

# The Effects of the Incorporation of Bioactive Glasses and Zinc Oxide Fillers of Dental Provisional Light-polymerized Composite

Watcharapon Intra<sup>1</sup>, Jinthana Lapidattanakul<sup>2</sup>, Nisanart Traiphol<sup>3,4</sup>, Naruporn Monmaturapoj<sup>5</sup>, Pong Pongprueksa<sup>1</sup>

<sup>1</sup>Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

<sup>2</sup>Department of Oral Microbiology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

<sup>3</sup>Laboratory of Advanced Chromic Materials, Department of Materials Science, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

<sup>4</sup>Center of Excellence on Petrochemical and Materials Technology, Chulalongkorn University, Bangkok, Thailand

<sup>5</sup>Assistive Technology and Medical Devices Research Center, National Science and Technology Development Agency, Pathumtani, Thailand

## Abstract

This study aims to evaluate the effects of the addition of bioactive glasses (45S5, S53P4) and zinc oxide fillers on the mechanical, physical, and antibacterial properties of resin composite. Resin composites incorporated with various fillers (15wt% 45S5, 15wt% S53P4, and 1wt% ZnO) were produced. The mechanical properties (flexural strength, flexural modulus, and fracture toughness), physical properties (water sorption, and water solubility), depth of cure, and antibacterial property of these experimental composites were compared with a SiO<sub>2</sub> composite and a commercial Systemp onlay. Both 15wt% bioactive glass sample groups showed significantly lower flexural strength, flexural modulus, and fracture toughness than the 1wt% ZnO and SiO<sub>2</sub> groups. The incorporated 45S5 bioactive glass composite had the highest water sorption. The Systemp onlay displayed the highest water solubility, while the S53P4 bioactive glass group indicated a negative solubility. The depth of cure was deepest in the Systemp onlay and the shallowest in the ZnO composite. All experimental composites did not demonstrate antibacterial effects against *Streptococcus mutans*. The addition of 15wt% bioactive glasses negatively impacted the mechanical properties, physical properties, and depth of cure of resin composite. None of the filler materials enhanced the antibacterial properties of resin composites.

**Keywords:** 45S5 bioactive glass, Flexural strength, Fracture toughness, S53P4 bioactive glass, Zinc oxide

**Received Date:** Feb 22, 2021

**Revised Date:** Mar 12, 2021

**Accepted Date:** May 4, 2021

**doi:** 10.14456/jdat.2022.1

### Correspondence to:

Pong Pongprueksa, Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University, 6 Yothi Rd., Ratchathewi, Bangkok, 10400 Thailand. Tel: 096-745-9977 E-mail: pong.pon@mahidol.edu

## Introduction

Visible light-cured composite is a recent development which is gaining popularity in the field of dentistry. The goals of this innovation are to improve the mechanical, antibacterial, and biocompatibility properties, which are essential in various applications of dental work. Provisional composite materials have evolved rather extensively with advancements in their handling, controllable working time,<sup>1</sup> addition of antimicrobial agents, and ease of placement and removal in one piece.<sup>2</sup>

Resin composite with antibacterial properties has been a subject of interest, with attempts to incorporate various antibacterial agents in the material.<sup>3</sup> One example of such agents is triclosan which displays broad-spectrum antimicrobial activity with a favourable safety profile. Unfortunately, there has been some evidence suggesting that triclosan is linked to hormonal disruption and an increased risk of breast cancer.<sup>4</sup>

To address these concerns, researchers have been looking into bioactive glass, which has widely been studied and used in oral surgery as a bone replacement. A couple of recent studies that investigated the addition of bioactive glass in resin composite showed favourable biocompatibility, stable mechanical properties, the ability to inhibit bacterial growth, and the potential to promote dentin remineralization.<sup>5,6</sup>

Besides bioactive glass, zinc oxide (ZnO) nanoparticles have also received much interest in multiple fields of research. Materials incorporated with this metal oxide have exhibited superior antibacterial and disinfecting properties, which has led to them being widely used in numerous kinds of medicines, temporary dental fillings, and dental root canal sealants.<sup>7</sup>

For these promising reasons, it was decided to produce experimental provisional resin composite incorporated with bioactive glasses (45S5 and S53P4) and ZnO nanoparticles. Hence, the purpose of this study was to evaluate the influence of the addition of bioactive glasses and zinc oxide in resin composite on its mechanical, physical, and antibacterial properties, as compared to silicate-filled composite and a commercially available Systemp onlay material. Therefore, the null hypotheses were no significant difference in mechanical, physical and

antibacterial properties among the experimental provisional composites and commercial provisional composite.

## Materials and Methods

### *Experimental resin composite preparation*

The 45S5 bioactive glass (45% SiO<sub>2</sub>, 6% P<sub>2</sub>O<sub>5</sub>, 24.5% Na<sub>2</sub>O, 24.5% CaO) and the S53P4 bioactive glass (53% SiO<sub>2</sub>, 4% P<sub>2</sub>O<sub>5</sub>, 23 Na<sub>2</sub>O, 20% CaO) were fabricated by the National Metal and Materials Technology Center, Thailand. The raw materials were mixed together according to the specified ratio, melted in a covered Pt-10%Rh crucible at 1450°C for two hours and then quenched in cold water to produce frit. Fine bioactive glass powder was produced by milling with a zirconia ball to achieve an average particle size (D0.5) of 5.81 µm according to the results of laser diffraction (Mastersizer 2000, Malvern Instruments, UK).

A prepolymerized dimethacrylate was prepared from a mixture of 98 wt% urethane dimethacrylate (UDMA) and 2 wt% trimethylbenzoyl diphenylphosphine oxide (TPO). The resin monomer was placed in a polyethylene mould (3x5x14 mm) and polymerized in a light furnace (Lumamat 100, Ivoclar Vivadent, Schaan, Liechtenstein) for 60 seconds. The polymerized resin was then ball milled in a high energy milling machine (Emax, Retsch, Haan, Germany) and sieved to an average particle of 62.2 µm as determined by laser diffraction (Mastersizer 2000, Malvern Instruments, UK).

Four experimental light-curing composites were produced, each consisting of a proportion of silicon dioxide (SiO<sub>2</sub>) fillers (0.7µm silanized glass, Esstech, Essington, PA, USA) to make up a total of 25 wt% of fillers as follows: 1) 15 wt% 45S5 bioactive glass (45S5), 2) 15 wt% S53P4 bioactive glass (S53P4), 3) 1 wt% ZnO nanoparticles (particle size 40 nm), and 4) 25 wt% SiO<sub>2</sub>. The fillers were mixed with 42 wt% of the prepolymerized dimethacrylate (UDMA). The final resin matrix composed of UDMA and triethyleneglycol dimethacrylate (TEGDMA) in a 80:20 mixture, TPO, a camphorquinone (CQ) photoinitiator, and ethyl 4-(dimethylamino)benzoate (EDMAB). The individual composition of the experimental composites is shown in Table 1.

**Table 1** Composition breakdown of the experimental resin composites and a commercially available resin (Systemp onlay) used in this study

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

Abbreviations: BAG-45S5, 45%SiO<sub>2</sub>, 6%P<sub>2</sub>O<sub>5</sub>, 24.5%Na<sub>2</sub>O, 24.5%CaO; BAG-S53P4, 53%SiO<sub>2</sub>, 4%P<sub>2</sub>O<sub>5</sub>, 23%Na<sub>2</sub>O, 20%CaO; ZnO, 40nm Zinc Oxide, CAS: 1314-13-2; SiO<sub>2</sub>, 0.7µm silanized glass filler, CAS:65997-17-3; UDMA, urethane dimethacrylate, CAS:72869-86-4; TEGDMA, triethylene glycoldimethacrylate, CAS:109-16-0; CQ, Camphorquinone, CAS:10373-78-1; EDMAB, ethyl 4-(dimethylamino)benzoate, CAS:10287-53-3; TPO, diphenyl (2,4,6-trimethylbenzoyl)phosphine oxide, CAS:75980-60-8.

All monomers and photoinitiators were purchased from Sigma-Aldrich (Steinheim, Germany). The experimental resin composite containing 25 wt% SiO<sub>2</sub> filler as well as a commercially available Systemp onlay composite (Ivoclar Vivadent, Schaan, Liechtenstein) were used as controls.

#### Flexural strength and flexural modulus

Ten samples were prepared for each group by filling a bar-shaped polyethylene mould of the dimensions 2x2x25 mm with the material according to ISO 4049:2008 specifications,<sup>8</sup> covered with a transparent celluloid strip, compressed with a glass plate attached tightly to the mould surface, and excess material was removed. The specimens were light-cured for 20 seconds using a polywave LED curing unit (Bluephase N; Ivoclar Vivadent, Schaan, Liechtenstein) set to high with an output of around 1100 mW/cm<sup>2</sup>, and followed by further polymerization in a light furnace (Lumamat 100; Ivoclar Vivadent, Schaan, Liechtenstein) for 60 seconds. Finally, all specimens were incubated in water at 37°C for one week, after which their

dimensions were verified using a digital caliper (Mitutoyo, Tokyo, Japan).

Samples from each group were subjected to a three-point bending test on a universal testing machine (Model LF Plus; AMETEK Lloyd Instrument, Hampshire, UK), with the distance between the supporting bars set at 20 mm and a crosshead speed of 1 mm/min. The flexural strength (FS) and flexural modulus (FM) were calculated according to the following equations, where  $F_{max}$  = ultimate force (N),  $l$  = distance between support bars (mm),  $w$  = width of the specimen (mm),  $h$  = height of the specimen (mm),  $F_{lin}$  = force in the linear part of the stress-strain curve (N) and  $d_{lin}$  = deflection at  $F_{lin}$  (mm):

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

#### Fracture toughness

Another set of ten samples of each material was prepared in a similar manner to that described in the

previous section, with different dimensions measuring 2x1.5x 16 mm. A mini-interfacial Fracture Toughness (mini-iFT) notch was prepared in each sample using a 150 µm ultra-thin diamond blade (M1DO8; Struers, Ballerup, Denmark) at a feed speed of 0.015mm/s and a wheel speed of 1000 rpm water cooling under a stereo-microscope following the protocol from an earlier study.<sup>9</sup> The specimen was transferred to the universal testing machine, with the notch tip facing downwards in the test fixture. The mini-iFT ( $K_{QVM}$ ) was carried out using a four-point bending test setup with a crosshead speed of 0.05 mm/min.<sup>9</sup> After testing, all fractured specimens were evaluated by scanning electron microscopy (SEM) and the dimensions of the mini-iFT notch were measured (Image-Pro Plus, Media Cybernetics, MD, USA).  $K_{QVM}$  was then calculated according to the equations as used in a previous study.<sup>9</sup>

#### Water sorption and solubility

For this section, seven samples of each group were prepared in the 2x2x25 mm mould and polymerized in the same way as mentioned above. Instead of immediate incubation in water, this group of samples was first dry stored in a desiccator at 37°C for 24 hours, and each sample was weighed until a constant mass ( $m_1$ ) at an accuracy of  $\pm 0.1$  mg was obtained. Next, the dried samples were placed in distilled water at 37°C for seven days, after which they were removed, air-dried, and weighed to record their mass after saturation with water ( $m_2$ ). The specimens were then placed in the desiccator again until a final constant dry mass ( $m_3$ ) was reached.<sup>10</sup>

The volume ( $V$ ) of each specimen was determined as follows:  $V = \text{length} \times \text{height} \times \text{width}$

Water sorption (WS) and water solubility (SL) in  $\mu\text{g}/\text{mm}^3$  were calculated according to the equations below:

$$WS = \frac{m_2 - m_3}{V}$$

$$SL = \frac{m_1 - m_3}{V}$$

#### Depth of cure

The solvent dissolution technique was used for testing the depth of cure of the composite samples. Six

cylindrical specimens per group were prepared in a mould of 5 mm diameter and 10 mm depth, light-cured for 20 seconds through a glass slide tightly attached on top of the specimens, then immersed immediately in tetrahydrofuran (Ajax Finechem, Inc., Auckland, New Zealand) and stored in the dark at 22°C for 48 hours. After storage, the specimen was dried and the maximum length of the remaining material was measured using a digital caliper and divided by two according to the ISO/DIS 4049 (2008) standard.<sup>8</sup>

#### Antibacterial property (Direct contact method)

The antibacterial property of the composite materials against *Streptococcus mutans*, a cariogenic pathogen, were tested at two different bacterial concentrations ( $1 \times 10^5$  CFU/ml and  $1 \times 10^7$  CFU/ml), based on a previously reported protocol<sup>11</sup> with some modifications. Disc-shaped samples of each group were prepared in a mould of 10 mm diameter and 1 mm thickness. Both the top and bottom surfaces were light-cured for 20 seconds using Bluephase N with an output of around 1100 mW/cm<sup>2</sup> and the samples were then sterilized by ultraviolet light.

*S. mutans* UA159 strain was chosen and grown in brain heart infusion (BHI) broth for 24 hours. 500 µl of the bacterial suspension was transferred into 5 ml of fresh BHI broth and incubated at 37°C, 5% CO<sub>2</sub> until the exponential phase of growth was reached (confirmed by measuring optical density at 590nm; OD<sub>590nm</sub>=0.40). This *S. mutans* suspension was diluted with BHI broth to two concentrations of viable cells at  $1 \times 10^5$  CFU/ml and  $1 \times 10^7$  CFU/ml.

Four disc samples from each group were placed in a 24-well plate (Thermo Fisher Scientific, Jiangsu, China) and 20 µl of the prepared bacterial suspension was added to the surface of each specimen. The same amount of *S. mutans* suspension was also placed in four blank wells to act as the control. Besides, the disks covered with sterilized BHI broth were included to test the sterilization.

To obtain a thin layer of bacteria on the disc samples in order to ensure direct contact between the bacterial cells and the tested surfaces, the suspensions were evaporated by opening the 24-well plate and placing

it in a laminar flow clean bench (NuAire, Inc., Plymouth, USA) for one hour. Upon drying, 520 µl of BHI broth was added to each well and gently mixed for ten minutes. After mixing, 20 µl of the solution from each well was transferred to a new 24-well plate containing 480 µl of BHI broth per well. These two 24-well plates, i.e. plates with and without the specimens, were incubated at 37°C, 5% CO<sub>2</sub> for 18 hours. Lastly, the optical density at 590 nm (OD<sub>590nm</sub>) for each well was measured using a microplate reader (BioTek ELx800; BioTek Instruments, Inc., Vermont, US).

This entire procedure was independently performed three times to obtain averaged data at OD<sub>590nm</sub> for each experimental group.

### Statistical analysis

All data was subjected to Shapiro-Wilk tests to determine the normality of distribution and the Levene's test to assess the equality of variances. Flexural modulus,

water sorption, and depth of cure were analyzed by one-way ANOVA and Tukey's HSD test at a 95% confidence interval ( $p < 0.05$ ), while Kruskal Wallis tests were run for the results of water solubility, antibacterial property. These statistical analyses were carried out using IBM SPSS for Windows, version 18.0 (IBM Corp., Armonk, NY, USA). Measurements of flexural strength and fracture toughness were evaluated by Weibull distribution (with 95% confidence bounds as calculated from R version 3.6.0 with WeibullR and Weibulltools packages).

## Results

### Flexural strength, flexural modulus, and fracture toughness

The data regarding flexural strength, flexural modulus, and fracture toughness are summarized in Table 2.

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

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

Different letters indicate statistical differences within a column ( $p < 0.05$ ).

1 Beta, shape, slope or modulus of Weibull parameter.

2 Eta, Characteristic life or scale of Weibull parameter.

3 95% confidence interval at Characteristic strength (=63.2% unreliability)

The results of all three tests showed similar trends, where the measurements were significantly higher in the SiO<sub>2</sub> and the ZnO groups compared to both bioactive glass composites 45S5 and S53P4, while the commercial Systemp onlay had the lowest readings ( $p < 0.001$ ). However, the mini-iFT of the Systemp onlay could not be evaluated

as the specimen did not break under testing. The images from the SEM failure analysis of the representative mini-iFT specimens are presented in Fig. 1.

### Water sorption, water solubility, and depth of cure

The measurements of water sorption, water solubility, and depth of cure are shown in Table 3.

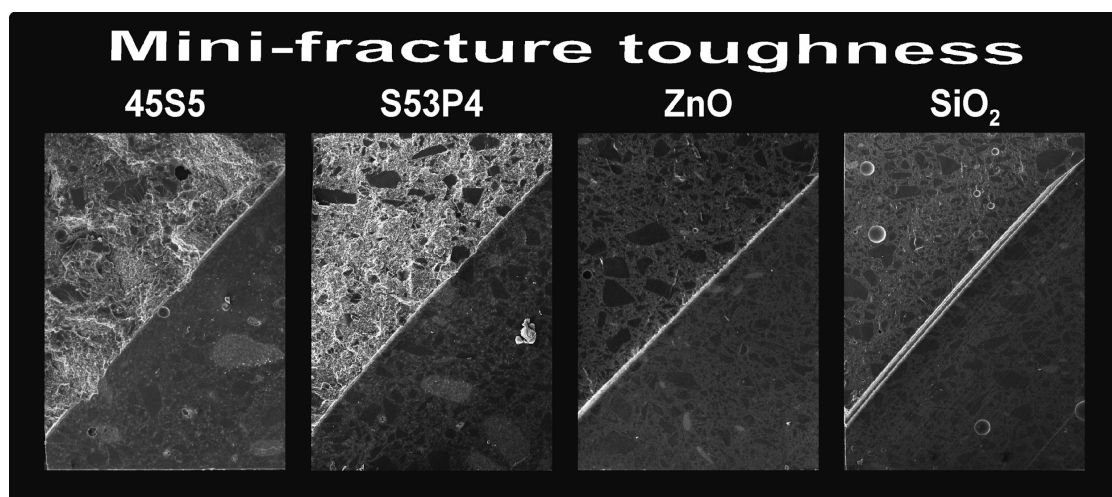


Figure 1 SEM surface analysis of mini-interfacial fracture toughness specimens

Table 3 Values of water sorption, water solubility and depth of cure of the tested materials

Material	Water sorption ( $\mu\text{g}/\text{mm}^3$ )	Water solubility ( $\mu\text{g}/\text{mm}^3$ )	Depth of cure (mm)
45S5	63.2 (2.5) <sup>A</sup>	5.5 (1.7) <sup>a,b</sup>	3.0 (0.2) <sup>l,III</sup>
S53P4	56.5 (3.3) <sup>B</sup>	-10.1 (0.9) <sup>c</sup>	3.2 (0.3) <sup>II</sup>
ZnO	20.1 (1.1) <sup>C</sup>	2.1 (0.8) <sup>a,b,c</sup>	2.7 (0.2) <sup>III</sup>
SiO <sub>2</sub>	20.8 (1.5) <sup>C</sup>	0.5 (0.7) <sup>b,c</sup>	3.9 (0.3) <sup>I</sup>
Systemp onlay	20.4 (1.7) <sup>C</sup>	27.9 (2.8) <sup>a</sup>	4.4 (0.5) <sup>I</sup>

Different letters indicate statistical differences within a column ( $p < 0.05$ )

The highest water sorption was seen in the 45S5 bioactive glass group ( $63.2 \pm 2.5 \mu\text{g}/\text{mm}^3$ ), which was significantly higher than the S53P4 bioactive glass, the SiO<sub>2</sub>, the Systemp onlay, and the ZnO composite in decreasing order, but the latter three composites did not differ significantly between each other.

The highest water solubility was displayed by the Systemp onlay ( $27.9 \pm 2.8 \mu\text{g}/\text{mm}^3$ ), while the lowest was found in the the S53P4 bioactive glass group which recorded negative water solubility values ( $-10.1 \pm 0.9 \mu\text{g}/\text{mm}^3$ ).

Measurements of the depth of cure decreased starting from the Systemp onlay ( $4.4 \pm 0.5 \text{ mm}$ ), followed by the SiO<sub>2</sub> ( $3.9 \pm 0.3 \text{ mm}$ ), the S53P4 bioactive glass ( $3.2 \pm 0.3 \text{ mm}$ ), the 45S5 bioactive glass ( $3.0 \pm 0.2 \text{ mm}$ ), and finally the ZnO ( $2.7 \pm 0.2 \text{ mm}$ ), though the differences between

the first two groups and the last two groups were not statistically significant.

#### Antibacterial properties

The concentration of bacterial cells in suspension was assessed by measurements of optical density. Growth of *S. mutans* in BHI broth after direct contact with the specimens would produce a cloudy appearance in the medium. Hence the absorbance value of each liquid medium at 590 nm would indicate the bacterial turbidity around each sample.

The optical density data are presented in Table 4. For both bacterial concentrations of  $10^5 \text{ CFU}/\text{ml}$  and  $10^7 \text{ CFU}/\text{ml}$ , although the readings in all tested groups were lower than the control, no statistically significant difference was observed between them ( $p > 0.05$ ), regardless of whether the samples were present or not.

**Table 4** Optical density readings (at 590 nm) of composite materials after incubation with *S. mutans* at  $10^5$  CFU/ml and  $10^7$  CFU/ml

Material	Optical density (OD <sub>590nm</sub> )			
	<i>S. mutans</i> $10^5$ CFU/ml.		<i>S. mutans</i> $10^7$ CFU/ml.	
	Presence of tested materials	Absence of tested materials	Presence of tested materials	Absence of tested materials
45S5	0.25 ± 0.02 (0.25)	0.25 ± 0.03 (0.26)	0.30 ± 0.01 (0.29)	0.30 ± 0.02 (0.30)
S53P4	0.25 ± 0.01 (0.25)	0.25 ± 0.01 (0.25)	0.29 ± 0.01 (0.29)	0.30 ± 0.01 (0.30)
ZnO	0.25 ± 0.01 (0.25)	0.25 ± 0.01 (0.25)	0.29 ± 0.01 (0.30)	0.30 ± 0.01 (0.30)
SiO <sub>2</sub>	0.27 ± 0.02 (0.26)	0.26 ± 0.01 (0.26)	0.30 ± 0.02 (0.30)	0.30 ± 0.03 (0.29)
Systemp onlay	0.27 ± 0.01 (0.28)	0.27 ± 0.01 (0.27)	0.30 ± 0.01 (0.30)	0.31 ± 0.02 (0.31)
Control	0.30 ± 0.02 (0.30)	0.29 ± 0.03 (0.30)	0.31 ± 0.01 (0.31)	0.32 ± 0.003 (0.32)

mean ± SD (median)

## Discussion

This study was carried out to compare the mechanical, physical, and antibacterial properties between provisional composites incorporated with three types of experimental fillers (45S5, S53P4, and zinc oxide nanoparticles) with a SiO<sub>2</sub> composite and a commercial composite Systemp Onlay. The results showed significant differences in terms of flexural strength, flexural modulus, water sorption, water solubility, and depth of cure between the groups. Therefore, all null hypotheses were rejected except for antibacterial properties.

The SiO<sub>2</sub> and the ZnO composites showed equally superior flexural strength, flexural modulus, and fracture toughness than the 45S5 and the S53P4 composites, with the Systemp onlay displaying the lowest values. (Table 2) Flexural strength and flexural modulus do not seem to be influenced by increasing concentrations of ZnO fillers (particle size 20 nm) at 0-5 wt%,<sup>12</sup> which would explain the insignificant differences in these properties between the SiO<sub>2</sub> and the ZnO samples.

The addition of bioactive glass fillers appeared to reduce the flexural strength of the composite material. These results are in accordance with previous studies that found a negative linear correlation between the amount of bioactive glass fillers with the flexural strength and modulus.<sup>13,14</sup> Par *et al* reported that the flexural strength and modulus decreased when the amount of bioactive glass fillers (particle

size 4-13 µm) was increased from 5 to 40 wt%.<sup>13</sup> In contrast, another study showed that the flexural strength and fracture toughness were unaffected by increasing concentrations of bioactive glass (particle size 0.04-3.0 µm) by up to 15 wt%.<sup>5</sup> These conflicting observations could be due to the different manufacturing process and particle size of the bioactive glass fillers.<sup>15,16</sup> This study utilized a melt-quenching process to produce bioactive glass particles of 5.81 µm in diameter. Nevertheless, the previous studies investigated the experimental composite containing a high concentration of SiO<sub>2</sub>,<sup>5,13</sup> while the present study investigated a provisional composite containing only 25 wt% of filler, including 10 wt% of SiO<sub>2</sub> with 15 wt% of bioactive glass. Thus, the low concentration of SiO<sub>2</sub> filler incorporated with bioactive glasses could lead to lower mechanical properties (flexural strength, flexural modulus and fracture toughness).

The lowest values of flexural strength and flexural modulus were displayed by the commercially available Systemp onlay. Interestingly, the fracture toughness could not be recorded as the specimen was bendable and did not break under the test conditions. The fracture toughness test is representative of indentation-induced cracking to characterize the toughness of brittle materials.<sup>17</sup>

To interpret these results, the composition of resin composites should be analyzed. Resin composites

are made up of a diverse range of components which function as monomers, initiators, and fillers. In general, a resin composite with higher filler volume results in higher flexural strength and flexural modulus values,<sup>18</sup> the exception being for bioactive glass fillers as previously mentioned. The filler composition in the Systemp onlay is lower than in the other experimental composites, at 19.4% and 25% respectively.

According to the manufacturer, the Systemp onlay is composed of a dimethacrylate monomer (polyester urethane dimethacrylate) and a monomethacrylate monomer (monofunctional ethyl triglycol methacrylate) but the experimental composites consisted of two dimethacrylate monomers (urethane dimethacrylate and triethylene glycol dimethacrylate) instead. Dimethacrylates differ structurally from monomethacrylates, where the former is made up of cross-linked molecules while the latter is a linear structure.<sup>19</sup> These differences give the Systemp onlay its unique characteristics of being a highly flexible material.

The types and concentrations of initiators in resin composites can also affect their mechanical properties. The Systemp onlay is comprised of 1 wt% of initiators which included a catalyst, a stabilizer, and triclosan. This study chose to use 1.5 wt% of a different variety of initiators (CQ, Lucirin TPO, and EDMAB) in the experimental composites in order to enhance the initial polymerization process. This increased polymerization could have contributed further to the improved mechanical properties in the experimental group.<sup>20</sup>

Water sorption and solubility of resin composites refer to the uptake of water by the material and the dissolution of the material components respectively.<sup>21</sup> Various factors that could affect these properties include the filler content (type, content, concentration, particle size, nature), coupling agent, monomer hydrophobicity and hydrophilicity, solvent type, and polymerization mechanism.<sup>22,23</sup>

Water sorption was significantly higher in the sample of bioactive glass composites compared to the other groups. The results are similar to a previous study that showed increasing water sorption with increasing concentrations of bioactive glass fillers.<sup>24</sup> It can be concluded

that this could be due to the highly hydrophilic bioactive glass fillers as compared to  $\text{SiO}_2$ .<sup>24</sup>

Conversely, water solubility was significantly higher in the Systemp onlay than in the other composites. This could be related to the extent of polymerization as previously discussed. A lower degree of polymerization could lead to a greater amount of leachable monomer components<sup>25</sup> or to more unreacted functional groups suspended from the network.<sup>26</sup> For the S53P4 composite group, a negative water solubility value was recorded. Though this negative value suggests that the water content was not completely removed during the dry storage period, it cannot be concluded that no components eluted from the material either. A plausible explanation would be that the glass particles and ions in the composite material participated in silica gel formation, which often occurs in glass ionomer-based<sup>27,28</sup> and bioactive glass materials.<sup>29</sup>

Even though these mechanisms should theoretically also occur in the other bioactive glass group of 45S5, a negative water solubility value similar to that of S53P4 was not shown in 45S5. This could simply be due to the higher solubility of the 45S5 particles,<sup>30</sup> or that when water was absorbed by the highly hydrophobic composite, part of the water was bound and entrapped in the resin network.<sup>31</sup>

The parameters that can influence the depth of cure of resin composite are chemical composition (filler and monomer), curing intensity, and curing time.<sup>32</sup> In the present study, standardized monomers, curing intensity, and curing time were used, while only the fillers varied. The filler and monomer compositions are associated with the opacity of the material, which consequently affects its refractive index. A mismatched refractive index between the filler and resin monomer would promote light scattering at the filler-resin interface.<sup>33</sup> The more well-matched the refractive index between the filler and resin monomer, the higher the translucency and light transmittance of the composite material.<sup>34</sup> To illustrate this point, the material refractive indexes of the composite material components in this study are: TEGDMA (1.461), UDMA (1.485), silanized  $\text{SiO}_2$  (1.553), and ZnO powder

(1.989).<sup>35</sup> As it was found that the depth of cure was highest in the Systemp onlay and SiO<sub>2</sub> composite groups, this would suggest that the components of the commercial material are of well-matching refractive indices. The groups with bioactive glasses displayed lower curing depths compared to SiO<sub>2</sub>, which is in agreement with previous studies.<sup>36</sup> The shallowest depth of cure was seen in the ZnO composite and this can be related to the large difference in the refractive indices between ZnO and the resin monomer as was also noted by other researchers.<sup>12,35,37</sup>

Regarding the antibacterial property testing against *S. mutans* at 10<sup>5</sup>CFU/ml and 10<sup>7</sup>CFU/ml in this study, though the optical density readings which indicate bacterial growth were lower in the experimental composites compared to the control, they did not reach statistical significance. Bacterial growth was also similar in the presence or absence of the composite samples. (Table 4) Thus, it would seem that the addition of bioactive glasses or ZnO fillers to the resin composite has no effect on its antibacterial properties against *S. mutans*. However, many studies have shown that ZnO has an inhibitory effect on oral bacteria.<sup>38,39</sup> For example, Hojati *et al* reported that their flowable composite samples containing 1 wt% ZnO resulted in reduced colony-forming units of *S. mutans* while 4 wt% ZnO successfully inhibited bacterial growth completely.<sup>12</sup> The possible reasons behind the contrasting results of this study should be explored.

Antibacterial behaviour of resin composites depends on their degradation and ion release. Silver-doped bioactive glass experiments suggest that composite materials containing 2.1 wt% Ag<sub>2</sub>O (2.1wt%) exhibited antibacterial properties due to the release of silver ions and did not depend on changes in pH.<sup>3</sup>

The commercial Systemp onlay contains triclosan which is intended to reduce bacterial adhesion on the surface of the material. Again, other authors have validated this property. Using the direct contact method, Slutzky *et al* demonstrated a significant inhibitory effect of Systemp onlay against *S. mutans* growth in suspension even after ageing the material for seven days.<sup>40</sup> It can only be deduced

that there were dissimilarities in the methodology regarding the bacterial preparation and result measurement that caused this data to not be in accordance with other studies.

In summary, the addition of bioactive glasses or ZnO filler particles might negatively impact the mechanical and physical properties and not have a positive improvement on the antibacterial properties of resin composite. Therefore, this experimental provisional composite still needs to be improved for achieving an antibacterial property.

## Conclusion

1) Resin composite incorporated with 15wt % bioactive glasses (45S5, S53P4) had significantly reduced mechanical properties, physical properties, and depth of cure.

2) The addition of 1 wt% ZnO in the resin composite did not improve its mechanical and physical properties but significantly decreased the depth of cure.

3) All experimental composites and the commercial Systemp onlay did not display antibacterial properties towards *S. mutans*.

## Acknowledgements

Our research was partially supported by the Thailand Research Fund (TRF Research Scholar; Project No. RSA6080090). We would also like to express our gratitude to the National Metal and Materials Technology Center (MTEC) for synthesizing the bioactive glasses (45S5 and S53P4) used in our study. The authors declare no potential conflicts of interest with respect to the authorship and publication of this article.

## References

1. Balkenhol M, Feger P, Mautner MC, Wöstmann B. Provisional crown and fixed partial denture materials: mechanical properties and degree of conversion. *Dent Mater* 2007;23(12):1574-83.
2. von Fraunhofer JA, editor.. Dental materials at a glance. 2<sup>nd</sup> ed. Oxford, England: Wiley-Blackwell; 2013.
3. Chatzistavrou X, Fenno JC, Faulk D, Badyak S, Kasuga T, Boccaccini AR, *et al*. Fabrication and characterization of bioactive and antibacterial composites for dental applications. *Acta biomaterialia* 2014;10(8):3723-32.

4. Dinwiddie MT, Terry PD, Chen J. Recent evidence regarding triclosan and cancer risk. *Int J Environ Res Public Health* 2014;11(2):2209-17.
5. Khvostenko D, Mitchell J, Hilton T, Ferracane J, Kruzic J. Mechanical performance of novel bioactive glass containing dental restorative composites. *Dent Mater* 2013;29(11):1139-48.
6. Chatzistavrou X, Velamakanni S, DiRenzo K, Lefkelidou A, Fenno JC, Kasuga T, *et al.* Designing dental composites with bioactive and bactericidal properties. *Mater Sci Eng C* 2015;52:267-72.
7. Kołodziejczak-Radzimska A, Jesionowski T. Zinc oxide—from synthesis to application: a review. *Materials* 2014;7(4):2833-81.
8. International Organization for Standardization. ISO 4049 Dentistry - Polymer-based restorative materials. Geneva: ISO; 2008.
9. Pongprueksa P, De Munck J, Karunratanakul K, Barreto B, Van Ende A, Senawongse P, *et al.* Dentin bonding testing using a mini-interfacial fracture toughness approach. *J Dent Res* 2016;95(3):327-33.
10. Pieper C, Zanchi C, Rodrigues-Junior S, Moraes R, Pontes L, Bueno M. Sealing ability, water sorption, solubility and toothbrushing abrasion resistance of temporary filling materials. *Int Endod J* 2009;42(10):893-9.
11. Huang Qt, He Jw, Lin Zm, Liu F, Lassila LV, Vallittu PK. Physical and chemical properties of an antimicrobial Bis-GMA free dental resin with quaternary ammonium dimethacrylate monomer. *J Mech Behav Biomed Mater* 2016;56:68-76.
12. Hojati ST, Alaghemand H, Hamze F, Babaki FA, Rajab-Nia R, Rezvani MB, *et al.* Antibacterial, physical and mechanical properties of flowable resin composites containing zinc oxide nanoparticles. *Dent Mater* 2013;29(5):495-505.
13. Par M, Tarle Z, Hickel R, Ilie N. Mechanical properties of experimental composites containing bioactive glass after artificial aging in water and ethanol. *Clin Oral Investig* 2019;23(6):2733-41.
14. Yang SY, Piao YZ, Kim SM, Lee YK, Kim KN, Kim KM. Acid neutralizing, mechanical and physical properties of pit and fissure sealants containing melt-derived 45S5 bioactive glass. *Dent Mater* 2013;29(12):1228-35.
15. Fiume E, Migneco C, Verné E, Baino F. Comparison between Bioactive Sol-Gel and Melt-Derived Glasses/Glass-Ceramics Based on the Multicomponent  $\text{SiO}_2\text{-P}_2\text{O}_5\text{-CaO-MgO-Na}_2\text{O-K}_2\text{O}$  System. *Materials* 2020;13(3):540.
16. Wajda A, Goldmann WH, Detsch R, Boccaccini AR, Sitarz M. Influence of zinc ions on structure, bioactivity, biocompatibility and antibacterial potential of melt-derived and gel-derived glasses from  $\text{CaO-SiO}_2$  system. *J Non-Cryst Solids* 2019;511:86-99.
17. Feng Y, Zhang T. Determination of fracture toughness of brittle materials by indentation. *Acta Mechanica Solida Sinica* 2015;28(3):221-34.
18. Kim KH, Ong JL, Okuno O. The effect of filler loading and morphology on the mechanical properties of contemporary composites. *J Prosthet Dent* 2002;87(6):642-9.
19. Astudillo-Rubio D, Delgado-Gaete A, Bellot-Arcís C, Montiel-Company JM, Pascual-Moscardó A, Almerich-Silla JM. Mechanical properties of provisional dental materials: A systematic review and meta-analysis. *PLoS One* 2018;13(2):e0193162.
20. Grohmann CVS, Soares EF, Souza-Junior EJC, Brandt WC, Puppim-Rontani RM, Geraldini S, *et al.* Influence of different concentration and ratio of a photoinitiator system on the properties of experimental resin composites. *Braz Dent J* 2017;28(6):726-30.
21. Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. *Dent Mater* 2006;22(3):211-22.
22. Mese A, Burrow MF, Tyas MJ. Sorption and solubility of luting cements in different solutions. *Dent Mater J* 2008;27(5):702-9.
23. Van Landuyt K, Nawrot T, Geebelen B, De Munck J, Snauwaert J, Yoshihara K, *et al.* How much do resin-based dental materials release? A meta-analytical approach. *Dent Mater* 2011;27(8):723-47.
24. Par M, Spanovic N, Bjelovucic R, Marovic D, Schmalz G, Gamulin O, *et al.* Long-term water sorption and solubility of experimental bioactive composites based on amorphous calcium phosphate and bioactive glass. *Dent Mater J* 2019;38(4):555-64.
25. Pongprueksa P, Miletic V, Janssens H, Van Landuyt KL, De Munck J, Godderis L, *et al.* Degree of conversion and monomer elution of CQ/amine and TPO adhesives. *Dent Mater* 2014;30(6):695-701.
26. Boaro LC, Goncalves F, Guimaraes TC, Ferracane JL, Pfeifer CS, Braga RR. Sorption, solubility, shrinkage and mechanical properties of “low-shrinkage” commercial resin composites. *Dent Mater* 2013;29(4):398-404.
27. Gladys S, Van Meerbeek B, Braem M, Lambrechts P, Vanherle G. Comparative physico-mechanical characterization of new hybrid restorative materials with conventional glass-ionomer and resin composite restorative materials. *J Dent Res* 1997;76(4):883-94.
28. Toledano M, Osorio R, Osorio E, Aguilera FS, Romeo A, de la Higuera B, *et al.* Sorption and solubility testing of orthodontic bonding cements in different solutions. *J Biomed Mater Res B Appl Biomater* 2006;76(2):251-6.
29. Renno AC, Bossini PS, Crovace MC, Rodrigues AC, Zanotto ED, Parizotto NA. Characterization and *in vivo* biological performance of biosilicate. *Biomed Res Int* 2013;141427.
30. Oonishi H, Hench LL, Wilson J, Sugihara F, Tsuji E, Matsuura M, *et al.* Quantitative comparison of bone growth behavior in granules of Bioglass®, A-W glass-ceramic, and hydroxyapatite. *J Biomed Mater Res* 2000;51(1):37-46.
31. Wei YJ, Silikas N, Zhang ZT, Watts DC. Diffusion and concurrent solubility of self-adhering and new resin-matrix composites during water sorption/desorption cycles. *Dent Mater* 2011;27(2):197-205.
32. Rueggeberg FA, Caughman WF, Curtis JW, Jr. Effect of light intensity and exposure duration on cure of resin composite. *Oper Dent* 1994;19(1):26-32.

33. Yoshimura HN, Goldenstein H. Light scattering in polycrystalline alumina with bi-dimensionally large surface grains. *J Eur Ceram Soc* 2009;29(2):293-303.
34. Yoshimura HN, Chimanski A, Cesar PF. Systematic approach to preparing ceramic-glass composites with high translucency for dental restorations. *Dent Mater* 2015;31(10):1188-97.
35. Wang Z, Lu Z, Mahoney C, Yan J, Ferebee R, Luo D, *et al.* Transparent and high refractive index thermoplastic polymer glasses using evaporative ligand exchange of hybrid particle fillers. *ACS Appl Mater Interfaces* 2017;9(8):7515-22.
36. Kattan H, Chatzistavrou X, Boynton J, Dennison J, Yaman P, Papagerakis P. Physical properties of an ag-doped bioactive flowable composite resin. *Materials* 2015;8(8):4668-78.
37. Demir MM, Koynov K, Akbey Ü, Bubeck C, Park I, Lieberwirth I, *et al.* Optical properties of composites of PMMA and surface-modified zincite nanoparticles. *Macromolecules* 2007;40(4):1089-100.
38. Wanitwisutchai T, Monmatrapoj N, Srisatjaluk R, Subannajui K, Anuwongnukroh N, Dechkunakorn S, *et al.* Antibacterial Properties among Different Concentration of Bioactive Glasses. *Key Eng Mater* 2019;814:349-53.
39. Sergi R, Bellucci D, Salvatori R, Maisetta G, Batoni G, Cannillo V. Zinc containing bioactive glasses with ultra-high crystallization temperature, good biological performance and antibacterial effects. *Mater Sci Eng C Mater Biol Appl* 2019;104:109910.
40. Slutzky H, Slutzky-Goldberg I, Weiss E, Matalon S. Antibacterial properties of temporary filling materials. *J Endod* 2006;32(3):214-7.