อิทธิพลของ 3-เมทาคริโลซีโพรพิวไตรเมท็อกซีไซเลนปรับสภาพซิงค์ออกไซด์นาโนพาทิเคิล ที่ใส่ใน
วัสดุโพลิเมทิลเมทาคริเลตต่อคุณสมบัติการต้านเชื้อรา การมองเห็นและเชิงกล

นายกฤช กมลขันติกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Effect of 3-methacryloxypropyltrimethoxysilane modified zinc oxide nanoparticles incorporated in polymethylmethacrylate material on antifungal, optical and mechanical properties

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Thesis Title   Effect of 3-methacryloxypropyltrimethoxysilane modified zinc oxide nanoparticles incorporated in polymethylmethacrylate material on antifungal, optical and mechanical properties

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กฤช กมลขันติกุล : อิทธิพลของ 3-เมทาคริโลซีโพรพิวไตรเมท็อกซีไซเลนปรับสภาพซิงค์ออ
อกไซด์นาโนพาทิเคิล ที่ใส่ในวัสดุโพลิเมทิลเมทาคริเลตต่อคุณสมบัติการต้านเชื้อรา การ
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ทพ. ดร.แมนสรวง อักษรนุกิจ, หน้า.

การศึกษานี้มีจุดมุ่งหมายเพื่อหา 1) ลักษณะของอนุภาคนาโนซิงค์ออกไซด์ที่เคลือบด้วยสาร
สามเมธาครีลอกซีโพรพิวไตรเมธอกซีไซเลน (เอ็มพีเอส) ในปริมาณต่างกันด้วยเครื่องฟลูเรียร์ทรานส์
ฟอร์ม อินฟราเรดสเปคโทรมิเตอร์ (เอฟทีไออาร์) และเครื่องวิเคราะห์การเปลี่ยนแปลงน้ำหนักของ
สารโดยอาศัยความร้อน (ทีจีเอ) ก่อนและหลังการล้างด้วยสารเตตระไฮโดรฟูแรน 2) อิทธิพลของ
สารเอ็มพีเอสในปริมาณต่างกัน ซึ่งเคลือบบนอนุภาคนาโนซิงค์ออกไซด์ที่ใส่ในวัสดุพีเอ็มเอ็มเอต่อ
คุณสมบัติเชิงกล และ 3) อิทธิพลของอนุภาคนาโนซิงค์ออกไซด์ในปริมาณต่างกัน ที่เคลือบหรือไม่
เคลือบด้วยสารเอ็มพีเอส ซึ่งใส่ในวัสดุพีเอ็มเอ็มเอ ต่อคุณสมบัติการต้านเชื้อรา การมองเห็นและ
เชิงกล ผลจากเอฟทีไออาร์และทีจีเอแสดงถึงความสำเร็จในการเคลือบอนุภาคนาโนซิงค์ออกไซด์จาก
การไซลาไนเซชั่น โมเลกุลของสารไซเลนแบบชั้นเดี่ยว มีการเรียงตัวแบบแนวนอนกับพื้นผิวอนุภาคนา
โนซิงค์ออกไซด์ เมื่อวัดคุณสมบัติเชิงกล พบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญระหว่างกลุ่มที่มี
ปริมาณสารเอ็มพีเอสที่ต่างกัน (0-14.4 เปอร์เซ็นต์โดยน้ำหนัก) ซึ่งเคลือบบนอนุภาคนาโนซิงค์ออก
ไซด์ที่ใส่ในวัสดุพีเอ็มเอ็มเอ ปริมาณที่ต่างกัน (1.25, 2.5 และ 5 เปอร์เซ็นต์โดยน้ำหนัก) ที่เคลือบ
หรือไม่เคลือบอนุภาคซิงค์ออกไซด์ด้วยสารไซเลน ที่ใส่ในวัสดุพีเอ็มเอ็มเอ ถูกประเมินคุณสมบัติการ
ต้านเชื้อรา การมองเห็น และเชิงกลด้วยวิธีสัมผัสเชื้อแบบโดยตรง การมองเห็น และการโค้งงอแบบ
สามจุด พบว่าในปริมาณอนุภาคซิงค์ออกไซด์ที่เท่ากัน กลุ่มที่มีไซเลนจะมีการลดปริมาณเชื้อแคนดิดา
อัลบิแคนได้มากกว่ากลุ่มที่ไม่ไซเลน ความแตกต่างของสีและความทึบของกลุ่มที่ไม่ไซเลนจะมากกว่า
กลุ่มที่มีไซเลน ความแข็งแรงต่อการโค้งงอของกลุ่มที่มีไซเลนจะมากกว่ากลุ่มที่ไม่ไซเลนอย่างมี
นัยสำคัญ ยกเว้นกลุ่ม 1.25 เปอร์เซ็นต์โดยน้ำหนัก สรุปได้ว่า วัสดุพีเอ็มเอ็มเอที่มีการเคลือบอนุภาค
นาโนซิงค์ออกไซด์ โดยเฉพาะในปริมาณ 2.5 เปอร์เซ็นต์โดยน้ำหนัก ที่มีการเคลือบด้วยสารไซเลน จะ
มีผลในการต้านเชื้อรา มีความต่างของสีและความทึบที่น้อยกว่า เมื่อเปรียบเทียบกับกลุ่มอนุภาคซิงค์ออ
อกไซด์ที่ไม่ได้มีการเคลือบด้วยสารไซเลน โดยยังคงคุณสมบัติเชิงกลไว้

ภาควิชา	ทันตกรรมประดิษฐ์	ลายมือชื่อนิสิต	
สาขาวิชา	ทันตกรรมประดิษฐ์	ลายมือชื่อ อ.ที่ปรึกษาหลัก	
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KEYWORDS: ANTIFUNGAL EFFECT / OPTICAL PROPERTIES / MECHANICAL PROPERTIES / SILANIZED ZINC OXIDE NANOPARTICLES

KRID KAMONKHANTIKUL: Effect of 3-methacryloxypropyltrimethoxysilane modified zinc oxide nanoparticles incorporated in polymethylmethacrylate material on antifungal, optical and mechanical properties. ADVISOR: ASSOC. PROF. MANSUANG ARKSORNNUKIT, DDS., MS., Ph.D., pp.

This study aimed to evaluate 1) the characteristics of different amount of 3-methacryloxypropyltrimethoxysilane (MPS) deposited on zinc oxide nanoparticles (ZnOnps) by Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA) before and after tetrahydrofuran washing, 2) the effect of different MPS amount deposited on ZnOnps incorporated in PMMA material on mechanical properties, and 3) the effect of different ZnOnps amounts with or without MPS incorporated in PMMA material on antifungal, optical and mechanical properties. The FTIR and TGA results showed the success of silanization. The monolayered silane molecules were parallelly oriented on the ZnOnps surface. The different MPS amounts (0-14.4 wt%) deposited on ZnOnps incorporated in PMMA material demonstrated no significant differences on mechanical properties. The different amount (1.25, 2.5 and 5 wt%) of non-silanized (Nosi) or silanized (Si) ZnOnps incorporated in PMMA material on antifungal, optical and mechanical properties were evaluated by direct contact test, optical test and three-point bending test. With the same ZnOnps amount, the Si groups demonstrated a greater reduction in C. albicans compared with the Nosi groups. The color difference and opacity of the Nosi groups were greater when comparing with the Si groups. The flexural strength of Si groups, except for the 1.25% groups, was significantly greater compared with the Nosi groups. It was concluded that PMMA material incorporated with silanized ZnOnps, particularly 2.5% of silanized ZnOnps, had a greater antifungal effect, less color changes and opacity compared with non-silanized ZnOnps, while retaining its mechanical properties.

Department:	Prosthodontics	Student's Signature	....
Field of Study:	Prosthodontics	Advisor's Signature	....
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จุฬาลงกรณ์มหาวิทยาลัย

CHULALONGKORN UNIVERSITY

Chapter I

Introduction & Literature review

Background and Rationale

At present, the ratio of senescent population continuously increased. In 2030, the older Thai people ratio was estimated to be around 40.97% of total population that tripled from 14.3% in 2000.[1] The elderly people generally have physiological changes and are susceptible to chronic diseases such as diabetes mellitus, and oral diseases, etc. Poor oral health in elderly people is considered in affecting quality of life. Recently, the national oral health survey collected the Decayed, Missing, and Filled teeth (DMFT) score of the Thai elderly people (60-74 years) and demonstrated that the highest proportion of score was the missing teeth which averaged 13.38 per older adults. The percentage of the elderly who had loss all of the tooth varied from 5.9% in the North to 19.7% in the Central.[2] All of these problems are the challenge to public health system which must be solved by the appropriate method in order to improve the quality of life.

Denture has been used to rehabilitate patients with missing teeth. It enhances masticatory function, esthetics, phonetic and particularly improves the quality of life. Material mostly used for denture fabrication is polymethylmethacrylate (PMMA). The daily toothbrushing is recommended to denture wearers to maintain the hygiene of the denture and to reduce the quantity of microorganism on the denture.[3,4] However, PMMA can be

reservoirs of microorganisms which are the etiology of many oral diseases. A cross-sectional study demonstrated that the elderly denture wearer showed a 6.9-times higher probability of developing oral candidiasis compared with non-wearers.[5] *Candida albicans* (*C. albicans*) is the most common causative pathogen of oral candidiasis[5]; the disease associated with fungal infection. Denture induced stomatitis is one form of candidiasis.[6] The topical and/or systematic antifungal drugs are used in treating an oral fungal infection. Several studies suggested methods to disinfect the infected denture, such as ultrasonic soaking[7], microwave energy[8], and incorporation of metal/metal oxide into PMMA.[9-13]

Zinc oxide (ZnO), a metal oxide, is widely used in dentistry as a restorative luting material, root canal filling material and temporary restoration. Many studies demonstrated the effectiveness of these particles on inhibiting the growth of fungi[11,14] and bacteria.[15-19] This metal oxide varies in particle size ranging from microns to nanometers.[12,14,17] However, nano-sized particles with a high surface energy result in the aggregation of the particles. Particle aggregation generates weak points in the material. However, surface modification of these particles prevents the formation of aggregated nanoparticles.[20-22]

To date, there are limited information about the antifungal, optical and mechanical properties of PMMA incorporated with surface modified ZnOnps.

Literature review

<u>Denture induced stomatitis</u>

Oral candidiasis is the disease associated with fungal infection which can be categorized into 5 forms as follows;

1. Pseudomembranous candidiasis (oral thrush)

2. Acute atrophic (erythematous) candidiasis (antibiotic sore mouth)

3. Chronic erythematous (atrophic) candidiasis

4. Chronic hyperplastic candidiasis (candida leukoplakia)

5. Angular cheilitis

Denture induced stomatitis or denture sore mouth or chronic erythematous (atrophic) candidiasis is one form of candidiasis.[6] *C. albicans* is the most common causative pathogen. Moreover, *C. glabrate, C. tropicalis* and *C. krusei* can also cause this disease. The prevalence of *Candida* species increases in denture wearers because dentures decrease the flow of oxygen and saliva especially in the maxillary denture which cover the whole palate. Generally, clinical sign of denture stomatitis exhibits diffuse erythema of denture-covering mucosa and papillary hyperplasia.

Denture induced stomatitis mostly occurs in patients with predisposing factors, such as poor denture hygiene, immunocompromised disorder, xerostomia and age of the denture.[6] Moreover, elderly patient is also a risk

factor for denture induced stomatitis due to their reduced cell mediated immunity that may lead to fungal infection.

The use of topical drugs, such as nystatin clotrimazole, and/or systematic antifungal drugs, such as ketoconazole, itraconazole, are the treatment of choice in treating an oral fungal infection. However, the primary fungal reservoir in contaminated denture may not be eliminated. The disinfection of the fungal reservoir within denture should be performed. Several studies suggested methods of disinfection as follows;

1. Mechanical removal by daily denture brushing:[3,4] This method is easy and cheap. An effective and nonabrasive cleanser should be used with daily denture brushing. However, due to the low abrasive resistance of PMMA material, the denture brushing can create deep scratches where act as the microbial reservoirs which unable to clean. Moreover, this method need compliances especially for handicapped patient.

2. Chemical agent soaking[23]: A previous study recommended that 100% white vinegar was an effective disinfection method for acrylic resins. However, only chemical soaking may be not effective in removing the denture plaque which highly adheres and has adverse effect. For example, sodium hypochlorite soaking can cause reduction in surface hardness, flexural strength and discoloration.

3. Ultrasonic cleaner[7]: This method is reliable and simple. It is recommended to use for 15 min after daily brushing without any detergents.

4. Microwave irradiation[8]: This method is recommended using 450 Watt of power setting for 3 min in 200 mL of deionized water. The irradiation can raise up the temperature. However, the final temperature of water is 77 °C which is less than the glass transition temperature of PMMA material (Tg = 100.4 °C)

5. Incorporated antimicrobial agents into denture base material such as incorporation of metal/metal oxide into PMMA.[9-13]

Many metal/ metal oxides incorporated into PMMA material have been studied. Silver nanoparticles are of interest because of their biocompatibility and antimicrobial effect. A previous study[9] demonstrated 1 μg/mL of silver nanoparticles, calculated from the volume of methyl methacrylate monomer, incorporated in microwave-cured PMMA material reduced the amount of *C. albicans* cell (American type culture collection (ATCC) 90026). Nam et al.[13] demonstrated that 20% (w/w) of silver nanoparticles, calculated from the weight of PMMA powder, reduced the amount of *C. albicans* cell (ATCC 60026). However, the incorporation of silver nanoparticles into PMMA caused discoloration of PMMA[13] and reduction in flexural strength[9].

Zinc oxide nanoparticles and its antimicrobial effect

Zinc oxide (ZnO), a metal oxide, is a white powder that is insoluble in water and odorless. This inorganic compound is widely used in dentistry by

adding into a restorative luting material, root canal filling material and temporary restoration. This metal oxide varies in particle size ranging from microns to nanometers.[12,14,17] Many studies demonstrated the effectiveness of these particles on inhibiting the growth of fungi[11,14] and bacteria.[15,16,18,19,24] Jones et al.[25] studied the antibacterial effect of six metal oxides nanoparticles; MgO, TiO_2, CuO, CaO, CeO_2 and ZnO and found that among nanoparticles, ZnOnps showed the inhibition of growth of various microorganisms in the visible light condition. The inhibition effect depended on particle size, concentration and type of microbes. Small particle size and high concentration of ZnO resulted in better antimicrobial activity.[11,14,17,24] Cierech et al.[11] demonstrated the antifungal effect of PMMA material coated with ZnOnps onto the surface by ultrasonic method. It was claimed as a quick and effective method of treating denture stomatitis. However, it should be noted that the denture must be daily brushed which could eliminate the coating layer resulted in the reduction of antifungal effect.

Antimicrobial mechanism of ZnOnps

The mechanism of antifungal activities of ZnOnps is still ambiguous. On the one hand, the mechanism of ZnOnps was the generation of reactive oxygen species such as hydrogen peroxide, superoxide anion and hydroxyl radical in water suspension as shown in Figure 1.[14,17] The generated oxygen species could penetrate into cell membrane and caused cell death.

Furthermore, the antifungal effect of ZnOnps suspension increased when activated by both UV and visible light.[14,17,24]

$$ZnO + h\upsilon \longrightarrow e^- + h^+ ; \qquad h^+ + H_2O \longrightarrow {}^\bullet OH + H^+$$

$$e^- + O_2 \longrightarrow {}^\bullet O^-_2 \quad ; \qquad {}^\bullet O_2 + H^+ \longrightarrow HO_2{}^\bullet$$
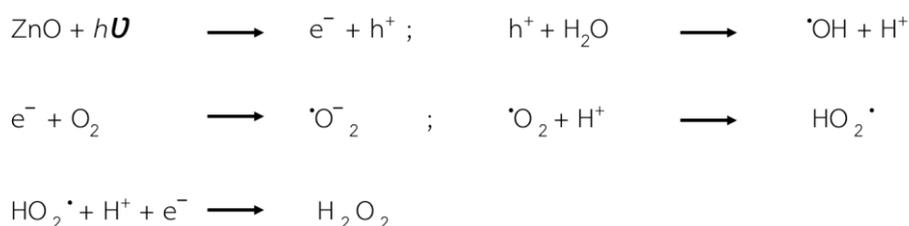
$$HO_2{}^\bullet + H^+ + e^- \longrightarrow H_2O_2$$

Figure 1 The reactive oxygen species production by zinc oxide particles.

On the other hand, the soluble Zn ion was released from the nanoparticles.[18] Then, the positive ion of Zn could interact with negative charge ion of lipopolysaccharide in cell membrane by electrostatic attraction resulting in disruption of the cell membrane. However, a previous study[17] mentioned that Zn ions at low concentration was not toxic and might be used as a supplement in promoting the metabolic action of bacteria. Raghupathi et al.[24] studied the antifungal effect of Zn ion using the atomic absorption spectrophotometry and found that the amount of Zn ions releasing from ZnOnps were very low resulted in no antimicrobial effect. They believed that the antimicrobial effect of ZnOnps is mainly from the action of ZnOnps rather than free Zn ions. They proposed two mechanisms of antimicrobial effect of ZnOnps; (1) the production of reactive oxygen species (2) deposition of the ZnOnps on the microbial surface or cumulation of ZnOnps in the cytoplasm resulted in the disruption of cellular function and disorganization of microbial cell membrane. Dutta et al.[26] also explained the deposition of the ZnOnps in the microorganisms, so called

"internalization", that ZnOnps could be internalized via the transport channel on the cell membrane. Another possible mechanism was the adherence of ZnOnps on the microbial surface resulted in physical blockages of the transport channel of the cell membrane. The transportation of cellular nutrients from media were hindered, resulted in cell starvation and cell death.

Antimicrobial methods

Minimum inhibitory concentration (MIC) is the lowest concentration which could inhibit the growth of microorganism. It could be determined by serial dilution of antimicrobial concentration such as broth dilution test, agar dilution test. Minimum fungicidal concentration (MFC) is the lowest concentration which kill the fungi. The MFC value is determined by sub culturing the last clear MIC tube onto the medium agar until no colonies were found. Actually, the MFC value is higher than the MIC value. A previous investigation[12] studied the antifungal effect of ZnOnps (averaged particle size of 26nm) against *C. albicans* 14053 and reported that the MIC value was established at 0.75 mg/mL level. Lipovsky et al.[14] also reported that the ZnOnps concentration of 1 mg/mL exhibited the 99.5% reduction of *C. albicans* cell which is considered almost complete killing.

The agar diffusion or disc diffusion test is an antimicrobial method. The principle of this method is to place sample discs containing an antimicrobial on an agar plate which microbes were spread on. Then, the agar plate is

incubated. If an antibiotic can stop the bacteria from growing or kill the microbes, the halo, so-called a zone of inhibition (area of no microbes or no growth), around the sample will appear. A high antimicrobial effect results in a large inhibition zone while small zone means low antimicrobial effect.

Sevinc et al.[15] investigated the antibacterial effect of composite resin containing ZnOnps by disc diffusion test. The results indicated that no inhibition zones around the sample. They explained that insolubility of ZnOnps prevented the diffusion of a sufficient amount of Zn ion into the surrounding medium agar to express antibacterial effect. Hojati et al.[19] also studied the antimicrobial effect of ZnOnps. They mentioned that the results of disc diffusion test were in agreement with Sevinc et al.[15] as no inhibition zone was formed around the test specimens. They stated that the disc diffusion test and MIC were the important conventional methods for determining the antimicrobial effect of material. Mostly, these methods used to investigate the effect of antibiotics which was based on water-soluble antimicrobial substances. However, these methods might not be valid for ZnOnps testing because of its low solubility.

The direct contact test is a method for evaluating the solid material that has low solubility. The principle of this method is the measurement the antimicrobial effect in close contact between microbes and the tested sample. The small amount of microbial suspension was placed onto the sample surface and allowed the suspension to evaporate resulted in the decrease of suspension volume. The microbes were forced to be

approximate with the sample surface, which has an antimicrobial agent, resulted in either the growth inhibition or cell death.

Mechanical properties of PMMA material

The flexural strength (FS) and flexural modulus (FM) of heat-polymerizable material must not be lower than 65 MPa and 2 GPa, respectively according to ISO specification 20795-1.[27] A three-point bending test was used to evaluate FS and FM of the bar-shaped specimens (64×10×3.3 mm). A universal testing machine used at a crosshead speed of 5 mm/min with a support span width of 50 mm. FS and FM were calculated according to the following equations:

$$FS = 3FL/2BH^2$$

$$FM = F_1L^3/4BH^3D$$

where, F = the maximum load (N), F_1 = the load at a point in straight line portion of the load/defection curve (N), L = the support span width (mm), B = the width of the specimen (mm), H = the thickness of the specimen (mm), D = the deflection at load F_1 (mm).

Some studies examined the different amounts of ZnOnps that affected the mechanical properties of composite resin materials by mixing ZnOnps without any surface modification directly into the composite resins.[15,16,19] To date, there is no study about mechanical properties of denture base material incorporated with ZnOnps. The ZnOnps exposed on

the surface of the polymer might be hydrolyzed because of no chemical bond between resin matrix and metal nanoparticles. As a result, their antimicrobial activities are lost. Furthermore, nano-sized particles with a high surface energy easily aggregate among each other. Particle aggregation generates weak points within the material resulted in low mechanical properties. However, surface modification of these particles prevents the formation of aggregated nanoparticles.[20,21] Xia et al.[22] demonstrated that the FS and microhardness of composite resin containing modified TiO_2 nanoparticles with allyltriethoxysilane was greater than that of unmodified TiO_2 nanoparticles due to the better dispersion in the matrix and smaller clusters.

Silane coupling agent and Silanization

Silane coupling agents create a bond between organic and inorganic materials. These agents consist of two terminal functional groups; organofunctional groups, which establish bond with organic resin, and hydrolysable groups, which create bond with inorganic materials. The typical chemical structure of silane coupling agent is shown in Figure 2. A previous study[28] reported that only small amount of silane coupling agent could provide better mechanical properties and durability of composite resin after 28 days of aging.
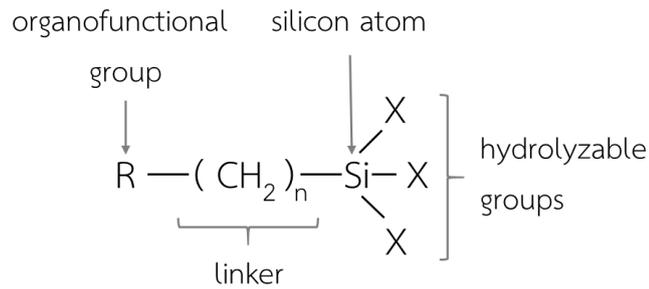
Figure 2 The chemical structure of silane coupling agent

A mechanism of silanization divided into two main processes[29] as shown in Figure 3. Hydrolysis process begins with the moisture or solvent, for examples water, ethanol, acetone, hydrolyzes the alkoxy group of the silane molecule and results in silanol group. In the closed system, the silanol group is reversible between attaching to and releasing from the hydroxyl group on the filler surface via hydrogen bond. This mechanism is a dynamic and reversible process. Liu et al.[30] studied the result of thermogravimetric analysis on silica particles, which were immediately rinsed with methanol after hydrolysis, and found that no weight loss from deposited silane molecules. It indicated that the silane molecule were physically adsorbed onto the silica surface and were completely rinsed off with methanol. In the open system, loss of water molecules occurs by increasing temperature or vacuum. The condensation process gradually rises and causes the less intermolecular distance. Finally, the silane molecule is absorbed onto the filler via covalent bond between the silanol group of silane molecules and the hydroxyl group of filler.
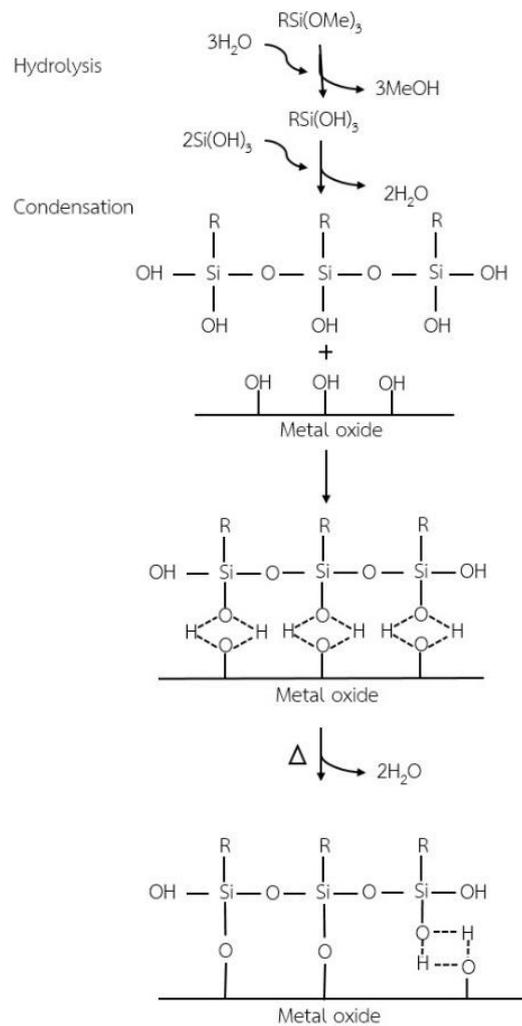
Figure 3 The silane molecules deposited onto the metal oxide surface

The first layer of silane molecule attached on the filler surface via covalent bond is called "Chemisorbed silane". While, the outer silane layer which bonds onto the first layer via Van der Waals force or hydrogen bond is called "Physisorbed silane". Moreover, silane molecules, especially in high concentration, can create hydrogen bond among each other so called "homocondensated silane".

Factors related to silanization

1. Type of silane coupling agent

Silane coupling agents are diversity depended on the functional group such as vinyl groups, methacryloxy groups, and amino group, etc. A previous study[31] demonstrated the different silane coupling agents and solvents affected the shear bond strength of PMMA bonding to alumina. They compared 3 types of the silane coupling agent namely 3-methacryloxypropyltrimethoxysilane (MPS), 3-aminopropyltriethoxysilane (APS) and N-2 (aminoethyl) 3-aminopropyltriethoxysilane (AAPS) and 3 types of solvent namely ethanol, toluene and isopropanol. They found that MPS and APS in ethanol groups provided the highest bond strength. AAPS has longer linker length than APS and higher MW than MPS and APS. They assumed that the longer silane molecules could not be effectively deposited onto the alumina filler due to the steric hindrance effect.

Linker length is a backbone in the silane structure which links between the hydrolysable group and organofunctional group. If the linker length is long, the organofunctional group can extend from the substrate surface resulted in greater mobility and steric hindrance effect. The typical linker length should be less than three carbon atoms.

3-methacryloxypropyltrimethoxysilane (MPS) is a common silane coupling agent used in dentistry due to its small molecular structure which cause less steric hindrance. The chemical structure is shown in Figure 4.
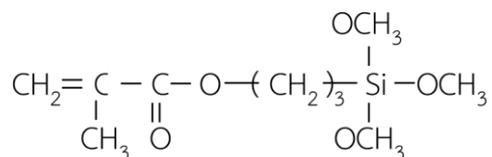
$$CH_2= C - C - O - (CH_2)_3 - Si - OCH_3$$

with CH_3, O below the chain and OCH_3 above and below the Si.

Figure 4 The chemical structure of 3-methacryloxypropyltrimethoxysilane

2. The amount of silane coupling agent

Nishiyama et al.[32] studied the amount of silane deposited on silica filler after drying at room temperature after 14 days and indicated that with lesser amount of monolayer-coverage, all silane molecules were deposited onto the silica filler as chemisorbed silane. The amount of physisorbed silane could not be observed from GPC chromatograms. The FTIR result at low concentration of MPS indicated the absorbance peak of carbonyl group (C=O) bond at 1706 cm^{-1} which corresponding to hydrogen bonded C=O group. However, at high concentration of silane coupling agent and when chemisorbed silane was saturated, the remaining silane molecules could deposit on the first layer of chemisorbed silane; physisorbed silane. The physisorbed silane could then be observed. By increasing the amount of silane, the high MW of physisorbed silane was formed as oligomer. The FTIR result at high concentration of MPS indicated the new absorbance peak of C=O bond at 1726 cm^{-1} which corresponding to free C=O group.

Arksornnukit et al.[28] studied the effect of the amount of MPS deposited on spherical-shaped silica filler (1.5 μm of averaged particle size), which incorporated in composite resin, on flexural properties after 28 days-

aging. By varying the amount of MPS from 0 to 5.45 mass% which exceeded the monolayer coverage (0.66 mass%), they denoted that the amount of MPS has a slight effect on the FS and hydrolytic durability. Moreover, they also mentioned that the existence of physisorbed silane did not affect the hydrolytic stability of the composite resin.

Sideridou et al.[33] studied the flexural properties of composite resin containing with 5 different amounts of MPS, 1-10 wt% deposited onto the surface of silica filler. The averaged silica particle size was 40 nm. They found that FS showed insignificant differences among groups.

Chaijareenont et al.[34] studied the effect of the amount of MPS (0-0.4%) deposited on spherical-shaped alumina particles (18-23 μm of particle size) on flexural properties. They advocated that 0.1 mass% of MPS was expected to form a monolayer of MPS on the alumina particle surface base on the calculation from Arkle's equation[35]. The silanized alumina particles were incorporated in PMMA material. They demonstrated that FS of PMMA material incorporated with 0.1-0.4% MPS showed insignificant difference.

Kanie et al.[36] studied the flexural properties of PMMA material containing various concentration of MPS without filler particle incorporation and reported that there was no difference among FS and FM in PMMA material that containing MPS from 0 to 6 mol%. This attributed to the molecular chain of MPS which is longer than that of methyl methacrylate monomer (MMA). Consequently, the PMMA material containing MPS might

be more flexible than that containing pure MMA due to increasing of specific volume.

3. Type of solvent

Many solvents were used in silanization such as methanol, ethanol, isopropanol, acetone, and water, etc. Liu et al.[30] indicated that silanization of silica particle using toluene was quite difficult to handle because silica particle mixed with toluene formed gel-like mixture and required high temperature (60 °C) while stirring. They also studied the amount of MPS deposited on the silica surface with different solvent using thermogravimetric analysis. They found that the total amount of deposited silane in acetone was greater than that of 95% aqueous ethanol. However, after methanol washing, the amount of attached silane (chemisorbed silane) in aqueous ethanol was greater than that of acetone.

4. Water content in silane solution

Water for hydrolysis can come from many sources such as by adding, on the substrate surface, and atmosphere. Mostly, adding the water in silane solution play an important role in the hydrolysis process. The hydrolysis rate of the hydrolysable group in the silane molecule is controlled by the amount of water in the silane solution. A previous study[37] investigated the characteristics of chemisorbed and physisorbed silane which deposited on silica filler in one day in terms of the amount of water content in silane solution. They demonstrated that the amount of water content should be slightly less than or equivalent to 30 volume%, calculated from ratio

between water and ethanol in silane solution. The amount of chemisorbed silane increased by increasing the amount of water content up to 30% and then remained unchanged even though increasing the water content over 30%. Mostly, low MW of physisorbed silane occurred when water content was less than 30%. When, the amount of water content exceeded 30%, silane molecules was rapidly hydrolyzed. The amount of chemisorbed silane adsorbed onto the filler surface was limited. Thus, the remaining hydrolyzed silane molecules could homo-condensate and became high molecular weight (MW) physisorbed silanes such as tetramer as observed by gel permeation chromatography (GPC) chromatograms. The high MW of physisorbed silane can cause the degradation of silane bi-layer (chemisorbed and physisorbed silane) leading to the low hydrolytic stability because high MW silane has less silanol groups resulted in low probability to form bond. This decrease the cohesional force between silane bi-layer.

5. pH of silane solution

In the solution system, the acidic condition accelerated the hydrolysis process and decelerated the intermolecular condensation by stabilizing the Si-OH groups. This resulted in low MW silane species which were chemisorbed onto the filler surface.[32] However, the deposition behavior was quite different between the solution system and the deposited system. In the deposited system, an acid hastened the hydrolysis mechanism of the hydrolyzable group in the silane molecule. In the meantime, the solvent of silane solution such as ethanol was evaporated resulted in the pH changes

and less intermolecular distance. The hydrolyzed silane molecules could self-condense and form the high MW silane species. Therefore, the MW of the silane molecules under acidic condition was higher than under non-acidic conditions. When condensation mechanism began, in acidic condition, high MW silane molecule such as tetramer species was probably chemisorbed on the filler surface and hindered the deposition of other molecules on the filler surface resulted in a low amount of chemisorbed silane. While in non-acidic condition, monomer and dimer was adsorbed onto the filler surface resulted in a high amount of chemisorbed silane. Within the same amount of silane molecules, the remaining silane molecules or physisorbed silane under acidic condition was greater than under non-acidic. The large amount of physisorbed silane could homocondense and form the multilayer silane which led to low hydrolytic stability. Therefore, the addition of hydrochloric acid to the silane solution lower the hydrolytic stability of the silane bi-layer in the silane deposited system.[38] Furthermore, cross-polarization/ magic-angle spinning [29]silicone nuclear magnetic resonance (CP/MAS ^{29}Si NMR) result demonstrated that most of the chemisorbed silane under acidic condition was vastly hydrolyzed until no silanol group were left which leading to less chance to create hydrogen bonding with physisorbed silane resulted in low hydrolytic stability. The same investigators also studied the tensile bond strength of resin on the silica plate before and after degradation in hot water (90 °C). Before degradation, tensile strength of both condition increased with increasing the amount of MPS. However, after degradation, tensile strength in acidic condition was less than that in non-acidic condition.

They concluded that the hydrolytic stability of the silane multilayer (chemisorbed and physisorbed silane) depended on the adsorption characteristics of the silane molecules. Nakornchai et al.[39] studied the pH effect of solvent on flexural properties of heat-cured acrylic resin containing silanized fluoride-releasing filler and reported that the FS of non-adjusted pH was greater than that of acidic-adjusted pH group.

In contrast, a previous study[40] investigated the different pH of silanization (pH 1, 4, and 6) and reported that there was no effect of pH on hydrolytic durability of composite in terms of flexural properties. The different result might be explained by the different silica particle size and evaluation method.

6. Drying temperature

Liu et al.[30] stated that the drying process promoted the formation of covalent bonding between either silane/silane or silane/filler. A previous study[37] focused on the effect of drying temperature (heat treatment) in condensation mechanism. They found that by increasing the drying temperature from 25 up to 80 °C, the MW of physisorbed silane increased due to the homocondensation, but the amount of chemisorbed silane was normally steady. However, when the drying temperature was raised up to 110 °C, the amount of chemisorbed silane is dramatically increased. This phenomenon is explained by the high MW of physisorbed silane was condensed and turned to chemisorbed layer resulted in the occurrence of multilayer of chemisorbed which was easily degraded and resulted in low

hydrolytic stability. Moreover, chemisorbed silane after heating up to 100 ºC was very stable by tri-dentate bonding and no silanol group available to create hydrogen bond with silanol group of the physisorbed silane as shown by CP/MAS ^{29}Si NMR result. This resulted in low cohesional force which mostly was Van der Waals attraction force. It demonstrated in low tensile strength. This report was also in line with Arksornnukit et al.[40] that by heating up to 110 ºC in drying process might promote the chemisorbed silane, the condensation of the silane molecules and also polymerization of the vinyl group (organofunctional group, C=C) which led to the reduction of FS. The scanning electron microscope (SEM) image of the fracture surface of 110 ºC demonstrated the obviously poor bonding between filler and resin matrix.

7. Drying time

Nishiyama et al.[32] studied the drying time of MPS deposited on silica particle at room temperature and found that the amount of chemisorbed silane deposited on the silica surface dramatically increased within 1 day and continued gradually increased until 14 days and then reached the plateaus. With the increased of the drying time, the amount of physisorbed silane decreased while the proportional of high MW of physisorbed silane molecules increased.

8. Type of filler

Ishida and Miller[41] demonstrated that filler particles have unique surface acid/base functionalities. For example, the clay sample has an acidic surface at pH 4.1, zinc oxide sample has a neutral surface at pH 7.4, and the calcium carbonate sample has a basic surface at pH 9.4. They concluded from the FTIR data that the neutral-surfaced substrate has high retention of MPS after washing the substrate with tetrahydrofuran which indicating a high amount of chemisorbed silane. While, the acidic and basic-surfaced substrate has less amount of chemisorbed silane. Even though, the same type of filler can have different aqueous slurry pH values and the amount of hydroxyl group on the surface, depending on the nature of production techniques.

Bressy et al.[20] studied the amount of hydroxyl group on the ZnOnps surfaces by the calculation of percent weight loss from thermogravimetric analyzer. They compared the results between commercially ZnOnps and the synthesized ZnOnps and found that the amount of hydroxyl group of the synthesized ZnOnps was greater than that of the commercially ZnOnps. The explanation of the low amount of hydroxyl group was that the commercially ZnOnps are produced at high temperature in synthetic process, which the annealing treatment could reduce the defects of the crystalline structure of oxides leading to a decrease of hydroxyl group concentration. The amount of hydroxyl groups on the filler surface have an influence on the amount of deposited silane. By increasing the amount of hydroxyl group, the probability of creating bond between the hydrolyzed silane molecules and hydroxyl

group of filler particles increased. The MPS deposited onto the synthesis ZnOnps was preferentially perpendicularly oriented while the MPS deposited onto the commercially ZnOnps was parallelly oriented.

Karabela et al.[42] studied the amount of MPS deposited on silica filler with different averaged particle size of fumed amorphous silica, ranged from 7 to 40 nm and indicated that with decreasing the silica particle size resulted in an increase of the amount of MPS deposited on filler surface.

Characteristics instruments

1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a method to characterize the chemical structure of the substance. It demonstrates specific bands which result from molecular vibration when the substance absorbs an IR radiation. The principle of FTIR is that a bond of each chemical structure or functional group have different frequencies of molecular vibration.

FTIR have many handling techniques such as transmission (conventional), diffuse reflectance (DRIFTS), etc. Attenuated total reflectance (ATR) is one of the handling techniques. The advantages of this techniques are minimal sample preparation which no need to grind or press into pellets, fast and easy for cleaning after measurement. The ATR technique works by measuring the changes of an internally reflectance infrared (IR) beam. When IR beam is emitted from the IR source and passed through the crystal which

contact to the sample, the sample absorbs some energy. The internally reflectance IR beam is attenuated and exits the opposite site of the crystal and the detector in the spectrometer. The detector will translate the signal of attenuated IR beam into an IR spectral band.

The MPS deposited on oxide surface was studied by FTIR spectroscopy as shown in Figure 5. The absorption bands around 498, 1110, 1634, 2800-3000 cm^{-1} represented the functional group of Zn-O, Si-O-Si, C=C, hydrocarbon chain, respectively.
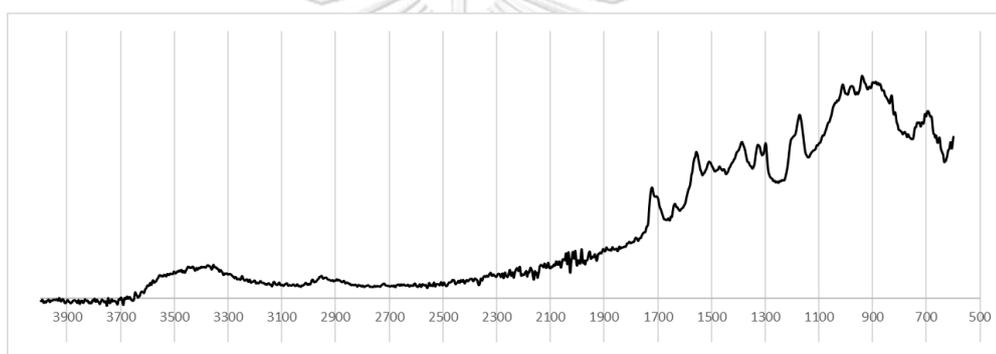

Figure 5 The spectrum of MPS deposited ZnOnps

Two important absorption bands in the MPS study should be notified.[20,30,33,43,44] The band at 1700-1702 cm^{-1} represents the hydrogen bonded C=O group while another at 1718-1720 cm^{-1} represents the free C=O group. In monolayer silane absorbed onto filler, two orientations of silane molecules was proposed. The perpendicular orientation occurs at high concentration of MPS absorbed on the filler surface which only silanol group of MPS created bond with hydroxyl group on the filler surface. The C=O of MPS that does not create bond with hydroxyl group is called "free C=O

bond" as shown in Figure 6A. While, the parallel orientation mostly occurs at low concentration of MPS absorbed on the filler surface which silanol group and C=O of MPS created bond with hydroxyl group on the filler surface. This C=O created bond is known by "Hydrogen bonded C=O bond" as shown in Figure 6B. Therefore, FTIR can be used to explain the orientation of the MPS molecule on the filler surface. However, in terms of multilayer silane, both absorption band can be observed as shown in Figure 6C. Miller and Ishida[43] demonstrated that the surface area occupied by a silane molecule is 0.55 nm^2, corresponding to 1.8 molecules MPS/nm^2 when MPS parallelly oriented on the filler surface. While, MPS perpendicularly oriented on the filler surface, it occupied less area, 0.24 nm^2/molecule, corresponding to 4.16 molecules MPS/nm^2.
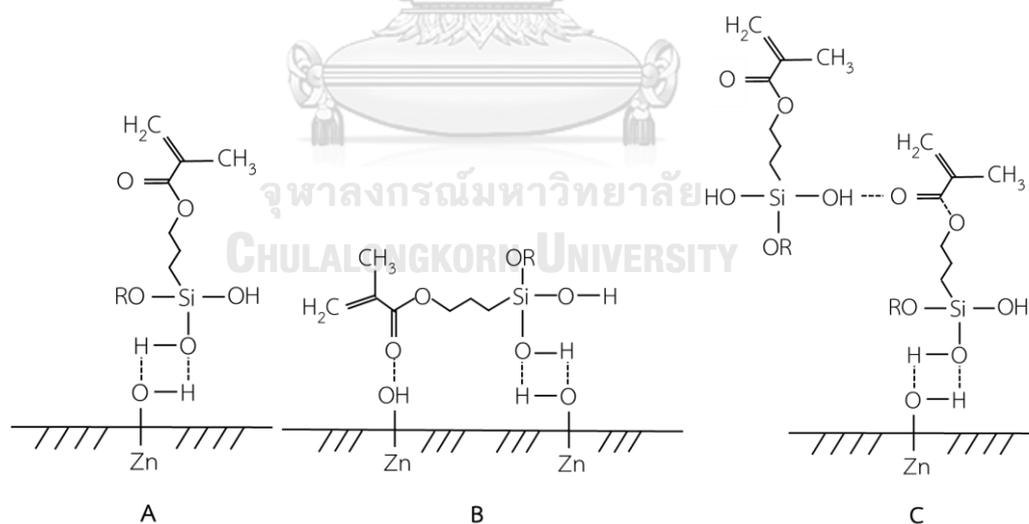

Figure 6 The characteristics of deposited silane molecule onto the zinc oxide surface. A. free C=O bond found in perpendicular orientation of deposited silane, B. hydrogen bonded C=O bond found in parallel orientation of deposited silane, C. multilayer of silane molecules

2. Thermogravimetric Analysis (TGA)

The principle of TGA is that a measurement of the weight change of a sample using a programmed precision balancer when increasing temperature with time in a furnace, under a controlled environment such as heating rate, gas atmosphere, and flow rate. The weight change is the decomposition of the sample. The results are displayed by a thermogravimetric (TG) curve which is normally plotted between the percent of weight change on the Y-axis and temperature or time on the X-axis as shown in Figure 7.
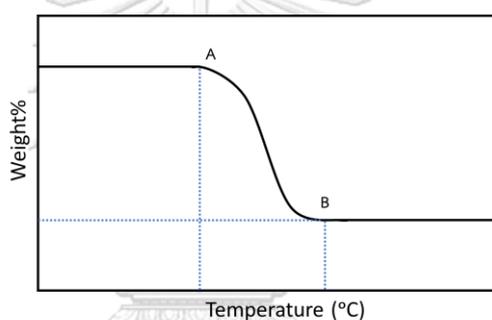

Figure 7 A thermogravimetric (TG) curve; A. represents the initial temperature that a sample starts decomposition which the thermobalance can detect the weight change, B. represents the final temperature at which the weight change reaches a maximum.

A previous study[20] investigated the MPS deposited onto the ZnO particles and claimed that the thermal degradation of the MPS could divided into two main steps of weight loss. The first step was physisorbed silane which generally removing within a temperature range of 30-250 °C. The second step was chemisorbed silane which is removed in higher temperature

range (250-550ºC). Furthermore, the homocondensated silane could also be decomposed at temperature of 420-430 ºC.

Optical evaluation

Many methods have been introduced to evaluate the color characteristics of a material. Visual measurement using shade tab in Munsell color system is convenience and low cost.[45] However, this method is subjective, depending on various factors such as the mood and experiences of the observer, and illumination. A spectrophotometer is a reliable method to quantify the color characteristics of a material. The most commonly utilized color system for color assessment is the CIELAB system, which defines the color characteristics as L*, a* and b*.[46-53] L* represents brightness, a* represents the red-green coordinates and b* represents the yellow-blue coordinates. The CIELAB system can mathematically express the color difference (ΔE) between the two objects. The following equations were used:[54]

$$\Delta E = [(L^*_1 - L^*_2)2 + (a^*_1 - a^*_2)2 + (b^*_1 - b^*_2)^2]^{1/2}$$

Where, L^*_1, a^*_1, b^*_1 = the mean value of the first group, L^*_2, a^*_2, b^*_2 = the mean value of the second group.

Opacity is another optical property of a material, representing the ability of a material to block the passage of light.[46] Opacity is based on the

ratio of the reflectance of the material when backed by a black background compared with a white background. The following equations were used:[54]

$$Opacity = (Y_1/Y_2) \times 100$$

Where, Y_1 = brightness of specimen backed by black background, Y_2 = brightness of specimen backed by white background.

According to an increased demand in esthetic, especially color appearance, researchers have attempted to find a threshold to determine a color difference for standardize in dental field. Mostly, thresholds of visual color differences were developed from evaluations of the color of denture teeth[47,48] or ceramic specimens within the tooth color range[52]. The threshold presented as 50/50% perceptibility and 50/50% acceptability. The 50/50% perceptibility threshold, which 50% of observers could detect a difference in color between two objects, was 1.2[52], 1.9[47], and 2.6[48]. While 50/50% acceptability threshold, which 50% of observers accept the difference of the color into the mouth, was 2.7[52], 4.2[47], and 5.5[48]. However, the color parameters especially L*, between tooth and gingiva might not correlate[25]. The same study demonstrated that the color of gingiva depended on type (attached gingiva or alveolar mucosa) and location in the mouth. A previous study demonstrated the threshold for detecting of gingival color differences (ΔE =3.1±1.5)[53]. A previous study[49] measured the L*, a* and b* of healthy human gingiva and reported that most subjects had L* ranged from 50.6 to 57.2, a* ranged from 22.6 to 27 and b* ranged from 12.4 to 15.6.

Chapter II

Research objectives, Research hypotheses, Expected benefits and Limitations

Research objectives and research hypotheses;

1. To find the amount of non-silanized or silanized ZnOnps incorporated in PMMA material which provided antifungal effect

First hypothesis

$H1_0$: There would be no significant differences on the antifungal effect among groups (PMMA incorporated with different amounts of ZnOnps with or without silanization).

$H1_a$: There would be significant differences on the antifungal effect among groups (PMMA incorporated with different amounts of ZnOnps with or without silanization).

2. To find the optimum amount of MPS deposited on ZnOnps incorporated in PMMA material which provided better mechanical properties

Second hypothesis

$H2_0$: There would be no significant differences on the mechanical properties among groups (PMMA incorporated with different amount of MPS deposited on ZnOnps).

$H2_a$: There would be significant differences on the mechanical properties among groups (PMMA incorporated with different amount of MPS deposited on ZnOnps).

3. To find the optimum amount of non-silanized or silanized ZnOnps incorporated in PMMA material which provided antifungal effect and maintaining optical and mechanical properties

Third hypothesis

$H3_0$: There would be no significant differences on the optical characteristics among groups (PMMA incorporated with different amounts of ZnOnps with or without silanization).

$H3_a$: There would be significant differences on the optical characteristics among groups (PMMA incorporated with different amounts of ZnOnps with or without silanization).

Forth hypothesis

$H4_0$: There would be no significant differences on the mechanical properties among groups (PMMA incorporated with different amounts of ZnOnps with or without silanization) between storage times (48 h and 1 month).

$H4_a$: There would be significant differences on the mechanical properties among groups (PMMA incorporated with different amounts of ZnOnps with or without silanization) between storage times (48 h and 1 month).

Expected benefits

This research is the frontier in utilizing silanized ZnOnps in PMMA material to accomplish new denture base material. This modified material has an antifungal effect which might be use in treating fungal infection in denture wearers. By adding the particles, the material should maintain its optical and mechanical properties. The insight investigation of MPS deposited ZnOnps might be applied to other restorative materials. This also reflects the new knowledge in silane coupling agent.

Limitations

This experimental design limited only in vitro and a short-term evaluation. The long-term evaluation of the antifungal effect of PMMA material incorporated with silanized ZnOnps both in vitro and in vivo is recommended.

Chapter III

The optimum amount of MPS deposited on ZnOnps which showed better mechanical properties

Introduction

ZnO is a water insoluble white powder. The addition into the material is to enhance the material properties, especially antimicrobial effect. ZnOnps becomes popular as the additive in the material due to its high antimicrobial effect. However, the nano-sized particle which is easy to aggregate among each other becomes the weak point in the material. Surface modification of ZnOnps by silanization is necessary to increase the distance between ZnOnps to prevent aggregation which might enhance mechanical properties. In addition, this perhaps renders in the better antimicrobial material.

Many factors influence the silanization such as type, amount of silane coupling agent, pH, temperature during silanization, etc. These factors lead to different characteristics of the deposited silane molecule on the oxide surface and affect the mechanical properties. An excess amount of silane coupling agent over monolayer coverage results in the physisorbed silane deposited over the chemisorbed silane. It is believed that physisorbed silane which create a weak bond with chemisorbed silane might responsible to the hydrolytic instability of the material. Thus, the optimum amount of silane molecules should be used to enhance the mechanical properties of material. Moreover, FTIR spectroscopy and TGA instrument were used, to understand the characteristics of the deposited silane molecules.

Therefore, the objectives of the present study were to find the optimum amount of MPS deposited on ZnOnps which showed better mechanical properties and to understand the characteristics of MPS deposited on the ZnOnps surface by FTIR spectroscopy and TGA instrument. The null hypothesis was that there would be no significant differences on the mechanical properties among groups (PMMA incorporated with different amount of MPS deposited on ZnOnps).

Materials and methods

Silanization of ZnOnps

Table 1 Materials used and their compositions

Material/Product name	Manufacturer	Batch Number	Composition
Zinc oxide nanoparticles/ ZoNoP	Nano Materials Technology Co., Ltd. Chonburi, Thailand	190411	Zinc oxide: (purity > 99.5%, average particles size 20-40 nm, specific surface area 26.22 m^2/g)
Heat-cured denture base material/ SR Triplex Hot	Ivoclar Vivadent, Schaan, Liechtenstein	R84665	Powder: Polymethylmethacrylate Liquid: Methyl methacrylate, Ethylene glycol dimethacrylate
Silane coupling agent/ 3- trimethoxysilyl propyl methacrylate	Sigma-Aldrich, St. Louis, MO, USA	SHBD3265V	Methacryloxypropyltrimethoxysilane (MPS)

The materials used and their compositions in this study are listed in Table 1. The amount of MPS, varied from 1.04 (x/8), 2.06 (x/4), 4.03 (x/2), 7.75 (1x) and 14.4 (2x) weight%, were calculated from Arkle's equations[35] as demonstrated below:

The amount of MPS = (the amount of ZnOnps x specific surface area of ZnOnps)/ specific surface area of MPS

The specific surface area of MPS = 314 m^2/g

The solution consisting of different amount of MPS and 70 volume% ethanol, was magnetically-stirred for 60 min. ZnOnps, without any purification, were dispersed into the solution and manually triturated using mortar and pestle until the solution was almost completely evaporated. The silanized ZnOnps were then left to dry at room temperature for 14 days before use.
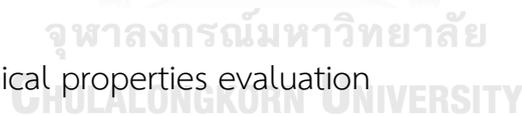
Part 1.1: Mechanical properties evaluation

Specimen preparation

Non-silanized (Nosi) or 1.04, 2.06, 4.03, 7.75, 14.4 wt%-silanized (Si) ZnOnps of 2.5 wt%, calculated from the weight of polymethylmethacrylate (PMMA) powder, were homogeneously mixed with liquid monomer using a vortex mixer (VTX-3000L, LMS, Tokyo, Japan) for 10 min. A mixture of pure PMMA powder and liquid monomer without ZnOnps served as the control group. Fifty-six bar-shaped (64×10×3.3 mm) specimens (n=8) were fabricated.

Polyvinylsiloxane material was injected into the molds to prepare silicone patterns. The patterns were invested in flasks with dental stone. After the dental stone set, the flasks were opened and the silicone patterns were removed. The PMMA powder and liquid monomer suspension (with and without silanized ZnOnps) were mixed at a powder/liquid ratio of 23.4 g:10 mL and left until the mixture reached the dough stage. The PMMA powder and liquid monomer without additives were mixed according to the recommendation of manufacturer and used as control. The mixture was then placed into the flask and hydraulic-pressed using a conventional method. The flasks were placed in a water bath, heated up to 100 °C and let boil for 45 min to achieve polymerization according to the recommendations. After cooling to room temperature, the specimens were deflasked, the excess material was removed using a carbide bur in a low-speed rotary instrument and wet-polished using 320 and 600-grit silicon carbide paper with a polishing machine (Nano 2000, Pace Technologies, Tucson, AZ, USA). The dimensions of the specimens were measured using a digital micrometer (minimum reading: 0.001 mm, Digimatic Micrometer, Mitutoyo Corp., Kanagawa, Japan). The specimens were ultrasonically cleaned for 5 min to remove any debris and dried with compressed air.

A three-point bending test was used to evaluate FS and FM of the bar-shaped specimens according to ISO 20795-1.[27] A universal testing machine (EZ-SX, Shimadzu, Kyoto, Japan) was used at a crosshead speed of 5 mm/min

with a support span width of 50 mm. FS and FM were calculated according to the following equations:

$$FS = 3FL/2BH^2$$

$$FM = F_1L^3/4BH^3D$$

where, F = the maximum load (N), F_1 = the load at a point in straight line portion of the load/defection curve (N), L = the support span width (mm), B = the width of the specimen (mm), H = the thickness of the specimen (mm), D = the deflection at load F_1 (mm).

Statistical analysis

The FS and FM were separately analyzed by one-way ANOVA with groups (control, Nosi, 1.04, 2.06, 4.03, 7.75, and 14.4) at $P<0.05$. Tukey HSD's comparison test was used when homogeneity of variance was observed while Games-Howell comparison test was used when homogeneity of variance was rejected.

Part 1.2: The characteristics of silanized ZnOnps

The characteristics of silanized ZnOnps were studied. After 14-day storage time, 0.4 g of each ZnOnps groups (1.04, 2.06, 4.03, 7.75, and 14.4) was placed in two 1.5-mL micro-centrifuge tubes. The Nosi groups was used as baseline. The first tube of each group was labelled as "dried" groups. The second tube of each group, one milliliters tetrahydrofuran (THF) was added

and vortexed for 1 min. THF was selected because the Hildebrand solubility parameter of THF[55] is 9.52 $cal^{1/2}\ cm^{-3/2}$ which was close to MPS (8.66)[56]. It is also easy to evaporate at room temperature. The suspension was centrifuged using high speed centrifuge machine (6K15, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 10,000 rpm for 10 min. Then, the supernatant solution was discarded into quartz cuvette to measure an absorbance peak at a wavelength of 250 nm using UV-VIS spectrophotometer (Nicolet Evoluation 500, Thermo Electron Corp., Madison, WI, USA). This procedure was repeated until no absorbance peak was detected to ensure complete removal of physisorbed silane deposited on the ZnOnps. After the last decant, ZnOnps after THF washing was left to dry at room temperature for 1 day. The second tube was labeled as "washed" groups. All tubes were investigated to observe the characteristics of MPS that deposited on ZnOnps using the instruments as follows;

Fourier Transform Infrared Spectroscopy (FTIR)

An alpha ATR-FTIR spectroscopy (Equinox 170, Bruker Optik GmbH, Ettlingen, Germany) was used to understand chemical structure of the ZnOnps. The data were recorded in the spectral range from 500 to 4000 cm^{-1}, with 64 scans and a resolution of 4 cm^{-1} in absorbance mode.

Thermogravimetric Analysis (TGA)

A thermogravimetric analyzer (TG 209 F3 Tarsus, NETZSCH Gerätebau GmbH, Selb, Germany) was used to measure the weight loss (wt%) of MPS that deposited on ZnOnps. The data were recorded in the temperature range between 35-700 °C with a heating rate of 10 °C/min and nitrogen flux of 100 mL/min.

Result

One-way ANOVA of the flexural strength data demonstrated significant differences among experimental groups (P=0.003) while that of the flexural modulus results indicated insignificant differences among experimental groups (P=0.094). The flexural strength of Nosi group shows a significant lower than that of the control group. While, the flexural strength of Si groups show insignificant differences when compared with the control group (Table 2).

Table 2 Mean (Standard deviation) of flexural strength and flexural modulus of each experimental group.

Group	Flexural strength	Flexural modulus
Control	91.93 (3.29)[b]	2.41 (0.07)[a]
Nosi	80.21 (2.84)[a]	2.38 (0.06)[a]
1.04	85.23 (2.06)[a,b]	2.39 (0.04)[a]
2.06	85.48 (5.06)[a,b]	2.35 (0.11)[a]
4.03	85.16 (5.96)[a,b]	2.31 (0.10)[a]
7.75	86.10 (6.34)[a,b]	2.43 (0.06)[a]
14.4	84.69 (6.58)[a,b]	2.36 (0.08)[a]

Note: The value with the same superscript letters in the same column were not significantly different ($P<0.05$).

The FTIR spectra of dried and washed groups are shown in Figure 8-11. The absorption band at 1110 and 2800-3000 cm^{-1} of dried groups (Figure 8) increased with increasing the MPS amount. In contrast, the absorption band at 1110 and 2800-3000 cm^{-1} of washed groups (Figure 9) increased with increasing the MPS amount until 2.06 wt% of MPS and then remained constant. Figure 10 and 11 shows the enlarged section of FTIR spectra of dried and washed groups in the spectral range of 1600-1800 cm^{-1}. The single absorption bands at 1702 cm^{-1} was observed at 1.04 wt% of MPS amount. The absorption band at 1634 and 1720 cm^{-1} was observed at higher MPS

amount than 1.04 wt%. In dried groups, the band at 1634 and 1720 cm^{-1} increased with increasing the MPS amount, while, the band at 1634 and 1720 cm^{-1} in washed groups remained no change.
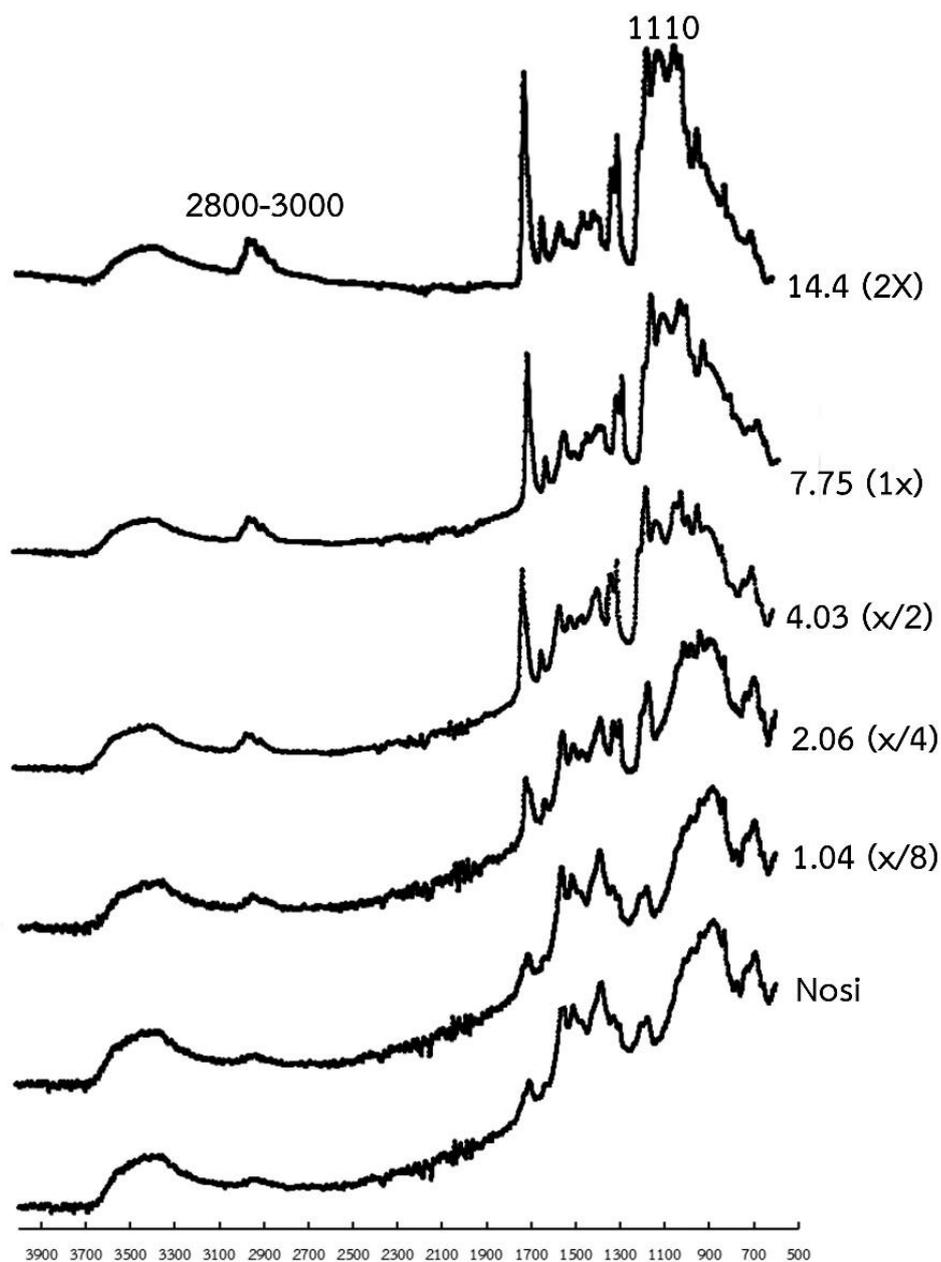

Figure 8 The FTIR spectra (range from 500-4000 cm^{-1}) of different amount of MPS (0-14.4 wt%) which deposited on ZnOnps in dried groups.
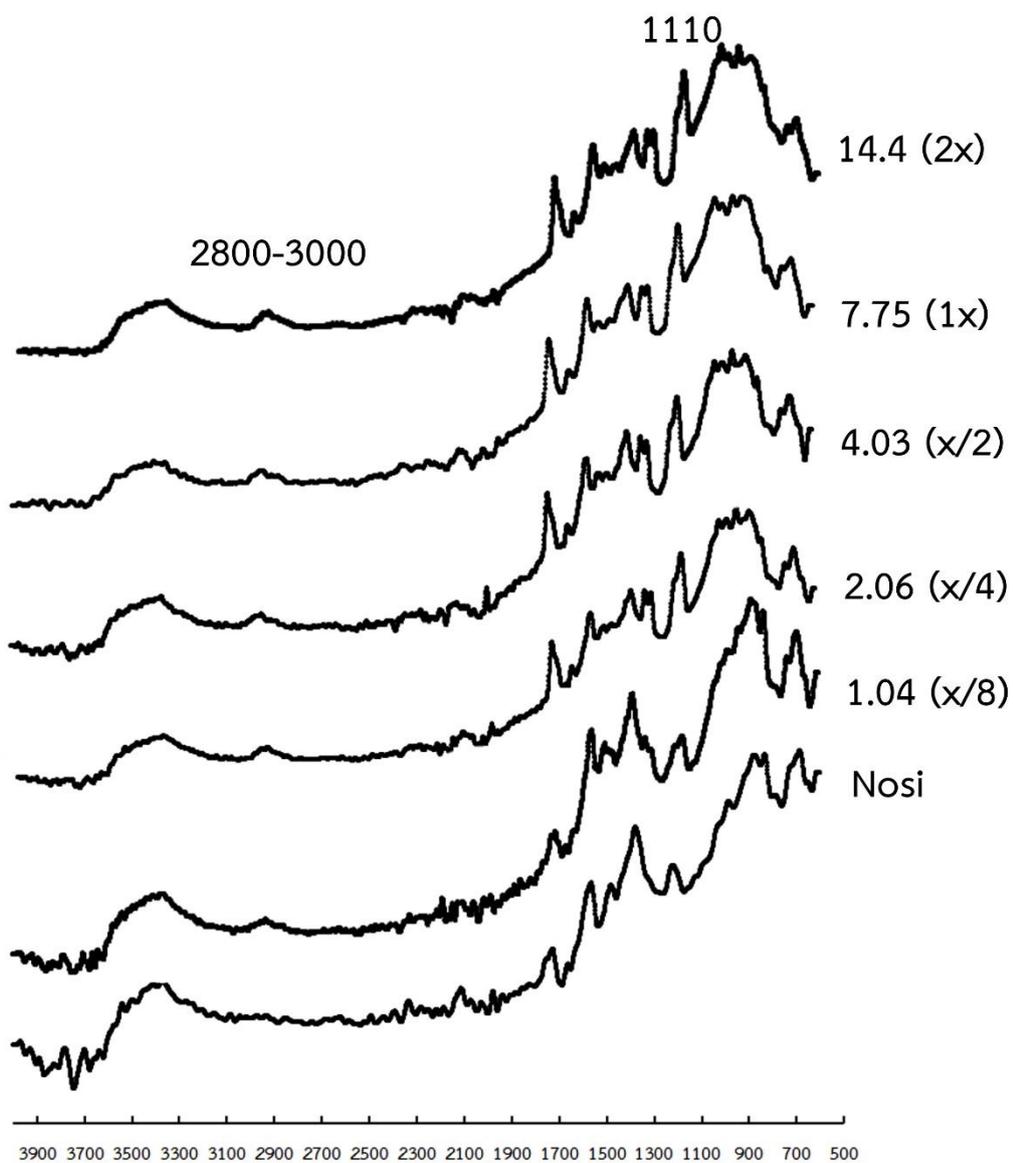
Figure 9 The FTIR spectra (range from 500-4000 cm^{-1}) of different amount of MPS (0-14.4 wt%) which deposited on ZnOnps in washed groups.
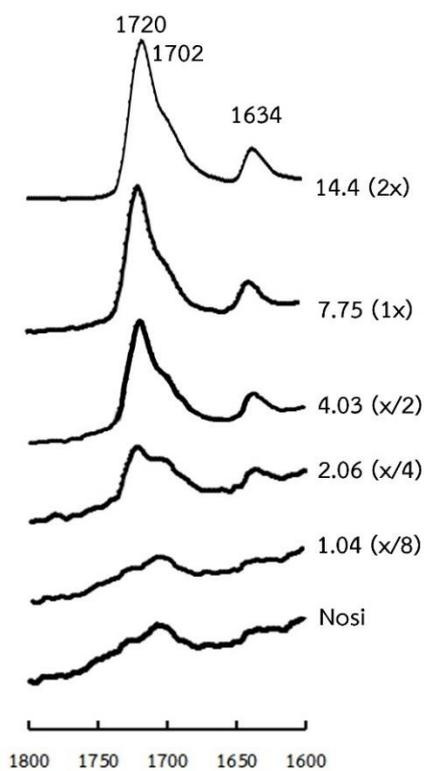
Figure 10 An enlarged section (range from 1600-1800 cm^{-1}) of the FTIR spectra shown in Figure 10 (dried groups).
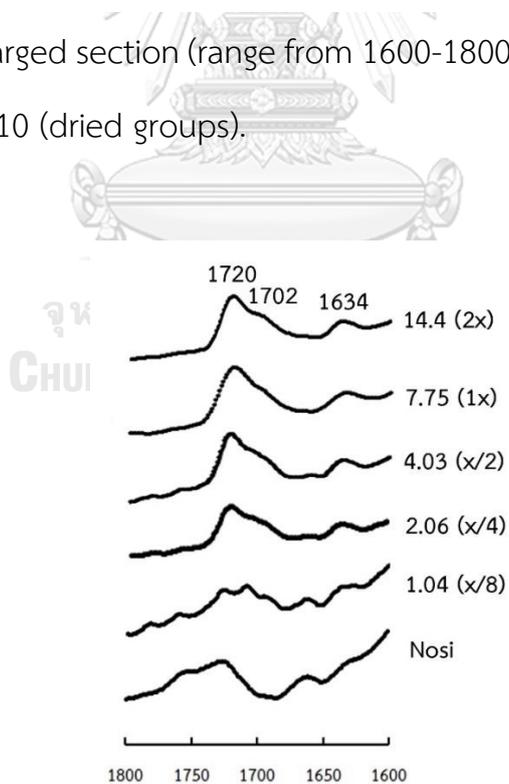

Figure 11 An enlarged section (range from 1600-1800 cm^{-1}) of the FTIR spectra shown in Figure 11 (washed groups).

The TG curve of Nosi, 1.04, 2.06, 4.03, 7.75 and 14.4 wt% of MPS deposited on ZnOnps before and after THF washing are shown in Figure 12-19, respectively. All groups show two-step weight loss, while only the Nosi group shows one-step weight loss that occurred around 250 °C. The first step of all groups, except the Nosi group, occurred between 220 and 320 °C followed by the second step occurred between 320 and 500 °C. In the dried groups, the second step increased a reduction with increasing the MPS amount, while, the second step in the washed groups gradually declined at the same level.
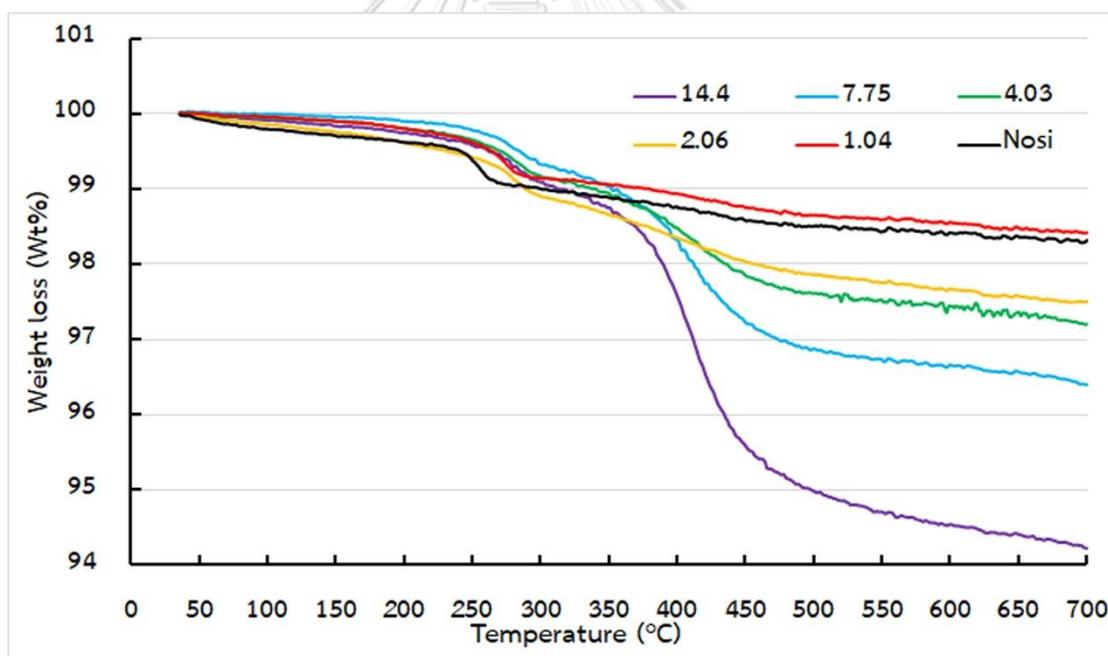

Figure 12 The TG curve of different amount of MPS (0-14.4 wt%) which deposited on ZnOnps before THF washing (dried groups).
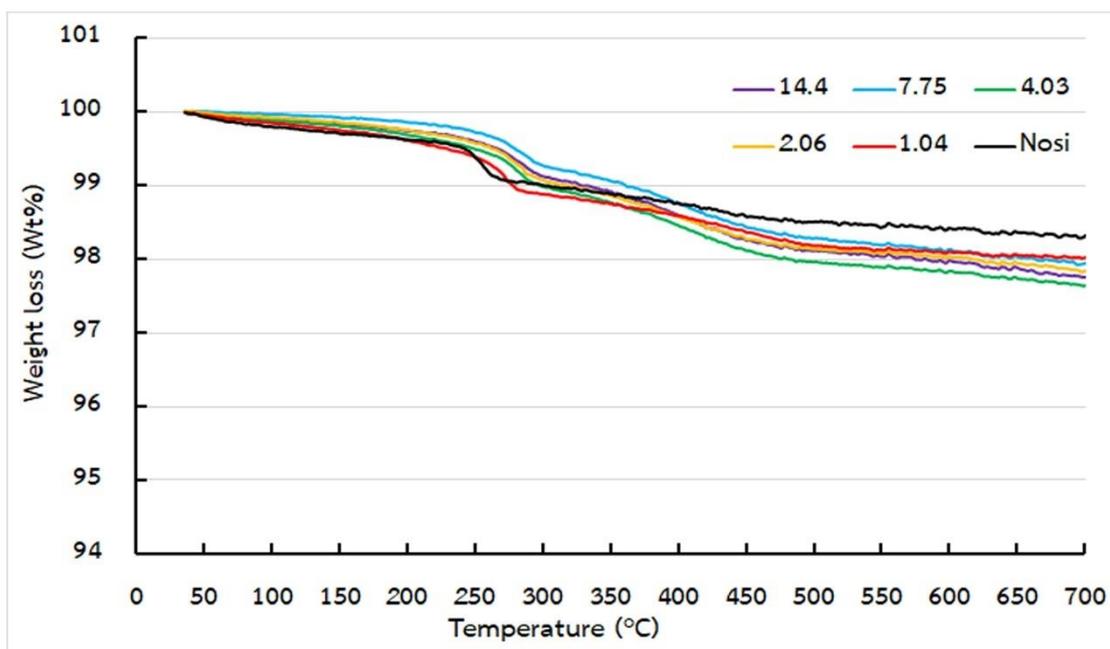
Figure 13 The TG curve of different amount of MPS (0-14.4 wt%) which deposited on ZnOnps after THF washing (washed groups).

Figure 14 The TG curve of the Nosi group.

Figure 15 The TG curve of 1.04 wt% of MPS deposited on ZnOnps in dried and washed group.
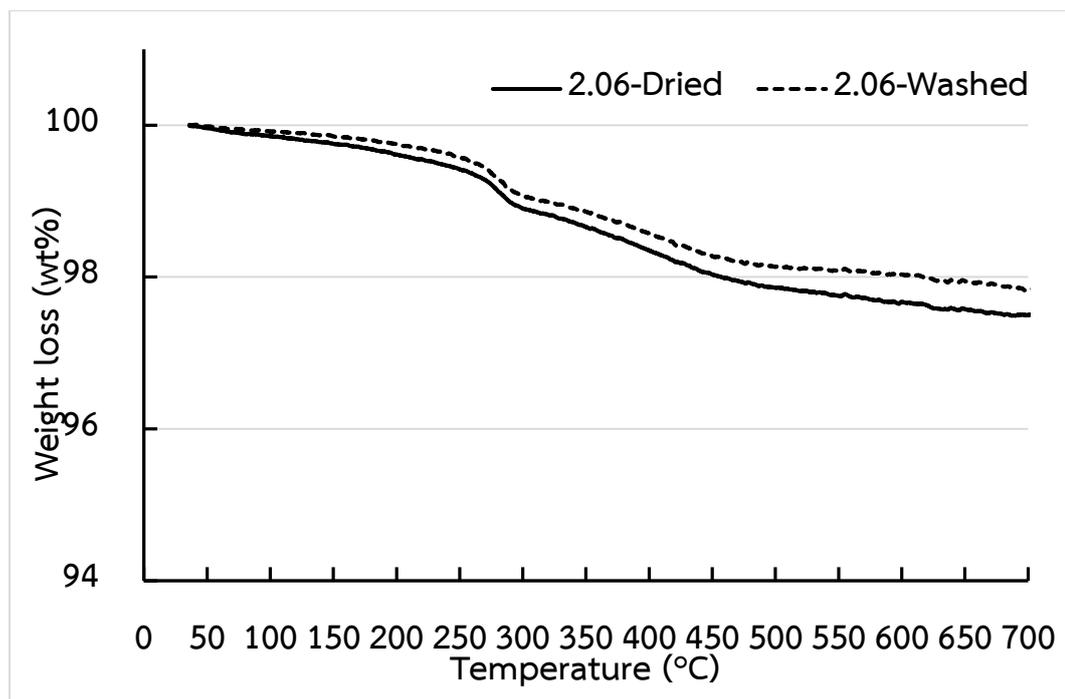
Figure 16 The TG curve of 2.06 wt% of MPS deposited on ZnOnps in dried and washed group.
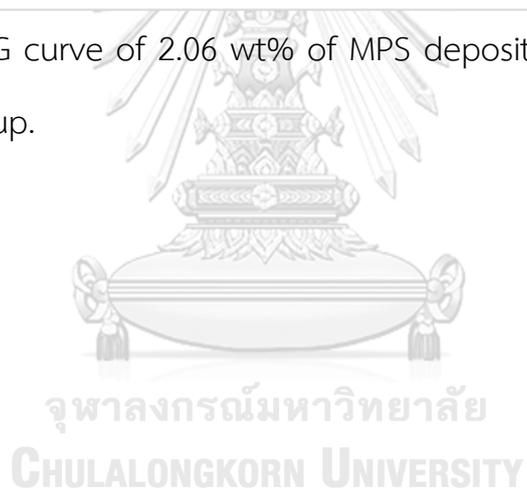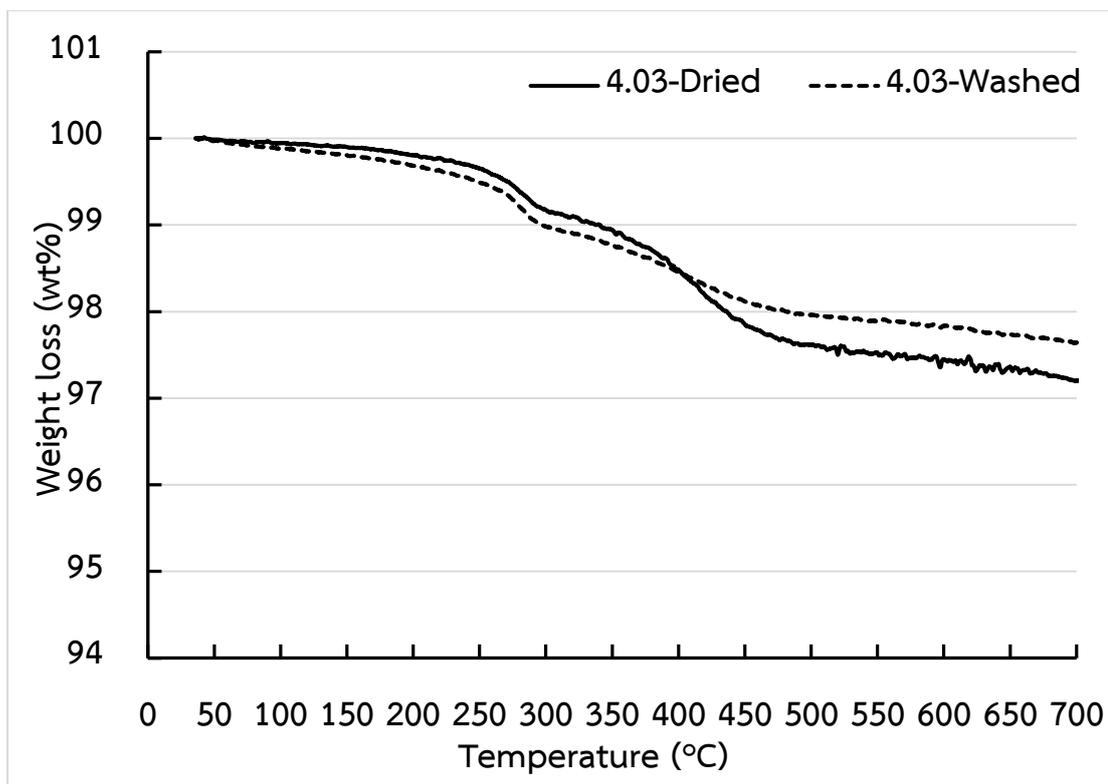
Figure 17 The TG curve of 4.03 wt% of MPS deposited on ZnOnps in dried and washed group.
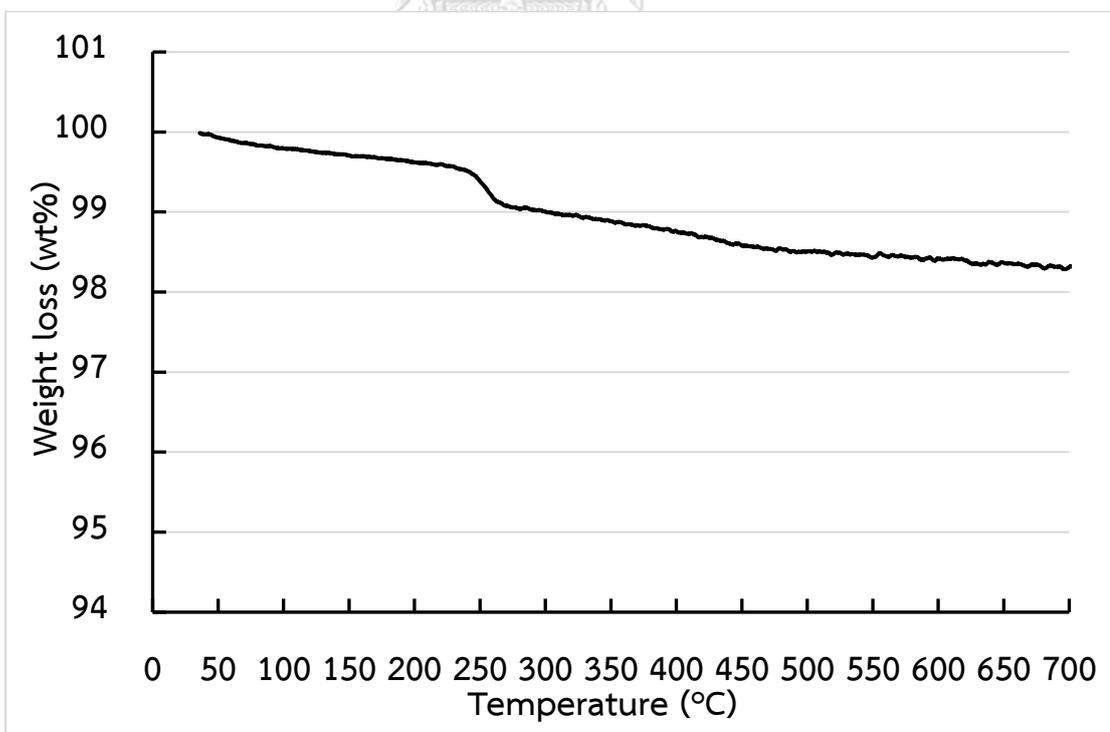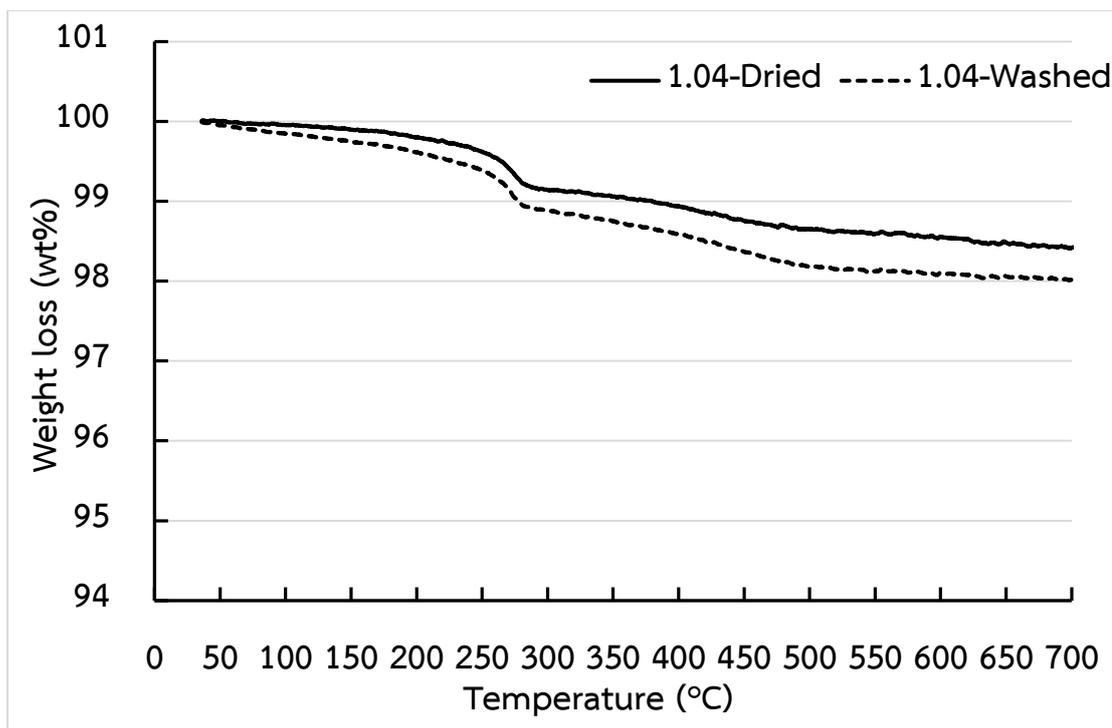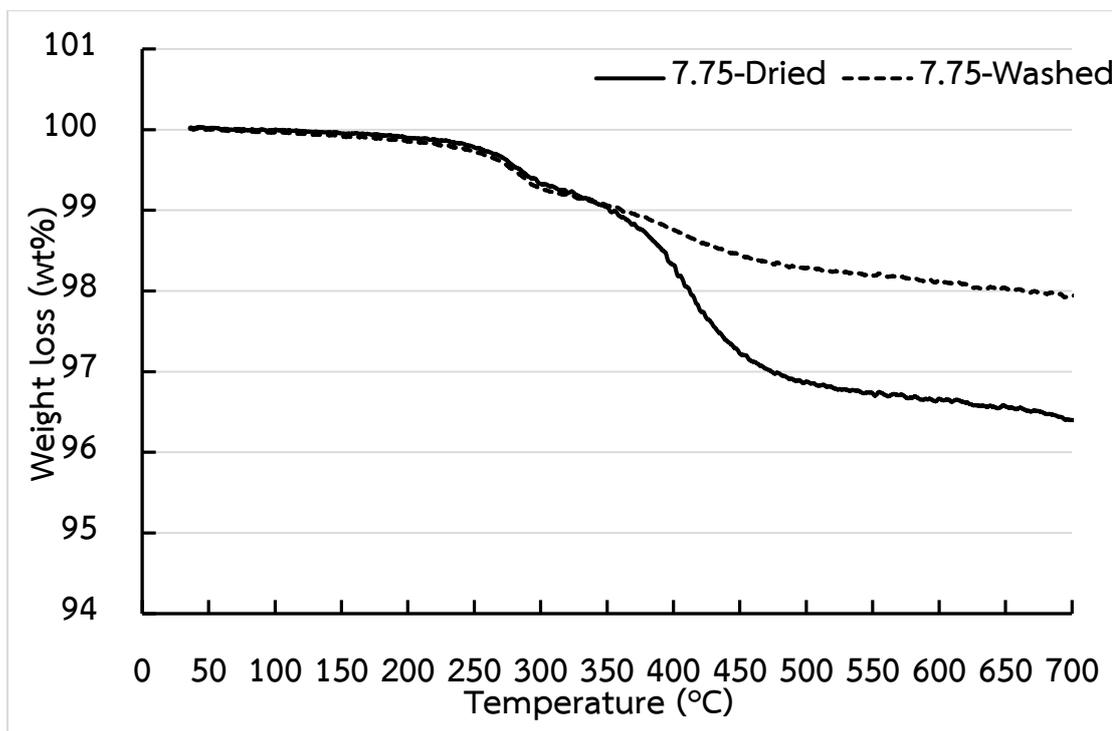
Figure 18 The TG curve of 7.75 wt% of MPS deposited on ZnOnps in dried and washed group.
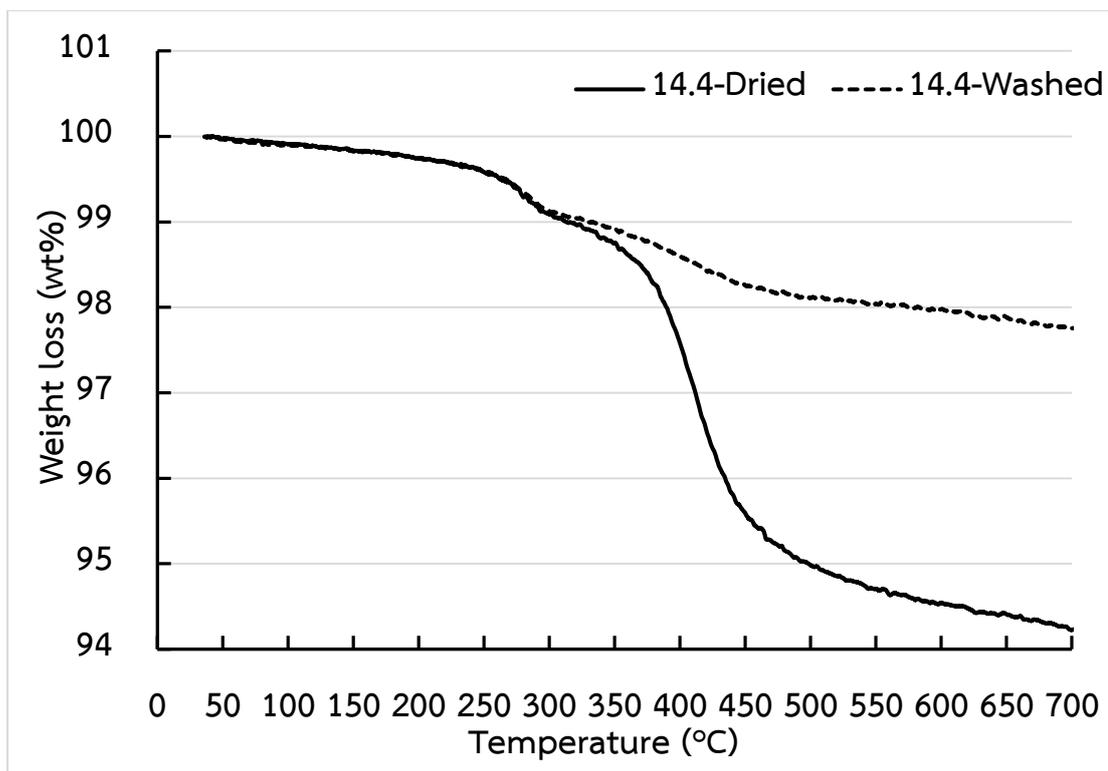
Figure 19 The TG curve of 14.4 wt% of MPS deposited on ZnOnps in dried and washed group.

Discussion

The aim of the present study was to investigate the flexural properties of PMMA material incorporated with different amounts of MPS deposited on ZnOnps. Statistical analysis indicated that there were significant differences on flexural strength data among groups while there were insignificant differences on flexural modulus data among groups. Therefore, the null hypothesis was rejected for flexural strength while accepted for flexural modulus.

FTIR spectroscopy was used to understand the characteristic of functional groups of substances. In the present study, the various amount of

MPS molecules deposited on the ZnOnps surface before and after THF washing were studied in the FTIR spectral range of 500-4000 cm^{-1}. The FTIR spectra after THF washing confirmed the existence and effectiveness of silanization of ZnOnps. The absorption bands at 1110, 1634, 1702, 1720 and 2800-3000 cm^{-1} represented the functional group of Si-O-Si, vinyl (C=C bond), hydrogen bonded carbonyl, free carbonyl and hydrocarbon chain (C-H bond). At low MPS amount (1.04 wt%), only absorption band at 1702 cm^{-1} was found in the spectral range of 1600-1800 cm^{-1}. This indicated that not only the silanol group of MPS molecules and the hydroxyl group of ZnOnps created Si-O-Zn bond, the carbonyl group of MPS molecules also created hydrogen bond on the hydroxyl group on the ZnOnps surface. It could imply that the chemisorbed silane molecules were parallelly oriented. At higher MPS amount, two absorption bands at 1702 and 1720 cm^{-1} were found. The absorption band at 1720 cm^{-1} of dried groups was found and increased with increasing of the MPS amount. Moreover, the absorption band at 1110, 1634 and 2800-3000 cm^{-1} also increased. This result indicated that the multilayer of silane molecules were formed as the amount of free carbonyl group, Si-O-Si, vinyl group and hydrocarbon chain of MPS molecules increased. While, the absorption band at 1720 cm^{-1} of washed groups, ranged from 2.06-14.4 wt%, steadily constant. This result indicated that THF washing was effective to remove physisorbed silane molecules. At the MPS amount of 2.06 wt% was hypothesized to be the optimum amount of monolayer chemisorbed silane which could deposited on the ZnOnps surface in the present study. However, the MPS amount of monolayer coverage that calculated from

Arkle's equation (7.75 wt%) was greater than the MPS amount from the FTIR result. It might attribute to the steric hindrance effect. The Arkle's equation might suitable for the filler in microns scales as a previously described.[28] However, It was interesting that at 2.06 wt% of MPS after THF washing had two absorption bands (1702 and 1720 cm^{-1}). This result might be explained by a high molecular weight silane molecule which had both hydrogen bonded carbonyl and free carbonyl group deposited on the ZnOnps surface.

According to Miller and Ishida[43], the silane molecule oriented parallelly to the oxide surface occupied 0.55 nm^2, while the silane molecules oriented perpendicularly occupied less area (0.24 nm^2). In this study, it was found that monolayer silane molecule occupied 0.51 nm^2 of ZnOnps surface which could imply that chemisorbed silane molecule in this study parallelly oriented. This result also correspondence with Bressy et al.[20] that the commercial ZnOnps had low hydroxyl group density on the surface resulted in a parallel orientation of the monolayer silane molecules. In contrast, the synthesized ZnOnps had higher amount of hydroxyl group, therefore, a perpendicular orientation of silane molecules was mostly found.

The characteristics of the deposited MPS was studied by TGA instrument. The TG curve also confirmed the existence and the effectiveness of ZnOnps silanization. The TG curve of the Nosi group, ZnOnps without silanization, revealed one-step weight loss around 250 °C which corresponded to the impurities of the commercial ZnOnps. The TG curve of all dried and washed groups of different amounts of MPS revealed two-step weight loss at 220-320 °C and 320-500 °C. The first step might be due to the

impurities, that also found in the Nosi group. The physisorbed silane was not obviously observed in the TG curve. This might be due to the homocondensation among physisorbed silane molecules during an increase of temperature in the furnace. In the dried groups, the second step show greater reduction with increasing the MPS amount. This result indicated the decomposition of chemisorbed silane and homocondensated silane as shown in the previous study[20] which indicated that the homocondensated silane was decomposed at 420-430 °C. After THF washing, the physisorbed silane and homocondensated silane were totally rinsed off. Therefore, the second step of TG curve in all washed groups demonstrated almost the same reduction, which corresponded to the decomposition of chemisorbed silane. When compared between dried group and washed group of the same MPS amount, it was found that the second-step weight loss of the MPS amount of 1.04 and 2.06 wt% showed the same trend in weight reduction which might be the decomposition of chemisorbed silane. In contrast, the second-step weight loss of the higher MPS amount in dried groups showed the greater reduction when compared with the washed group. This might attribute to the decomposition of physisorbed silane or homocondensated silane which deposited on the chemisorbed silane. Therefore, the MPS amount of 2.06 wt% might be the complete monolayer coverage.

At 2.5 wt% of ZnOnps, the addition of non-silanized or silanized ZnOnps as filler did not result in an improvement in the flexural properties of PMMA material. This finding is likely due to the particles' spherical shaped and soft filler which allows stress to pass directly through the ZnOnps

compared with other hard fillers such as silica or glass. However, the flexural strength and flexural modulus of the experimental groups were greater than 65 MPa and 2 GPa, respectively, as required by ISO 20795-1.[27]

The flexural strength of the Si groups was greater than that of the Nosi group, although, the flexural strengths of the Si groups were not significant different compared with that of the Nosi group. Silanization enhanced the dispersion of ZnOnps in the liquid monomer and decreased particle aggregation. This might attribute to the higher flexural strength in the Si groups compared to the Nosi group.

When the MPS amount exceeded the monolayered coverage amount (2.06 wt%), it was believed that the physisorbed silane was deposited on the chemisorbed silane by Van der Waals force or hydrogen bond resulted in low hydrolytic stability and a decrease in mechanical properties. In the present study, the flexural strength of various amount (1.04-14.4 wt%) of MPS deposited on ZnOnps surface which incorporated in PMMA material did not show significant differences among groups. This result was in an agreement with the previous studies.[28,33,34,36] This finding could be explained by the physisorbed silane, which had double bond at the terminal group, could act as a copolymer with MMA resulted in a crosslinked matrix.[30] Thus, it was hypothesized that the physisorbed silane might not be responsible for a negative effect on flexural strength. Other factors such as the size, shape, type and amount of filler might play a crucial role on flexural strength.

The flexural modulus of the Nosi and Si groups did not significantly change compared with the control group. The flexural modulus was

influenced by filler content. However, the 2.5 wt% ZnOnps added into PMMA in this study was considered a small amount. Therefore, it did not show significant effect on flexural modulus.

Conclusion

Within the limitation of this study, it was concluded that there were no significant differences on mechanical properties among the various amounts (0-14.4 wt%) of MPS deposited on ZnOnps which incorporated in PMMA material. Two points zero six wt% of MPS amount was the optimum amount of monolayer chemisorbed silane which deposited on the ZnOnps surface.

Chapter IV

The amount of non-silanized or silanized ZnOnps incorporated in PMMA material which provided antifungal effect

Introduction

Zinc oxide (ZnO) is widely used in many industries. By adding into the material, the antimicrobial effect of the material is observed. The effectiveness of these particles as the antimicrobial in inhibiting the growth of fungi and bacteria is on the particle size and amount. Small particle size and high concentration of ZnO resulted in better antimicrobial effect. However, ZnOnps can easily aggregated among themselves and cause a reduction in the antimicrobial effect. The MPS grafting was a useful process to reduce the aggregation of ZnOnps.

From the result of the previous experiment, the flexural properties of PMMA materials which were incorporated with different amount of MPS deposited onto ZnOnps were not significantly different among groups. The result of FTIR and TGA indicated that 2.06 wt% of MPS (x/4) was the beginning point at which MPS could deposit and became saturated onto the ZnOnps surface. Therefore, this amount of MPS was applied in the next experiment.

Many antimicrobial testings were introduced to evaluate the effect of inhibition or killing microorganism such as Minimum inhibitory concentration, Minimum fungicidal concentration and agar diffusion. Mostly, these methods were used to investigate the effect of water-soluble antimicrobial substances.

However, these methods might not be valid for ZnOnps testing because of the low solubility. Therefore, the direct contact test, a method for evaluating the solid material that has low solubility, was used. There is little information about antifungal effect of non-silanized or silanized ZnOnps incorporated in PMMA material.

Therefore, the objective of the present study was to find the amount of non-silanized or silanized ZnOnps incorporated in PMMA material which provided antifungal effect. The null hypothesis was that there would be no significant differences on the antifungal effect among groups (PMMA incorporated with different amounts of ZnOnps with or without silanization).

Materials and methods

Specimen preparation

The optimum amount (2.06 wt%) of silanized ZnOnps from part 1 was used in this part. Non-silanized (Nosi) or silanized (Si) ZnOnps of 1.25, 2.5 and 5% (w/w), calculated from the weight of polymethylmethacrylate (PMMA) powder, were homogeneously mixed with liquid monomer using a vortex mixer (VTX-3000L, LMS, Tokyo, Japan) for 10 min. A mixture of pure PMMA powder and liquid monomer without ZnOnps served as the control group. Forty-two disc-shaped (12x2 mm) specimens (n=6) were fabricated as previously described in the materials and methods part 1.1.

Antifungal assay

Candida albicans, ATCC 90028, was obtained as a stock culture from the Department of Microbiology, Faculty of Dentistry, Chulalongkorn University. The colonies were picked and transferred into a glass tube containing 3 mL of Sabouraud Dextrose broth (SDB) (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated overnight at 37°C. After incubation, the *C. albicans* suspension was transferred into new glass tube containing 3 mL of SDB and adjusted to achieve an optical density of 0.5-McFarland suspension using a spectrophotometer (Nicolet Evolution™ 500, Thermo Electron Corp., Madison, WI, USA) at 530 nm.

0.5-McFarland standard preparation[57]

A $BaSO_4$ turbidity standard was used to standardize the inoculum density. The standard solution consisted of 0.5 mL of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2 \cdot 2H_2O$) to 99.5 mL of 0.18 mol/L H_2SO_4 (1%v/v). With this turbidity, the spectrophotometer should show the absorbance peak at 530 nm equal to 0.3 as the 0.5-McFarland standard.

The suspension, consisting of approximately 1.5×10^6 cell/mL was diluted 100 times and 100 µL was spread on the Sabouraud Dextrose Agar (HiMedia Laboratories Pvt. Ltd.) to confirm the initial amount of *C. albicans*.

The fungal assay was modified from a previous study.[13] The specimens were sterilized using low temperature steam and formaldehyde.[58] Disc-shaped specimens from each group were placed at the bottom of 24-well

cell culture plate (Costar, Corning, NY, USA). A 50 μL *C. albicans* suspension was inoculated on the surface of the specimen in each well and left in the incubator with the lid slightly opened in a sterilized condition for 2 h. After a 2 h incubation, each well was filled with 950 μL of SDB. A mixture of the suspension and SDB served as the positive control while SDB alone served as the negative control. The 24-well culture plate was shaken using an orbital shaker placed in an incubator at 37°C for 24 h. The colony-forming unit (CFU) of *C. albicans* in each well culture plate was determined using serial dilution method, followed by spreading on an agar plate. When determining the amount of *C. albicans*, only the agar plates with 20-200 CFU were counted. The amount of *C. albicans* was independently determined 6 times and averaged.

Statistical analysis

The *C. albicans* growth data were analyzed using one-way ANOVA with groups (control, 1.25Si, 1.25Nosi, 2.5Si, 2.5Nosi, 5Si and 5Nosi) at P<0.05. Tukey HSD's comparison test was used when homogeneity of variance was observed while Games-Howell comparison test was used when homogeneity of variance was rejected.

Result

One-way ANOVA of the *C. albicans* growth results demonstrated significant differences among experimental groups (P<0.001). The Si groups

showed a significant reduction in *C. albicans* compared with the control group, and in the Nosi groups, only the 5Nosi group showed a significant reduction (Figure 20). The reduction in *C. albicans* in the Si groups was greater than those of the Nosi groups with the same amount of ZnOnps.

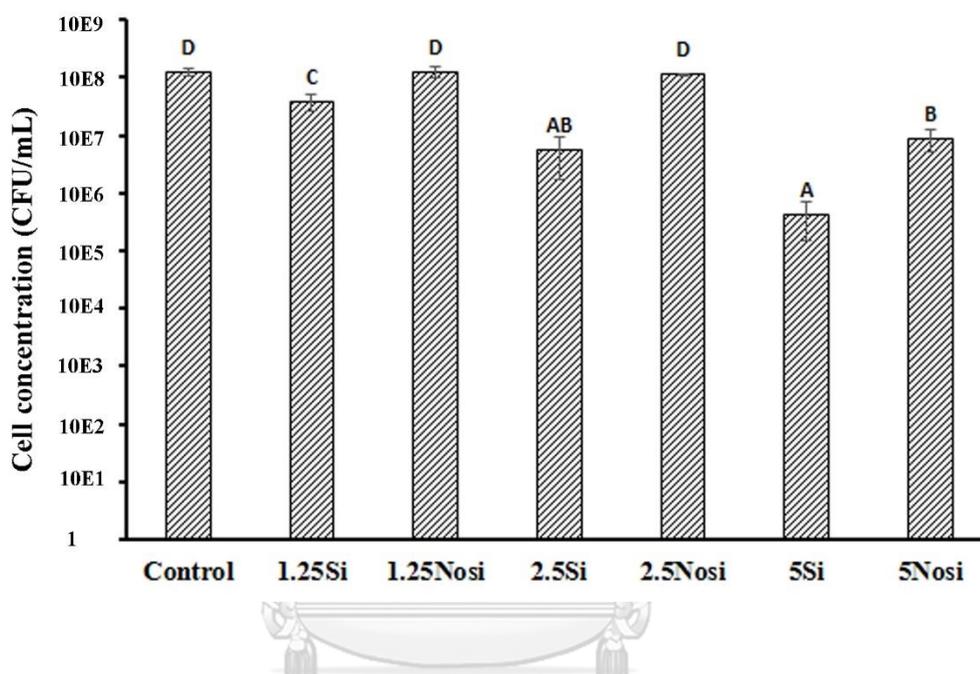Figure 20 Mean and standard deviation of the growth of C. albicans in each experimental group. Groups with the same letter were not significantly different. (P<0.05)

Discussion

The aim of the present study was to investigate the antifungal effect of PMMA material incorporated with different amounts of silanized or non-silanized ZnOnps. Statistical analysis indicated that there were significant

differences on antifungal effect among groups. Therefore, the null hypothesis was rejected.

This study found that increased amount of silanized ZnOnps added into PMMA resulted in a significantly increased reduction in *C. albicans*, while among non-silanized groups, a significant antifungal effect was only found in the 5Nosi group. Compared with the control group, the 5Si group demonstrated the highest reduction in *C. albicans* (99%), followed by the 2.5Si group (95%). The antifungal effect of the Si groups was greater than that of the Nosi groups at the same amount of ZnOnps. The differences in antifungal effect between the Nosi and Si groups might be attributed to the distribution of ZnOnps in PMMA. It is likely that the non-silanized nanoparticles aggregated due to their high surface energy into microns scale particles resulting in a low surface-to-volume ratio and were poorly distributed in PMMA material. In contrast, during silanization, the hydroxyl groups of the ZnOnps react with the hydrolysable groups of the silane coupling agent, resulting in the silane coupling agent surrounding the ZnO particle, thus increasing the distance among ZnOnps. Therefore, the silanized ZnOnps will be well-distributed in PMMA material and maintain theirs nano-particle size, resulting in a high surface-to-volume ratio.[14,21] This increased ratio led in a greater exposure area of ZnOnps on the surface in the Si groups compared with the Nosi groups. An increased contact area between the ZnOnps and *C. albicans* might contribute to the increased antifungal effect. The antifungal effect might also attribute to the dissociation of Zn ions from the ZnOnps. Zn ions can dissociate from the silane molecules because of

the hydrolysable group of the silane coupling agent, which has a hydrophilic terminal. The hydrophilic terminal attracts water molecules and undergoes hydrolysis. This reaction may explain the finding that increased silane coupling agent in PMMA resulted in more water absorption.[36] Moreover, the dissociation energy of the Si-O bond in the silane coupling agent is higher than that of Zn-O.[59] Therefore, unbound Zn ions that have an antifungal effect can easily dissociated due to water absorbing into the material.

Conclusion

Within the limitations of this study, it was concluded that increased amount of silanized ZnOnps incorporated in PMMA material resulted in a significantly increased reduction in *C. albicans*, while among non-silanized groups, a significant antifungal effect was only found in the 5Nosi group.

Chapter V

The optimum amount of silanized ZnOnps incorporated in PMMA material which provided antifungal effect and maintaining optical and mechanical properties

Introduction

Denture has been used to rehabilitate patients with missing teeth. It enhances masticatory function, phonetic and esthetic. However, denture can be reservoirs of microorganisms which are the etiology of many oral diseases. Many cleaning methods were introduced to denture wearer such as mechanical brushing, chemical soaking, microwave irradiation. The incorporation of antimicrobial agents into denture base material such as metal/ metal oxide was a possible method to reduce the number of microorganisms in the denture base material.

From the result of the previous experiment, we found that 1.25Si, 2.5Si, 5Si and 5Nosi groups were significantly reduced the amount of fungal cell. However, the incorporation of ZnOnps might affect other properties of denture material because ZnOnps easily aggregate among each other. Then, we continued to evaluate the optical and mechanical properties of ZnOnps incorporated in PMMA material for better understanding.

Therefore, the objective of the present study was to find the optimum amount of silanized ZnOnps incorporated in PMMA material which provided antifungal effect and maintaining optical and mechanical properties. The null hypotheses were that 1) there would be no significant differences on the

optical characteristics among groups (PMMA incorporated with different amounts of ZnOnps with or without silanization). 2) there would be no significant differences on the mechanical properties among groups (PMMA incorporated with different amounts of ZnOnps with or without silanization) and storage times (48 h and 1 month).

Materials and methods

Specimen preparation

The optimum amount (2.06 wt%) of silanized ZnOnps from part 1 was used in this part. Non-silanized (Nosi) or silanized (Si) ZnOnps of 1.25, 2.5 and 5% (w/w), calculated from the weight of polymethylmethacrylate (PMMA) powder, were homogeneously mixed with liquid monomer using a vortex mixer (VTX-3000L, LMS, Tokyo, Japan) for 10 min. A mixture of pure PMMA powder and liquid monomer without ZnOnps served as the control group. Fifty-six disc-shaped (12x2 mm) specimens for the optical properties test and 112 bars (64×10×3.3 mm) for the mechanical properties test were fabricated as previously described in the experimental part 1.

Optical properties evaluation

A spectrophotometer (Ultrascan XE, Hunter Associates Laboratory, Inc., Reston, VA, USA) was used to observe the differences in color as determined by the CIELAB system and opacity of disc-shaped specimens from CIEXYZ

system using D65 illuminant and 10-degree observer. The specimens in each group (n=8) were measured three times against the white and black background and averaged. L* (brightness), a* (red-green coordinate) and b* (yellow-blue coordinate) of the CIELAB system were used to calculate the color differences of the specimens (ΔE), and Y (brightness) of the CIEXYZ system was used to evaluate the opacity of the specimens. The following equations were used:[54]

$$\Delta E = [(L^{*}_{1} - L^{*}_{2})2 + (a^{*}_{1} - a^{*}_{2})2 + (b^{*}_{1} - b^{*}_{2})^{2}]^{1/2}$$

Where, L^{*}_{1}, a^{*}_{1}, b^{*}_{1} = the mean value of the first group, L^{*}_{2}, a^{*}_{2}, b^{*}_{2} = the mean value of the second group.

$$\text{Opacity} = (Y_{1}/Y_{2}) \times 100$$

Where, Y_{1} = brightness of specimen backed by black background, Y_{2} = brightness of specimen backed by white background.

Mechanical properties evaluation

A three-point bending test was used to evaluate FS and FM of the bar-shaped specimens according to ISO 20795-1.[27] Sixteen specimens from each experimental group were randomly divided into two subgroups (n=8) based on a storage time in 37°C deionized water for 48 h or 1 month before testing. A universal testing machine (EZ-SX, Shimadzu, Kyoto, Japan) was used as previously described in the materials and method part 1.1.

Scanning electron microscope (SEM) observation and energy dispersive X-ray (EDX) analysis

Fractured surfaces of the bar-shaped specimens of the control, 5Si and 5Nosi groups after the three-point bending test were randomly selected, gold-sputter coated and observed using SEM (Quanta 250, FEI Company, Eindhoven, The Netherlands) under acceleration of 20 kV and magnification of 35,000x.

One specimen was selected from the 5Si and 5Nosi groups, carbon-sputter coated and observed using the EDX detection system built into the SEM instrument (JSM-5410LV, JEOL Ltd., Tokyo, Japan). Zn-mapping images of the surfaces were captured at an acceleration voltage of 20 kV and a magnification of 2,000x.

Statistical analysis

The L*, a*, b* and opacity data were analyzed using one-way ANOVA at $P<0.05$. The FS and FM were separately analyzed by two-way ANOVA with groups (control, 1.25Si, 1.25Nosi, 2.5Si, 2.5Nosi, 5Si and 5Nosi) and storage time (48 h and 1 month) as main factors at $P<0.05$. Tukey HSD's comparison test was used when homogeneity of variance was observed while Games-Howell comparison test was used when homogeneity of variance was rejected.

Result

One-way ANOVA of L*, a*, b* and opacity demonstrated significant differences among the experimental groups (*P*<0.001) (Table 3). Photograph of the experimental groups demonstrated color change of the specimens as the amount of non-silanized or silanized ZnOnps increased (Figure 21). Increasing amounts of ZnOnps added into PMMA resulted in increased L*, ΔE, and opacity in both the Nosi and Si groups. In contrast, a greater decrease in a* and b* was observed as the amount of ZnOnps increased. ΔE and opacity of the Nosi groups were greater than those of the Si groups containing the same amount of ZnOnps.
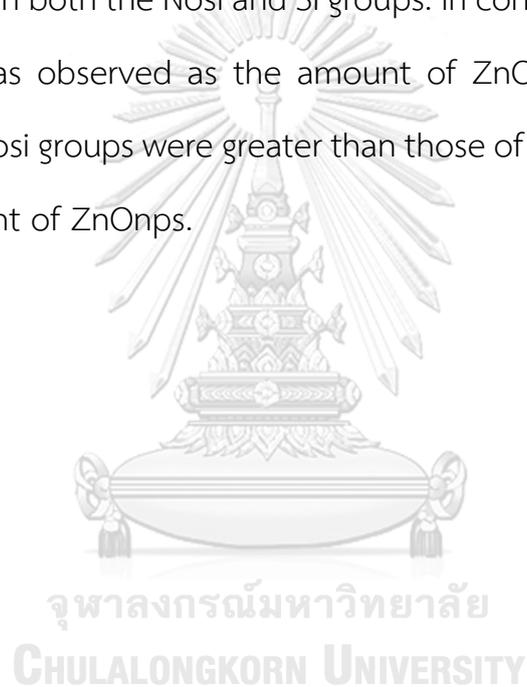
Table 3 Mean (Standard deviation) of color characteristics of each experimental group.

Group	L*	a*	b*	ΔE	Opacity
Control	59.86 (0.30)[a]	21.48 (0.24)[g]	14.95 (0.13)[f]	-	71.3 (1.0)[a]
1.25Si	66.06 (0.29)[b]	20.55 (0.28)[f]	8.58 (0.26)[e]	8.9	87.0 (0.8)[b]
1.25Nosi	69.48 (0.24)[c]	19.77 (0.14)[e]	6.36 (0.33)[c]	13.0	91.6 (0.6)[c]
2.5Si	70.04 (0.34)[d]	19.39 (0.18)[d]	7.33 (0.17)[d]	12.9	91.6 (0.9)[c]
2.5Nosi	74.54 (0.23)[e]	17.25 (0.14)[c]	5.11 (0.15)[b]	18.2	95.8 (0.5)[d]
5Si	75.42 (0.30)[f]	16.54 (0.31)[b]	5.30 (0.22)[b]	19.0	96.7 (0.7)[d]
5Nosi	78.43 (0.22)[g]	14.94 (0.09)[a]	4.74 (0.15)[a]	22.2	98.0 (0.3)[e]

Notes: The values with the same superscript letters in the same column were not significantly different ($P<0.05$). "-" indicates no data.

Abbreviation: ΔE=the color differences between corresponding group (non-silanized or silanized ZnOnps) and control group.

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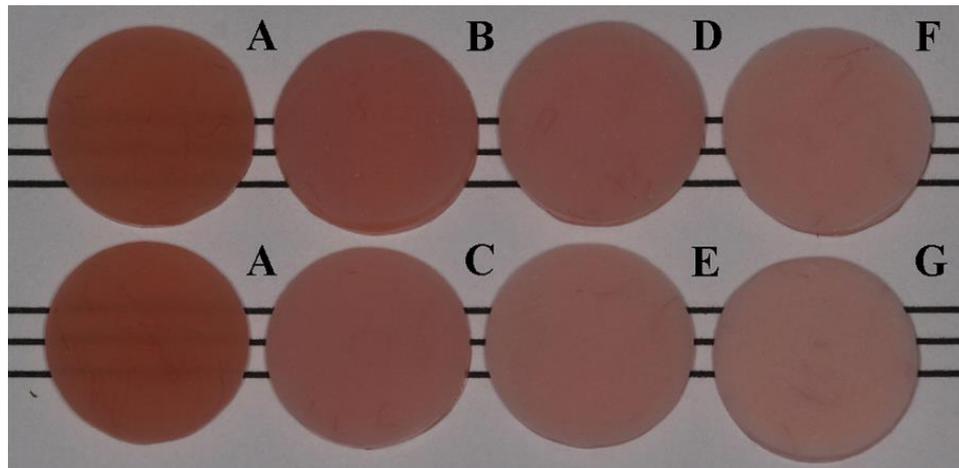Figure 21 Photograph of the experimental groups; A. control, B. 1.25Si, C. 1.25Nosi, D. 2.5Si, E. 2.5Nosi, F. 5Si, G. 5Nosi.

Two-way ANOVA of flexural strength data demonstrated significant differences only among the experimental groups ($P<0.001$) while that of the flexural modulus results indicated significant differences on storage time ($P=0.015$) and interactions ($P=0.006$) as demonstrated in Table 4 and 5.

Table 4 Two-way ANOVA of flexural strength of the experimental groups for two main factors and interactions.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7064.9[a]	13	543.5	24.0	.000
Intercept	820347.6	1	820347.6	36265.9	.000
Group	6910.7	6	1151.8	50.9	.000
Storage time	2.8	1	2.8	.1	.724
Group * Storage time	151.3	6	25.2	1.1	.359
Error	2216.8	98	22.6		
Total	829629.3	112			
Corrected Total	9281.7	111			

a. R Squared = .761 (Adjusted R Squared = .729)

Table 5 Two-way ANOVA of flexural modulus of the experimental groups for two main factors and interactions.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.2[a]	13	.0	2.3	.010
Intercept	681.1	1	681.1	93458.8	.000
Group	.0	6	.0	.7	.649
Storage time	.0	1	.0	6.1	.015
Group * Storage time	.1	6	.0	3.3	.006
Error	.7	98	.0		
Total	682.1	112			
Corrected Total	.9	111			

a. R Squared = .234 (Adjusted R Squared = .133)

Therefore, the flexural strength data of the two storage times were pooled and analyzed. Post hoc's multiple comparison tests with means and standard deviation of the flexural strength and flexural modulus are shown in Table 6.

Table 6 Mean (Standard deviation) of flexural strength and flexural modulus of each experimental group.

Group	Flexural strength	Flexural modulus	
		48 h	1 month
Control	92.92 (5.08)^c	2.51 (0.05)^{ab}	2.39 (0.09)^a
1.25Si	92.85 (3.26)^c	2.52 (0.08)^{ab}	2.44 (0.06)^{ab}
1.25Nosi	89.57 (3.78)^c	2.48 (0.06)^{ab}	2.41 (0.06)^a
2.5Si	89.81 (5.35)^c	2.43 (0.13)^{ab}	2.48 (0.07)^{ab}
2.5Nosi	81.78 (5.52)^b	2.50 (0.04)^{ab}	2.47 (0.06)^{ab}
5Si	83.03 (3.84)^b	2.49 (0.11)^{ab}	2.51 (0.05)^{ab}
5Nosi	69.13 (5.79)^a	2.54 (0.04)^b	2.44 (0.06)^{ab}

Note: The values with the same superscript letters in the same column were not significantly different ($P<0.05$).

Between 48 h and 1 month of water storage, the flexural strength and flexural modulus of the experimental groups did not significantly change. Compared with the control group, the flexural strengths of the 1.25Si, 1.25Nosi, and 2.5Si groups were not significantly different, and the flexural moduli of the experimental groups were not significantly different at the same storage time. The flexural strength of the Si groups was significantly greater than those of the Nosi groups with the same amount of ZnOnps except for the 1.25% groups. Increased amount of ZnOnps resulted in increased reduction in the flexural strength; however, the flexural modulus did not show the same trend.

The fractured surface of the bar-shaped specimens of the control and 5% groups were observed using SEM (Figure 22). The Si group image demonstrated well distributed ZnOnps on its surface (Figure 22B), while that of the Nosi group showed aggregated ZnOnps on the fractured surface as indicated by white arrows (Figure 22C).
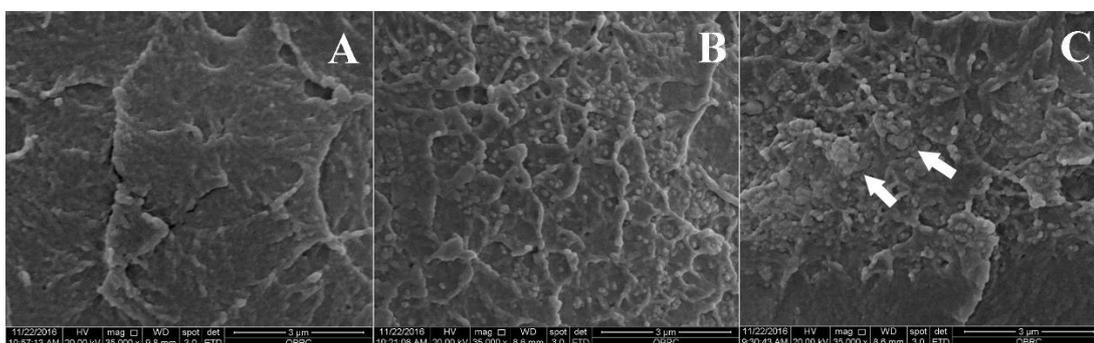

Figure 22 SEM images of the fractured surface of specimen (35,000x); A. control, B. 5Si, C. 5 Nosi.

The Zn-mapping images from EDX analysis demonstrated the distribution of Zn on the surface of the 5% groups (Figure 23). In the 5Si group, the ZnOnps were evenly dispersed (Figure 23A), however, in the 5Nosi group, the ZnOnps were densely aggregated in some areas (Figure 23B).
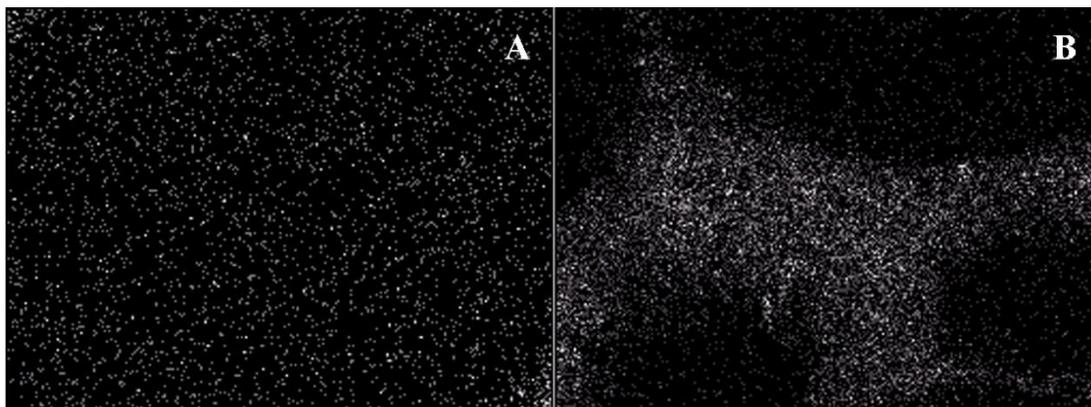
Figure 23 Zn-mapping images of the surface of specimen using EDX on SEM (2,000x); A. 5Si, B. 5 Nosi.

Discussion

The aim of the present study was to investigate the optical and mechanical properties of PMMA material incorporated with different amounts of non-silanized or silanized ZnOnps that provided antifungal effect. Statistical analysis indicated that there were significant differences on optical and mechanical properties among groups. Therefore, the null hypotheses were rejected.

This study clearly shown that adding ZnOnps caused drastic color changes as also demonstrated in a previous study of silver nanoparticles.[13] A previous study demonstrated that the threshold for detecting gingival color differences was 3.1±1.5.[53] The ΔE of all groups exceeded the threshold established for color acceptability in dentistry (ΔE=2.7).[52] Furthermore, the ΔE and opacity of the Nosi and Si groups increased as the amount of ZnOnps added into PMMA. These changes in the optical characteristics of a material

are a common disadvantage of incorporating metal oxide into PMMA. Therefore, the use of metal oxide should take into account the possible color change, especially in the esthetic zone. However, the color of the gingiva of the denture can be clinically corrected by extrinsic coloration. Interestingly, it is found that adding ZnOnps resulted in increased L*, indicating that the material became brighter. Therefore, it may be possible to clinically correct the color by staining PMMA denture base material.[60] Extrinsic coloration can be easily performed clinically to reduce the brightness of the material than with low-brightness material such as denture base incorporated with silver. Discoloration of silver particles due to oxidative reaction causes a metallic appearance of the denture base.

At the same amount of ZnOnps, the ΔE and opacity of the Nosi groups was greater than those of the Si groups. This result might be explained by the even distribution of the silanized ZnOnps. Aggregation of the non-silanized ZnOnps resulted in almost completely blocking the transmission of light and a drastic color change. In contrast, silanization enhanced the distribution of the ZnOnps. Thus, the ZnOnps were homogeneously blended into the mixture between PMMA powder and liquid monomer resulting in less color and opacity changes. Notably, the ΔE and opacity between the Nosi and Si groups decreased as the amount of ZnOnps increased. This result suggests that silanization has little effect on the ΔE and opacity when large amounts of ZnOnps were added.

The flexural strength and flexural modulus of the experimental groups after 48 h and 1 month of water storage were greater than 65 MPa and 2

GPa, respectively, as required by ISO 20795-1.[27] Flexural strength is influenced by various factors such as the size, shape, and amount of filler; and especially silanization.[36,61] In the present study, flexural strength was ZnOnps amount-dependent, especially in the Nosi groups. The flexural strength of the Si groups was greater than that of the Nosi groups at the same amount of ZnOnps except for the 1.25% groups, which were not significantly different. This indicated that a small amount of ZnOnps did not affect the mechanical properties of PMMA. However, increased amount of ZnOnps resulted in more filler aggregations that were weak points in the specimen. Silanization enhanced the dispersion of ZnOnps in the liquid monomer and decreased particle aggregation. This may be why the flexural strength of the Si groups was higher compared with the Nosi groups. Furthermore, fillers play an important role in improving the mechanical properties of a material. As demonstrated in a previous study, whisker shaped aluminum borate filler was tailored to confront the breakage stress, improving the mechanical properties of PMMA.[61] However, the addition of ZnOnps as filler in the present study did not result in an improvement in the mechanical properties of PMMA. This finding is likely due to the particles' spherical shaped which results in stress being evenly transferred through the resin matrix of the specimen. Moreover, ZnO is a soft filler, which allows stress to pass directly through the ZnO compared with other hard fillers such as silica or glass filler.

The flexural modulus of the Nosi and Si groups did not significantly change compared with the control group at each water storage time in this study. The flexural modulus was influenced by filler content. However, the

5% ZnOnps added into PMMA in this study was considered a small amount. Therefore, it did not show significant effect on flexural modulus.

Conclusion

Within the limitation of this study, it was concluded that the ΔE of non-silanized and silanized groups exceeded the threshold established for color acceptability in dentistry (ΔE=2.7). Only the flexural strengths of the 1.25Si, 1.25Nosi, and 2.5Si groups were not significantly different when compared with the control group. While, the flexural moduli of the experimental groups were not significantly different at the same storage time.

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Chapter VI

General discussion and conclusion

General discussion

This research is the first to use silanized ZnOnps incorporated into PMMA to create the material that has an antifungal effect while maintaining its mechanical properties. It has been also demonstrated that the additional of ZnOnps in PMMA had a detrimental effect on the optical properties of PMMA. It was felt that the increased antifungal effect outweighs the color change because the color change can be adjusted using extrinsic coloration. However, future studies can determine the optimal concentration of ZnOnps incorporated in PMMA that results in a balance between the antifungal effect and the detrimental effect on opacity. Moreover, the addition of secondary filler which has refractive index close to PMMA and harder than ZnOnps may help in reinforcing the mechanical properties without causing more color change. This aspect should also be investigated in the future studies. The result of this study suggests that 2.5% Si ZnOnps incorporated into PMMA might fulfill the requirement in terms of antifungal and mechanical properties. Although the color differences of the PMMA might be an esthetic concern, this can be corrected by denture characterization using extrinsic coloration. Therefore, this nanocomposite material might be a material to inhibit the growth of *C. albicans* in the PMMA that causes denture-induced stomatitis. This material might be used as a treatment denture, a therapeutic denture used for a specific period, or for treating denture induced stomatitis

in patients, especially in the elderly patient with limited manual dexterity. However, the long-term evaluation of the antifungal effect of PMMA denture base material incorporated with silanized ZnOnps both in vitro and in vivo is recommended

Conclusions

Within the limitations of this study, it was concluded that PMMA incorporated with silanized ZnOnps, particularly with 2.5% silanized ZnOnps, had a greater antifungal effect, less color differences and opacity compared with non-silanized ZnOnps, while retaining its mechanical properties.

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APPENDIX

VITA

Krid Kamonkhantikul was born on October 31th, 1985 in Nakorn Ratchasima Province, Thailand. He graduated the degree of Doctor of Dental surgery with Honors from Faculty of Dentistry, Chulalongkorn University in 2010. Then, he had worked as a dentist at Na Kae Hospital at Nakorn Pranom Province for 1 year. After that, he attended the course of Graduate Diploma of Clinical Sciences Program and graduated in 2012. After graduation, he started working as the lecturer in Department of Prosthodontics, Faculty of Dentistry, Chulalongkorn University and was assigned to attend the Doctor of Philosophy Program in Prosthodontics, Department of Prosthodontics, Faculty of Dentistry, Chulalongkorn University.