

Research Article

Bond Strength of Reinforced Autopolymerized Acrylic Resin to Denture Base Resin

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Received 19 January 2023; Revised 18 May 2023; Accepted 27 June 2023; Published 7 July 2023

Academic Editor: Kaliannan Durairaj

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The aim of this study was to analyze the effect of applying short E-glass fiber (SEG) and ultrahigh molecular weight polyethylene (UWPE) filler on the shear bond strength between the repaired surface of the rapid heat-cured and the reinforced autopolymerized acrylic resin. Fifty-six circular ($15 \times 3.3 \text{ mm}^2$) rapid heat-cured resins were made and assigned equally to seven different groups. These were later bonded to fifty-six circular-reinforced autopolymerized acrylic resin specimens. Every test group included a control group, along with applying 1% and 2% SEG, 0.5% SEG/UWPE, 1% SEG/UWPE, and 1% and 2% UWPE. The universal testing machine was utilized to conduct the shear bond strength test. The repaired surface of these samples was assessed. Besides, the mode of failure was visualized under stereo microscope. The shear bond strength of all experimental groups was significantly higher than that of the control group. Group 3 with the addition of 1% SEG demonstrated the highest significance (12.86 MPa). The mode of failure for most of specimens was a mixed failure. Thus, it is indicated that enhancing the resin with 1% short E-glass fiber significantly improves the shear bond strength between repaired surface of the rapid heat-cured acrylic resin denture base and the reinforced autopolymerized acrylic resin.

1. Introduction

In the fabrication of denture bases, poly(methyl) methacrylate, known as PMMA, remains the preferred material of choice and has been used in the dental industry ever since its introduction in the 1930s, up until today. PMMA resin has gained immense popularity in the past centuries due to its ease of manipulation and processing, affordability and pleasing aesthetics, adequate strength, and dimensional stability, as well as its biocompatibility [1]. Despite its popularity, conventional PMMA is far from ideal in meeting the mechanical requirements of a dental prosthesis. The conventional PMMA denture bases are shown to be brittle and weak, and therefore it has insufficient strength to withstand high stress of masticatory forces [2-4]. This is reflected in the unresolved problem of denture fracture and the accompanying costs to repair [5].

The majority of fractured dentures is repaired with autopolymerizing acrylic resin. This is a simple, quick, and low-cost method that can be executed chairside at the clinic [6, 7]. Despite the fact that autopolymerized acrylic resin has lower transverse strength than standard heat-cured acrylic resin, knowing that it is frequently fractured again at the mended site, it is widely used and applied as a denture repair material [8]. Many factors influence the success of PMMA denture repair, including the repair surface design, the surface treatment, the combination of denture base resin, and the repair material applied, as well as the use of adhesives and reinforcements [9]. The ideal denture repair materials should have adequate strength, good dimensional stability with color match, ease of manipulation, and cost effectiveness. Researchers have made numerous attempts to modify PMMA resins in order to improve mechanical performance as well as bond strength between conventional heat-cured denture base resin and the repair material [7, 10]. Adhesion between materials can be improved through mechanical and chemical surface modification. Chemical treatment by proper wetting and changes in surface morphology of the repair surface with monomer, acetone, methylene chloride, or chloroform makes a crucial contribution to the strength of repaired acrylic resin [11, 12]. Mechanical surface treatment with abrasive air blasting showed significant improvement in the denture repairs [13, 14].

Particulate fillers are added into the polymer matrix to modify the physical and mechanical properties of the polymers. Adhesion between fillers and matrix is critical for achieving optimal properties because it allows the load to be transferred from the weak matrix to the fillers, providing the necessary reinforcement [1, 6, 15]. The bonding is typically based on the silanization of glass fibers, particle sizes and shapes, and particles' surface treatments. The short fiberreinforced resin was investigated and presented as a viable option for the use as a denture material since it is capable of enduring high stress-bearing forces [16]. A previous study showed that adding 1% short E-glass fiber significantly improved the flexural strength of the autopolymerized acrylic resin [17]. Stipho concluded that the highest transverse strength was found when the denture acrylic resin was enhanced by adding 1% glass fiber before and after repair, and that the inclusion of higher than 5% glass fiber content yielded no significant mechanical benefits [18]. Another study by Deb et al. 2020 revealed that using glass fiberreinforced autopolymerized acrylic resin as a repair material showed to have the highest flexural strength compared to unreinforced autopolymerized and light-cured acrylic resin [19]. The E-glass fiber has undergone extensive research, leading it to be widely recognized as one type of PMMA reinforcement. Nonetheless, the optimal concentration for reinforcements related to the dental field as well as fiber sizes remains undetermined. Moreover, UWPE is one of the most durable and versatile materials that have been recently introduced in dentistry as it also possesses significant potential applications in many areas especially as fillers in resin polymers [20]. This is due to UWPE's high wear-resistance, toughness, ductility, and biocompatibility [21]. UWPE is white in color, allowing it to be implemented in aesthetic applications in the dental field [22]. However, one documented disadvantage of this material is its inertness. Gutteridge indicated that the addition of 1% UWPE fiber yielded promising results for acrylic resin reinforcement, but there was no significant effect between surfaces treated with UWPE fiber [23]. According to Alla et al., concentrations as low as 1% UWPE can significantly improve denture base resin impact strength [24]. The study by Ranade et al. also

stated that inclusion of UWPE improved both the composites' toughness and modulus while decreasing the flexural strength [25].

Although reinforcing fillers and particles help improves the properties of denture repair, there have been insufficient studies, and more research studies are required [26]. To date, the effect of SEG and UWPE as hybrid reinforcement in terms of shear bond strength has not been investigated. As a result, the goal of this research was to determine the influence of SEG and UWPE filler particle additions on the shear bond strength and mode of failure of repaired autopolymerized PMMA denture base. It was hypothesized that there are no statistically significant differences with respect to the shear bond strength between the repaired surface of autopolymerized acrylic resin with different percentages of SEG and UWPE filler particles and rapid heat-cured acrylic resin.

2. Materials and Methods

2.1. Reinforced Autopolymerized Acrylic Resin Preparation. The autopolymerized acrylic resin used in this experiment was Unifast Trad (GC Corporation). A commercial discontinuous short E-glass fiber with diameters of $16 \,\mu\text{m}$ and $220\,\mu\text{m}$ in length, known as microglass milled fibers (asreceived silanized), was obtained and used as received from Fibertec (Bridgewater). The UWPE filler particles (\emptyset 150 μ m, PSD X50; lot no. 200410018) were obtained from IRPC Public Company Limited, performing further surface treatment with Chromic acid solution (K2Cr207:H2SO4: H₂O) of (7:150:12 wt%) following Li et al.'s method [27, 28] before being used as a reinforcing filler in this study. The reinforced autopolymerized acrylic resin was obtained with a combination of resin polymer powder, along with preweighed SEG and UWPE filler particles in the proportions specified by weight (Table 1). A magnetic stirrer machine set to 450 rpm for a duration of 30 minutes was used to reach an even and consistent dissemination of reinforcement particles that are spread throughout the resin polymer powder mixture.

2.2. Specimen Preparation. This experiment was conducted following the Standard Test Method for Shear Strength of Adhesive Bonds Between Rigid Substrates by the Block-Shear Method (ASTM D4501-01(2014)).

A circular stainless-steel mold of diameter $15 \pm 0.2 \text{ mm}$ and thickness of $3.3 \pm 0.2 \text{ mm}$ was used to fabricate the specimens with rapid heat-cured resin (Vertex-Dental B.V) according to manufacturer's recommendations (curing for 20 minutes at 100°C). The specimens were standardized with digital caliper and fixed into PVC mold with autopolymerized acrylic resin (Kerr Corporation). All specimens were polished with an automatic polishing machine (Future- Tech Corp.) with abrasive silicon carbide paper 600 grit (Waterproof abrasive paper DCC; TOA Paint Co., Thailand). Specimens were immersed in an ultrasonic machine (Crest Ultrasonics Corp.) for 1 minute to remove contaminated

Group		Repair material description (% weight)	Monomer	
1	Control	100% autopolymerized acrylic resin		
2	2% SEG	98% autopolymerized acrylic resin + 2% SEG		
3	1% SEG	99% autopolymerized acrylic resin + 1% SEG		
4	0.5% SEG/UWPE	99% autopolymerized acrylic resin + 0.5% SEG + 0.5% UWPE	Powder: liquid 0.5 ml/1 g	
5	1% SEG/UWPE	98% autopolymerized acrylic resin + 1% SEG + 1% UWPE		
6	1% UWPE	99% autopolymerized acrylic resin + 1% UWPE		
7	2% UWPE	98% autopolymerized acrylic resin + 2% UWPE		

SEG = short E-glass fiber. UWPE = ultrahigh molecular weight polyethylene filler.

particles and then were randomly divided into seven groups (n=8). After that, they were adhered to autopolymerized acrylic resin integrated with various percentages by weight of SEG and UWPE filler particles, according to each test group.

2.3. Bonding Procedures. The boundaries of the area of repaired surface were set by using a masking tape with a center hole of diameter 5 mm. The adhered surface was treated by application of MMA monomer for 180 seconds. Then, silicone mold with a center diameter hole of 5 ± 0.2 mm and 2 ± 0.2 mm height was placed over the PVC mold, ensuring that the center hole of the silicone mold and the masking tape is aligned together (Figure 1). The reinforced autopolymerized acrylic resin powder was simply mixed with liquid monomer and poured into the silicone mold, achieving minimal excess to account for polymerized shrinkage. The polyethylene film and weight pendulum of 1 kg were then placed over the silicon mold. All specimens were immersed in water at 50°C for 5 minutes to achieve a full polymerization reaction. Specimens were then stored in distilled water in an incubator at $37 \pm 1^{\circ}$ C for 50 ± 2 hours. This was then followed by placing all specimens in thermocycling machine (Medical and Environmental Equipment Research Laboratory) at 5°C and 55°C for 5,000 cycles prior to the test.

2.4. Shear Bond Strength Test. The shear bond strength tests were conducted on all fifty-six prepared specimens under UTM (Shimadzu). The axis of the specimen was placed in a position where the knife-edge shearing blade was in contact with the junction of rapid heat-cured acrylic resin and reinforced autopolymerized acrylic resin interface, securing a parallel location. Shear force was applied at a crosshead speed of 1.26 mm/min with a 50 N load cell. The needed forces (N) for the separation of the resin interface were noted.

The shear bond test (Figure 2) was used to determine the adhesives' bond strength in a given direction or under a type of stress. The aim of the shear bond test between the conventional heat-cured acrylic resin and the repair self-cured acrylic resin was to determine the bonding ability between two types of acrylic resins together while under stress, simulating denture repair procedures performed at the clinic. The resulting force was calculated using the following formula:

$$S = \frac{T}{A},$$
 (1)

where S is the shear bond strength (MPa), T is the tension applied (N), and A is the bonded area (mm^2).

2.5. Mode of Failure. All of the test specimens were visually analyzed using a stereo microscope at 20× magnification for examining mode of failure. Failure was defined according to three types: (1) cohesive failure, (2) adhesive failure, or (3) mixed failure. The percentage of retained reinforced autopolymerized acrylic resin or dislodged rapid heat-cured acrylic resin on the repaired surface determined the type of failure. The failure modes were defined as adhesive when appeared up to 25% on the repaired surface, as cohesive where fracture of reinforced autopolymerized acrylic resin or rapid heat-cured acrylic resin exceeding 75%, and lastly as mixed when cohesive and adhesive failures were between 25 and 75%.

2.6. Scanning Electron Microscope (SEM) Analysis. The repaired surface topography was visualized using a scanning electron microscopy (Oxford X-Max 50). SEM at ×15 and ×1000 examines the mode of failure (adhesive, cohesive, or mixed).

2.7. Statistical Analysis. Analysis of the study's findings was conducted using statistical software (SPSS Statistics 22.0). Kolmogorov–Smirnov's test was used for testing of normality, and Levene's test was used for homogeneity of variances. Both tests revealed no violation of assumption. To explore statistically significant means among the tested groups, a parametric of one-way ANOVA was utilized, accompanied by the Tukey HSD multiple comparison post hoc test. A *P* value of 0.05 represented a statistical significance ($\alpha = 0.05$).

3. Results

Table 2 summarizes the means, standard deviations, and statistical significances of the shear bond strength (MPa) of the tested groups. The ANOVA results indicated statistically

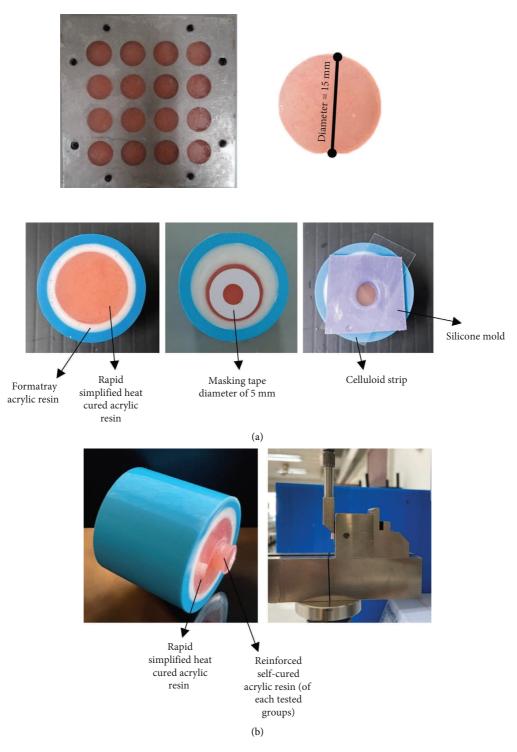


FIGURE 1: Specimen preparation for shear bond strength test.

significant differences between the groups ($p \le 0.001$, F = 22.670); thus, Tukey HSD was used to compare the tested groups. When all the reinforced groups of autopolymerized acrylic resin were analyzed and compared, group 3 (1% SEG) indicated the strongest shear bond, followed by group 5 (1% SEG/UMPE), group 7 (2% UMPE), group 2 (2%S EG), group 6 (1% UMPE), and group 4 (0.5% SEG/UMPE) (12.86 ± 0.51, 11.12 ± 0.42, 10.80 ± 0.63, 10.80 ± 0.63, 10.74 ± 0.94, and

10.57 \pm 0.29, respectively). The control group has the lowest shear bond strength (9.37 \pm 0.66 MPa).

As opposed to the control group (p < 0.005), supplying with different concentration of SEG and UWPE significantly increased the shear bond strength for all reinforced groups. There were no significant differences seen in the reinforced groups, considering group 2 (2% SEG), group 4 (0.5% SEG/ UMPE), group 5 (1% SEG/UMPE), group 6 (1% UMPE), and

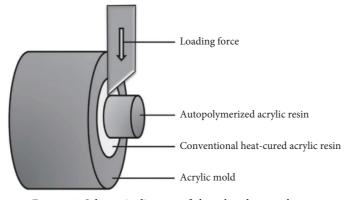


FIGURE 2: Schematic diagram of shear bond strength test.

TABLE 2: Mean values and standard deviations of the shear bond strength test (MPa) between reinforced autopolymerized acrylic resin and rapid heat-cured acrylic resin of tested groups.

	Shear bond strength test (MPa)						
	Group	N	Mean	Std. deviation			
1	Control	8	9.37 ^a	0.66			
2	2% SEG	8	10.80^{b}	0.63			
3	1% SEG	8	12.86 ^c	0.51			
4	0.5% SEG/UWPE	8	10.57 ^b	0.29			
5	1% SEG/UWPE	8	11.12 ^b	0.42			
6	1% UWPE	8	10.74^{b}	0.94			
7	2% UWPE	8	10.80^{b}	0.63			

Groups with the same lowercase superscripted letter indicated no significant differences between groups at p values <0.05. SEG = short E-glass fiber. UWPE = ultrahigh molecular weight polyethylene filler.

group 7 (2% UMPE). Moreover, group 3 (1% SEG) showed significantly highest values for shear bond strength compared to the control group and all other reinforced groups ($p \le 0.001$).

As shown in Figures 3 and 4, all of the shear bond strength tested specimens were visually analyzed using a stereo microscope at 20× magnification and SEM at ×15 and ×1000 to examine the mode of failure. The images revealed that the majority of mode of failure was a mixture of cohesive and adhesive failures. The control group, group 6 (1% UMPE), and group 7 (2%UM PE) were noted to have 25% of adhesive failure between interface of reinforced autopolymerized and rapid heat-cured acrylic resin. Another 75% of tested specimens were noted to be of cohesive failure of PMMA within the acrylic resin itself. These cohesive failures were seen to be of reinforced autopolymerized acrylic resin. On the other hand, 100% mixed failures occurred for group 2 (2% SEG), group 3 (1% SEG), group 4 (0.5% SEG/UMPE), and group 5 (1% SEG/UMPE). In addition, groups 2 and 3 cohesive failures were noted to be of rapid heat-cured crylic resin while groups 4 and 5 cohesive failures were noted to be of both autopolymerized and rapid heat-cured acrylic resin, as summarized in Figure 5.

4. Discussion

This research was performed to assess the impact of adding SEG and UWPE fillers on the shear bond strength of the repaired autopolymerized acrylic denture base in improving the strength of the repaired denture. Two commonly employed techniques for evaluating the bond strength of dental materials are the shear bond strength (SBS) and the flexural bond strength (FBS) [29, 30]. Shear bond strength is not influenced by the strength of the adhesive itself and provides a straightforward assessment of the adhesive's bond strength, owing to its simple preparation of the specimen and relatively easy testing protocol, as well as the low occurrence of the pretest failure [30]. Considering the previously mentioned advantages of this type of test along with the fact that reinforced acrylic resin has been tested with regards to flexural strength in earlier studies [17], evaluating the strength and durability of the acrylic denture base when shear force is applied would be of high value, in order to further explore and validate the previous outcomes. Out of these points, this study was conducted, revealing that the addition of SEG and UWPE significantly affects the shear bond strength between reinforced autopolymerized and rapid heat-cured acrylic resin. Thus, the null hypothesis was rejected.

The results of the current investigation showed that the SEG and UWPE fillers significantly improved the shear bond strength between reinforced repaired acrylic resin and rapidsimplified heat-cured acrylic resin in comparison to the control group. Moreover, the filler concentration had a direct influence on increased shear bond strength for UWPE but inversely proportion for SEG. What is noted from the present experiment is also similar to Stipho's study, where the strength and deflection of repaired acrylic resin joints, enhanced with various several fiber concentrations, were evaluated. The authors revealed that after repair, 1% glass fiberreinforced autopolymerized acrylic resin could recover 65% of the strength of intact fracture load [18]. The flexural and adhesive properties of conventional resin along with the joint of repair resin directly influence the deflection and strength of the repair units. It takes less energy to break the repair units as

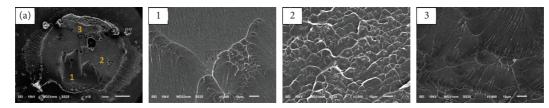


FIGURE 3: (a) Representative SEM images (x15, x1000) showing a mixed failure at bonding interface between rapid heat-cured acrylic resin and reinforced autopolymerized acrylic resin: (1) area of dislodged rapid heat-cured acrylic resin, (2) area of adhesive failure between two types of PMMA resin, and (3) area of intact reinforced autopolymerized acrylic resin.

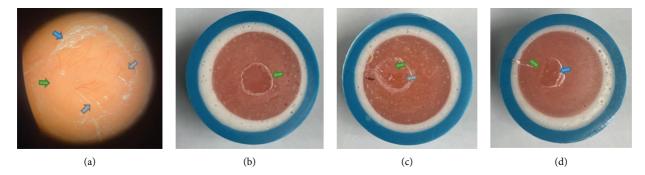
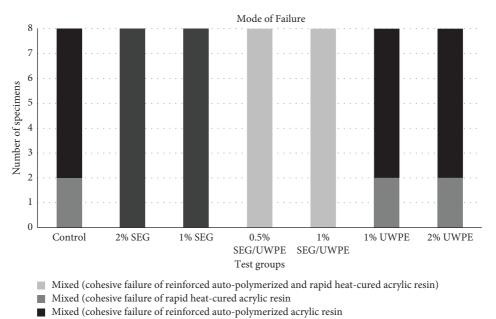


FIGURE 4: (a) Image under stereo microscope at 20× magnification showing a mixed failure at bonding interface between rapid heat-cured acrylic resin and reinforced autopolymerized acrylic resin. Representative photographic images of (b) adhesive failure and (c, d) mixed failure. Green arrow: adhesive failure. Gray arrow: cohesive failure of rapid heat-cured acrylic resin. Blue arrow: cohesive failure of reinforced autopolymerized acrylic resin.



Adhesive

FIGURE 5: Mode of failure in each group of all specimens.

they become more rigid. Specimens with 1% glass fiber reinforcement had a larger mean deflection at failure than those that were not reinforced. The increased filler loads may result in a more surface area between the filler and the resin matrix. The decreased in strength could be from additional sites where failure may occur. However, because of the morphology of the UWPE particles being quite irregular, the mechanical interlocking between the resin and the particles could play an additional role in enhancing the mechanical characteristics, which resulted in significant superior shear bond strength over the control group. Furthermore, groups with hybrid reinforcement of SEG and UWPE fillers to PMMA did not add up the properties but rather significantly lowered the strength of the PMMA resin compared to the group reinforced with SEG alone.

The current research findings also demonstrated significant increase of the shear bond strength for the inclusion of 1% SEG compared to all tested groups while the incorporation of 2% SEG lowered the shear bond strength. Krause et al. suggested that due to the rod shape of glass fibers, greater energy levels are required to dislodge the particles from the matrix [31]. The outcomes of this experiment could be explained by the fact that the low concentration of fillers is due to the homogeneous dissemination of the particles and their capability to occupy the interpolymeric chain spaces, whereas high concentration can result in agglomeration, which creates spaces [32]. The spaces could provide an explanation for the material's decreased strength and nonhomogeneous mixing within the resin. These hollow spaces impair the stress distribution, resulting in structural weak points that eventually weaken the material [33]. As reported by Gad et al., glass fibers' reinforcement could potentially improve the mechanical properties when utilized in low percentages. Alhotan et al. also stated the importance of homogeneous filler distribution and good adhesion between fiber and matrix within the resin matrix, as this has a major effect in stress transfer between the matrix and the fibers. The transverse strength of the material will certainly be influenced by strong adhesion. He concluded that to provide a desirable reinforcement for PMMA denture base resins, a filler of E-glass fiber with a concentration of 3-7% by weight is recommended [34]. Matinlinna et al. reported that the use of silane coupling agents helps promote the adhesion between dental restorative materials [35]. The UWPE filler used in this study has been surface treated with potassium dichromate and short E-glass fiber used was presilanized by the manufacturer. The use of a silane coupling agent improved the chemical bond between the filler and the PMMA matrix, thus requiring more energy to disintegrate the bonds that formed. The presilanized E-glass has the potential to form chemical bonds between the fiber and matrix. Superior repaired strength can be seen in this study with the addition of 1% SEG, considering that an optimum level of filler to the matrix is reached. Abushowmi et al. also claimed that incorporation of nanofillers (nano-ZrO₂ and nano-SiO₂) is advantageous over microfiller (glass fiber), owing to its even distribution, strong bonding with resin matrix, and ability to fill gaps between polymeric chains [36]. Therefore, the incorporation of nanofillers proved to be an effective method for increasing repair bond strength and avoiding repeated denture fractures.

Kumar et al. tested different acrylic resins for denture repair. The authors demonstrated the heat-cured denture base repaired with autopolymerizing repair resin obtained higher mean shear bond strength, compared to visible lightcured resin [13]. The interface between the two resins is typically the weakest point of repaired dentures. Several attempts were made to overcome this problem of increasing the bond strength by performing surface modification using chemical and mechanical treatments. Mechanical surface

treatments prior to denture repair were recommended by many studies [37, 38], as these treatments could promote higher debonding force at the interface of two PMMA materials. In this study, abrasive paper 600 grit was implemented for all specimens' bonding interface with the same speed and time, under automatic polishing machine. Artificial aging via thermocycling was also simulated in this denture repair study. This helped to determine the longevity of acrylic resin mimic oral cavity environment. Overtime, penetration of water molecules can cause the softening of the denture base and significantly influence the mechanical properties of the repaired acrylic resin [37]. From the current study, the addition of UWPE and SEG as hybrid reinforcement did not show to have synergy benefits in improving shear bond strength of acrylic resin. It is noteworthy that previous investigations also demonstrated the benefits of chemical treatment of the denture base prior to repairing with autopolymerizing acrylic resin, indicating higher shear strength when this was done, and different chemicals suitable for different acrylic resins [11]. Therefore, future studies using various chemical treatments, accompanied by the addition of 1% SEG to autopolymerized PMMA as a repair material, which is concluded from this study, would be of great interest in order to reveal whether this combination would give optimal outcomes in denture base repair.

Bonding performance has been evaluated using the mode of failure. Adhesive failures have always been viewed as the least acceptable, followed by mixed failures and cohesive failures [39]. Prpić et al. evaluated the shear bond strength of 10 groups, consisting of combinations of several types of denture teeth with cold-/heat-polymerized, as well as CAD/CAM denture base resin. The authors also concluded that higher cohesive and mixed failure rates were present with higher shear bond strength values, which is in accordance with this study [40]. The mode of failure for the majority of specimens was a mixed failure. Group 2 (1% SEG) and group 3 (2% SEG) 100% failure were mixed failure with cohesive breakdown of rapid heat-cured acrylic resin. This indicated that the reinforced autopolymerized acrylic resin has superior mechanical properties over that of rapid heat-cured acrylic resin. The addition of E-glass fiber to autopolymerized acrylic resin enhances its strength by serving as a reinforcement. These fibers effectively distribute stress across the material, preventing the occurrence of cracks or fractures [17]. Previous reports demonstrated remarkable benefits from adding glass for the reinforcement of PMMA [1]. One essential advantage is that glass fibers offer excellent mechanical enhancement and aesthetics, compared to other types of fibers [41]. It has been shown that adding glass elevates the strength and the toughness, Vickers hardness, as well as the flexural strength of the denture base [42-45]. Moreno-Maldonado et al. also concluded that the deformation was also reduced significantly (<1%) in fiber-reinforced PMMA [42]. Consequently, the autopolymerized PMMA resin will continue to be among the first options for repairing denture prostheses. Clinically, the simple mixing procedure of E-glass fiber to autopolymerized PMMA resin to repair fractured dentures

may be an effective means and efficient solution, considering the absence of any additional lab work, especially for patients who are waiting for new dentures to be made and with longer denture service life.

Some limitations of this study include its in vitro nature, which did not fully replicate clinical conditions. Furthermore, the more percentages of reinforced materials, the more color change of acrylic denture base. Although different proportions of reinforced materials were tested, surface treatment was standardized and the same method was implemented for all groups as mentioned earlier, i.e., other surface treatments were not examined. This could have some influence on the results, based on the chemical treatment used. Consequently, future research is necessary to evaluate the repair bond strength of reinforced acrylic resin under more realistic clinical conditions and to determine the most suitable surface treatment.

5. Conclusion

Within the limitations of this research, it can be concluded that

- In comparison to the control group, the addition of SEG and UWPE filler to autopolymerized PMMA denture base significantly improved shear bond strength
- (2) The addition of 1% SEG to autopolymerized PMMA denture base significantly improved the shear bond strength with rapid heat-cured acrylic resin, and this ratio is recommended as the reinforcement for chairside repair denture base material

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

NA conceptualized the study; NA, CA, and PN proposed the methodology; NA and NW validated the data; NA and CA performed formal analysis; NA, BM, and PN performed data curation; NA, CA, and PN prepared the original draft; NA, CA, and BM reviewed and edited the manuscript; and NA, SW, and NW supervised the study.

Acknowledgments

The study was supported by Srinakharinwirot University, Bangkok, Thailand (grant number 544-2564).

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