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The Influence of Amine Contents in Experimental Acrylic Resin on The Flexural Strength and Cell Cytotoxicity

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Abstract
Background/Objectives: The purpose of this study was to evaluate the influence of benzoyl peroxide(BPO) and amine ratio on the flexural strength and cell cytotoxicity of acrylic resin.
Methods/Statistical analysis: To prepare the specimens, six experimental acrylic resin groups were prepared depending on BPO:amine ratio followed by 1:0.25, 1:0.5, 1:0.75, 1:1, 1:1.25, 1:1.5 mol%. To measure mechanical properties, three point bending test was carried out according to ISO 4049 specification. The cytotoxicity test was carried out according to ISO 10993-5 specification. The residual monomers of fully polymerized resin materials were identified using gas chromatography/mass spectrometry. All data were statistically analyzed by a one-way ANOVA.
Findings: Groups with A0.25 and A0.5 showed significantly lower level of flexural strength that other experimental and control groups ($p < 0.05$). Flexural strengths were increased according to the increasing proportions of amine. Groups with A0.75, A1, A1.25 and A1.5 had no statistically significant difference compared to control group ($p > 0.05$). Flexural strengths were increased as the proportion of amine was increased. The cytotoxicity of each group exhibited increasing cell viability as more amine was added (Figure 2). Groups with A0.25, A0.5, and A0.75 had a significantly higher cytotoxicity than other experimental and control groups ($p < 0.05$). Groups with A1, A1.25 and A1.5 had no statistically significant difference compared to control group ($p > 0.05$). The cytotoxicity of each group exhibited increasing cell viability as more amine was added. The residual monomer tended to decrease as the proportion of amine increased. It was considered that the amount of BPO:amine ration should be carefully adjusted in order to minimize the harmful effect of residual monomer.
Improvements/Applications: Based on these results, the distribution of amine content in the liquid should be uniform when used in clinical practice as the amine content affects the properties of acrylic resin.

Keywords: Acrylic resin, Amine, Benzoyl peroxide, Cell cytotoxicity, Flexural strength, Residual

monomer methyl methacrylate.

1. Introduction

Since acrylic resin was first used in dentistry as a denture base material in 1935, it has been used in diverse fields due to its convenience of use, short polymerization time, and good manipulability [1, 2]. Acrylic resin has been widely used for denture repair and relining, temporary crown and bridge materials. Acrylic resin is used by mixing polymethyl methacrylate (PMMA) and methyl methacrylate (MMA)[3].

PMMA is mainly used to have a molecular weight between 5-50 million, and is used by adding benzoyl peroxide (BPO) as a polymerization initiator[4]. In general, the use of PMMA with high molecular weight can produce products with strong strength. However, there is a disadvantage in that the reaction is insufficient due to the low dissolution rate of monomer[3,5]. In the monomer-polymer reaction, the monomer penetrating into the PMMA causes the PMMA to be plasticized. Thereafter, free radicals by benzoyl peroxide and amine initiate polymerization of the vinyl group[6].

MMA is one of the monomer used in the polymerization process of acrylic resins for dental application, which is known to be a highly volatile, flammable, and toxic component[4,5]. MMA is used as various dental materials despite the potential for harmful effects on the biocompatibility[6]. If complete polymerization was not performed during the polymerization process, residual monomers are produced, which adversely affects biocompatibility[7, 8]. In particular, acrylic resin for denture or temporary crown is a material in direct contact with the oral mucosa[1]. Accordingly, if unreacted monomer remains in the resin, it may have a local and direct effect on the human body due to moisture in saliva in the oral cavity, causing skin irritation and erythema[9]. Previous studies on unreacted monomer have been conducted on cytotoxicity and hypersensitivity[9, 10]. In order to investigate the cytotoxicity of MMA, the cell cytotoxicity test was carried out at different concentrations of MMA[11]. As a result, the higher the concentration of MMA, the higher the cytotoxicity[12, 13].

Also, the results of cell morphology in previous study have shown that the shape of attached cells was changed due to toxicity of MMA, resulting in damage to various cells[10, 14]. Accordingly, it is thought that there is a demand to develop acrylic resins that had lower amounts residual monomer released MMA[15]. However, research on reducing the amount of unreacted monomer elution is still lacking.

The hypotheses of this study were as follows: Under the same amounts of BPO, (1) reduction of the BPO:amine ratio would increase flexural strength of acrylic resin, (2) reduction of the BPO:amine ratio would decrease cytotoxicity of acrylic resin, (3) reduction of the BPO:amine ratio would decrease residual monomer of acrylic resin. The purpose of this study was to investigate the influence of BPO:amine ratio in acrylic resins on flexural strength and cytotoxicity, and to minimize the amount of residual monomer of acrylic resin by evaluating the degree of reduction of eluted unreacted monomers.

2. Materials and Methods

2.1. *Materials*

Conventional acrylic resin (GC Unifast, GC Co., Tokyo, Japan) was used as control group. Experimental acrylic resins were divided into six groups according to BPO:amine ratio followed by 1:0.25, 1:0.5, 1:0.75, 1:1, 1:1.25, and 1:1.5 mol%. Experimental conditions are presented in Table 1.

Table 1: Summary of experimental conditions

Experimental group code	BPO (mol%)	Amine (mol%)	BPO:Amine ratio
A0.25	1	0.25	1:0.25
A0.5		0.5	1:0.5
A0.75		0.75	1:0.75
A1		1	1:1
A1.25		1.25	1:1.25
A1.5		1.5	1:1.5

2.2. *Methods*

2.2.1. Flexural strength

To measure flexural strength, the specimens were fabricated with 25 mm length x 2 mm width x 2 mm height. After 24 hours storage in 37 °C water, three-point bending test was carried out according to ISO 4049 specification using Instron (crosshead speed 0.5 mm/min)[16]. Measurements were performed twice for each sample (n=6).

2.2.2. Cell cytotoxicity

The cytotoxicity test was carried out according to ISO 10993-5 specification using the L929 fibroblasts, which was evaluated using a MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay[17]. The specimens were extracted in culture medium with serum for 24 hours at 37 °C. The L929 cells (1×10^5 cells/mL) were seeded onto a 96-well plate (SPL, Pochen-Si, Gyeonggi-Do, Korea) and incubated in a humidified incubator at 37 ℃. The culture medium was removed from the well, 100 µL extracts were dispensed on to the cell, and incubated for 24 hours. Following this treatment, the extracts solution was extracted and changed with 50 µL of MTT solution. After incubation of the cell with MTT solution for 3 h, the MTT solution was extracted and 100 µL isopropanol was added to each well. The absorbance was estimated at 570 nm by ELISA reader (Epoch, BioTek, Winooski, VT, USA).

2.2.3. Residual monomer

The specimens were eluted for 24 hours to measure the amount of residual monomer. The residual monomers of fully polymerized resin materials were identified using gas chromatography (GC/MS).

2.2.4. Statistical analysis

All the results were analyzed using one-way ANOVA (PASW 21.0, IBM Co., NY, USA), and a Tukey test was conducted as a post-hoc test ($p = 0.05$).

3. Results and Discussion

In dentistry, acrylic resin has been extensively used for a long time due to their various applications[18, 19]. Numerous types of acrylic resin have been developed to improve physical and mechanical properties using polymerization reactions[20]. Amine have been used long time with BPO as an effective initiation system for free radical polymerization of acrylic resin[21]. However, acrylic resin may have unreacted monomers that adversely affect the human body if polymerization is not completed[9, 22]. When directly exposed to MMA at the dental clinic, dentists and support staff often experience discomfort due to hypersensitivity, dermatological reactions, and asthmatic reactions, [8, 23]. Although MMA have various harmful effects on the human body, they were used as various materials in dental clinics[7]. Residual monomer can lead to inflammation, allergic reaction, and irritation of oral tissue[10]. Therefore, it is necessary to minimize this residual monomer. The initiation of the polymerization of MMA:BPO from the powder and DMPT(N,N-Dimethyl-p-toludine) from the liquid react to from radicals, starting the curing of acrylic resins[21, 24]. The concentration of BPO and amine play a key role in the setting parameters[24]. In this study, we tried to reduce unreacted monomer by adjusting the amine content[25].

The first hypothesis; Under the same conditions of BPO, reduction of the BPO:amine ratio would increase flexural strength was accepted. The results of flexural strength are shown in figure 1. Groups with A0.25 and A0.5 showed significantly lower level of flexural strength that other experimental and control groups ($p < 0.05$). Flexural strengths were increased according to the increasing proportions of amine. There is no significant difference in groups with A0.75, A1, A1.25 and A1.5 compared with control group ($p > 0.05$). Flexural strength of acrylic resin highly affected by BOP/amine ratio, which is related to the polymerization reaction of acrylic resin[20]. Flexural strength of acrylic resin affects final product[16]. General requirements for acrylic resin are flexural strength, durability, biocompatibility, and ease of fabrication[26].

The second hypothesis; Under the same conditions of BPO, reduction of the BPO:amine ratio would decrease cytotoxicity was accepted. The results of cell viability are shown in Figure 2. The cytotoxicity of each group exhibited increasing cell viability as more amine was added. Groups with A0.25, A0.5, and A0.75 had a significantly higher cytotoxicity than other experimental and control groups ($p < 0.05$). There is no significant difference in groups with A1, A1.25 and A1.5 compared with control group ($p > 0.05$). In particular, A1.5 group showed higher cell viability than control group.

The third hypothesis; Under the same conditions of BPO, reduction of the BPO:amine ratio would decrease residual monomer was accepted. Considering the above results, this study measured the amount of residual monomer. The results of residual monomer are shown in Figure 3. The residual monomer of A0.25 group showed significantly higher than that of control group. However, residual monomer showed decreasing trend with increasing proportions of amine added. Acrylic resin is known to exhibit toxic reactions in cell culture, and previous studies have shown that toxicity is associated with unreacted residual monomer of material[27, 28]. Additionally, the results of this study showed that acrylic resin properties were influenced by residual monomer depending on amine content[9, 10, 29].

Based on the above results, residual monomer has been shown to adversely affect flexural strength and cytotoxicity of acrylic resin. Hence, it was considered that the amount of BPO:amine ratio should be carefully adjusted in order to minimize the harmful effect of residual monomer.

In this study, experiments were conducted on the flexural strength, cell cytotoxicity, and elution of residual monomer of acrylic resin. It was considered that further studies on the effects of various polymerization methods over time would be necessary. In addition, this study has limitation of *in vitro* study. Further study should be conducted to confirm the results of long-term clinical use of acrylic resin.

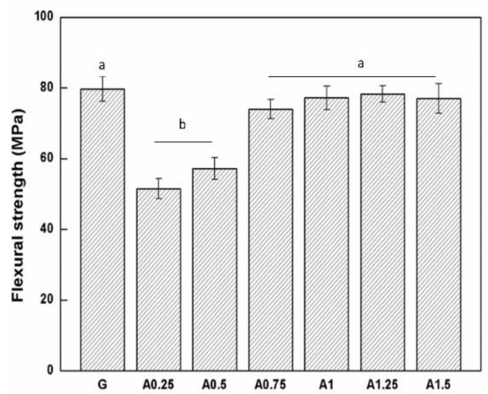

Figure 1. Flexural strength of the control group and experimental groups. Different alphabets showed statistically significant differences.

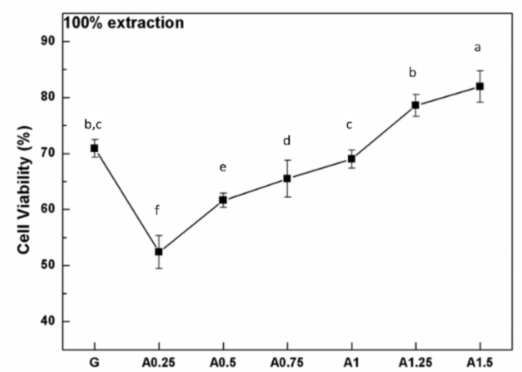

Figure 2. Cell viability of the control group and experimental groups. Different alphabets showed statistically significant differences.

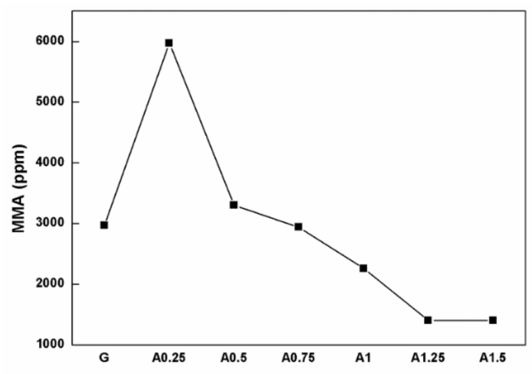

Figure 3. Evaluation of released Methyl methacrylate (MMA).

4. Conclusion

Within the limits of this study,

1. As the BPO:amine ratio decreased, the flexural strength of acrylic resin increased.
2. As the BPO:amine ratio decreased, the cytotoxicity of acrylic resin increased.
3. As the BPO:amine ratio decreased, the residual monomer of acrylic resin decreased.

Therefore, the results of this study showed that the properties of acrylic resin were influenced by residual monomer depending on amine content.

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