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Cytotoxic effects of TEGDMA on THP-1 cells *in vitro*

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Abstract

Objective: Resin based dental materials are not stable in the oral environment and may release their components into biological media. These components may include substances such as triethylene glycol dimethacrylate (TEGDMA), which is a major co-monomer of dental resin materials. This release can trigger host immune and inflammatory responses against foreign materials, mediated by monocytes. The aim of this study was to investigate the possible cytotoxic effects of TEGDMA on human THP-1 monocytes.

Material and Methods: THP-1 cells were exposed to various concentrations of TEGDMA (0.5 mM, 1 mM, 2 mM, 4 mM, or 8 mM) for 48 hours. An untreated group was used as control. The effects of TEGDMA on cell proliferation, cell viability and apoptosis were analyzed by light microscopy.

Results: Cell proliferation was inhibited by 4 mM and 8 Mm TEGDMA. Increasing TEGDMA concentrations caused a decrease in cell viability. All TEGDMA concentrations used in this study had an apoptotic effect on THP-1 cells when compared with the control group.

Conclusions: The dental monomer TEGDMA had an adverse effect on cell proliferation and exerted an apoptotic and toxic effect on THP-1 cells in a concentration-dependent manner.

Key words: *TEGDMA, cytotoxicity, apoptosis, necrosis, cell proliferation, monocyte.*

Introduction

Tooth colored, resin-based restorative materials are very popular in today's dentistry as they provide pleasing aesthetic and sufficient mechanical properties. These materials have been routinely used in dental practice as a direct-indirect filling material, a fissure-sealing agent, and a bonding agent. Even though the use of resin-based materials offers a beautiful smile, they may also carry some biological risks (1). Studies have shown that the polymerization processes involved in use of resin-based dental materials could be incomplete under clinical conditions and that, even after polymerization, monomers and other components of dental materials may leak into the oral environment as a result of degradation processes or erosion (2,3).

Triethylene glycol dimethacrylate (TEGDMA) is a dental co-monomer that has been used to dilute viscous polymers to obtain suitable viscosities for dental purposes (4). Significant quantities of TEGDMA can be released from polymerized resin composites (5). Due to its hy-

drophilic character, TEGDMA may manage to diffuse trough dentin to pulp tissue in sufficient concentrations to cause detrimental effects (6).

In recent years, cell culture systems have seen increased use in biocompatibility tests and a number of test methods have been introduced (7). Since innate immune system cells mediate host immune and inflammatory responses against foreign objects, monocyte activities have been popular for use as cell culture models to study biocompatibility (8,9). Cytotoxicity is one biocompatibility test procedure that is often used to ascertain the toxic effects of an agent on living cells. A cytotoxic material can stop cell growth and division or can trigger processes such as apoptosis or necrosis (10).

Apoptosis is a genetically programmed cell death that is characterized by a series of distinct morphological changes, such as shrinkage of the cell, fragmentation of the cytoplasm into membrane-bound apoptotic bodies and rapid phagocytosis by neighboring cells (10). Under physiological conditions, apoptosis rarely occurs in tissues (10). However, an injury, such as an exposure to a toxic chemical, may activate the apoptotic response, which rapidly causes cell death (11). In contrast, necrosis is a non-physiological process and is considered to be the general appearance of cell death following rapid loss of cell homeostasis due to an injury (10). The cause of necrosis could be ischemia, heat, toxins, mechanical trauma, or even apoptosis. (12) Necrosis does not indicate a specific form of cell death but refers to changes secondary to cell death caused by any mechanism, including apoptosis. Consequently, it would be better to refer to the cell death displaying the morphological changes of apoptosis as "apoptotic necrosis" (12). The aim of this study was to examine the potential cytotoxic effects and mode of cell death in caused by TEGDMA in a human monocyte cell line.

Materials and Methods

Reagents:

Triethylene glycol dimethacrylate (TEGDMA; CAS-No. 109-16-0) and β-mercaptoethanol were obtained from Sigma Aldrich (Taufkirchen, Germany). RPMI 1640 medium, containing 2 mM L-glutamine, 10 mM Hepes, 1 mM sodium pyruvate, 1.5 g/l NaHCO3 and 4.5 g/l glucose was purchased from PAN Biotech (Aidenbach, Germany). Fetal Bovine Serum (FBS) and Penicillin-Streptomycin were purchased from Gibco (Eggenstein, Germany). Osmium (VIII) oxide for microscopy and toluidine blue was obtained from Merck (Darmstadt, Germany).

Cell Culture:

Human THP-1 monocytes (ATCC TIB 202) were grown in RPMI 1640 cell culture medium containing 50 µmol/L β-mercaptoethanol, 100 µg/ml penicillin-streptomycin and 10 % fetal bovine serum. 3 ml of THP-1 cell suspensions (300000 cells/ml) were seeded into wells of 24 well plates and exposed to TEGDMA at different concentrations (0.5 mM, 1 mM, 2 mM, 4 mM, or 8 mM). An untreated group was used as a control. All groups were incubated for 48 hours at 37°C with 5% CO_2.

Histological sample preparation:

In this study, we investigated the cytotoxic effect of the dental monomer TEGDMA on a monocyte cell line. Monocyte suspensions were divided into 6 groups, as shown in (Table 1).

Table 1. Triethylene glycol dimethacrylate concentrations used in this study.

Group 1	Untreated
Group 2	TEGDMA 0,5 mM
Group 3	TEGDMA 1 mM
Group 4	TEGDMA 2 mM
Group 5	TEGDMA 4 mM
Group 6	TEGDMA 8 mM

.(mM: milimolar)

After a 48 hour incubation period, cell suspensions were centrifuged for 5 minutes at 300 x g and supernatants were removed. For microscopic examination, cell pellets were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer, dehydrated in a graded acetone series, and transferred to propylene oxide. After dehydration, specimens were embedded in Araldite CY212. Sections were cut using an ultramicrotome (LKB NOVA, Bromma, Sweden). Then, semi-thin sections were stained with toluidine blue. After this histological procedure, cells specimens were examined under a light microscope and photographed with an attached camera (Olympus BH 40; Olympus®, Tokyo, Japan).

Histological Evaluation:

Cells in prophase, metaphase, anaphase and telophase stages were evaluated as mitotic cells. Apoptotic cells were characterized by their condensed chromatin, contracted cell morphology, and retention of membrane integrity and organelles. Swelling of the cell, lack of membrane integrity and disintegration of organelles and cellular contents were the criteria to determine apoptotic necrotic (non-vital) cells. Relevant cell types are shown in (Fig.1).

The numbers of viable cells, mitotic cells, apoptotic cells, and apoptotic necrotic cells were determined microscopically by examining 1000 cells/slide of two parallel cultures per concentration (Stereo Investigator 7.00 Bioscience-Microbright Field Inc. USA). At least four slides derived from two independent experiments were analyzed, and differences between median values

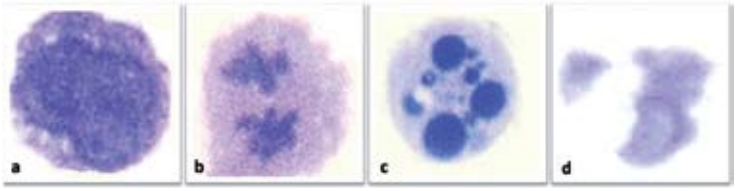

Fig.1. (a) Substantial monocyte (b) Mitotic monocyte in anaphase phase (c) Apoptotic Monocyte (d) Necrotic Monocyte.

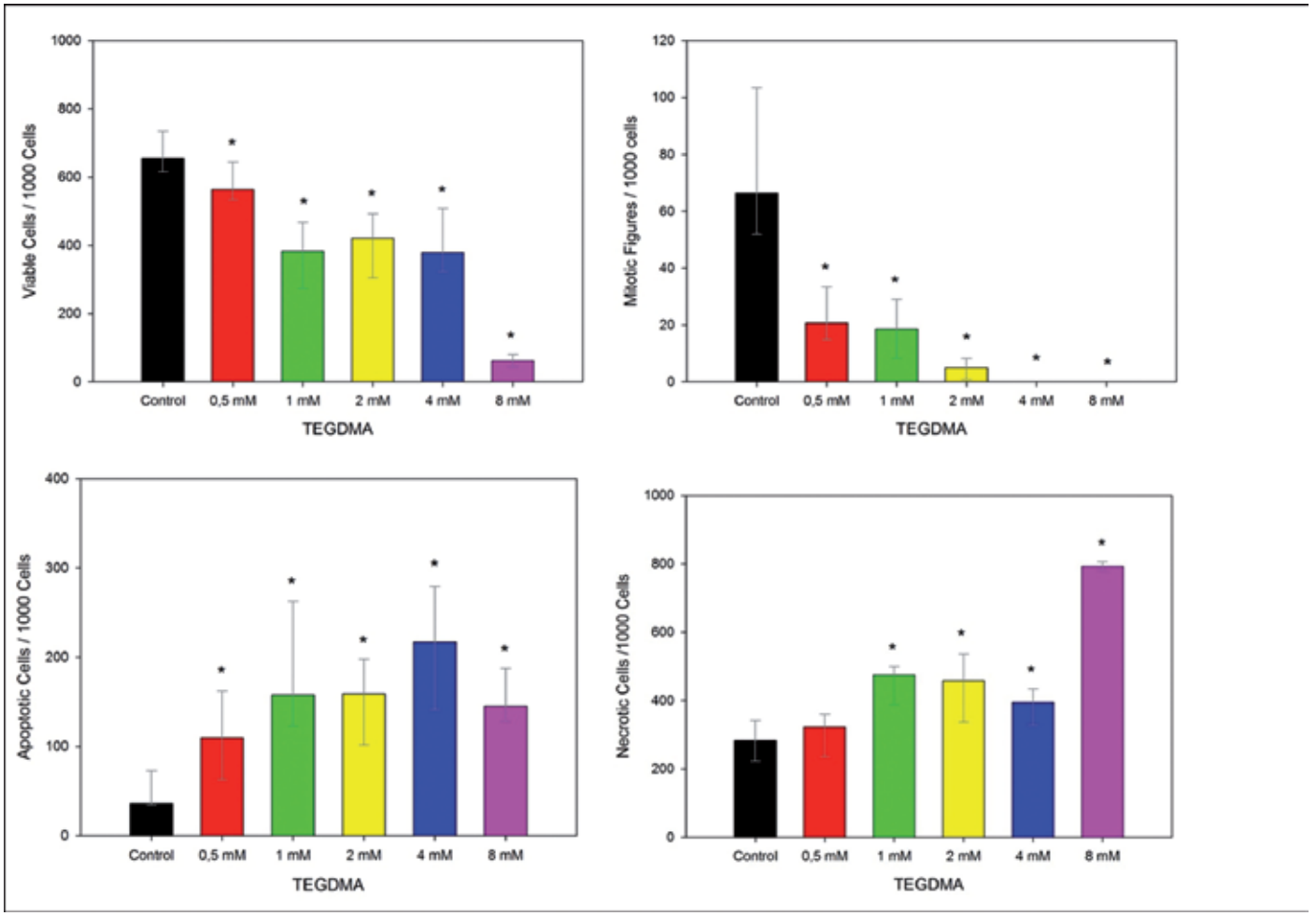

Fig. 2. The numbers of viable cells, mitotic cells, apoptotic cells, necrotic cells (necrotic and/or apoptotic necrotic cells) induced by various concentrations of TEGDMA after 48 hours. Bars represent medians (25% and 75% percentiles) calculated from independent experiments. Statistically significant differences between untreated and TEGDMA treated cell cultures are indicated by asterisks.

with the control group were statistically analyzed using the Mann–WhitneyU-test (α=0.05) (SPSS, Version 16.0; SPSS, Chicago, IL, USA). The values of the median, upper and lower quartile were also computed (SigmaPlot 10.0, Rock Ware, Golden, CO, USA).

Results

Cytotoxic effects of TEGDMA on THP-1 cells were analyzed after a 48 hour exposure period. As demonstrated in (Fig.2), increasing TEGDMA concentrations caused a noticeable dose-related decrease in living cells. When compared with the control group, 0.5 mM TEGDMA significantly reduced cell viability. A concentration of 8 mM TEGDMA was severely toxic to THP-1 cells and caused an approximately 10-fold decrease in numbers of viable cells.

A similar effect was observed in mitotic activity (Fig.2). The number of mitotic cells was noticeably reduced with increasing TEGDMA concentrations. A significant decrease in mitotic activity was seen in cells treated with 0.5 mM TEGDMA compared with the control group. Concentrations of 4 mM and 8 mM TEGDMA strongly inhibited cell proliferation.

TEGDMA at all concentrations used in this study caused an apoptotic effect on THP-1 cells after a 48 hour incubation period when compared with the control group (Fig.2). The maximum apoptotic effect was seen at a concentration of 4 mM TEGDMA.

The number of necrotic cells (necrotic and/or apoptotic necrotic cells) was significantly increased following treatment with 1 mM TEGDMA (Fig.2). Although the number of apoptotic cells were reduced at 8 mM TEGDMA, the number of nonviable cells was increased about 2-fold over that seen in the 4 mM group.

Discussion

The aim of this investigation was to analyze possible cytotoxic effects of TEGDMA in human monocytes. The dental monomer tested in this study indicated substantial evidence of cytotoxicity, such as inhibition of cell proliferation, and increases in apoptosis and necrosis. These effects were induced in a concentration-dependent manner.

A biocompatible dental material should remain stable in the oral environment and must provide maximum benefit with the minimum risk (1). Analyses of eluted components from composite filling materials have revealed that significant quantities of triethylene glycol dimethacrylate (TEGDMA) can be released, depending on to the degree of curing (3,13). In the immune system, monocytes direct many of the inflammatory responses to foreign materials. It has been pointed out that the THP-1 monocyte cell line could be 3 to 25 times more sensitive than human peripheral monoycytes in cytotoxic evaluations (14). For this reason, the ability of TEGDMA to alter THP-1 cell viability or other cellular functions may have significant relevance to the overall biological responses to this monomer (14).

Cell proliferation is directly associated with cell growth, differentiation, and tissue turnover (15). In this study, 0.5 mM TEGDMA only reduced cell proliferation when compared with non- treated group. Higher concentrations of 4 mM and 8 mM TEGDMA strongly inhibited cell proliferation and also induced other cellular responses. These findings corroborate with other studies that have concluded that TEGDMA may cause cell cycle delays and may influence physiological processes such as cell growth and differentiation (15,16). Due to its low molecular weight and relatively high hydrophilicity, TEGDMA may penetrate into all biological compartments, including cell nuclei and other membrane-bounded compartments (17). Recent findings indicate that TEGDMA is likely to generate cellular stress via formation of reactive oxygen species (ROS) and could cause apoptosis in a time and dose-dependent manner (11,18,19). In the current study, TEGDMA concentrations over 0.5 mM caused significant alterations that were indicative of apoptosis, including reduced cell volume and chromatin condensation. The apoptotic effect peaked at a 4 mM TEGDMA concentration and a higher concentration of 8 mM TEGDMA was severely toxic to monocytes. In a previous study, human gingival fibroblast cells exposed to 5 mM and 7.5 mM TEGDMA for 24 h showed symptoms of apoptosis (11). In human primary pulp cells, a two-fold increase in the percentage of apoptotic cells was induced by 1 mM TEGDMA (20).

Although there are limitations in this study, it provides new insights into biocompatibility of tooth colored resin materials. This monomer carries the risk of cytotoxicity in THP-1 monocytes in a concentration dependent manner. Consequently, there may be a risk that leaching of this compound may prevent monocytes from initiating a proper first response to pathogens or products of pathogenic organisms following dental treatments. A clear understanding of the complex interactions between immune cells and resin monomers is needed. For this reason, further studies should be conducted to address the possible risks posed by the use of tooth colored resin materials in dental practice.

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