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# Biological Property of HDDMA Based Resin Matrix System for Dentistry

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**Abstract:** Recently, there is a great interest in the replacement of commercial bis-GMA resin matrix system for dentistry. Bis-GMA is considered to be relatively cytotoxic and allergenic to human. A novel resin matrix system based on HDDMA is under investigation. This study aimed to determine the biological property especially the cytotoxicity property of proposed HDDMA-based resin matrix systems of FRCs material toward fibroblast cells. Fifteen FRCs specimens were prepared and divided into 3 groups. Two groups were based on HDDMA matrix systems and one group was bis-GMA-based. The cytotoxicity property was determined by MTT method. The result revealed significant differences in cells viability between HDDMA-based matrices and bis-GMA-based, whilst no significant difference between the two proposed HDDMA-based matrices. In conclusion, proposed resin matrix systems based on HDDMA exhibited a less cytotoxic material than bis-GMA-based.

**Key words:** HDDMA, bis-GMA, cytotoxicity, cells, FRCs

## INTRODUCTION

Now a days, there has been a great interest in the application of Fiber-Reinforced Composites (FRCs) materials in dentistry. Fiber-reinforced composites have been used in removable prosthodontic (Vallittu, 1997) as Fixed Partial Dentures (FPD) (Vallittu, 1998), periodontal splints and in orthodontic treatment (Vallittu, 1997). Fiber-reinforced composites consist of a resin matrix reinforced by fibers which induce relatively high strength and modulus (Mallick, 1993; Zhang and Matinlinna, 2012) and exhibit good biocompatibility in general (Mallineni *et al*., 2013).

The resinous matrix of FRCs has the function of binding the sized fibers, transferring strength to fibers and preventing fibers from the outside environment (chemicals, temperature fluctuation, moisture and mechanical attack). Silanes are needed for bonding dissimilar material types (Lung and Matinlinna, 2012). Matrix influences also mechanical properties such as compressive strength, interlaminar and in-plane shear properties and interaction between matrix and fiber (Mallick, 1993).

There are two major types of polymer matrices used in FRCs, namely cross-linked and linear polymers. The cross-linking polymer refers to multifunctional di-methacrylate resins. The linear polymer refers to a monofunctional methacrylate polymer. In FRCs with the so-called IPN (Interpenetrating Network) structure, usually the matrix consists of a cross-linking polymer, a linear polymer and a photoinitiator (Mallick, 1993).

The composition of a resin matrix in FRCs is usually complex since it contains a great variety of different monomer and additives (Geurtsen *et al*., 1998). Some studies reported that residual monomers, additives or polymerization products were released from set resin matrix into adjacent tissues in the oral cavity over time (Ferracane and Condon, 1990; Ferracane, 1994). The first release of free monomers occurred during the monomer to polymer conversion and a long-term release of leachable substances was generated by erosion and degradation over a longer time period (Goldberg, 2008; Gupta *et al*., 2012). The release of leachable components into surrounding tissues may cause adverse local reactions or systemic effects on patients.

Bis-GMA-MMA (Bisphenol-A-Glycidyl Methacrylate -Methyl Methacrylate) resin combination as the basic resin matrix is widely used in commercial resin-based dental materials. It was reported, however that MMA became an allergen in denture base materials, especially for dental technicians (Pfeiffer and Rosenbauer, 2004).

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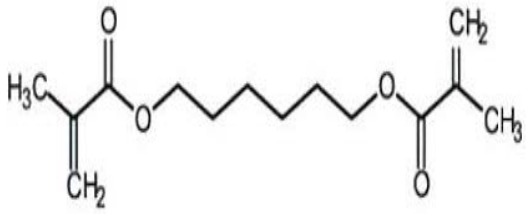

Fig. 1: Chemical structure of HDDMA (Vallittu and Sevelius, 2000)

The release of residual monomers of MMA was said to be the primary cause of irritation to the mucous membrane in the oral cavity (Urban *et al*., 2007). Bis-GMA as a key component in dental resin composites was considered to be relatively more cytotoxic and allergenic than the other dental resin monomers studied (Schmalz and Bindslev, 2009). It was also reported that bis-GMA was the most cytotoxic monomer among 35 studied dental resin composite monomers. The study assessed monomers such as bis-GMA, GMA, HDDMA, BPA, CQ, TEGDMA, HEMA and MMA (Moharamzadeh *et al*., 2009). In addition, some previous studies revealed that bis-GMA affected the vitality of dental pulp and induced pulp inflammation and it was able to disturb normal differentiation procedures of pulp fibroblasts (Imazato *et al*., 2009), moreover induced allergic contact stomatitis in the oral cavity. In fact, the commercial resin composite matrix systems employ widely the bis-GMA-based system as the basic matrix components despite the fact that it may cause pseudo-estrogenic effects though this aspect is disputed (Rathee *et al*., 2012). To reduce the harmful effect of this matrix system, it may be necessary to look for a new and safer matrix system for human instead of using bis-GMA-MMA.

Resin matrix system consisting of 1, 6-Hexanediol Di-Methacrylate (HDDMA), Fig. 1, is under current investigation. It has the characteristic of a similar reactive group as bis-GMA. It has also the property of low viscosity, fast curing with low volatility, hydrophobic backbone and good applicability for use in free radical polymerization. Its linear chemical structure affects its performance not to be viscous as bis-GMA (Vallittu and Sevelius, 2000).

The hydrophobic HDDMA is currently used as a functional monomer and as a cross-linking agent between the molecular chains of certain polymers. Technical applications of HDDMA include adhesives, elastomers sealants, photopolymers, electronics and coatings, when improved adhesion, hardness and abrasion resistance are sought (Vallittu, 2014). Interestingly, the biological properties of HDDMA were reported being not embryotoxic, mutagenic, teratogenic or inducing reproductive effects in humans. It is noteworthy that none of HDDMA precursor components are listed by IARC, NTP, OSHA or ACGIH as carcinogens (Vallittu and Sevelius, 2000).

The objective of this recent study was to determine the cytotoxicity property of proposed HDDMA-based matrix systems for E-glass FRCs on fibroblast cells by MTT method. The cytotoxicity property of the proposed HDDMA-based systems was compared and contrasted with the bis-GMA-based system.

## MATERIALS AND METHODS

**Experimental:** The materials used for the FRCs specimens and their manufacturers are listed in Table1. Fibroblast cell line was obtained from Universitas Gadjah Mada, Indonesia. The culture medium of RPMI 1640, DMEM, penicillin, streptomycin, amphotericin and trypsin were obtained from Gibco (USA). Other materials used in this research were obtained from Sigma-Aldrich (Belgium).

E-glass fibers were kept in desiccators for 24 h. Next, the fibers were sized by immersion in a sizing solution for 1 min and gently pressed dry of the excess sizing solution (Zhang and Matinlinna, 2011). The size fibers were cut into 25 mm long roving with a surgical steel knife (Matinlinna *et al*., 2009).

Fifteen FRCs specimens were prepared with the dimension of 2×2×25 mm (Zhang *et al*., 2014). Two bundles of fibers were placed into a custom-made brass mold and embedded into the experimental resin matrix with 3 different compositions. The experimental resin matrix groups were: HDDMA-based1: 78.4 wt% HDDMA+19.6 wt% MMA+1.0 wt% CQ+1.0 wt% CEMA; HDDMA-based2: 49.0 wt% HDDMA+49.0 wt% MMA+1.0 wt% CQ+1.0 wt% CEMA and bis-GMA-based: 78.4%wt bis-GMA+19.6 wt% MMA+1.0 wt% CQ+1.0 wt% CEMA. All specimens were light-cured with a light-curing unit (Woodpecker, USA) for the 40s each. The light output was 650 mW $cm^{-2}$ and wavelength of 520 nm. After light-curing, specimens were powdered then diluted in a culture medium (0.1 mg powder/1 mL medium).

The cytotoxicity determination was carried out by MTT method which was based on ISO 10993-5 Part 5 (ISO, 2009). An amount of 100 μL of the specimen solution was added into a 96-well plate containing fibroblast cells of $2\times10^4$ cells/100 μL and incubated for 24 h. Next, 10 μL of MTT was added to the well incubated for 4h then 100 μL of the so-called stop solution was added. The Optical Density (OD) of the cells viability was determined

by using the ELISA reader with a wavelength of 550 nm. Cell viability was expressed as the % of cytoviability using the formula (ISO, 2009):

$$\text{Percentage of cytoviability} = \frac{100\% \times OD_{550\text{control}}}{OD_{550\text{control}}}$$

Where:

$OD_{550\,\text{treated}}$ = The mean value of the measured optical density of the treated cell

$OD_{550\,\text{control}}$ = The mean value of the measured optical density of the control cell

The cytoviability data were statistically analyzed by one-way ANOVA followed by post hoc LSD. By the data of % cytoviability, it was further analyzed for cytotoxicity property. The cytotoxicity property was determined based on ISO 10993. It was stated that the lower viability percentage, the higher the cytotoxic potential of the test item is. If viability is reduced to <70%, it has a cytotoxic potential (ISO, 2009). The experimental flow of the study was as Fig. 2.

## RESULTS AND DISCUSSION

Cytotoxicity characteristic as a primary factor of biocompatibility is generally assessed by in vitro cell cultures. In general, *in vitro* studies are more easily controlled. In vitro methods allow the evaluation of various parameters in a simple system, i.e., by decreasing variables and allowing more specific determination of cytotoxic mechanisms. Although in vitro evaluation cannot be quantitatively correlated with in vivo results, there are several clinical reports demonstrating tissue cytotoxicity when tissue is exposed to components deriving from the resins. Oral tissue in direct contact with in situ polymerized resin composite may suffer from higher concentrations of chemicals that may lead to greater tissue damage.

Two proposed resin matrix systems of HDDMA-based and a bis-GMA-based matrix system had been tested on fibroblast cells. Table 2 showed that HDDMA-based1 group exhibited the highest value of fibroblast cells viability percentage while bis-GMA-based group showed the lowest value. The viability value of the HDDMA-based1 group and the HDDMA-based 2 group were nearly similar. By the data obtained, it was seen that the HDDMA-based groups showed less cytotoxic effect than the bis-GMA-based matrix to fibroblast cells.

Structurally thinking, an HDDMA monomer has the reactive groups similar to a bis-GMA monomer. HDDMA configuration is linear without the benzene groups as is

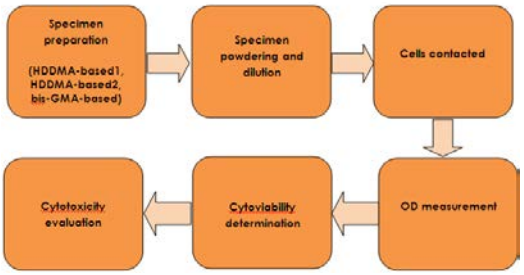


Fig. 2: Flow diagram of the experimental study

the case of bis-GMA. This condition probably contributed to the less viscosity property of HDDMA than bis-GMA. Moreover, structurally HDDMA does not possess the hydroxyl groups as bis-GMA which might have affected the relatively high water sorption. By this condition, it was assumed that the resin matrix system based on HDDMA contributed less plasticization by water and thereby caused hydrolytic degradation. This might have affected the leachable component of matrix resin to the surrounding tissue and as a result there would be less-cytotoxicity property in HDDMA-based matrices than in bis-GMA-based matrix to fibroblast cells. This phenomenon is parallel as it was found in research about the relationship of monomer structures and cytotoxicity (Yoshii, 1997). In that research, it was said that the hydroxyl group of acrylates and methacrylates seemed to enhance cytotoxicity. In the current study, the HDDMA-based1 group exhibited the highest value of fibroblast cells viability percentage. This fact was might be caused by the higher HDDMA percentage content than the MMA in the experimental matrix composition. The HDDMA was reported less cytotoxic than the MMA (Stoeva *et al*., 2008). This aspect merits further studies.

The normality analysis of the data by Kolmogorov-Smirnov test showed that all of the groups exhibited the significant value of $p>0.05$. By this result, it could be said that the data had a normal distribution and could be analyzed by ANOVA. The ANOVA revealed a significant difference of cell viability percentage among the groups (F-value of 14.149, $p<0.01$). The HDDMA-based matrix systems (i.e., the HDDMA-based1 group and HDDMA-based2 group) and the bis-GMA-based matrix system had different monomer compositions. The difference in the monomer compositions might affect the cell viability. Hiyasat *et al.* (2005) reported that a change in the chemical structure of resin composites and variations in the ratio of fillers and monomers significantly affected the element release and cytotoxic level of these materials.

Table 1: Materials used in the current study

| Material and abbreviation | Manufacturer |
|---|---|
| Bis-phenol-A-glycidyl methacrylate (bis-GMA) | Sigma-Aldrich, USA |
| Methyl Methacrylate (MMA) 1,6-hexanediol | ProSciTech, Australia |
| d- methacrylate (HDDMA) | Esstech, USA |
| Camphorquinone (CQ) | Esstech, USA |
| N,N-Cyanoethyl Methyl Aniline (CEMA) | Esstech, USA |
| Unidirectional E-glass fibers | Ahlstrom Fiberglass, Karhula,Finland |

Table 2: Average of fibroblast cells viability in percentage

| Experimental groups | Percentage of cells viability (mean±SD) |
|---|---|
| HDDMA-based 1 | 67.73±0.71 |
| HDDMA-based 2 | 66.78±0.20 |
| Bis-GMA-based | 64.36±1.63 |

Further analysis by LSD showed a significant difference between HDDMA-based1 group and bis-GMA group and also between the HDDMA-based2 group and bis-GMA group ($p<0.01$). There was no significant difference between HDDMA-based1 group and HDDMA-based 2 group ($p>0.05$). By this result, it could be concluded that the percentage of HDDMA composition in HDDMA-based matrix systems did not significantly influence the cells viability although it was observed that HDDMA-based 1 group showed higher cell viability than the HDDMA-based 2 group. In fact, the handling property of HDDMA-based 1 group matrix system was easier and more convenient than HDDMA-based 2 group (because of the lower viscosity). Moreover, a previous study on biomechanical strength of the matrix systems suggested that HDDMA-based 1 group performed better strength than HDDMA-based 2 group and comparable to the bis-GMA-based properties (Sunarintyas *et al*., 2016). Other study said the higher flexural and hardness properties of the HDDMA-based 1 group were observed in comparison with the HDDMA-based 2 group after 4 weeks in water storage. By this finding, it is concluded that the HDDMA-based 1 group would merit further evaluation as an alternative matrix system to replace bis-GMA in the FRCs material. However, more studies are vital to carrying out.

Table 2 revealed the percentages average of fibroblast cells viability of the three groups were <70%. According to the ISO 10993, it was stated that If the viability of material was reduced to <70%, it had a cytotoxic potential effect. By this fact, it can be said although HDDMA-based matrix systems showed better cytotoxicity property than bis-GMA-based, it still needed further improvement to find out such a matrix system which did not have the potential cytotoxicity property to human. One alternative was by replacing the co-monomer of MMA which was reported became an allergen to the dental technician (Pfeiffer and Rosenbauer, 2004) and an irritant to the mucous membrane in the oral cavity (Urban *et al*., 2007) by other monomer which was reported more biocompatible.

## CONCLUSION

The current study aimed to replace the resin matrix system of FRCs based on bis-GMA with HDDMA. It was reported that the hydroxyl groups of current commercial bis-GMA were the main source of not only the high viscosity of the monomer but also contributed to the relatively high water sorption. Structurally, HDDMA is linear without benzene group and does not possess hydroxyl group. By this condition, HDDMA is not viscous and is assumed contributing less plasticization by water. This might have affected less leachable component to the surrounding environment and as a result, there would be less cytotoxicity property than the bis-GMA matrix system.

The pilot study suggested that a novel resin matrix system based on HDDMA (i.e., the HDDMA-based 1 group and HDDMA-based 2 group) revealed a significant difference in fibroblast cells viability with the commercial bis-GMA matrix system. The HDDMA matrix system might have the potential to substitute commercial bis-GMA matrix. The HDDMA-based 1 matrix system would merit evaluation as an alternative to replace bis-GMA in the matrix system of dental FRCs.

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