	.994	-.20725	.13075

Based on observed means.

The error term is Mean Square(Error) = .032.

3.Tensile bond test statistics.

Tests of Normality

	Concentration	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Maxforce	Control	.110	20	.200*	.951	20	.382
	10 ppm	.130	20	.200*	.956	20	.461
	20 ppm	.202	20	.032	.940	20	.238
	30ppm	.139	20	.200*	.914	20	.076
	40 ppm	.182	20	.082	.916	20	.082
	50 ppm	.122	20	.200*	.970	20	.758
	60 ppm	.127	20	.200*	.961	20	.559

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Tests of Between-Subjects Effects

Dependent Variable: Maxforce

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	3457.994 ^a	13	266.000	28.345	.000	.745
Intercept	105557.613	1	105557.613	11248.222	.000	.989
Treatment	51.213	1	51.213	5.457	.021	.042
Concentration	3253.608	6	542.268	57.784	.000	.733
Treatment * Concentration	153.173	6	25.529	2.720	.016	.115
Error	1182.432	126	9.384			
Total	110198.039	140				
Corrected Total	4640.426	139				

a. R Squared = .745 (Adjusted R Squared = .719)

Multiple Comparisons Dependent Variable: Maxforce

Tukey HSD

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	10 ppm	-7.32125*	.968730	.000	-10.22408	-4.41842
	20 ppm	-10.28125*	.968730	.000	-13.18408	-7.37842
	30ppm	-10.68500*	.968730	.000	-13.58783	-7.78217
	40 ppm	-11.84250*	.968730	.000	-14.74533	-8.93967
	50 ppm	-13.54875*	.968730	.000	-16.45158	-10.64592
	60 ppm	-16.24250*	.968730	.000	-19.14533	-13.33967
10 ppm	Control	7.32125*	.968730	.000	4.41842	10.22408
	20 ppm	-2.96000*	.968730	.043	-5.86283	-.05717
	30ppm	-3.36375*	.968730	.012	-6.26658	-.46092
	40 ppm	-4.52125*	.968730	.000	-7.42408	-1.61842
	50 ppm	-6.22750*	.968730	.000	-9.13033	-3.32467
	60 ppm	-8.92125*	.968730	.000	-11.82408	-6.01842
20 ppm	Control	10.28125*	.968730	.000	7.37842	13.18408
	10 ppm	2.96000*	.968730	.043	.05717	5.86283
	30ppm	-.40375	.968730	1.000	-3.30658	2.49908
	40 ppm	-1.56125	.968730	.675	-4.46408	1.34158
	50 ppm	-3.26750*	.968730	.017	-6.17033	-.36467
	60 ppm	-5.96125*	.968730	.000	-8.86408	-3.05842
30ppm	Control	10.68500*	.968730	.000	7.78217	13.58783
	10 ppm	3.36375*	.968730	.012	.46092	6.26658
	20 ppm	.40375	.968730	1.000	-2.49908	3.30658
	40 ppm	-1.15750	.968730	.895	-4.06033	1.74533
	50 ppm	-2.86375	.968730	.056	-5.76658	.03908
	60 ppm	-5.55750*	.968730	.000	-8.46033	-2.65467
40 ppm	Control	11.84250*	.968730	.000	8.93967	14.74533
	10 ppm	4.52125*	.968730	.000	1.61842	7.42408
	20 ppm	1.56125	.968730	.675	-1.34158	4.46408
	30ppm	1.15750	.968730	.895	-1.74533	4.06033
	50 ppm	-1.70625	.968730	.576	-4.60908	1.19658
	60 ppm	-4.40000*	.968730	.000	-7.30283	-1.49717

50 ppm	Control	13.54875*	.968730	.000	10.64592	16.45158
	10 ppm	6.22750*	.968730	.000	3.32467	9.13033
	20 ppm	3.26750*	.968730	.017	.36467	6.17033
	30ppm	2.86375	.968730	.056	-.03908	5.76658
	40 ppm	1.70625	.968730	.576	-1.19658	4.60908
	60 ppm	-2.69375	.968730	.088	-5.59658	.20908
60 ppm	Control	16.24250*	.968730	.000	13.33967	19.14533
	10 ppm	8.92125*	.968730	.000	6.01842	11.82408
	20 ppm	5.96125*	.968730	.000	3.05842	8.86408
	30ppm	5.55750*	.968730	.000	2.65467	8.46033
	40 ppm	4.40000*	.968730	.000	1.49717	7.30283
	50 ppm	2.69375	.968730	.088	-.20908	5.59658

Based on observed means.

The error term is Mean Square(Error) = 9.384.

*. The mean difference is significant at the .05 level.



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