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Research article

Biocompatibility and mechanical properties of an experimental E-glass fiber-reinforced composite for dentistry

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ABSTRACT

Objectives: To evaluate the biocompatibility and mechanical properties of experimental *bis*-phenol-A and *bis*-GMA free E-glass fiber-reinforced composites (FRCs) prepared with hexanediol dimethacrylate (HDDMA) based resin.
Methods: Two ratios of HDDMA/TEGDMA resin were evaluated: exp-1 (70/30 wt.%) and exp-2 (50/50 wt.%) with two *bis*-GMA resin control groups (*bis*-GMA/MMA and *bis*-GMA/TEGDMA resins, both 70/30 wt.%). E-glass fibers were embedded into the resins to prepare FRCs specimens. Biocompatibility was assessed for cytoviability and biofilm formation with *Streptococcus mutans*, *Streptococcus sanguinis*, *Enterococcus faecalis*, and *Candida albicans*. Mechanical properties were evaluated for flexural strength and hardness (24 h, water storage 1 and 28 days), water sorption (1, 7, 14, and 28 days), contact angle, and surface roughness. The data were analyzed statistically by one-way and two-way ANOVA ($p < 0.05$).
Results: Cytoviability of the experimental groups was significantly higher than the control groups ($p < 0.05$). The exp-1 cytoviability ($98.2 \pm 1.3\%$) met the ISO 10993-5 standard requirement for noncytotoxic materials. The adherence of bacteria to the experimental FRCs was visibly less than the controls, while *Candida albicans* adhered visibly more to the experimental groups than the controls ($p < 0.05$). Flexural strength showed slightly higher values for controls than for the experimental groups. The exp-1 hardness value was significantly higher in the control groups for all storage conditions ($p < 0.05$). The water sorption of the experimental groups was significantly higher than the controls. The surface roughness indicated no significant difference ($p = 0.87$). The exp-1 showed a higher contact angle with the control groups.
Conclusion: The experimental HDDMA/TEGDMA-based FRCs might be potential alternatives for *bis*-GMA-based FRCs.
Clinical significance: The HDDMA/TEGDMA E-glass FRCs might provide biocompatible restorations.

1. Introduction

Fiber-reinforced composites (FRCs) are today widely used in many clinical dental treatments such as fixed dental prostheses, periodontal splinting, filling materials, endodontic posts, and orthodontic retainers [1]. The benefits of FRCs are mainly related to their aesthetics, time efficiency, metal-free dentistry, and economic concerns. FRCs are less expensive than dental ceramics, possess better aesthetics than metallic restorations, and can be prepared and finished on one visit only at the chairside. In contrast, ceramic materials are inherently brittle and hard, owing to the potential to fracture and wear the antagonist's teeth. By using FRC materials, the aesthetically pleasing-looking bridge can be finished in one chairside session only [2].

Clinical failures related to dental splints in stabilizing teeth have been reported due to mastication forces during the normal function [3]. Attempts and innovations have introduced first polyethylene and subsequently E-glass fibers to reinforce the resin matrices to create *e.g.,* a thin splint with high strength and easiness to maintain oral hygiene. The preferred choice of FRC posts was mostly related to the dentin-like modulus of elasticity of the FRC post, allowing better distribution of forces along the length of the root [4]. The modulus of elasticity of resin composites (5.7–25 GPa) and FRC posts (16–40 GPa) have revealed

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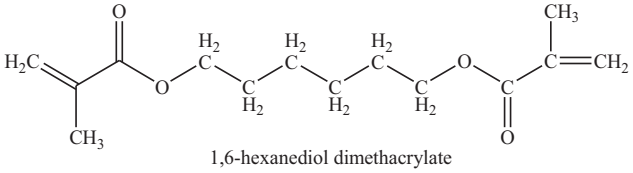
increasing shock resistance, lower mobility, shock absorption, and fatigue resistance [5]. The other reasons for choosing FRCs as endodontics are due to the easy shaping and placement, minimal requirement of tooth structure removal, and improved mechanical longevity [6].

Bis-GMA resin is a cross-linking polymer with high viscosity, and it is still the most used basis for dental resin composites [7]. Some previous laboratory studies have used methyl methacrylate (MMA) as a comonomer and diluent of *bis*-GMA [8]. Most commercial resin composite products employ tri(ethyleneglycol) dimethacrylate (TEGDMA) and urethane dimethacrylate (UEDMA) as a copolymer; and camphorquinone (CQ) as a photosensitizer. Moreover, some dental products also include reducing agents such as *N,N*-cyanoethyl methylanilide (CEMA), or 2-(dimethylamino) ethyl methacrylate (DMAEMA) [9]. However, *bis*-phenol-A (BPA) cleaved from the *bis*-GMA resin can cause inflammation or allergy contact dermatitis, or even the so-called pseudo-estrogenic effect [10]. It was also reported that *bis*-GMA is the most cytotoxic monomer of 35 examined used in dental materials [11]. Related to the BPA exposure from dental resins, some other possible effects have been reported, such as metabolic disease, endometrial disorders, cardiovascular disease, diabetes, DNA double-strand breaks, mammary, and prostate cancer. Moreover, another study reported that the co-monomer MMA might cause allergy and contact dermatitis [12]. The release of residual monomers of MMA was reported to be the primary cause of irritation to the mucous membrane [13]. That said, to reduce the harmful effects of *bis*-GMA and MMA resin, it is necessary to look for alternative resins.

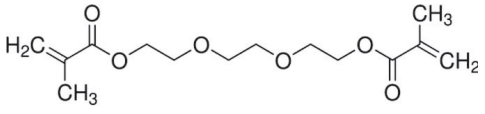
One potential alternative monomer for replacing *bis*-GMA could be 1,6-hexanediol dimethacrylate, HDDMA (Figure 1), because it has some similar characteristics to the *bis*-GMA molecular structure, especially in its functional groups. Moreover, HDDMA is less viscous than *bis*-GMA because of its linear structure and HDDMA has low volatility, possesses a hydrophobic backbone, yet is structurally rigid enough and it is fast curing [14]. Some laboratory research has already been conducted on HDDMA resin: it had been combined with MMA for an experimental resin matrix for E-glass FRC and had been evaluated for flexural strength and hardness [15]. The results suggested that the HDDMA/MMA matrix provided comparable flexural strength and hardness to the *bis*-GMA/MMA resin. Moreover, HDDMA/MMA resin has exhibited significant differences in cell viability. It was also concluded that HDDMA-based resin provides less cytotoxic effects than *bis*-GMA. In addition, even the viability of the fibroblast cells induced by HDDMA/MMA resin matrix was less than 70% [16]: this aspect might need to be improved because the ISO 10993-5 standard requires the viability of a biomaterial to be more than 70%.

Triethyleneglycol dimethacrylate (TEGDMA) exhibits excellent viscosity and copolymerization (Figure 1) [17], and it is reported that



1,6-hexanediol dimethacrylate

1,6-hexanediol dimethacrylate (HDDMA).



Tri(ethyleneglycol) dimethacrylate (TEGDMA)

Figure 1. Structures of 1,6-hexanediol dimethacrylate (HDDMA) and tri (ethylene glycol) dimethacrylate (TEGDMA).

40–50% of monomers of modern commercial dental resins include TEGDMA [18]. Therefore, it would be scientifically interesting to evaluate HDDMA combined with TEGDMA. The mechanism of adhesion of bacteria and colonization of biofilm formation on dental materials is important: adhesion and biofilm growth are related to the material surface characteristics including surface roughness and surface free energy [19]. Bacteria over the teeth and restorative materials are potentially etiological factors for causing secondary caries [2]. A previous study also reported that a dental resin composite showed disadvantages including being prone to plaque accumulation. Bacteria indicated for major causative dental pathogenesis in the oral cavity include *Streptococcus mutans* (dental caries) [20], *Enterococcus faecalis* (endodontic infection) [21], and *Streptococcus sanguinis* (dental plaque accumulation in periodontal splints and orthodontic retainers) [22]. Oral fungi, such as the yeast *Candida albicans* may cause candidiasis in some immunocompromised patients [23]. Knowing the effect of new material on the biofilm formation by these microorganisms could predict the biocompatibility of the said material.

This laboratory research was aimed to evaluate the biocompatibility and some mechanical properties of an experimental E-glass FRC with an HDDMA/TEGDMA resin matrix, compared and contrasted to experimental *bis*-GMA-based E-glass FRCs. The schematic diagram of the study design is seen in Figure 2. The two hypotheses tested were: a) HDDMA/TEGDMA E-glass FRCs have more favorable biocompatibility, and b) HDDMA/TEGDMA E-glass FRCs have better mechanical properties than *bis*-GMA-based FRCs.

2. Materials and methods

2.1. Resin matrix preparation

The materials utilized in this laboratory research are shown in Table 1. The start materials for experimental resins were used as such without redistillation. The resins were prepared by using an analytical balance (AG 285, Mettler Toledo, Switzerland) for the weight to percentage ratio. The prepared experimental resin groups were a combination of HDDMA/TEGDMA with the ratios of 70/30 wt.% (exp-1 group) and 50/50 wt.% (exp-2 group). The resin matrices were set up into four groups: two control groups of *bis*-GMA/MMA and *bis*-GMA/TEGDMA FRCs with a weight ratio of 70/30 wt.% for both groups; and two experimental groups of HDDMA/TEGDMA FRCs with the weight ratio of 70/30 wt.% and 50/50 wt.%. CQ and DMAEMA were added to each resin matrix system at 1 wt.% [8, 9].

2.2. FRCs specimen preparation

Experimental FRC specimens for mechanical evaluation were made as described by Zhang *et al.* [8]. The unidirectional E-glass fiber bundles were prepared by first keeping them in a desiccator for 24 h to avoid humidity. The E-glass fibers had already been silanized by the manufacturer and they were next immersed in a sizing solution for 1 min. The sizing solution for *bis*-GMA FRCs consisted of *bis*-GMA/TEGDMA or *bis*-GMA/MMA for 50/50 wt.% respectively, whereas for HDDMA/-TEGDMA FRCs it was HDDMA/TEGDMA for 50/50 wt.%. After the sizing treatment, the fibers were cut into 5 mm long bundles by steel scissors and kept in a petri-dish in dark [8, 9].

FRCs specimens were prepared as 5 specimens in a group ($n = 5$) (Table 1) and randomly assigned to 4 study groups. This samples size calculation was estimated by using statistical software PASS11 (Power Analysis and Sample Size, NCSS, Kaysville, UT, USA), which is today a leading sample size calculation software [24]. It is noteworthy that PASS11 software has become popular software that provides sample size tools for various statistical tests in health sciences [25]. We have estimated the minimum sample size based on the different values of the mean and standard deviation of the preliminary study from the evaluated dependent variables. Moreover, the power is set to be at least 80% and $p < 0.05$ [26].

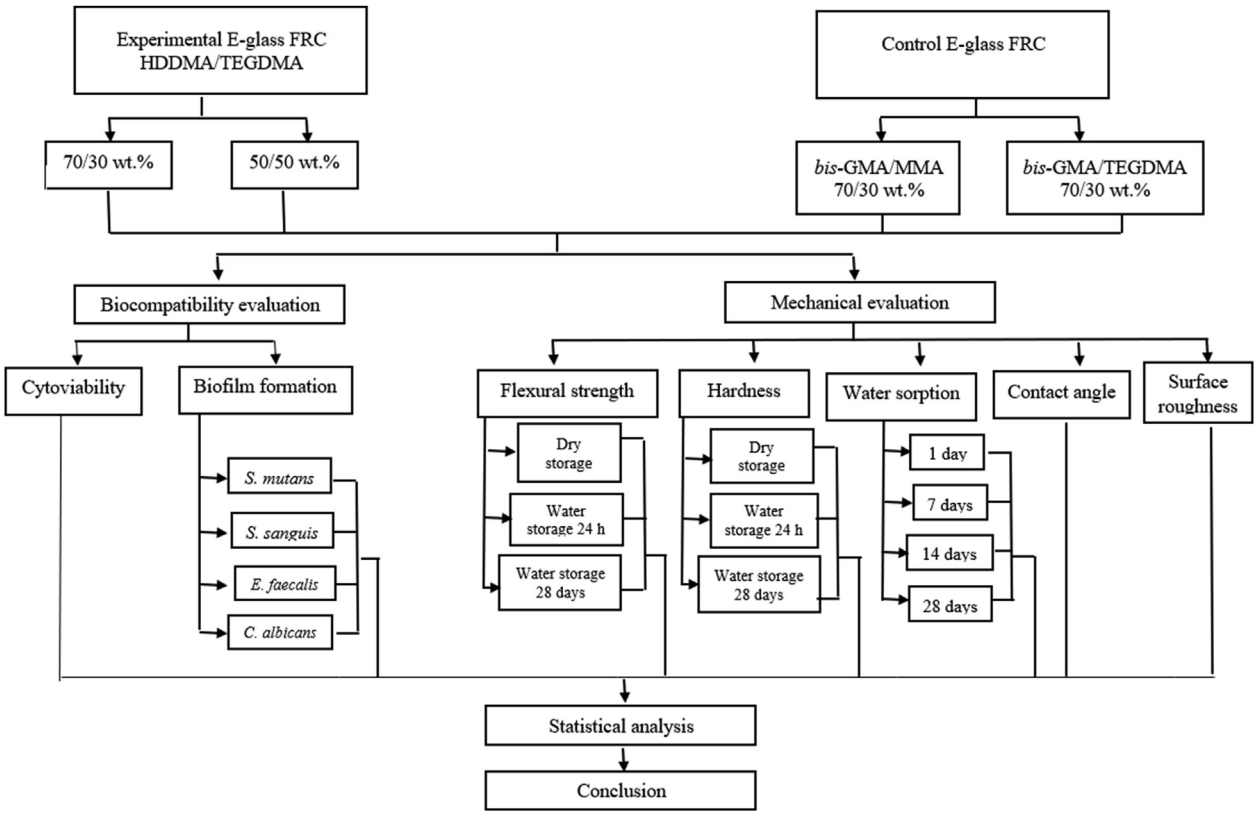


Figure 2. Flow diagram of the study design.

Table 1. List of materials utilized in the current laboratory study.

Materials	Manufacturer	Lot number	City and country	Purity
Bis-phenol-A-glycidyl methacrylate (*bis*-GMA)	Sigma-Aldrich	MKBF2771	St Louis, MO, USA	N/A
Methyl methacrylate (MMA)	Merck	S6033190022	Hohenbrunn, Germany	>99%
Tri(ethyleneglycol) dimethacrylate (TEGDMA)	Sigma-Aldrich	STBC5193V	St Louis, MO, USA	>95%
1,6-Hexanediol dimethacrylate (HDDMA)	Esstech	719–05	Essington, PA, USA	Purified
Camphorquinone (CQ)	Esstech	688–50	Essington, PA, USA	N/A
2(Dimethylamino) ethyl methacrylate	Sigma-Aldrich	1437599	St Louis, MO, USA	>98%
Silanized E-glass fibers bundles (R338-2400/V/P)	Ahlström	091301167703	Karhula, Finland	N/A

All FRC specimens for biocompatibility tests (Table 2) were prepared by mixing a total amount of 22.5 wt.% by the manufacturer silanized E-glass fibers (2 mm in length) with the resin matrix, respectively, into discs (2 mm thick, 6.5 mm diameter). Specimens were then polished with SiC paper (360 grit). At last, the specimens were cleansed for 20 min in an ultrasonic bath containing distilled water, then rinsed, immersed in distilled water again, and kept in an incubator at a temperature of 37 °C before the cytoviability test and biofilm evaluation [19].

All FRC specimens for mechanical testing were prepared as follows: two fiber bundles were arranged in a brass mold with the size of 2 mm × 2 mm x 25 mm, then the fiber bundles were placed unidirectionally and carefully covered with resin, avoiding air bubbles. All experimental resins (exp-1 and exp-2) and controls (*bis*-GMA/MMA and *bis*-GMA/TEGDMA) were embedded in the mold. These specimens were then polymerized on two opposite surfaces for 3 × 40 s by a light-curing unit (Woodpecker, USA) at an average output of 720 mW/cm^2. The excess resin material was removed by abrasive paper (360 grit). These rhombohedral specimens for flexural strength and hardness testing were kept in a desiccator for 24 h (dry storage) at room temperature, and in distilled water for 1 and 28 days in an incubator before testing at room temperature. Specimens for water sorption were stored for 1, 7, 14, and 28 days in an incubator at 37 °C [8].

Table 2. List of microorganisms and cells used in the current laboratory study.

Product name	Manufacturer	Lot number	City and country	Product number
Streptococcus mutans ATCC 25175 PK/5	Thermoscientific	116063	Waitham, MA, USA	R4607001
Enterococcus faecalis ATCC 29212 PK/5	Thermoscientific	215690	Waitham, MA, USA	R4607030
Streptococcus sanguinis type I ATCC 10556 PK/5	Thermoscientific	859173	Waitham, MA, USA	R4607023
Candida albicans ATCC 10231 PK/5	Thermoscientific	538381	Waitham, MA, USA	R4601503
Fibroblast cell line ATCC-CCL81	Thermoscientific	5848419	Waitham, MA, USA	84113001

2.3. Biocompatibility

2.3.1. Cytoviability

The FRCs specimens were eluted in RPMI medium for 24 h at 37 °C. The specimens were then removed and the elute extracts were filtered by a 0.22 μm Millipore membrane (Millipore; Billerica, MA, USA). Undiluted extracts were used in the cytoviability test (Table 2).

The cytoviability evaluation was adapted from ISO 10993-5 [27]. Fibroblast cells ATCC-CCL81 of 1×10^4 were put in a 96-well plate, then stored at 37 °C for 24 h. A stock solution was diluted into RPMI medium to the concentration of 0.01 μg/ml. The solution was then filtered (0.22 μm Millipore). The extracted medium (100 μl) was inserted into the well. For negative control, a fresh cell medium was used. The cytoviability of fibroblast cells was determined by the MTT method after 24 h. The Elisa reader (BioRad, California, USA) was used with a wavelength of 550 nm. The percentage of cytoviability was determined with the following formula:

$$\%\ \text{cytoviability} = \frac{100\% \times OD_{550\ \text{treated}}}{OD_{550\ \text{control}}} \quad (1)$$

where $OD_{550\ \text{treated}}$ is the average value of the measured OD of the treated cell, and $OD_{550\ \text{control}}$ is the average value of the measured OD of the control cell. The rate of cytotoxicity was determined by severe (viability 30%), moderate (viability 30–60 %), mild (viability 60–90 %), and noncytotoxic (>90%) [27].

2.3.2. Biofilm formation

Sterile saliva was used for biofilm formation. The Ethical Certificate (EC) for collecting saliva was issued by the Dentistry Faculty Ethics Committee (EC 00490/KKEP/FKG-UGM/EC/2020). The biofilm formation testing was adapted from previous research [19]. The whole of human saliva was used as pellicles. Saliva from 2 volunteers (females, aged 53–55 years) was cleared by centrifugation (8000 g, 10 min, 4 °C) and the supernatant was filtered (0.22 μm Millipore). The sterile saliva was stored in a refrigerator (4 °C) until used for biofilm formation. The FRCs specimens were autoclaved at 121 °C for 15 min, cooled down to room temperature, and then immersed in sterile saliva for 1 h to form pellicles. The three bacteria used were *S. mutans* ATCC 25175, *E. faecalis* ATCC 29212, and *S. sanguinis* ATCC 10556, grown anaerobically in a brain heart infusion (BHI) medium at 37 °C for 24 h. *C. albicans* ATCC 10231 was grown aerobically in the Sabourauds broth at 37 °C for 24 h.

The FRCs specimens were taken out from sterile saliva and inserted into *S. mutans*, *S. sanguinis*, *E. faecalis*, and *C. albicans* suspensions with the amount of the microorganism as a standard 1×10^7 cells/ml. The suspension with specimens was incubated at the temperature of 37 °C anaerobically for 24 h for bacteria, and aerobically for the yeast. The specimens were then washed with PBS to remove the non-adherent bacteria and then inserted into BHI suspension for bacteria, and into the Sabourauds broth for yeast. The microorganisms were separated by sonication of 30 s pulses at 25 W by three 30 s intermittent cooling stages. The cells were diluted serially, then placed into BHI or Sabourouds agar, and kept in an incubator at 37 °C for 48 h. The colonies were counted after 24 h [28].

2.4. Mechanical experiments

The flexural strength was measured by a three-point bending test (ISO 4049:2019) by using a universal testing machine (Torsee UTM AMU-10, Tokyo, Japan). The test span was 20 mm long and the crosshead speed was 1 mm/min. The maximum load of the load-deflection curve was recorded to determine the flexural strength by the formula [7]:

$$Ó = 3\ FL/2bd^2 \quad (2)$$

where Ó was the flexural strength (MPa) value, while F was the maximum load in the load-deflection curve (N). L was the span between the two supports (mm), while b was the specimen width (mm), and d was the specimen height (mm).

The surface hardness was determined by a microhardness testing machine (type MTX 70 Matsuzawa, Kanagawa, Japan) according to ISO 6507-2. A load of 0.245 N and a loading duration of 20 s were applied [7]. Three indentations were made randomly on each specimen surface. The Vickers hardness (VHN) was calculated as the means of 15 random indentations recorded in each group.

Water sorption determination was done by keeping the samples in a desiccator for 24 h. The dry weight sample was balanced and averaged (M_1) after three replication measurements using an electronic balance (AG 285, Mettler Toledo, Switzerland). Samples were then immersed in 15 ml of distilled water in a sealed tube wrapped with aluminum foil and incubated at 37 °C for 1, 7, 14, and 28 days. The specimens were wiped dry with tissue paper before weighing after each immersion. At the end of the period, the measured weights were recorded as M_2. Water sorption (WS) was calculated by the formula [7]:

$$WS = \frac{(M_2 - M_1)}{M_1} \times 100\% \quad (3)$$

The surface roughness of five specimens in each group was recorded by a roughness tester (Starret SR 300, Massachusetts, USA) which had a cut-off value of 0.8 mm. Each specimen was measured three times on random spots, and the R_a of each group was averaged.

The water contact angle was measured by the sessile drop method to evaluate hydrophilicity (or hydrophobicity) [29]: a 5 μl distilled water droplet was placed on the surface of the tested materials and the static contact angle was measured. The measurement of the contact angle was recorded by a digital camera of Canon 30 D with a macro lens EF 100 mm. The measurements were taken on three different random spots on the surface.

2.5. Scanning electron microscopy (SEM) and photo image evaluation

SEM images (magnifications 1000x and 2000x) were taken on representative specimens in each group for biofilm adherence and flexural strength. Specimens were mounted on an aluminum holder and sputter-coated with gold, then were recorded using SEM (JSM-T300 JEOL, Tokyo, Japan). For cytotoxicity imaging, inverted microscopy with a digital camera of Canon 30 D was used (magnifications 40x). The contact angle values were recorded by a digital camera (Canon 30 D).

2.6. Statistical analysis

Two-way ANOVA was carried out for biofilm formation, flexural strength, surface hardness, and water sorption. One-way ANOVA was used for the surface roughness, contact angle, and cytoviability evaluation. After the ANOVA analysis, a further *post hoc* test of LSD was carried out. The statistical significance was set as $p < 0.05$ (SPSS Statistics version 20.0, IBM, USA). The data normality was assessed by using the *Kolmogorov-Smirnov* test.

3. Results

3.1. Biocompatibility

3.1.1. Cytoviability

Table 3 represents the MTT results of the fibroblast cytoviability values after 24 h exposed to FRCs suspension extract. The exp-1 group provided the highest cytoviability value of 98.2 ± 1.6% whereas the control-1 showed the lowest. One-way ANOVA revealed the F value of 4.389 ($p < 0.05$), which meant the resin composition of FRC influenced

the fibroblast cytoviability. Further LSD *post hoc* analysis determined significant differences between the groups ($p < 0.05$).

3.1.2. Biofilm formation

The adherence of bacteria on the exp-1 and exp-2 was less than the control-1 (Table 4). *S. mutans* adherence on exp-1 was the lowest and exp-2 was the highest. The adherence of *S. sanguinis* and *E. faecalis* in the experimental groups was less than the control groups. The adherence of *C. albicans* to the FRCs was higher in the experimental groups than in the control groups. Statistical analysis revealed that various resin compositions, microbes, and interactions of resin and microbes significantly influenced the biofilm formation ($p < 0.05$). Further *post hoc* analysis by LSD indicated significant differences for all comparisons.

3.2. Mechanical tests

3.2.1. Flexural strength

Table 5 indicates that the flexural strength of FRCs was lower after water storage of 28 days. The exp-2 revealed the lowest flexural strength on various storage conditions. The flexural strength of control groups was higher than the experimental groups. Two-way ANOVA showed that the resin compositions, storage, and interaction of resin composition and storage influenced the flexural strength of FRCs ($p < 0.05$). The *post hoc* LSD test revealed there was a significant difference between groups for water storage for 28 days.

3.2.2. Surface hardness

The highest hardness was found in the exp-1 and the lowest was in the exp-2 (Table 6). Statistical analysis by two-way ANOVA revealed there was a significant influence of resin compositions, storage conditions, and interaction between resin matrix composition and storage composition ($p < 0.05$).

3.2.3. Water sorption

Table 7 describes the average percentage of water sorption of FRCs for 28 days. The water sorption seemed high for 14 days, then on the 28 days not too high. The water sorption of experimental groups appeared higher than in the two control groups. Statistical analysis by two-way ANOVA indicated there was a significant influence on resin compositions, storage condition, and interaction of resin and storage condition ($p < 0.05$). The *post hoc* LSD analysis indicated there were significant differences in water sorption between groups, except for control-1 and control-2.

Table 3. Cytoviability (%), 24 h, dry FRC specimens (n = 5).

Resin matrix composition of FRCs	Percentage of cell viability
exp-1: HDDMA/TEGDMA (70/30 wt.%)	98.2 ± 1.3^{a}
exp-2: HDDMA/TEGDMA (50/50 wt.%)	85.4 ± 2.6^{b}
control-1: *bis*-GMA/MMA (70/30 wt.%)	65.2 ± 2.8^{c}
control-2: *bis*-GMA/TEGDMA (70/30 wt.%)	67.6 ± 4.2^{c}

Mean values within the column with superscripts a-c indicate significantly different values ($p < 0.05$).

Table 5. Flexural strength of FRC specimens in MPa (n = 5).

Resin matrix composition of FRCs	Dry storage	Water storage 24 h	Water storage 28 days
exp-1: HDDMA/TEGDMA (70/30 wt.%)	669.2 ± 5.6^{aA}	671.0 ± 10.5^{aA}	401.8 ± 6.8^{aB}
exp-2: HDDMA/TEGDMA (50/50 wt.%)	618.4 ± 8.3^{bA}	618.4 ± 9.9^{bA}	342.6 ± 7.8^{bB}
control-1: *bis*-GMA/MMA (70/30 wt.%)	673.6 ± 5.3^{aA}	674.2 ± 3.9^{aA}	423.2 ± 4.5^{cB}
control-2: *bis*-GMA/TEGDMA (70/30 wt.%)	681.4 ± 4.4^{cA}	680.6 ± 3.4^{cA}	431.0 ± 2.2^{dB}

Mean values within columns with different superscripts a-d show significantly different values ($p < 0.05$).
Mean values within rows with different superscripts A-B show significantly different values ($p < 0.05$).

Table 6. Surface hardness of FRC specimens in VHN (n = 5).

Resin matrix composition of FRCs	Dry storage	Water storage 24 h	Water storage 28 days
exp-1: HDDMA/TEGDMA (70/30 wt.%)	57.4 ± 2.1^{aA}	57.0 ± 9.6^{aA}	56.8 ± 1.9^{aA}
exp-2: HDDMA/TEGDMA (50/50 wt.%)	41.6 ± 5.3^{bA}	41.2 ± 8.2^{bA}	35.8 ± 4.8^{bB}
control-1: *bis*-GMA/MMA (70/30 wt.%)	54.2 ± 6.2^{cA}	54.0 ± 2.5^{cA}	52.8 ± 5.4^{cB}
control-2: *bis*-GMA/TEGDMA (70/30 wt.%)	53.0 ± 5.2^{cA}	53.6 ± 9.2^{cA}	$52,6 \pm 9.4^{cA}$

Mean values within columns with different superscripts a-c show significantly different values ($p < 0.05$).
Mean values within rows with different superscripts A-B show significantly different values ($p < 0.05$).

3.2.4. Surface roughness and contact angle

The surface roughness of the exp-1 was the smallest (Table 8). Statistical analysis by one-way ANOVA showed there was no significant influence of resin composition of FRC on the surface roughness ($p = 0.87$). The contact angle of the exp-1 provided the highest value and the exp-2 the lowest. Statistical analysis showed a significant influence of resin matrix composition on the surface contact angle. The *post hoc* analysis showed the exp-2 group was not significantly different from the control-2 group ($p = 0.35$).

3.2.5. SEM and photo images

SEM images were taken at the sites of fracture on the tension sides after 3-point bending as seen in Figure 3. The images show there was some resin attached to the fibers. Determination of biofilm adherence was recorded by SEM and colony counting as seen in Figure 4. The cytoviability was recorded by photo images using a digital camera

Table 4. Biofilm adherence (CFU/ml), dry FRC specimens (n = 5).

Resin matrix composition of FRCs	*S. mutans*	*S. sanguinis*	*E. faecalis*	*C. albicans*
exp-1: HDDMA/TEGDMA (70/30 wt.%)	119.2 ± 3.0^{aA}	71.6 ± 4.0^{aB}	108.6 ± 5.9^{aC}	354.0 ± 7.6^{aD}
exp-2: HDDMA/TEGDMA (50/50 wt.%)	162.6 ± 6.9^{bA}	78.3 ± 6.3^{bB}	148.2 ± 7.8^{bC}	336.0 ± 9.3^{bD}
control-1: *bis*-GMA/MMA (70/30 wt.%)	156.2 ± 7.4^{cA}	171.0 ± 3.2^{cB}	169.0 ± 8.0^{cB}	271.6 ± 4.2^{cC}
control-2: *bis*-GMA/TEGDMA (70/30 wt.%)	152.6 ± 3.8^{dA}	229.2 ± 7.1^{dB}	202.0 ± 9.7^{dC}	219.6 ± 8.7^{dD}

Mean values within columns with different superscripts a-d show significantly different values ($p < 0.05$).
Mean values within rows with different superscripts A-D show significantly different values ($p < 0.05$).

Table 7. Water sorption of FRC specimens (%) (n = 5).

Resin matrix composition of FRCs	1 day	7 days	14 days	28 days
exp-1: HDDMA/TEGDMA (70/30 wt.%)	1.1 ± 0.7^{aA}	1.9 ± 0.4^{aB}	2.3 ± 0.8^{aC}	2.6 ± 0.4^{aD}
exp-2: HDDMA/TEGDMA (50/50 wt.%)	1.7 ± 0.4^{bA}	2.7 ± 0.7^{bB}	3.2 ± 0.6^{bC}	3.5 ± 0.7^{bD}
control-1: *bis*-GMA/MMA (70/30 wt.%)	0.8 ± 0.4^{cA}	1.3 ± 0.6^{cB}	1.8 ± 0.5^{cC}	2.2 ± 0.2^{cD}
control-2: *bis*-GMA/TEGDMA (70/30 wt.%)	0.9 ± 0.3^{cA}	1.4 ± 0.6^{cB}	1.9 ± 0.6^{cC}	2.4 ± 0.6^{dD}

Mean values within columns with different superscripts a-d show significantly different values ($p < 0.05$).
Mean values within rows with different superscripts A-D show significantly different values ($p < 0.05$).

Table 8. Surface roughness (μm) and contact angle (°), dry FRC specimens (n = 5).

Resin matrix composition of FRCs	Surface roughness (μm)	Contact angle (°)
exp-1: HDDMA/TEGDMA (70/30 wt.%)	1.67 ± 0.20^{a}	67.7 ± 1.3^{a}
exp-2: HDDMA/TEGDMA (50/50 wt.%)	1.74 ± 0.20^{b}	62.6 ± 2.6^{b}
control-1: *bis*-GMA/MMA (70/30 wt.%)	1.78 ± 0.20^{b}	65.2 ± 4.0^{c}
control-2: *bis*-GMA/TEGDMA (70/30 wt.%)	1.76 ± 0.30^{b}	63.0 ± 3.8^{b}

Mean values within columns with different superscripts a-c show significantly different values ($p < 0.05$).

(Canon 30 D) via an inverted microscopy facility (Figure 5). The contact angle measurement images are seen in Figure 6.

4. Discussion

Often a minimum of six samples have been employed in FRC laboratory studies. In this study, the selected specimen number 5 was based on the power analysis by PASS11 statistical software [24] and this was deemed appropriate because the power analysis aims to design experiments and detect the effect size (ES) which would be sufficiently large to be of scientific interest [26].

The cytoviability in all experimental groups is significantly higher than in the control groups (Table 3). This fact might be related to the use of a non-BPA resin in the experimental groups (HDDMA/TEGDMA). The control groups contain *bis*-GMA resin: the BPA cleaved from *bis*-GMA resin has been reported cytotoxic [10]. ANOVA revealed there were significant differences in the biological properties among all resins studied. The cell viability exhibited significant differences among the four resin groups ($p < 0.05$), and further *post hoc* statistical analysis with LSD noticed no significant difference between the exp-1 and the exp-2. The most interesting result was the finding in the HDDMA/TEGDMA resin influencing cell viability of 98.2% which is almost similar to the fibroblast cell negative control group (99%). This viability was also seen in the micrograph images through inverted microscopy which showed an almost similar number of fibroblast cells exp-1 and negative control groups (Figure 4). This finding might suggest excellent cell viability with HDDMA/TEGDMA.

The adherence of the three bacteria to the experimental FRCs was significantly less than onto the control *bis*-GMA-based FRCs (Table 4): the lowest adherence of *S. mutans* in the exp-1 might be related to the relatively higher contact angle than the other resins (Table 8). That said, it

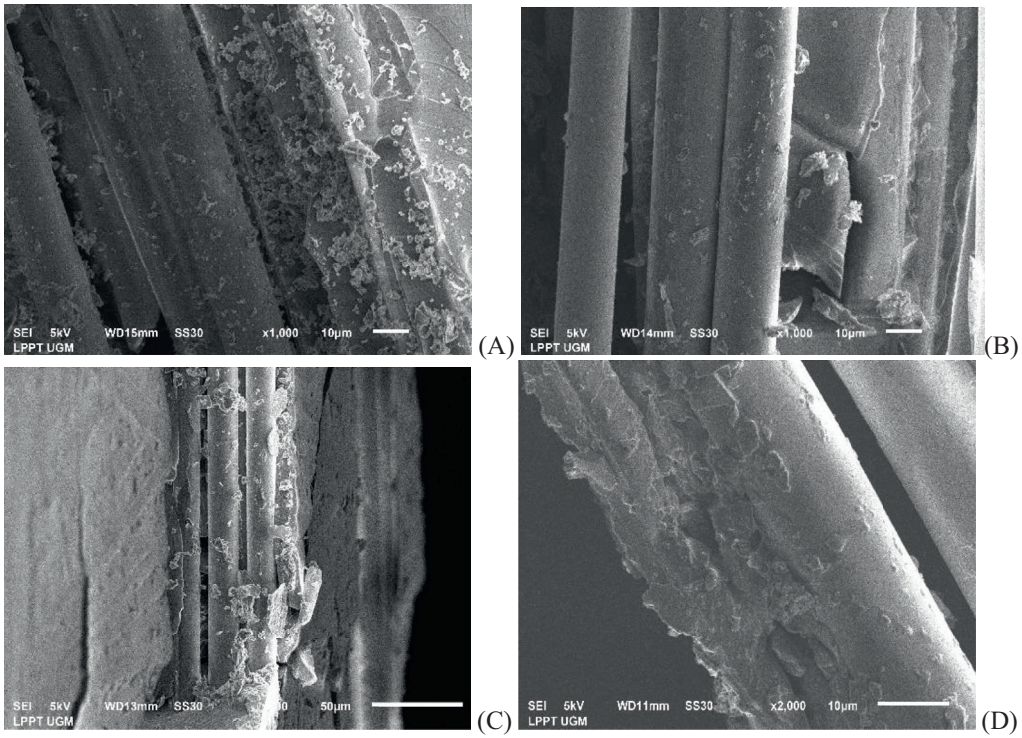


Figure 3. SEM micrographs of FRC specimens after the flexural test: (A) FRC exp-1, (B) FRC exp-2, (C) FRC control-1, (D) FRC control-2 (magnifications 1000x, 2000x).

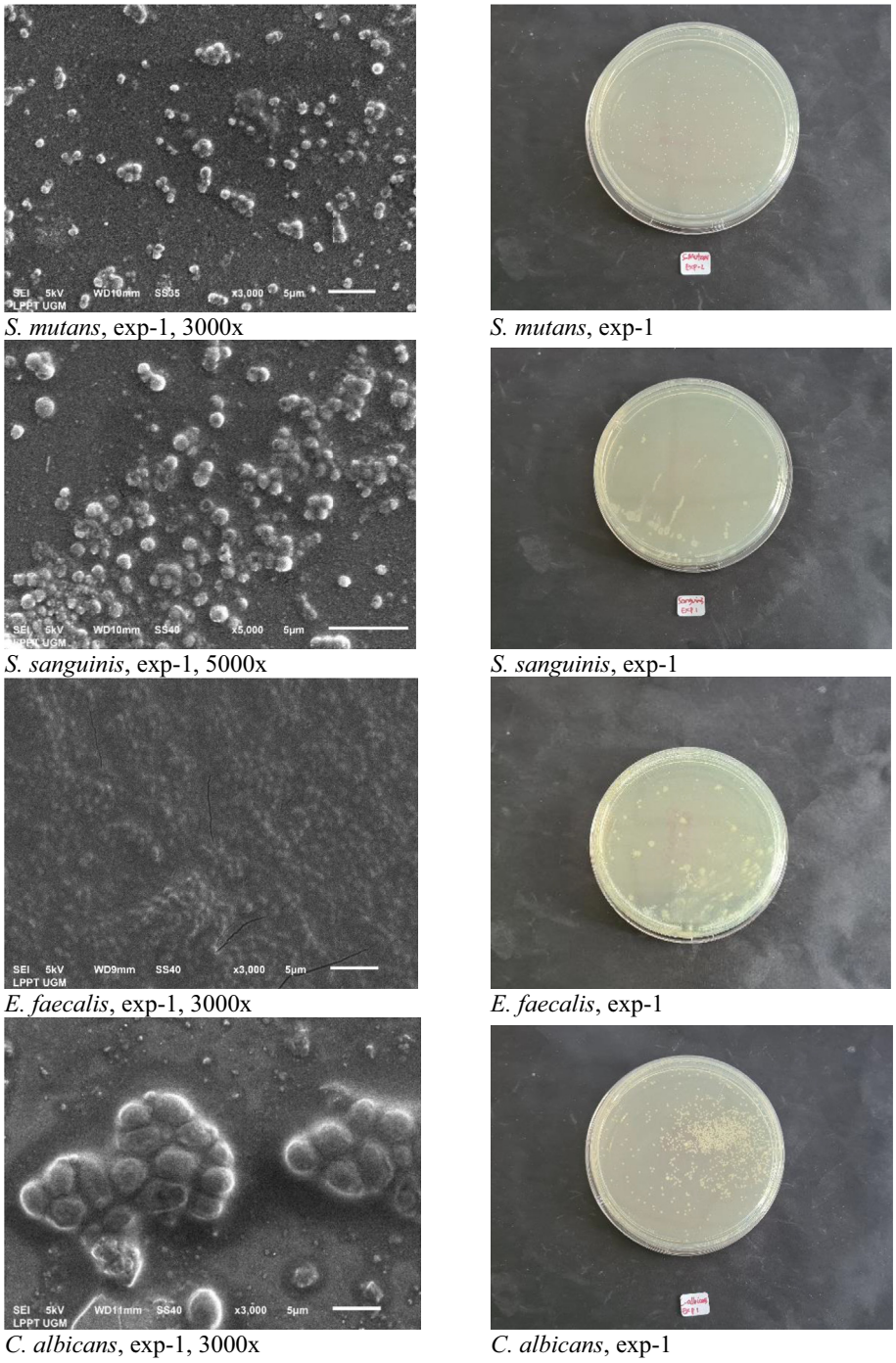


Figure 4. SEM micrographs (magnifications 3000x and 5000x) and photographs of the colony counting and the biofilm adherence.

was previously reported that *S. mutans* being a high surface energy strain should adhere preferentially to the hydrophilic substrate [30]. The *S. sanguinis* exhibits similar characteristics to *S. mutans*: it adheres preferably onto a hydrophilic surface [31]. However, it was shown that *S. sanguinis* adhered significantly less to the experimental groups than control groups (Table 4). This fact might be related to the HDDMA monomer, so the current non-BPA resin seemed to be more colonized by *S. sanguinis* than the control group resins. *E. faecalis* adhered significantly less onto relatively hydrophobic surfaces than hydrophilic surfaces (Table 4): this fact is similar to previous findings which reported that *E. faecalis* biofilm formation was stronger on the hydrophobic surface [32]. It was also shown that *C. albicans* adheres more to the experimental FRCs than the control groups (Table 4). *C. albicans* was reported as a hydrophobic strain of yeast adhering more to hydrophobic surfaces [33].

The flexural strengths of the control groups were slightly but not significantly higher than the experimental groups (Table 5). This might be related to the *bis*-GMA molecule which has a higher molecular weight with its benzene rings, whereas the HDDMA molecule is linear with a lower molecular weight. The longer immersion time of FRCs in water probably caused the flexural strength to decrease because more water was absorbed into the specimens. The hardness of the experimental resin exp-2 was significantly lower than the other 3 groups (Table 6). This was also the case during dry storage, water storage 24 h, and 28 days. The water sorption for the exp-2 was significantly higher in all storage

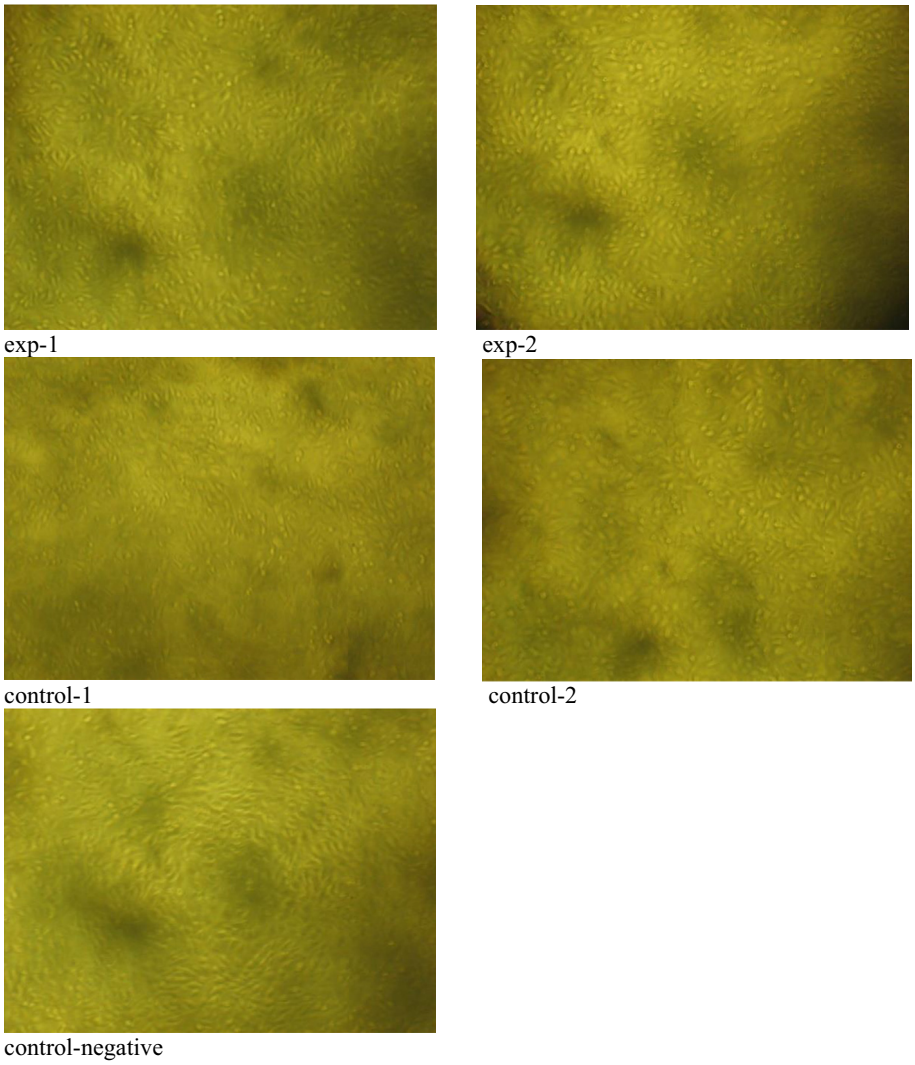


Figure 5. Fibroblast cells viability (magnification 40x).

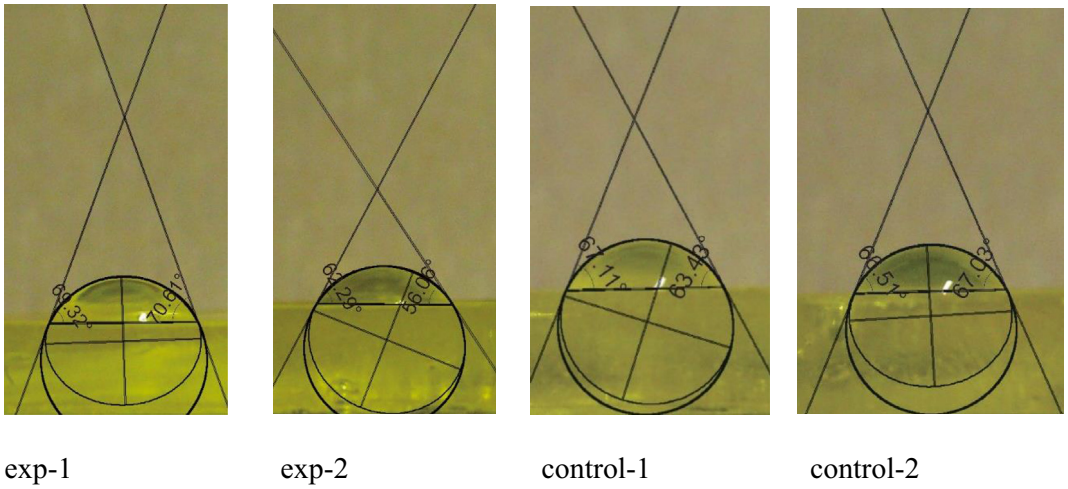


Figure 6. Contact angle measurement and results.

groups. These observations might be related to the smaller molecular weight and size of HDDMA and its wt.% in the specimen.

There were marginal differences in surface roughness for all FRCs groups (Table 8) and this was probably related only to the polishing of the FRC surfaces by the abrasive paper of 360 grit. The surface contact angle values suggest that the exp-1 had a significantly greater contact angle than the others: this might be related to the hydrophobic properties of HDDMA/TEGDMA.

The first hypothesis, which stated HDDMA/TEGDMA E-glass FRCs have better biocompatible properties compared to *bis*-GMA-based FRCs, can be accepted. However, the second hypothesis stating that the experimental FRCs have better mechanical properties than controls must be rejected. According to these findings, the evaluated novel resin matrix system of HDDMA/TEGDMA appears potentially promising for dental use. However, it is yet to be further evaluated *e.g.*, for other ratios of HDDMA and TEGDMA resins. In our future research, long-term evaluation of the FRCs in water storage, and the analysis of the monomer release will be included.

5. Conclusion

The microbiological properties suggest that the experimental HDDMA/TEGDMA FRCs have the potential to be future biocompatible dental materials. Moreover, HDDMA/TEGDMA FRCs showed similar mechanical properties to controls.

Declarations

Author contribution statement

Siti Sunarintyas: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Widowati Siswomihardjo: Performed the experiments; Analyzed and interpreted the data.

James K.H. Tsoi: Contributed reagents, materials, analysis tools or data.

Jukka P. Matinlinna: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data associated with this study has been deposited at Figshare, available at doi.org/10.6084/m9.figshare.17468546.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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