Cytotoxicity of three di-functional monomers used in dental composite resins

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Dental composite resins have been widely used in dentistry due to ease in handling and aesthetic appearance [1]. Residual monomers, however, are inevitably contained in most set restorations [2], often irritating neighboring tissues (i.e. pulp or mucosa) [3]. Bis-phenol A, a constituent of difunctional monomer, Bis-phenol A glycidyl dimethacrylate (Bis-GMA) most frequently used in dentistry, has been reported to elute into the oral fluid from set composites [4] and thus to be a potential estrogenic danger [5]. There have been, however, few reports which actually and directly measured the cytotoxicity of difunctional monomers contained in dental composite resins [6, 7]. The purpose of this investigation was to clarify the cytotoxicity of three difunctional monomers used in dental composite resins, namely triethyleneglycol dimethacrylate (TEGDMA, 99% pure, Tokyo Kasei Co., Tokyo, Japan), D-GMA® (70% pure Bis-GMA, Shin-Nakamura Kougyo Co., Wakayama, Japan) and Art Resin® (80% pure urethane di-methacrylate, Negami Kougyou Co., Ishikawa, Japan), using C3H mouse derived 10T1/2 fibroblast cells (C3H10T1/2) (JCRB0003, Japan Health Sciences Foundation, Osaka, Japan). C3H10T1/2 has been reported to be useful for evaluating both cytotoxicity and mutagenicity of biomaterials [8].

The medium for cell culture consisted of Dulbecco's modified Eagle (DME) medium (Cat. No. 11885-084, Life Technologies, NY, USA) supplemented with 10% (v/v) fetal bovine serum (Cat. No. 26140-079, Lot No.1008629, Life Technologies, NY, USA) and 2% (v/v) antibiotics (Penicillin-Streptomycin dissolved in 80 ml isotonic saline solution, Cat. No. 15145-014, Life Technologies, NY, USA). In the preliminary experiments, the solubility of three monomers such as TEGDMA, D-GMA and Art resin with DME medium were determined to be 10, 7 and 3 mM L^{-1}, respectively. TEGDMA was dissolved into DMEM medium with the concentration levels of 0.02, 0.2, 0.5, 1 and 2 mM L^{-1}, D-GMA was done with those of 0.01, 0.1, 1, 2.5 and 5 mM L^{-1}, whilst Art resin was done with those of 0.05, 0.1, 0.5, 1 and 2.5 mM L^{-1}.

The cells were grown in plastic dishes 100 mm in diameter filled with original DME medium at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cells were sub-cultured by trypsinization (0.05% trypsin and 0.025% EDTA) before confluence. Prior to cytotoxic studies, cells were seeded into 24-well microplates (Cat. No. 3820, Iwaki Glass Co., Tokyo, Japan) at a density of 5000 in 1 ml original DME medium and cultured for 1 day. Next day, the medium was exchanged with original medium (control) and five different monomer concentrations, and the cells had been cultured for 6 days. One, three and six days later, the cells were trypsinized and the number of each well were counted three times using inverted phase-contrast light microscopy (CK30, Olympus Co., Tokyo, Japan) and a haemocytometer (Neubauer 03-202-1, Erma Co., Tokyo, Japan). For each cell count, three samples were prepared, respectively.

Fig. 1 shows the effect of TEGDMA concentration on the cell number 1, 3 and 6 days after medium exchange. At 1st day, the addition of TEGDMA monomer concentration led to a decrease in the cell number. At 3rd and 6th days, the cell number remained almost constant up to 0.2 mM L^{-1} while the increment in TEGDMA concentration at more than 0.5 mM L^{-1} brought about the decline in the cell number.

Fig. 2 indicates the effect of D-GMA monomer concentration on the cell number 1, 3 and 6 days after medium exchange. At 1st day, the increment in D-GMA decreased the cell number. At 3rd day, up to 0.1 mM L^{-1} D-GMA, the cell number was identical. At 6th day, up to 1 mM L^{-1}, the cell number was similar. The cell damage due to D-GMA was restored with time when the D-GMA concentration was less than 1 mM L^{-1}.

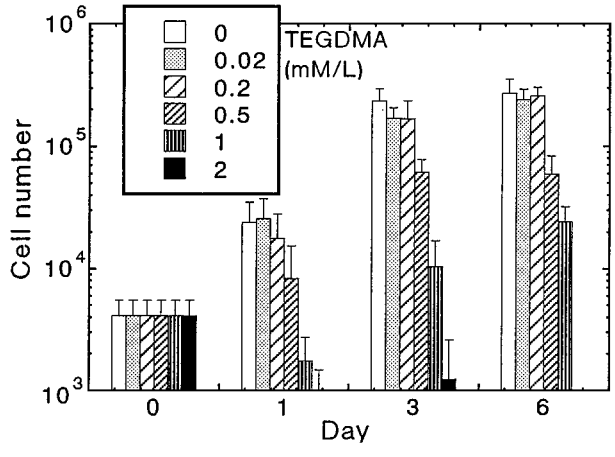

Figure 1 The effect of TEGDMA concentration on the cell number 1, 3 and 6 days after medium exchange.

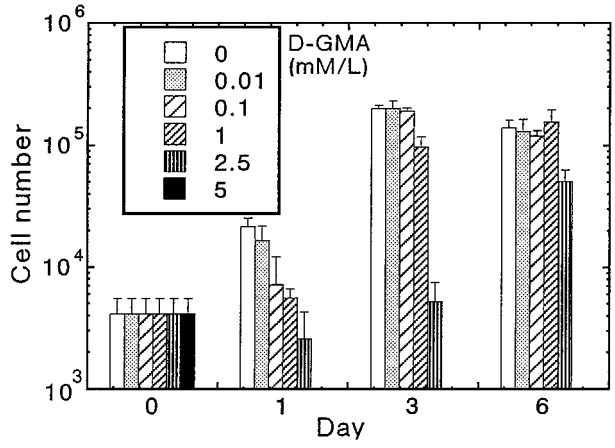

Figure 2 The effect of D-GMA monomer concentration on the cell number 1, 3 and 6 days after medium exchange.

Fig. 3 illustrates the effect of Art resin monomer concentration on the cell number 1, 3 and 6 days after medium exchange. At 1st day, the increment in Art resin monomer decreased the cell number. At 3rd day, up to 0.1 mM L^{-1} Art resin, the cell number was identical. At 6th day, up to 0.5 mM L^{-1}, the cell number was similar. The cell damage due to Art resin monomer was also restored with time when the Art resin monomer concentration was less than 0.5 mM L^{-1}.

Cytotoxicity by cell count has been judged by the amount of monomer concentration that caused 50% cell death (TC_{50}) with respect to the cell number of the control medium without monomer. Table I shows the (TC_{50}) monomer concentration which was graphically estimated in the monomer concentration versus cell number diagram, along with the maximum monomer concentration which allowed the cell recovery (Recovery) 6 days after medium exchange. The cytotoxicity of three difunctional monomers differed. One reason might be attributed to the molecular weight and mobility [4]. TEGDMA with the smallest molecular weight (226) appeared to most easily diffuse into the cells and disturb the metabolic function of the cells, resulting in the cytotoxicity with lowest TC_{50} and recovery monomer levels. Art resin with the second largest molecular weight (470) followed TEGDMA in the cytotoxicity order. D-GMA with the largest molecular weight (512) might diffuse into the cells in the most

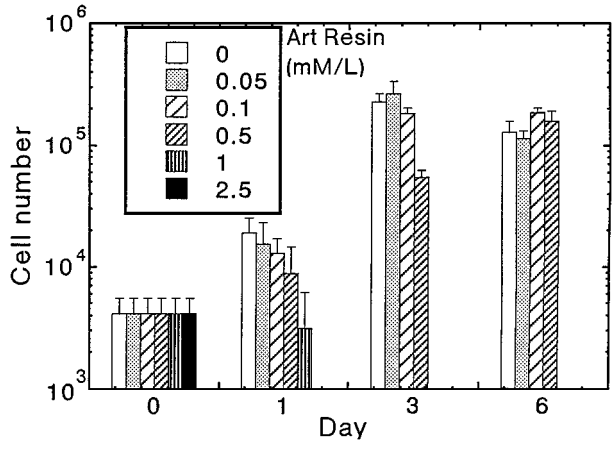

Figure 3 The effect of Art resin monomer concentration on the cell number 1, 3 and 6 days after medium exchange.

TABLE I TC_{50} and recovery monomer levels for C3H10T1/2 fibroblasts 6 days after medium exchange (mM L^{-1})

Monomer	TC_{50}	Recovery
TEGDMA	0.25	0.20
D-GMA	2.00	1.00
Art resin	0.80	0.50

sluggish fashion, producing the largest TC_{50} and recovery monomer levels with the least cytotoxicity. It should be cautioned here that the impurity (e.g. by products and raw chemicals) in D-GMA and Art Resin might increase the cytotoxicity levels to some extent.

If eluted monomers are accumulated in confined areas such as that just beneath the composite resin filling beyond the levels reported in Table I, tissue damage such as pulp irritation might be provoked. The bottom of visible-light-cured composite resin restorations, where polymerization are often premature, might release monomers beyond the concerned levels. Caution is needed to increase the degree of cure in the entire region of composite resin restorations in dental clinics.

Minute released monomers may exert the estrogenic damage in remote reproductive organs. The monomer levels which cause estrogenic cell damage might be astonishingly low and are now under investigation in other studies [9], but beyond the scope of this study.

Cancerous transformations were not observed in C3H10T1/2 cells which were exposed to three difunctional monomers, implying that DNA damage might be low by the three difunctional monomers examined.

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