Strength and Cytotoxicity in Glass-Fiber-Reinforced Denture Base Resin with Changes in the Monomer

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ABSTRACT: Glass fibers (GFs) are often used to strengthen denture base resins. An increase in the GF content can result in severe viscosity, bunching of the GFs, and difficulty in the handling and packing of the poly(methyl methacrylate) (PMMA) resin. To ensure better impregnation of the GFs and improvements in the workability, more methyl methacrylate is needed as the percentage of GFs is increased. The purpose of this study was to determine adequate powder/liquid ratios according to the GF ratios to reinforce with GFs and to evaluate the effect of probable residual monomer on the cytotoxicity and mechanical properties of PMMA denture base resin reinforced with different ratios of GFs. Five specimens were made according to the defined powder/liquid ratios. Ten specimens for each group were prepared with a stainless steel mold. The flexural strength (FS) and elastic modulus were measured with a three-point bending test in a universal testing machine. To examine changes in toxicity with time, three types of specimens for each group were fabricated and immersed for 0, 24, and 72 h in distilled water after polymerization. The cytotoxicity was evaluated with an agar overlay test. The workability was improved, and the FS and elastic modulus of the denture base resin reinforced with GFs were made significantly higher with increases in the amount of monomer. There was no difference between the control group and the GF-reinforced groups with regard to the cytotoxicity, despite the increasing monomer concentration. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: biocompatibility; dental polymers; fibers

INTRODUCTION

Poly(methyl methacrylate) (PMMA) is the material of choice for the fabrication of complete dentures. Although denture base resins are not ideal in all respects, their combination of properties, including their working characteristics, minimum expense, excellent aesthetics, accuracy of fit, stability in the oral environment, and ease of processing, account for their popularity and universal use.[1] However, mechanical failure in PMMA dentures often occur.[2,3] Many researchers have attempted to improve the mechanical properties of denture base resins by the addition of different types of fibers. Carbon[4] and aramid[5] fibers are not practical because of polishing difficulties and their undesirable aesthetic appearances.[6] The reinforcement of PMMA with ultrahigh-modulus polyethylene requires a complicated surface treatment and does not significantly improve the mechanical properties of denture base resins.[7] Glass-fiber (GF) reinforcement has advantages compared to other reinforcement methods, including improved aesthetics, enhanced bonding to the resin matrix, and ease of repair.[6]

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In this study, a conventional electrical mixer was used to mix commercial silanated GFs and resin powder. It was reported that optimal adhesion between the fibers and polymer matrix can be obtained by mixing with a silane-coupling compound.[8] To avoid the shortening of the fibers, a sharp blade was covered with a resin shield.[9] It divided the fiber bundles and produced an even mixture. Continuous GF-reinforced composites are anisotropic, with a high strength and stiffness in one direction parallel to the fibers. However, the placement of continuous GFs at the weak parts of the dentures can be difficult and requires additional technical procedures.[10] Chopped-GF-reinforced composites are isotropic and used easily with the conventional compression-molding technique. In a previous study, more than 6% GF inclusion deteriorated the plasticity, and packing was difficult. Specimens containing over 9 wt % fibers were excluded from the test as it proved very difficult to include them in the resin during mixing.[9] Particularly, long GFs deteriorated the plasticity more than short GFs did when some amount of fibers was added. The short fiber length represented a convenient size for manipulation and inclusion into acrylic resin dough.

The flexural strength (FS) and elastic modulus generally increase with higher volumetric content of GFs in a limited range.[11] However, there are some difficulties in the reinforcement of denture base

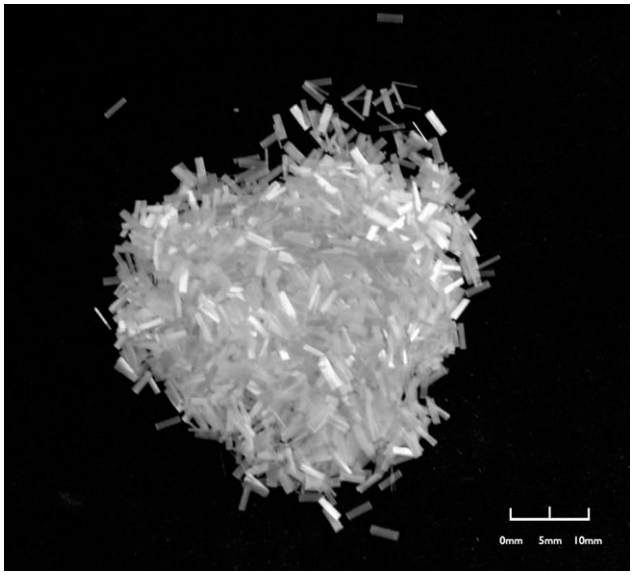

Figure 1 Commercially available chopped GF bundles (Hankuk Fiber Co.).

polymers with GFs. One of these is adequate impregnation of fibers in PMMA.[12] A higher content of GFs showed that the fibers tended to clump together when mixed with PMMA and that porosity was increased because of the formation of void spaces in the composites.[13] More methyl methacrylate (MMA) than that recommended in the powder/liquid ratio was needed as the percentage of GFs increased to ensure better impregnation of the GF and improvement in the workability.[14] However, a higher proportion of the monomer liquid in the mixture could increase the residual monomer content.[15] Excessive monomer can affect the mechanical properties and cytotoxicity of denture base resins.[16,17] Unfortunately, few reports have been issued on the topic of increasing monomer with increasing GF for the reinforcement of PMMA denture base resin. In this study, a minimal quantity of monomer and the powder/liquid ratio of that moment in each group were determined with increasing GF content until a similar viscosity and mixing appearance of powder and liquid with control group were evident. The aim of this study was to determine adequate powder/liquid ratios according to the GF ratios for the reinforcement of denture base resins and to evaluate the effect of probable residual monomer on the cytotoxicity and mechanical properties of PMMA denture base resins reinforced with different ratios of GFs.

EXPERIMENTAL

Materials

The acrylic resin used in this study was heat-polymerized PMMA (Vertex RS, Dentimex, Zeist, Netherlands). A 3-mm length of chopped electrical GF (Hankuk Fiber Co., Milyang, Korea) was used. The GF had a diameter of 11 μm; the bundle-form GFs, which consisted of about 100 single GFs, were commercially available (Fig. 1).

Determination of the powder/liquid ratio

The PMMA powder and GFs were mixed thoroughly at mass ratios of 0, 6, 9, 12, and 15 mass % with a conventional electrical mixer with a modified blunt blade. The manufacturer's recommendation for the PMMA powder/MMA liquid ratio (control) was 23 g/10 mL for routine clinical use. More MMA than the recommended powder/liquid ratio was needed as the percentage of GFs was increased to ensure better impregnation of the GFs and better mixing of the powder and liquid of the denture base resin. Through 10 repeated experiments, the average minimum quantity of added monomer was determined to reach a similar mixing viscosity and impregnation with the control group with increasing GF. The powder/liquid ratio of that moment in each group was determined. (Table I).

Specimen fabrication

Specimens were made according to the determined powder/liquid ratio at the point described previously.

Specimens for the three-point bending test

Ten specimens for each group were prepared with a stainless steel mold (Fig. 2) with dimensions of 65 × 10 × 3.3 mm^3. The PMMA powder and liquid were mixed by hand for 30 s and allowed to stand for 15 min. The unpolymerized acrylic resin dough was then packed and pressed slowly under 250 bar of pressure in the stainless steel mold incrementally to produce four specimens at a time. Two trial closures were made in the mold to remove excess material and each mold. The resin composite was cured in boiling water for 20 min according to the manufacturer's instructions. After processing, the specimens were polished in a polishing machine (EXAKT 400CS, EXAKT Apparatebau GmbH & Co., Norderstedt, Germany) with metallographic grinding paper with a 500 federation of european producers of abrasives

TABLE I
Classification of the Test Groups

Group	Fiber and resin type	Powder/liquid ratio
1	PMMA resin (control)	23 g/10.00 mL
2	PMMA resin + 6% GF	23 g/10.58 mL
3	PMMA resin + 9% GF	23 g/12.00 mL
4	PMMA resin + 12% GF	23 g/13.35 mL
5	PMMA resin + 15% GF	23 g/14.40 mL

number of specimens (n) = 10 per group.

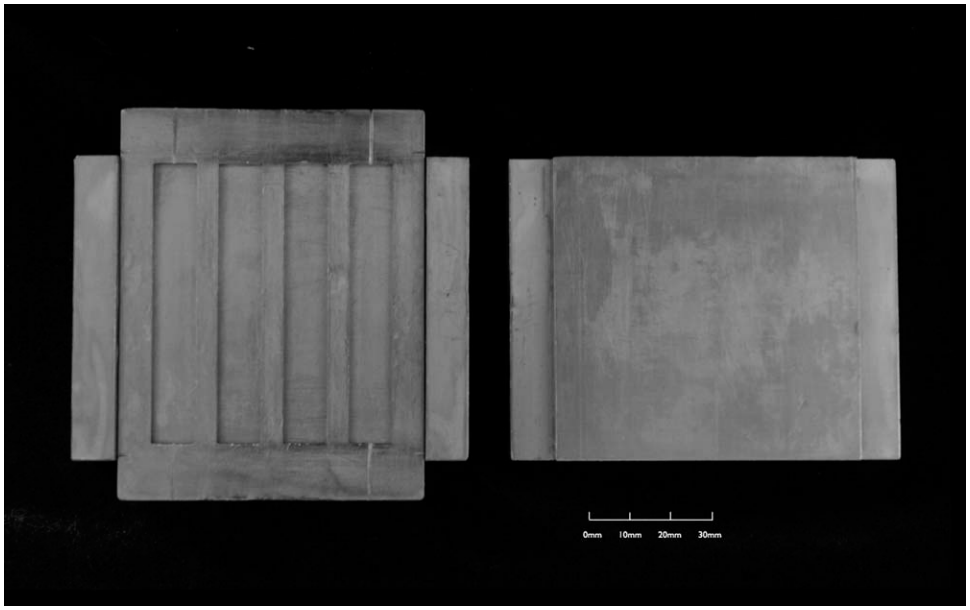

Figure 2 Stainless steel mold for making specimens.

(FEPA) and 1200 FEPA (grain size ≈ 30 and 14 μm) under running water. All specimens were stored in distilled water at 37°C for 48 h before testing.[18]

Specimen for the agar overlay test

A silicone mold (diameter = 10 mm, thickness = 2 mm) was used to fabricate the specimens. The specimens were prepared and polymerized. To examine the changes in toxicity with time, three types of specimens for each group were fabricated (fresh specimens and specimens immersed in distilled water for 24 or 72 h). Three specimens were prepared from each of the five groups and were immersed in distilled water for up to 72 h (72-h immersion). After 48 h of immersion, each of the three specimens was fabricated and soaked for another 24 h (24-h immersion). Twenty-four hours later, each of the three specimens was fabricated again (fresh specimens). Both planes of the specimens were disinfected by irradiation with ultraviolet light on each plane for 20 min. The agar overlay test was repeated three times.

TABLE II
Specification of the Materials Used in the Cytotoxicity Test According to ISO Technical Report 7405-1997

Type	Description	Batch no.
Mouse fibroblasts	American-type culture collection CCL 1	NCTC clone 929
Positive control	Poly(vinyl chloride)–plastic portex	499–300–000–000
Negative control	Polyethylene–plastic portex	800–100–680–100

Three-point bending test

The FS was measured with a three-point bending test in a universal testing machine (Instron model 3345, Instron, Massachusetts, USA) at crosshead speed of 5 mm/min. The distance between the centers of the support was 50 mm. The specimens were then loaded at the center until fracture occurred. The three-point bending test was carried out according to ISO 1567. A digital micrometer (Absolute Digimatic, Mitutoyo, Japan) was used to make a precise specimen measurement. The FS was calculated from the following formula:

TABLE III
Toxicity Evaluation by the Size of the Zone of Decoloration and the Degree of Cell Lysis with the Agar Overlay Method

Index	Zone description
Zone index	
0	No detectable zone around under the sample
1	Zone limited to the area under the sample
2	Zone not greater than 5 mm in extension from the sample
3	Zone not greater than 10 mm in extension from the sample
4	Zone greater than 10 mm in extension from the sample but not involving the entire plate
5	Zone involving the entire plate
Lysis index	
0	No observable lysis
1	Up to 20% of zone lysed
2	20–40% of zone lysed
3	40%–60% of zone lysed
4	60–80% of zone lysed
5	Over 80% lysed within zone

TABLE IV
FS of the Five Groups

Group		No. of specimens	FS(MPa) Mean	FS(MPa) Standard deviation	p^a	D^b
Group 1	0% GF	10	92.67	5.43	<0.05	a
Group 2	6% GF	10	104.10	6.65		b
Group 3	9% GF	10	109.19	8.36		b,c
Group 4	12% GF	10	115.44	10.89		c
Group 5	15% GF	10	112.50	7.09		c

[a] A one-way ANOVA indicated that there was a significant difference between the groups ($p < 0.05$).
[b] The same letters indicate a nonsignificant difference between groups on the basis of a Duncan's multiple-comparison test.

$$FS = 3WL/2bd^2$$

where W is the maximum load before fracture, L is the span between the two supports (50 mm), b is the width of the sample, and d is the thickness of the sample.

The elastic modulus (E) was calculated from the following formula:

$$E = WL^3/4\delta bd^3$$

where δ is the deformation.

Agar overlay test

The cytotoxicity test was an agar diffusion test carried out according to ISO Technical Report 7405-1997 in a quality-controlled testing laboratory.[19] The specification of the main materials used in the agar diffusion test are listed in Table II. Mouse fibroblast cells (L929) were plated at 1×10^6 cells in each 60×10 mm^2 plastic Petri dish. Dulbecco's Modified Eagle Medium (without phenol red, with L-glutamine, a 1% antibiotic–antimycotic mixture, and 10% fetal bovine serum) was used as the culture medium. When a confluent cell layer formed after 24 h of incubation (5% CO_2/95% air, 37°C), the medium was removed. Then, 6 mL of medium containing 1% agarose was added. When the agarose solidified, the specimens were applied on the agar and incubated for 24 h at 37°C (5% CO_2/95% air). Twenty-four hours later, the cells were stained with 0.5% neutral red in phosphate buffered saline with filtering. An optical microscope (400×) was used to investigate cellular changes and cytolysis. The results were evaluated according to the zone and lysis indices (Table III). There were three test specimens in each of the test groups, and the tests were run three times.

Statistical analysis

The statistical analysis was performed with SAS 9.1.3 (SAS Institute, Cary, North Carolina, USA). A one-way analysis of variance (ANOVA) was used to examine the statistical differences between the five groups of denture base resins. A Duncan's multiple-comarison test was used for the *post hoc* test. All analyses were performed at a 95% level of confidence.

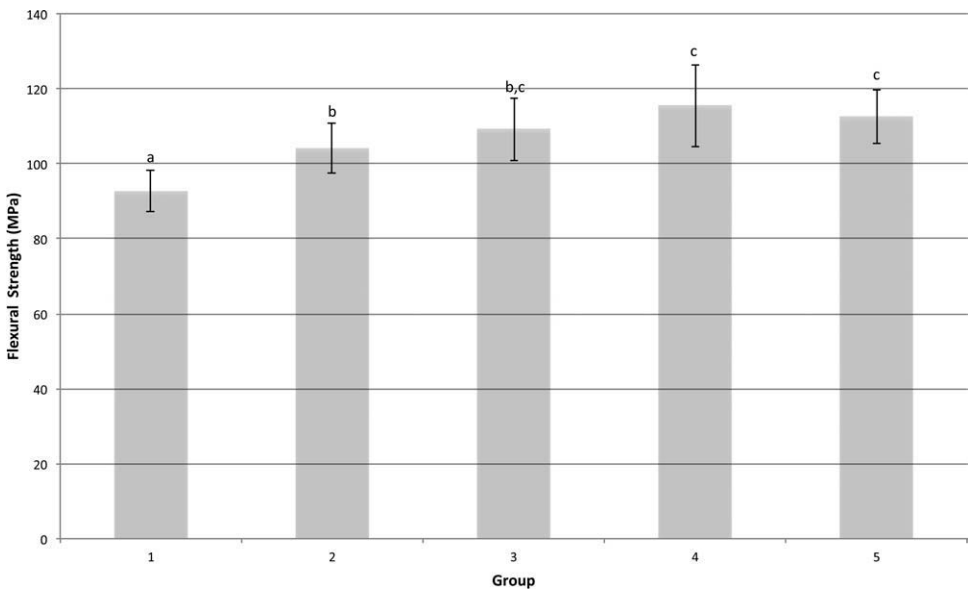

Figure 3 FS values in the experimental groups. The data were statistically analyzed with a one-way ANOVA at the 95% confidence level. When significant effects were detected, a Duncan's multiple-comarison test was used to determine which group means were different. The same letters indicate a nonsignificant difference between the groups on the basis of the Duncan's multiple-comarison test.

TABLE V
Elastic Moduli of the Five Groups

Group		No. of specimens	Elastic modulus (MPa)		p[a]	D[b]
			Mean	Standard deviation		
Group 1	0% GF	10	2046.97	173.18	<0.05	a
Group 2	6% GF	10	2895.57	305.35		b
Group 3	9% GF	10	3390.12	217.35		c
Group 4	12% GF	10	3425.69	258.16		c
Group 5	15% GF	10	3781.50	222.47		d

[a] A one-way ANOVA indicated there was a significant difference between the groups ($p < 0.05$).
[b] The same letters indicate a nonsignificant difference between groups on the basis of a Duncan's multiple-comparison test.

RESULTS

FS and elastic modulus

Table IV presents the FSs for each group. When all of the tested groups were compared, the FSs showed significant differences in the GF-reinforced groups with increasing monomer compared to the unreinforced group (control, $p < 0.05$; Fig. 3). The mean FSs of groups 4 and 5 were significantly higher than that of group 2. Table V presents the elastic modulus for each group. When all of the tested groups were compared, the elastic modulus was significantly higher in the GF-reinforced groups with increasing monomer compared to that in the unreinforced group (control, $p < 0.05$; Fig. 4).

Agar overlay test

The cytotoxicity results from the agar overlay test are shown in Table VI. Positive controls [poly(vinyl chloride)] induced a zone/lysis index of 1–4 [Fig. 5(a)]. Negative controls [polyethylene] showed a zone/lysis index of 0–0 [Fig. 5(b)]. All of the groups showed a zone/lysis index of 1–0 among samples that were tested immediately after polymerization, samples that were tested after immersion in distilled water for 24 h, and samples that were tested after immersion in distilled water for 72 h [Fig. 5(d)].

DISCUSSION

The FS and elastic modulus values of the GF-reinforced groups increased with increasing GF content, although the monomer content was increased. The FS of the 12% GF-reinforced group 4 increased by 24% compared to that of the control group. The elastic modulus of the 15% GF-reinforced group 5 increased by 84% compared to that of the control group. A high elastic modulus of the denture base materials is an important requirement for the resistance of denture deformation during mastication.[2] Stipho[13] reported that the FS increased by 18% with the addition of 1% silanated 2-mm short-rod GFs. Chen et al.[20] reported that the FS increased by 8% with the addition of 2% unsilanated 4-mm short-rod GFs. Lee et al.[9] reported that FS increased by 13% with the addition of 6% silanated 3-mm short-rod GFs and by 17% with the addition of 9% silanated 3-

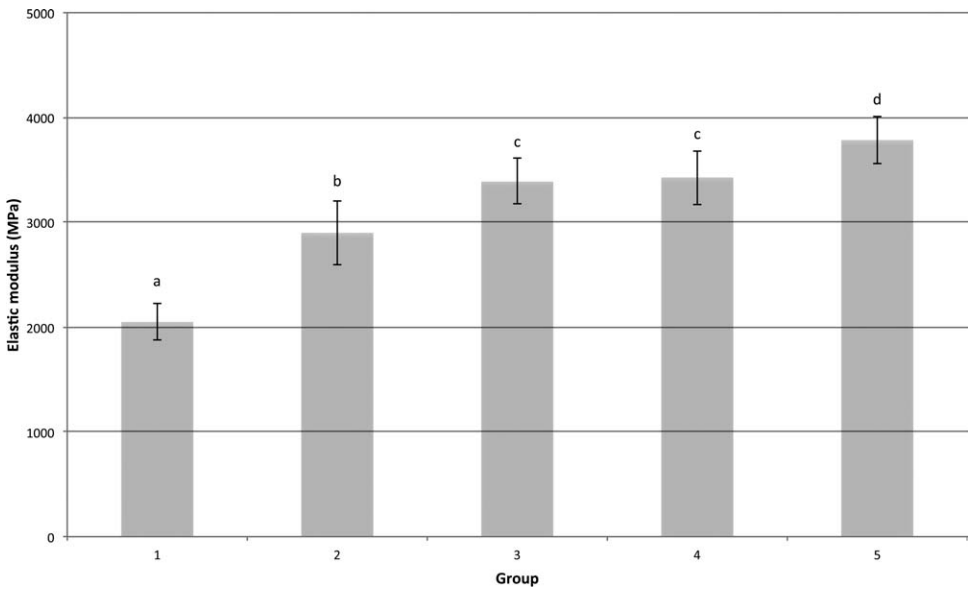

Figure 4 Elastic modulus in the experimental groups. The data were statistically analyzed with a one-way ANOVA at the 95% confidence level. When significant effects were detected, a Duncan's multiple-comarison test was used to determine which group means were different. The same letters indicate a nonsignificant difference between groups on the basis of the Duncan's multiple-comarison test.

TABLE VI
Cytotoxicity of the GF-Reinforced Denture Base Resin

Group	Fresh (Zi–Li)	24 h Immersion	72 h Immersion
1	1–0	1–0	1–0
2	1–0	1–0	1–0
3	1–0	1–0	1–0
4	1–0	1–0	1–0
5	1–0	1–0	1–0

Zi, zone index; Li, lysis index.

mm short-rod GFs. The increase in FS in our study was greater than those of previously reported studies. The poor wetting of the fibers appeared to be the explanation for the void space in acrylic–GF composites.[12] The void was taken as a factor that led to the reduction of the strength of the GF–resin composite. That is, the void was made when GF was not impregnated sufficiently, which was the reason the resins were weakened. To solve this problem, much more liquid or surface treatments have been tried. In this study, the PMMA powder and GFs were mixed thoroughly with a conventional electrical mixer with a modified blunt blade, and a limited quantity of monomer was increased when the GF amount was increased. Therefore, the workability of the denture base resin reinforced with higher contents of GF was improved, and no bunching of GFs was found.

A higher proportion of monomer liquid in a mixture for better impregnation and workability may increase the residual monomer content.[15] The monomer can act as an allergen, and the residual monomers in the prosthesis can cause irritation of the skin or the oral mucosa.[21] In this study, there was no difference between the cytotoxicity of the control group and that of the GF-reinforced group. As analyzed by the agar overlay test, there was no change in toxicity with time (fresh specimens and those immersed in distilled water for 24 or 72 h) in any of the groups. The agar overlay test is a rapid, standardized, sensitive, and inexpensive means and a good first step toward ensuring the biocompatibility of a medical device. However, the data can only be interpreted as characteristic of basic biological properties. No direct transformation of the data to usage tests or to man is possible, but by this simple method, basic biological information can be detected; this may help to explain phenomena observed in the usage tests or in patients.[22] Other cytotoxicity test methods, such as the determination of the protein synthesis of cells, mucous membrane irritation in hamsters, and sensitization of guinea pigs, are also needed for further study.[23] The results of our study suggest that the increase of the monomer amount for better impregnation of GFs did not increase the cytotoxicity of the PMMA resin.

It was interesting that the addition of 12% chopped GFs with 33.5% monomer resulted in an optimal increase in the FS, elastic modulus, and workability without any cytotoxicity change within the limitations of our *in vitro* study.

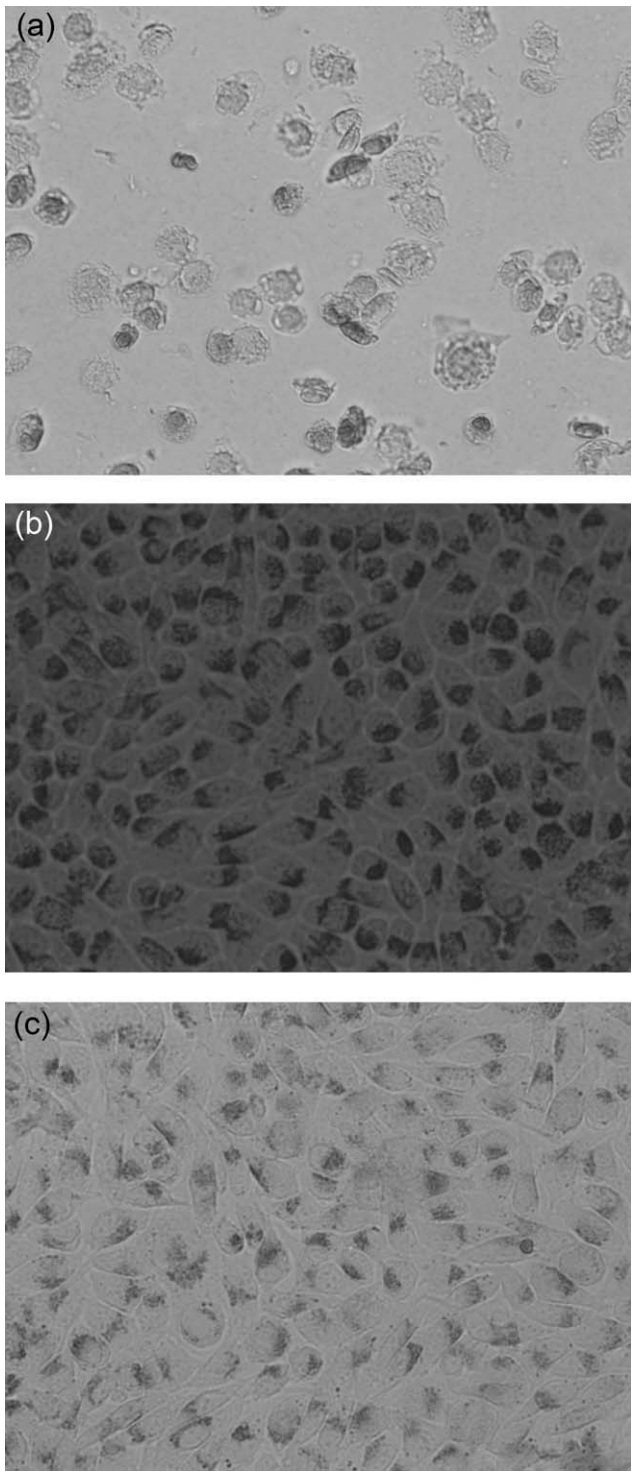

Figure 5 Photomicrographs of the cultured fibroblasts under the application of a (a) positive control, (b) negative control, and (c) GF-reinforced denture base resin (original magnification = 400×).

Further study is required to quantitatively assess the contents of the residual monomer with gas chromatography or other methods and the effects of excess monomer on the dimensional stability of the denture base.

CONCLUSIONS

In this study, it was shown that the workability of GF-reinforced denture base resin with significantly higher FS and elastic modulus values was improved by an increase in the amount of monomer, and there was no difference between the control group and the GF-reinforced groups with regard to cytotoxicity, despite the increasing monomer concentration. The use of GFs with the addition of more monomer is recommended as a method for the reinforcement of denture base resins.

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