CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

Genetic and Cellular Toxicology of Dental Resin Monomers

H. Schweikl[1]*, G. Spagnuolo[2], and G. Schmalz[1]

[1]Department of Operative Dentistry and Periodontology, University of Regensburg, D-93042 Regensburg, Germany; and [2]Department of Oral and Maxillofacial Sciences, University of Naples "Federico II", via S. Pansini 5, 80131-Naples, Italy; *corresponding author, helmut.schweikl@klinik.uni-regensburg.de

J Dent Res 85(10):870-877, 2006

ABSTRACT

Monomers are released from dental resin materials, and thus cause adverse biological effects in mammalian cells. Cytotoxicity and genotoxicity of some of these methacrylates have been identified in a vast number of investigations during the last decade. It has been well-established that the co-monomer triethylene glycol dimethacrylate (TEGDMA) causes gene mutations *in vitro*. The formation of micronuclei is indicative of chromosomal damage and the induction of DNA strand breaks detected with monomers like TEGDMA and 2-hydroxyethyl methacrylate (HEMA). As a consequence of DNA damage, the mammalian cell cycle was delayed in both G1 and G2/M phases, depending on the concentrations of the monomers. Yet, the mechanisms underlying the genetic and cellular toxicology of resin monomers have remained obscure until recently. New findings indicate that increased oxidative stress results in an impairment of the cellular pro- and anti-oxidant redox balance caused by monomers. It has been demonstrated that monomers reduced the levels of the natural radical scavenger glutathione (GSH), which protects cell structures from damage caused by reactive oxygen species (ROS). Depletion of the intracellular GSH pool may then significantly contribute to cytotoxicity, because a related increase in ROS levels can activate pathways leading to apoptosis. Complementary, cytotoxic, and genotoxic effects of TEGDMA and HEMA are inhibited in the presence of ROS scavengers like N-acetylcysteine (NAC), ascorbate, and Trolox (vitamin E). Elevated intracellular levels of ROS can also activate a complex network of redox-responsive macromolecules, including redox-sensitive transcription factors like nuclear factor kappaB (NF-κB). It has been shown that NF-κB is activated probably to counteract HEMA-induced apoptosis. The induction of apoptosis by TEGDMA in human pulp cells has been associated with an inhibition of the phosphatidylinositol 3-kinase (PI3-K) cell-survival signaling pathway. Although the details of the mechanisms leading to cell death, genotoxicity, and cell-cycle delay are not completely understood, resin monomers may be able to alter the functions of the cells of the oral cavity. Pathways regulating cellular homeostasis, dentinogenesis, or tissue repair may be modified by monomers at concentrations well below those which cause acute cytotoxicity.

KEY WORDS: dental materials, oxidative stress, genotoxicity, cell cycle, apoptosis.

Received February 1, 2005; accepted December 21, 2005

INTRODUCTION

The development of resin-based dental materials bonded to tooth structures has allowed for the application of new restorative techniques. The clinical success of these materials, however, depends not only on the physical and chemical properties of the materials, but also on their biological safety. The organic matrix of dental resin materials has been recognized as a source of compounds that cause a wide variety of adverse biological reactions. These analyses have been extensively reviewed in the literature (Schmalz, 1998; Geurtsen, 2000; Ortengren, 2000; Goldberg and Smith, 2004; Bouillaguet, 2004). Many of these compounds, mostly epoxy resins and acrylic monomers, have been identified as important occupational sensitizers, with an established potential for cross-reactivity. Work-related adverse effects—such as occupational skin disease (OSD), allergic contact dermatitis, or irritant contact dermatitis—have been frequently reported by dental personnel (Kanerva *et al.*, 1994; Kanerva, 2001; Alanko *et al.*, 2004). It has also been hypothesized that components from dental composite materials may alter cytokine secretion from human monocytes if applied at sublethal concentrations. Likewise, other tightly regulated pathways of cellular metabolism, like the induction of a cellular stress response or the alteration of lipid metabolism, were also modified (Schuster *et al.*, 2000; Noda *et al.*, 2002, 2003). Serious concerns about possible health problems have been raised since compounds like Bis-GMA, Bis-DMA, and bisphenol A (BPA) were identified as endocrine-disrupting chemicals capable of mimicking the effects of natural steroid hormones. These aromatic components are leached from commercial products like composites and sealants in concentrations at which biological effects have been described in experimental models. However, the clinical relevance of the exposure to estrogenic compounds is still controversial (Pulgar *et al.*, 2000; Ashby, 2002; Volkel *et al.*, 2002; Wada *et al.*, 2004).

To date, investigations into the genotoxicity and cytotoxicity of dental resin materials *in vitro* have been limited to the characterization of the alteration of various cellular endpoints. Damage to the cell membrane, inhibition of enzyme activities, or protein, RNA, and DNA synthesis, or simply the estimation of the number of surviving cells after treatment—all are indicators used to describe the modifications in basic cell function. These assays revealed a wide range of differences in the cytotoxic effects of the vast array of resin materials (Hanks *et al.*, 1991; Wataha *et al.*, 1999; Geurtsen, 2000; Schweikl *et al.*, 2005a). Considerable attention has been paid to the identification of the individual compounds responsible for the interaction with cellular structures. Primarily, the major monomers and co-monomers have been identified as the cytotoxic compounds of the organic matrix of these complex materials, and a relationship between the structural and biological activities of monomers has been reported (Hanks *et al.*, 1991; Yoshii, 1997). However, the molecular mechanisms underlying the genetic, as well as cellular, toxicity of resin monomers remain to be elucidated. Recently, resin monomers were also identified as chemicals that are able to disrupt the stable cellular redox balance, resulting in an increase in the levels of reactive oxygen species (ROS) and subsequent cell death *via* apoptosis. Furthermore, elevated levels of ROS are candidate agents for the mediation of genotoxic effects, since the genotoxicity of the monomers triethylene glycol dimethacrylate

(TEGDMA) and 2-hydroxyethyl methacrylate (HEMA) has been repeatedly demonstrated *in vitro* (Schweikl *et al.*, 2001). The transduction of redox imbalance to apoptosis or DNA damage is very complex and has been reviewed previously in the literature (Shackelford *et al.*, 2000; Barzilai and Yamamoto, 2004; Boonstra and Post, 2004).

The aim of this review is to present recent findings regarding the induction of genotoxic stress associated with alterations in the normal cell cycle as a reaction to resin monomers. We also focus on the role of ROS as a source of DNA damage and cell death *via* apoptosis. Finally, the influence of resin monomers on signal transduction pathways related to ROS and cell survival is discussed as well.

BIOAVAILABILITY OF RESIN MONOMERS

Composite restorative materials are a mixture of polymerized resin components reinforced by inorganic fillers (Peutzfeldt, 1997; Rueggeberg, 2002). Several studies have showed that monomers and other components were released from these materials into the oral environment even after polymerization. Among some 30 chemicals, the monomer 2-hydroxyethyl methacrylate (HEMA) and the co-monomer triethylene glycol dimethacrylate (TEGDMA) were detected (Santerre *et al.*, 2001; Michelsen *et al.*, 2003). Both HEMA and TEGDMA may diffuse through dentin in sufficient concentrations to cause cellular damage (Bouillaguet *et al.*, 1996; Hume and Gerzina, 1996). It has been estimated that the concentrations of HEMA and TEGDMA available from, for instance, dentinal adhesives would be in the millimolar range after diffusion through the dentin layer. HEMA leaching from dentin adhesives might reach concentrations as high as 1.5-8 mmol/L in the pulp. Likewise, the TEGDMA concentrations reaching the pulp after diffusion across dentin in deep cavities could be in the range of 4 mmol/L (Bouillaguet *et al.*, 1996; Noda *et al.*, 2002). As a consequence, these concentrations may be high enough to cause detrimental effects at subtoxic concentrations, such as modification of the normal inflammatory response or homeostasis of the pulpal tissues, including the disruption of the stable cellular redox balance and a disturbance of related redox-sensitive pathways.

INDUCTION OF GENOTOXIC EFFECTS BY MONOMERS OF DENTAL RESIN MATERIALS

Genomic DNA is a molecular target for components of dental resin materials. Genotoxic effects detected in bacteria and mammalian cells are indicative of the interaction between DNA and some monomers or associated metabolites. To date, it appears as if there is a clear difference among the genotoxic potencies of the various dimethacrylates. For instance, no induction of gene mutations was detected with the major monomers bisphenol A-diglycidyl dimethacrylate (Bis-GMA) and urethane dimethacrylate (UDMA) (Schweikl *et al.*, 1998). However, DNA damage may have occurred to some extent, because Bis-GMA tested positive in the DNA synthesis inhibition test (Heil *et al.*, 1996). Likewise, no gene mutations were detected with the monomers 2-hydroxyethyl methacrylate (HEMA) and methyl methacrylate (MMA). However, high concentrations of HEMA induced a large number of micronuclei, indicating chromosomal aberrations *in vitro* (Schweikl *et al.*, 1998, 2001). Therefore, it is likely that HEMA induced DNA damage. The bifunctional co-monomer TEGDMA caused dose-dependent mutagenic effects in

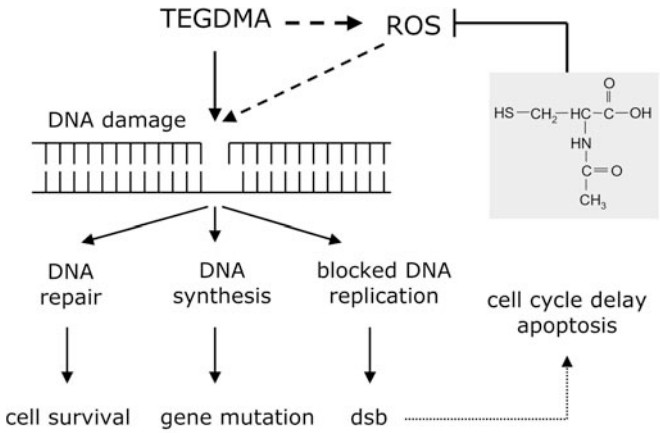

Figure 1. Model of the induction of genotoxicity in mammalian cells by triethylene glycol dimethacrylate (TEGDMA) and cellular responses. DNA damage by TEGDMA may occur either directly, *via* covalent binding to nucleophilic centers of double-stranded DNA, or indirectly, *via* the generation of reactive oxygen species (ROS). The replication of damaged DNA causes gene mutations, but the successful repair of damaged DNA will lead to cell survival. Incomplete repair, however, as well as DNA double-strand breaks (dsb) as a result of blocked DNA replication, will cause cell-cycle delay or apoptosis in the case of severe and irreversible damage. Genotoxicity and cell-cycle delay are inhibited in the presence of the ROS scavenger N-acetylcysteine (NAC).

mammalian cell cultures. The frequencies of gene mutations were increased more than ten-fold, and this monomer also induced the formation of micronuclei (Schweikl *et al.*, 1998, 2001). Furthermore, the initiation of DNA strand breaks by TEGDMA and HEMA was indicated in the comet assay (Kleinsasser *et al.*, 2004, 2006). Molecular analyses have shown that TEGDMA induced a characteristic spectrum of mutations in the *hprt* gene, which was used as a selection marker for the isolation of mutated V79 cells. Total deletions of the entire exon sequences of the *hprt* gene were detected in the majority of the TEGDMA-induced mutant cell colonies, although no point mutations were found (Schweikl and Schmalz, 1999).

The molecular mechanisms leading to mutations induced by resin monomers are unclear at present. Nonetheless, there are at least two possibilities for the generation of DNA lesions (Fig. 1). First, the spectrum of mutations induced by TEGDMA are similar to those caused in the genome of mammalian cells after exposure to x-rays and various chemical agents, including anticancer drugs (radiomimetic chemotherapeutic substances) (Lavin *et al.*, 2005; Pfeiffer *et al.*, 2005). The carbonyl moieties of acrylates and methacrylates adjacent to the carbon-carbon double bond function as electron-withdrawing groups. Consequently, the beta carbon of the double bond has a positive charge and can directly react with nucleophilic centers in molecules like DNA and proteins, as well as small cellular molecules like GSH, *via* the Michael addition reaction (Solomon, 1994). Structure-activity relationships of acrylates and methacrylates are consistent with reaction mechanisms *via* the Michael addition, and TEGDMA is a difunctional molecule with two sites for the Michael addition, since both α,β-unsaturated beta carbons are targets for nucleophilic attacks, which could subsequently result in the formation of intra-strand DNA cross-links (Marnett, 1994; Besaratinia and Pfeifer, 2005). Second, TEGDMA and related monomers could induce mutations by a secondary mechanism *via* the generation of ROS, as do agents such as ionizing radiation,

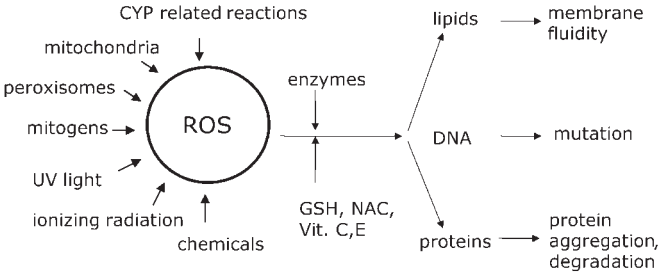

Figure 2. Production of reactive oxygen species (ROS) and cell response. ROS are generated within the cell in mitochondria and peroxisomes, and by cytochrome P450 enzymes (CYP). The intracellular amounts of ROS also increase after exposure to UV light, ionizing radiation, or chemical agents, including dental resin monomers. The defense system against ROS includes endogenous and exogenous anti-oxidants (glutathione GSH, vitamins, N-acetyl-cysteine NAC) plus enzymatic activities. Cellular macromolecules such as lipids, proteins, and DNA may be damaged when the production of ROS is higher than the anti-oxidant capacity of the cells.

UV, and certain chemicals (Achanta and Huang, 2004). ROS are the major agents responsible for endogenous DNA damage, which includes oxidation products of DNA bases, apurinic/apyrimidinic (AP) sites, and DNA strand breaks. Persistence of ROS-induced DNA damage could result in the generation of deleterious mutations. The base excision repair (BER) process is mainly responsible for the repair of ROS-induced DNA lesions, and BRCA1 is discussed as a key factor for the efficient repair of oxidative DNA damage generated by ROS (Adimoolam and Ford, 2003; Izumi *et al.*, 2003; Achanta and Huang, 2004).

It has recently been reported that camphorquinone (CQ), one of the most widely used initiators in modern light-cured dental resin systems, and a compound capable of ROS generation, induced DNA damage. Moreover, ROS scavengers like glutathione and elevated N-acetylcysteine (NAC) concentrations, but not mannitol treatment, reduced DNA damage caused by CQ (Pagoria *et al.*, 2005; Winter *et al.*, 2005). Similar experimental evidence has been presented for the resin monomers TEGDMA and HEMA, which increased the number of micronuclei in V79 cells. However, NAC protected these mammalian cells from monomer-induced genotoxicity, indicating that DNA damage was caused by ROS. These findings suggest that the clastogenic activity of TEGDMA and HEMA is, at least in part, a result of oxidative DNA damage (Schweikl *et al.*, 2006). The analyses of the TEGDMA-induced mutation spectra in the genome of mammalian cells revealed deletions of large DNA sequences, and therefore led to the hypothesis that the monomer might induce a delay in the cell cycle as a result of DNA damage. This assumption was based on the observations reported for agents like ionizing radiation or radiomimetic chemicals, which cause DNA strand breaks followed by cell-cycle arrest (Shackelford *et al.*, 1999; Kaina, 2003).

DNA DAMAGE AND REGULATION OF THE CELL CYCLE

Mitochondrial and genomic DNA are target molecules for endogenous and exogenous agents like ROS, alkylating substances, ultraviolet light (UV), ionizing radiation (IR), and chemotherapeutic substances. These genotoxic agents generate a variety of lesions, including the generation of DNA single or double strand breaks. As a response, an elaborate molecular regulatory system is activated to maintain cellular genomic integrity (Shackelford *et al.*, 1999). The successful completion of G1, S, and G2 phases of the eukaryotic cell cycle is precisely monitored at various cell-cycle checkpoints. These functional cell-cycle checkpoints activate cell responses upon DNA damage through the coordinated activities of sensor, transducer, and effector proteins (Nyberg *et al.*, 2002; Sancar *et al.*, 2004). The cell cycle might then be blocked to initiate repair of DNA damage or to activate programmed cell death. However, defects in cell-cycle checkpoint signaling are disastrous to genome integrity, because they can lead to irreversible cell damage like gene mutations or chromosomal damage. The regulation of the cell-cycle checkpoints is a network of complex mechanisms accomplished by p53-dependent as well as p53-independent pathways (Nyberg *et al.*, 2002; Bakkenist and Kastan, 2004).

The co-monomer TEGDMA has been shown to cause a reversible accumulation of V79 cells only in the G2 phase, probably due to the lack of a functional G1 cell-cycle checkpoint in this cell line (Schweikl *et al.*, 2005b). Since it was reported that the V79 cell line expresses a non-functional p53 protein, this block caused by TEGDMA was independent of normal p53 functions and pathways. In contrast, a fast and reversible G1 checkpoint response, to indicate cell response to DNA damage, was detected in p53-proficient normal human skin fibroblasts after exposure to TEGDMA. A prolonged G1 arrest caused by high TEGDMA concentrations was probably stabilized by a p53-dependent checkpoint (Schweikl *et al.*, 2005b). Most of the primary human pulp-derived fibroblasts in TEGDMA-treated cell cultures were delayed in the G2 phase. Although a clear response of the pulp cells at the G1 checkpoint was not detected, it appeared that this lack of an active checkpoint is most likely related to reduced vitality of primary human pulp cells beyond the fifth passage *in vitro* (Schweikl *et al.*, 2005b). Likewise, the induction of a cell-cycle delay by HEMA was shown with human gingival and pulp fibroblasts (Chang *et al.*, 2005; Schweikl *et al.*, 2006). The reports on apoptosis and cell-cycle arrest induced by a dental adhesive resin correspond to the observations found for the monomers TEGDMA and HEMA (Mantellini *et al.*, 2003; Chang *et al.*, 2005). Similar to the observation regarding the formation of micronuclei, N-acetylcysteine protected mammalian cells from the cell-cycle delay induced by TEGDMA and HEMA (Schweikl *et al.*, 2006). These findings show the relevance of ROS in the induction of genotoxicity as well as cell-cycle delay, and suggest a central function of these molecules in triggering pathways leading to cell death.

THE ROLES OF REACTIVE OXYGEN SPECIES AND GLUTATHIONE IN THE TOXICITY OF RESIN MONOMERS

Cell death is controlled by several factors within the cell, including the disruption of a stable redox balance between ROS, generated both endogenously and exogenously, and anti-oxidant protective systems. Such an imbalance is a result of the formation of partially reduced metabolites of cellular oxygen during electron transfer reactions. Primary sources of ROS (hydrogen peroxide, superoxide anion, hydroxyl radical) within the cell include mitochondria, cytochrome P450 enzymes, and peroxisomes. However, ROS are also generated from external sources as a result of exposure to UV light, ionizing radiation, and other environmental agents (Shackelford *et al.*, 2000;

Feinendegen, 2002; Droge, 2002) (Fig. 2). ROS are thought to contribute to the pathogenesis of several diseases, as well as to the promotion of early aging (Luchsinger and Mayeux, 2004; Mattson, 2004; Zecca *et al.*, 2004).

To prevent oxidative damage, mammalian cells have developed a complex defense system of redox-regulating molecules that include non-enzymatic anti-oxidants like glutathione (GSH) and enzymatic activities like superoxide dismutase (SOD), thioredoxin (Trx), thioredoxin reductase (TrxR), glutathione peroxidase 1 (GPx), glutathione reductase, catalase (CAT), and other related enzymes or regulators of their expression (Ames, 1983; Cerutti, 1985; Haddad, 2002; Levonen *et al.*, 2004; Mathers *et al.*, 2004; Watson *et al.*, 2004; Hayes *et al.*, 2005) (Fig. 2). The situation is even more complex in certain physiological circumstances, when radical scavenging by anti-oxidants like GSH results in elimination of a reactive radical at the expense of the formation of a radical from an anti-oxidant molecule. For instance, the scavenging of different reactive radicals by GSH yields sulfur-centered glutathione radicals (thiyl radicals). The reactivity of these secondary radicals toward essential biomolecules determines whether GSH functions as an effective anti-oxidant or contributes to free radical cell injury as a pro-oxidant (Sagrista *et al.*, 2002; Borisenko *et al.*, 2004).

Several studies have shown that the cytotoxicity of dental materials and monomers, such TEDGMA and HEMA, is associated with a rapid depletion of GSH (Engelmann *et al.*, 2002; Stanislawski *et al.*, 2003; Volk *et al.*, 2006). Because GSH plays an important role in protection and detoxification processes, the depletion of the intracellular glutathione pool by TEGDMA may contribute significantly to the cytotoxicity of this monomer (Geurtsen and Leyhausen, 2001). In addition, GSH depletion induced by TEGDMA is reported to be associated with the subsequent production of ROS, which in turn may contribute to the toxicity of the monomer in gingival and pulpal fibroblasts (Stanislawski *et al.*, 2003). ROS production in primary pulp and skin fibroblasts was also increased by the monomer HEMA (Spagnuolo *et al.*, 2004b; Chang *et al.*, 2005). The depletion of GSH in fibroblasts is not related to an increase in oxidized GSH (GSSG) (Lefeuvre *et al.*, 2004). Likewise, no significant change in the GSH-GSSG ratio was detected in THP-1 human monocytic cells after exposure to sublethal concentrations of HEMA and TEGDMA (Noda *et al.*, 2005). TEGDMA also modified the activity of glutathione transferase P1 (GSTP1) as a non-competitive antagonist of glutathione, the substrate of GSTP1. It is interesting to note that the toxicity of TEGDMA may be related to a polymorphic expression of GSTP1 (Lefeuvre *et al.*, 2004). Anti-oxidants such as N-acetylcysteine (NAC), ascorbate, and Trolox (water-soluble vitamin E) appear to be useful in preventing cell damage mediated by TEGDMA and HEMA (Stanislawski *et al.*, 2003; Walther *et al.*, 2004; Spagnuolo *et al.*, 2006). However, the question of why and how resin monomers may act *via* ROS remains unclear. There is evidence that anti-oxidants might also act as pro-oxidants under certain experimental conditions (Niki and Noguchi, 2004). Recently, it has been reported that NAC and glutathione reduced oxidative DNA damage caused by a dental resin compound. In contrast, a further study suggested that low concentrations (NAC < 2.5 mM and glutathione < 0.5 mM) of these cysteine-donating compounds even enhanced the extent of DNA damage (Winter *et al.*, 2005).

However, the situation with NAC is far more complex. NAC, as a source of sulfhydryl groups, acts as a scavenger of ROS such as hydroxyl radicals and hydrogen peroxide, and its many effects *in vitro* and in various tissues have been reviewed extensively (De Flora *et al.*, 2001; Zafarullah *et al.*, 2003; Wu *et al.*, 2004). NAC is a cysteine-donating compound that acts as a cellular precursor to GSH (Rahman and MacNee, 2000). The molecular mechanism of NAC activity on various redox-sensitive systems is far from being fully understood. It has been hypothesized that NAC may act directly on the sulfhydryl groups of cellular components without receptor-mediated signaling. Target proteins modulated by NAC might contain reactive cysteine residues that participate in a thiol-disulfide reaction through a redox status. Redox-sensitive cellular signal transduction components include Ras, Raf-1, and transcription factors such as AP-1 and NF-κB. Redox regulation of AP-1 and NF-κB probably occurs through a conserved cysteine residue. It has also been reported that reactive cysteine residues of proteins like Raf-1, MEK, and ERK change to a reduced state in the presence of NAC (Zafarullah *et al.*, 2003; Yu *et al.*, 2004). Thus, the imbalance of the cellular redox state due to the generation of reactive oxygen and sulfur species may activate major signal transduction pathways, leading to cell death *via* apoptosis.

THE INDUCTION OF APOPTOSIS BY DENTAL RESIN MATERIALS

Apoptosis is a programmed physiological process of cell death which plays a critical role not only in normal development, but also in the pathology of a variety of diseases and the activity of a large number of toxicants. The mechanisms leading to apoptosis have been extensively reviewed previously (Daniel *et al.*, 2003; Donovan and Cotter, 2004; Kaufmann *et al.*, 2004; Saelens *et al.*, 2004; Thorburn, 2004). In contrast to apoptosis, necrosis generally sets off a tissue inflammation process associated with clinical symptoms (Majno and Joris, 1995; Zhivotovsky, 2004).

Apoptosis has been described in several cell lines after exposure to eluates of composite materials and polymethacrylates (Cimpan *et al.*, 2000a,b; Gough and Downes, 2001; Quinlan *et al.*, 2002). It has been shown that adhesive-resin-induced apoptosis in mouse odontoblast-like cells (MDPC-23), undifferentiated pulp cells (OD-21), or macrophages is dependent on the degree of adhesive resin polymerization (Mantellini *et al.*, 2003). These findings suggest a relevant role for unpolymerized resin compounds in the induction of programmed cell death. Recently, the individual resin components (*e.g.*, TEGDMA and HEMA) capable of generating apoptosis or necrosis in normal human primary cells were identified. Depending on the exposure period, TEGDMA induced apoptosis in human pulp fibroblasts in a concentration-dependent manner (Spagnuolo *et al.*, 2004a). Moreover, the major monomer Bis-GMA affected the glutathione concentration and the percentage of apoptotic cells in cultured primary human gingival fibroblasts. Simultaneously with the induction of apoptosis, Bis-GMA caused a significant depletion of the intracellular GSH content (Engelmann *et al.*, 2004). Furthermore, it has been reported, more recently, that TEGDMA caused mitochondrial damage, as indicated by a collapse of the mitochondrial membrane potential (Lefeuvre *et al.,* 2005). This effect by TEGDMA was inhibited in the presence of Trolox (vitamin E), suggesting a role for the mitochondria in the generation of ROS, which ultimately leads to TEGDMA-induced apoptosis (Janke *et al.*, 2003; Engelmann

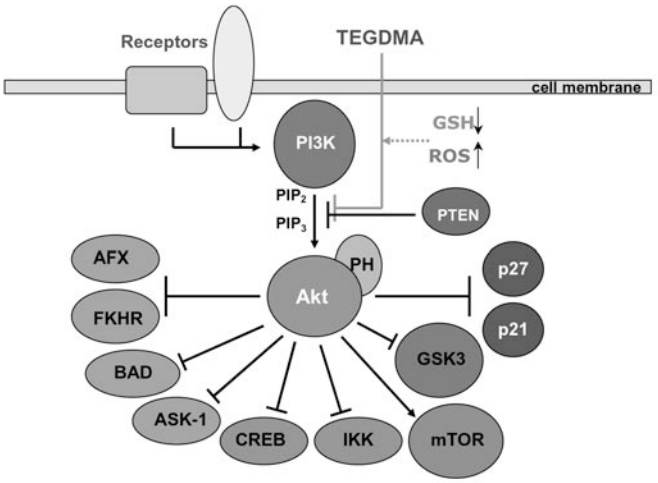

Figure 3. Description of the phosphatidylinositol-3 kinase (PI3-K) pathway and the possible modulatory effect of triethylene glycol dimethacrylate (TEGDMA). Membrane receptors (ligand-dependent tyrosine kinase receptor, G-protein-coupled receptor) activate PI3-K to form the second-messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3) by phosphorylating phosphatidylinositol-4,5-bisphosphate (PIP2). Then, PIP2 and PIP3 stimulate the phosphorylation (activation) of Akt (PKB/Akt). Akt is a key element in the control of substrates important for cell survival, cell cycle, protein synthesis, and metabolism. Activation of Akt by PI3-K is inhibited in the presence of TEGDMA. The possible links to elevated levels of reactive oxygen species (ROS) and a decrease in glutathione (GSH) levels remain to be elucidated. Tumor suppressor protein PTEN = phosphatase and tensin homologue; PH = pleckstrin homology; AFX, FKHR = forkhead transcription factors; BAD = pro-apoptotic factor of the Bcl-2 family; ASK-1 = apoptosis signal-regulating kinase 1; CREB = cAMP responsive element binding protein; IKK = inhibitor of NF-kappaB kinase; mTOR = mammalian target or rapamycin; GSK3 = glycogen synthase kinase 3; p21, p27 = cyclin-dependent kinase (cdk) inhibitors.

et al., 2004; Lefeuvre *et al.*, 2005). Cell death caused by HEMA in human primary skin fibroblasts is predominantly due to apoptosis rather than necrosis, as determined by flow cytometry and supported by the activation of caspases (Spagnuolo *et al.*, 2004b). It has recently been reported that HEMA induced apoptotic death in Peripheral Blood Mononuclear Cells (PBMCs) obtained from both healthy and HEMA-sensitized patients. It seemed that the induction of cell death by HEMA was lower in PBMCs obtained from patients compared with that in cells from healthy individuals (Paranjpe *et al.*, 2005).

CELLULAR SIGNALING IN MONOMER-INDUCED APOPTOSIS

Current evidence indicates that different stimuli use ROS as signaling messengers to activate redox-sensitive transcription factors like Nrf2 (NF-E2-related factor 2), a regulatory factor for the coordinated expression of cytoprotective genes under the regulation of the so-called anti-oxidant responsive element (ARE), activator protein-1 (AP-1), and nuclear factor kappa B (NF-κB), and induce gene expression as part of the oxidative stress response (Marshall *et al.*, 2000; Wenger, 2000; Michiels *et al.*, 2002; Haddad, 2004; Nioi and Hayes, 2004; Katoh *et al.*, 2005).

Currently, the NF-κB transcription factor family consists of a variety of Rel-domain-containing proteins, *e.g.*, RelA (p65), RelB, c-Rel, p50 (NF-κB1), and p52 (NF-κB2). In the cytoplasm, NF-κB consists of a heterotrimer of p50, p65, and IκBα (Beinke and Ley, 2004; Schmitz *et al.*, 2004). An important role is played by NF-κB in regulating the expression of anti-apoptotic proteins (*e.g.*, c-IAP-1/2, XIAP, cFLIP, Bfl-1/A1, Bcl-2, and Bcl-XL) and the cell-cycle regulators, cyclins D1 and E, which increase both cellular survival and proliferation (Karin and Lin, 2002; Karin *et al.*, 2002). Moreover, it has been shown that NF-κB activation prevents apoptotic oxidative stress by increasing thioredoxin and MnSOD levels, probably through up-regulation of target genes (Sakon *et al.*, 2003; Pham *et al.*, 2004; Djavaheri-Mergny *et al.*, 2004).

It appears that activation of nuclear factor-κB (NF-κB) is a cellular mechanism which fights against cell death induced by dental monomers like HEMA. An increase in ROS levels in primary skin fibroblasts caused by HEMA triggered NF-κB activation (Spagnuolo *et al.*, 2004b, 2006). Blocking ROS levels by pyrrolidine dithiocarbamate (PDTC), a specific anti-oxidant-NF-κB inhibitor, significantly increased the fraction of apoptotic cells after HEMA treatment. Accordingly, embryonic fibroblasts (MEF) derived from p65 knockout mice (p65-/-) were more susceptible to HEMA-induced apoptosis than were wild-type controls. These results indicate that exposure to HEMA triggers apoptosis, and that this mechanism is not directly dependent on ROS increase, since reduction of ROS did not reduce apoptosis. However, ROS production induced by HEMA was essential, because it activated NF-κB, which then exerted a protective role in counteracting apoptosis (Spagnuolo *et al.*, 2004b).

Once generated, ROS are also involved in other physiological processes, including acting as mediators in signal transduction pathways. The activation of a cascade of protein kinases is a key event in most of these pathways, to amplify extracellular signals. Signaling through the mitogen-activated protein kinase (MAPK) occurs *via* a cascade of protein phosphorylation steps. Currently, the MAPK family is divided into four subgroups: ERK (extracellular signal-regulated kinase), p38 MAPK, c-Jun NH(2)-terminal kinase (JNK)/stress-activated protein kinase (SAPK), and ERK5. Activation of MAP kinases induces a variety of cell responses, like activation of gene expression, cell proliferation, cell differentiation, cell-cycle arrest, or apoptosis. The activation of extracellular signal-regulated kinase (ERK1/2) is a well-studied MAPK pathway, discussed as being fundamental for the regulation of cell survival and apoptosis. The overall complexities of regulated cell responses to external factors through the various MAP kinase signaling pathways have been extensively discussed elsewhere (Dent *et al.*, 2003; Tanoue and Nishida, 2003; Torres and Forman, 2003; Engelberg, 2004; Kyosseva, 2004).

Signaling through phosphatidylinositol 3-kinase (PI3-K) is a cell survival pathway different from that of MAP kinases. After activation by ligand-dependent tyrosine kinase receptors, G-protein-coupled receptors, or integrins, PI3-K generates the second-messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3) by phosphorylating phosphatidylinositol-4,5-bisphosphate (PIP2). Then, PIP3 probably recruits protein kinase B/Akt (PKB/Akt) to the plasma membrane to allow for the subsequent phosphorylation by the phosphoinositide-dependent kinase-1 (PDK-1). PKB/Akt plays a central role in signaling as a downstream target for PI3-K (Leevers *et al.*, 1999; Downward, 2004). In several studies, the functional consequences of PKB/Akt phosphorylation have been defined, which has led to the conclusion that PKB/Akt is, among other processes, a component of the regulation of apoptosis and proliferation (Fig. 3). To date, it appears that PKB/Akt might both

negatively regulate proteins that promote the expression of death genes and positively regulate factors leading to cell survival (West *et al.*, 2002; Downward, 2004).

There is experimental evidence that the dental co-monomer TEGDMA induced cell death *via* apoptosis in a concentration-dependent manner by inhibiting the phosphorylation of PKB/Akt in human pulp cells. Furthermore, an elementary role of PI3-K in the signaling of environmental stress caused by the resin monomer was suggested after inhibition of PKB/Akt phosphorylation by the inhibitor LY294002. The blocking of phosphorylation amplified the effect of TEGDMA on the induction of apoptosis. However, activation of MAP kinase ERK1/2 by phosphorylation was not detected (Spagnuolo *et al.*, 2004a). These results suggest that the inhibition of PI3-K/Akt by TEGDMA (or its metabolites) might be a primary target in TEGDMA-induced cytotoxicity (Fig. 3). In addition, protein kinase B/Akt might protect pulp cells by counteracting TEGDMA-induced cytotoxicity. A recent study reported on the phosphorylation of ERK1/2 in a salivary gland cell line after exposure to HEMA or TEGDMA. Moreover, it appeared as if phosphorylation of both p38 and JNK was selectively increased by HEMA (Samuelsen *et al.*, 2006).

FUTURE CONSIDERATIONS

The clinical relevance of identifying the potential of dental materials and their components to induce damage in cells and tissues *in vitro* has been recently emphasized (Schmalz, 2002; Geurtsen, 2003). Moreover, advances in the analyses of the cellular toxicology of resin monomers, as discussed in this review, have also provided new insights into the interpretation of the risk factors for oral cavity tissue. For instance, the degree of the monomer diffusion across dentin is modified by parameters like the remaining dentin thickness, dentin permeability, or dentin location (Hume and Gerzina, 1996; Mjör and Ferrari, 2002). It has been estimated that sufficient amounts of the monomers HEMA and TEGDMA are probably eluted from clinically used bonding agents to cause cellular toxicity (Bouillaguet *et al.*, 1996; Noda *et al.*, 2002). Under such conditions, the modification of cell homeostasis by TEGDMA, HEMA, and related compounds might be of particular relevance. Resin monomers that gain access to signal transduction pathways that respond to the imbalance of the cellular redox state may also be able to change the effects of regulatory molecules like growth factors, and, thus, influence a variety of functions in tissue homeostasis of the dentin pulp complex (Goldberg and Smith, 2004). Cellular pathways—for instance, the secretory activity of odontoblasts or odontoblast-like cells—might be affected as well. Although the relationship between the degree of cell injury tolerated by odontoblasts and their survival is unclear, the concentrations of monomers in the pulp could be high enough to modify, indirectly, appropriate repair responses like the regulation of reactionary dentinogenesis (Goldberg and Smith, 2004; Qin *et al.*, 2004). The combined efforts being made in the field on cellular toxicology of resin monomers and related future findings derived from cell biology will improve our understanding of possible systemic disorders as well as the identification of potential chronic local adverse effects caused by dental materials.

ACKNOWLEDGMENTS

The authors are indebted to Dr. L.J. Nunez (Memphis, TN, USA) for critically reading the manuscript. This project was supported by the Medical Faculty of the University of Regensburg, Germany.

REFERENCES

Achanta G, Huang P (2004). Role of p53 in sensing oxidative DNA damage in response to reactive oxygen species-generating agents. *Cancer Res* 64:6233-6239.

Adimoolam S, Ford JM (2003). p53 and regulation of DNA damage recognition during nucleotide excision repair. *DNA Repair (Amst)* 2:947-954.

Alanko K, Susitaival P, Jolanki R, Kanerva L (2004). Occupational skin diseases among dental nurses. *Contact Dermatitis* 50:77-82.

Ames BN (1983). Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. *Science* 221:1256-1264.

Ashby J (2002). Scientific issues associated with the validation of in vitro and in vivo methods for assessing endocrine disrupting chemicals. *Toxicology* 181-182:389-397.

Bakkenist CJ, Kastan MB (2004). Initiating cellular stress responses. *Cell* 118:9-17.

Barzilai A, Yamamoto K (2004). DNA damage responses to oxidative stress. *DNA Repair (Amst)* 3:1109-1115.

Beinke S, Ley SC (2004). Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. *Biochem J* 382(Pt 2):393-409.

Besaratinia A, Pfeifer GP (2005). DNA adduction and mutagenic properties of acrylamide. *Mutat Res* 580:31-40.

Boonstra J, Post JA (2004). Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells. *Gene* 337:1-13.

Borisenko GG, Martin I, Zhao Q, Amoscato AA, Tyurina YY, Kagan VE (2004). Glutathione propagates oxidative stress triggered by myeloperoxidase in HL-60 cells. Evidence for glutathionyl radical-induced peroxidation of phospholipids and cytotoxicity. *J Biol Chem* 279:23453-23462.

Bouillaguet S (2004). Biological risks of resin-based materials to the dentin-pulp complex. *Crit Rev Oral Biol Med* 15:47-60.

Bouillaguet S, Wataha JC, Hanks CT, Ciucchi B, Holz J (1996). In vitro cytotoxicity and dentin permeability of HEMA. *J Endod* 22:244-248.

Cerutti PA (1985). Prooxidant states and tumor promotion. *Science* 227:375-381.

Chang HH, Guo MK, Kasten FH, Chang MC, Huang GF, Wang YL, *et al.* (2005). Stimulation of glutathione depletion, ROS production and cell cycle arrest of dental pulp cells and gingival epithelial cells by HEMA. *Biomaterials* 26:745-753.

Cimpan MR, Matre R, Cressey LI, Tysnes B, Lie SA, Gjertsen BT, *et al.* (2000a). The effect of heat- and auto-polymerized denture base polymers on clonogenicity, apoptosis, and necrosis in fibroblasts: denture base polymers induce apoptosis and necrosis. *Acta Odontol Scand* 58:217-228.

Cimpan MR, Cressey LI, Skaug N, Halstensen A, Lie SA, Gjertsen BT, *et al.* (2000b). Patterns of cell death induced by eluates from denture base acrylic resins in U-937 human monoblastoid cells. *Eur J Oral Sci* 108:59-69.

Daniel PT, Schulze-Osthoff K, Belka C, Guner D (2003). Guardians of cell death: the Bcl-2 family proteins. *Essays Biochem* 39:73-88.

De Flora S, Izzotti A, D'Agostini F, Balansky RM (2001). Mechanisms of N-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking-related end-points. *Carcinogenesis* 22:999-1013.

Dent P, Yacoub A, Fisher PB, Hagan MP, Grant S (2003). MAPK pathways in radiation responses. *Oncogene* 22:5885-5896.

Djavaheri-Mergny M, Javelaud D, Wietzerbin J, Besancon F (2004). NF-kappaB activation prevents apoptotic oxidative stress via an increase of both thioredoxin and MnSOD levels in TNFalpha-treated Ewing sarcoma cells. *FEBS Lett* 578:111-115.

Donovan M, Cotter TG (2004). Control of mitochondrial integrity by Bcl-2 family members and caspase-independent cell death. *Biochim Biophys Acta* 1644:133-147.

Downward J (2004). PI 3-kinase, Akt and cell survival. *Semin Cell Dev Biol* 15:177-182.

Droge W (2002). Free radicals in the physiological control of cell function. *Physiol Rev* 82:47-95.

Engelberg D (2004). Stress-activated protein kinases-tumor suppressors or tumor initiators? *Semin Cancer Biol* 14:271-282.

Engelmann J, Leyhausen G, Leibfritz D, Geurtsen W (2002). Effect of

TEGDMA on the intracellular glutathione concentration of human gingival fibroblasts. *J Biomed Mater Res* 63:746-751.

Engelmann J, Janke V, Volk J, Leyhausen G, von Neuhoff N, Schlegelberger B, *et al.* (2004). Effects of BisGMA on glutathione metabolism and apoptosis in human gingival fibroblasts in vitro. *Biomaterials* 25:4573-4580.

Feinendegen LE (2002). Reactive oxygen species in cell responses to toxic agents. *Hum Exp Toxicol* 21:85-90.

Geurtsen W (2000). Biocompatibility of resin-modified filling materials. *Crit Rev Oral Biol Med* 11:333-355.

Geurtsen W (2003). Toxicology of dental materials and 'clinical experience' [editorial]. *J Dent Res* 82:500.

Geurtsen W, Leyhausen G (2001). Chemical-biological interactions of the resin monomer triethyleneglycol-dimethacrylate (TEGDMA). *J Dent Res* 80:2046-2050.

Goldberg M, Smith AJ (2004). Cells and extracellular matrices of dentin and pulp: a biological basis for repair and tissue engineering. *Crit Rev Oral Biol Med* 15:13-27.

Gough JE, Downes S (2001). Osteoblast cell death on methacrylate polymers involves apoptosis. *J Biomed Mater Res* 57:497-505.

Haddad JJ (2002). Antioxidant and prooxidant mechanisms in the regulation of redox(y)-sensitive transcription factors. *Cell Signal* 14:879-897.

Haddad JJ (2004). Oxygen sensing and oxidant/redox-related pathways. *Biochem Biophys Res Commun* 316:969-977.

Hanks CT, Strawn SE, Wataha JC, Craig RG (1991). Cytotoxic effects of resin components on cultured mammalian fibroblasts. *J Dent Res* 70:1450-1455.

Hayes JD, Flanagan JU, Jowsey IR (2005). Glutathione transferases. *Annu Rev Pharmacol Toxicol* 45:51-88.

Heil J, Reifferscheid G, Waldmann P, Leyhausen G, Geurtsen W (1996). Genotoxicity of dental materials. *Mutat Res* 368:181-194.

Hume WR, Gerzina TM (1996). Bioavailability of components of resin-based materials which are applied to teeth. *Crit Rev Oral Biol Med* 7:172-179.

Izumi T, Wiederhold LR, Roy G, Roy R, Jaiswal A, Bhakat KK, *et al.* (2003). Mammalian DNA base excision repair proteins: their interactions and role in repair of oxidative DNA damage. *Toxicology* 193:43-65.

Janke V, von Neuhoff N, Schlegelberger B, Leyhausen G, Geurtsen W (2003). TEGDMA causes apoptosis in primary human gingival fibroblasts. *J Dent Res* 82:814-818.

Kaina B (2003). DNA damage-triggered apoptosis: critical role of DNA repair, double-strand breaks, cell proliferation and signaling. *Biochem Pharmacol* 66:1547-1554.

Kanerva L (2001). Cross-reactions of multifunctional methacrylates and acrylates. *Acta Odontol Scand* 59:320-329.

Kanerva L, Estlander T, Jolanki R (1994). Occupational skin allergy in the dental profession. *Dermatol Clin* 12:517-532.

Karin M, Lin A (2002). NF-kappaB at the crossroads of life and death. *Nat Immunol* 3:221-227.

Karin M, Cao Y, Greten FR, Li ZW (2002). NF-kappaB in cancer: from innocent bystander to major culprit. *Nat Rev Cancer* 2:301-310.

Katoh Y, Iida K, Kang MI, Kobayashi A, Mizukami M, Tong KI, *et al.* (2005). Evolutionary conserved N-terminal domain of Nrf2 is essential for the Keap1-mediated degradation of the protein by proteasome. *Arch Biochem Biophys* 433:342-350.

Kaufmann T, Schinzel A, Borner C (2004). Bcl-w(edding) with mitochondria. *Trends Cell Biol* 14:8-12.

Kleinsasser NH, Wallner BC, Harreus UA, Kleinjung T, Folwaczny M, Hickel R, *et al.* (2004). Genotoxicity and cytotoxicity of dental materials in human lymphocytes as assessed by the single cell microgel electrophoresis (comet) assay. *J Dent* 32:229-234.

Kleinsasser NH, Schmid K, Sassen AW, Harreus UA, Staudenmaier R, Folwaczny M, *et al.* (2006). Cytotoxic and genotoxic effects of resin monomers in human salivary gland tissue and lymphocytes as assessed by the single cell microgel electrophoresis (Comet) assay. *Biomaterials* 27:1762-1770.

Kyosseva SV (2004). Mitogen-activated protein kinase signaling. *Int Rev Neurobiol* 59:201-220.

Lavin MF, Birrell G, Chen P, Kozlov S, Scott S, Gueven N (2005). ATM signaling and genomic stability in response to DNA damage. *Mutat Res* 569:123-132.

Leevers SJ, Vanhaesebroeck B, Waterfield MD (1999). Signalling through phosphoinositide 3-kinases: the lipids take centre stage. *Curr Opin Cell Biol* 11:219-225.

Lefeuvre M, Bourd K, Loriot MA, Goldberg M, Beaune P, Perianin A, *et al.* (2004). TEGDMA modulates glutathione transferase P1 activity in gingival fibroblasts. *J Dent Res* 83:914-919.

Lefeuvre M, Amjaad W, Goldberg M, Stanislawski L (2005). TEGDMA induces mitochondrial damage and oxidative stress in human gingival fibroblasts. *Biomaterials* 26:5130-5137.

Levonen AL, Landar A, Ramachandran A, Ceaser EK, Dickinson DA, Zanoni G, *et al.* (2004). Cellular mechanisms of redox cell signalling: role of cysteine modification in controlling antioxidant defences in response to electrophilic lipid oxidation products. *Biochem J* 378(Pt 2):373-382.

Luchsinger JA, Mayeux R (2004). Dietary factors and Alzheimer's disease. *Lancet Neurol* 3:579-587.

Majno G, Joris I (1995). Apoptosis, oncosis, and necrosis. An overview of cell death. *Am J Pathol* 146:3-15.

Mantellini MG, Botero TM, Yaman P, Dennison JB, Hanks CT, Nör JE (2003). Adhesive resin induces apoptosis and cell-cycle arrest of pulp cells. *J Dent Res* 82:592-596.

Marnett LJ (1994). DNA adducts of alpha,beta-unsaturated aldehydes and dicarbonyl compounds. *IARC Sci Publ* 125:151-163.

Marshall HE, Merchant K, Stamler JS (2000). Nitrosation and oxidation in the regulation of gene expression. *FASEB J* 14:1889-1900.

Mathers J, Fraser JA, McMahon M, Saunders RD, Hayes JD, McLellan LI (2004). Antioxidant and cytoprotective responses to redox stress. *Biochem Soc Symp* 71:157-176.

Mattson MP (2004). Pathways towards and away from Alzheimer's disease. *Nature* 430:631-639

Michelsen VB, Lygre H, Skalevik R, Tveit AB, Solheim E (2003). Identification of organic eluates from four polymer-based dental filling materials. *Eur J Oral Sci* 111:263-271.

Michiels C, Minet E, Mottet D, Raes M (2002). Regulation of gene expression by oxygen: NF-kappaB and HIF-1, two extremes. *Free Radic Biol Med* 33:1231-1242.

Mjör IA, Ferrari M (2002). Pulp-dentin biology in restorative dentistry. Part 6: Reactions to restorative materials, tooth-restoration interfaces, and adhesive techniques. *Quintessence Int* 33:35-63.

Niki E, Noguchi N (2004). Dynamics of antioxidant action of vitamin E. *Acc Chem Res* 37:45-51.

Nioi P, Hayes JD (2004). Contribution of NAD(P)H:quinone oxidoreductase 1 to protection against carcinogenesis, and regulation of its gene by the Nrf2 basic-region leucine zipper and the arylhydrocarbon receptor basic helix-loop-helix transcription factors. *Mutat Res* 555:149-171.

Noda M, Wataha JC, Kaga M, Lockwood PE, Volkmann KR, Sano H (2002). Components of dentinal adhesives modulate heat shock protein 72 expression in heat-stressed THP-1 human monocytes at sublethal concentrations. *J Dent Res* 81:265-269.

Noda M, Wataha JC, Lockwood PE, Volkmann KR, Kaga M, Sano H (2003). Sublethal, 2-week exposures of dental material components alter TNF-alpha secretion of THP-1 monocytes. *Dent Mater* 19:101-105.

Noda M, Wataha JC, Lewis JB, Kaga M, Lockwood PE, Messer RL, *et al.* (2005). Dental adhesive compounds alter glutathione levels but not glutathione redox balance in human THP-1 monocytic cells. *J Biomed Mater Res B Appl Biomater* 73:308-314.

Nyberg KA, Michelson RJ, Putnam CW, Weinert TA (2002). Toward maintaining the genome: DNA damage and replication checkpoints. *Annu Rev Genet* 36:617-656.

Ortengren U (2000). On composite resin materials. Degradation, erosion and possible adverse effects in dentists. *Swed Dent J Suppl* 141:1-61.

Pagoria D, Lee A, Geurtsen W (2005). The effect of camphorquinone (CQ) and CQ-related photosensitizers on the generation of reactive oxygen species and the production of oxidative DNA damage. *Biomaterials* 26:4091-4099.

Paranjpe A, Bordador LC, Wang MY, Hume WR, Jewett A (2005). Resin monomer 2-hydroxyethyl methacrylate (HEMA) is a potent inducer of apoptotic cell death in human and mouse cells. *J Dent Res* 84:172-177.

Peutzfeldt A (1997). Resin composites in dentistry: the monomer systems. *Eur J Oral Sci* 105:97-116.

Pfeiffer P, Feldmann E, Odersky A, Kuhfittig-Kulle S, Goedecke W (2005). Analysis of DNA double-strand break repair by nonhomologous end joining in cell-free extracts from mammalian cells. *Methods Mol Biol* 291:351-371.

Pham CG, Bubici C, Zazzeroni F, Papa S, Jones J, Alvarez K, *et al.* (2004). Ferritin heavy chain upregulation by NF-kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species. *Cell* 119:529-542.

Pulgar R, Olea-Serrano MF, Novillo-Fertrell A, Rivas A, Pazos P, Pedraza V, *et al.* (2000). Determination of bisphenol A and related aromatic compounds released from bis-GMA-based composites and sealants by high performance liquid chromatography. *Environ Health Perspect* 108:21-27.

Qin C, Baba O, Butler WT (2004). Post-translational modifications of sibling proteins and their roles in osteogenesis and dentinogenesis. *Crit Rev Oral Biol Med* 15:126-136.

Quinlan CA, Zisterer DM, Tipton KF, O'Sullivan MI (2002). In vitro cytotoxicity of a composite resin and compomer. *Int Endod J* 35:47-55.

Rahman I, MacNee W (2000). Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches. *Free Radic Biol Med* 28:1405-1420.

Rueggeberg FA (2002). From vulcanite to vinyl, a history of resins in restorative dentistry. *J Prosthet Dent* 87:364-379.

Saelens X, Festjens N, Vande Walle L, van Gurp M, van Loo G, Vandenabeele P (2004). Toxic proteins released from mitochondria in cell death. *Oncogene* 23:2861-2874.

Sagrista ML, Garcia AE, Africa De Madariaga M, Mora M (2002). Antioxidant and pro-oxidant effect of the thiolic compounds N-acetyl-L-cysteine and glutathione against free radical-induced lipid peroxidation. *Free Radic Res* 36:329-340.

Sakon S, Xue X, Takekawa M, Sasazuki T, Okazaki T, Kojima Y, *et al.* (2003) NF-kappaB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. *EMBO J* 22:3898-3909.

Samuelsen JT, Dahl JE, Karlsson S, Morisbak E, Becher R (2006). Apoptosis induced by the monomers HEMA and TEGDMA involves formation of ROS and differential activation of the MAP-kinases p38, JNK and ERK. *Dent Mater,* available online 20 January [E-pub ahead of print].

Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S (2004). Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem* 73:39-85.

Santerre JP, Shajii L, Leung BW (2001). Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. *Crit Rev Oral Biol Med* 12:136-151.

Schmalz G (1998). The biocompatibility of non-amalgam dental filling materials. *Eur J Oral Sci* 106(2 Pt 2):696-706.

Schmalz G (2002). Materials science: biological aspects. *J Dent Res* 81:660-663.

Schmitz ML, Mattioli I, Buss H, Kracht M (2004). NF-kappaB: a multifaceted transcription factor regulated at several levels. *ChemBioChem* 5:1348-1358.

Schuster GS, Caughman GB, Rueggeberg FA (2000). Changes in cell phospholipid metabolism in vitro in the presence of HEMA and its degradation products. *Dent Mater* 16:297-302.

Schweikl H, Schmalz G (1999). Triethylene glycol dimethacrylate induces large deletions in the hprt gene of V79 cells. *Mutat Res* 438:71-78.

Schweikl H, Schmalz G, Rackebrandt K (1998). The mutagenic activity of unpolymerized resin monomers in *Salmonella typhimurium* and V79 cells. *Mutat Res* 415:119-130.

Schweikl H, Schmalz G, Spruss T (2001). The induction of micronuclei in vitro by unpolymerized resin monomers. *J Dent Res* 80:1615-1620.

Schweikl H, Hiller KA, Bolay C, Kreissl M, Kreismann W, Nusser A, *et al.* (2005a). Cytotoxic and mutagenic effects of dental composite materials. *Biomaterials* 26:1713-1719.

Schweikl H, Altmannberger I, Hanser N, Hiller KA, Bolay C, Brockhoff G, *et al.* (2005b). The effect of triethylene glycol dimethacrylate on the cell cycle of mammalian cells. *Biomaterials* 26:4111-4118.

Schweikl H, Hartmann A, Hiller KA, Spagnuolo G, Bolay C, Brockhoff G, *et al.* (2006). Inhibition of TEGDMA and HEMA-induced genotoxicity and cell cycle arrest by N-acetylcysteine. *Dent Mater,* available online 7 August 2006.

Shackelford RE, Kaufmann WK, Paules RS (1999). Cell cycle control, checkpoint mechanisms, and genotoxic stress. *Environ Health Perspect* 107(Suppl 1):5-24.

Shackelford RE, Kaufmann WK, Paules RS (2000). Oxidative stress and cell cycle checkpoint function. *Free Radic Biol Med* 28:1387-1404.

Solomon JJ (1994). DNA adducts of lactones, sultones, acylating agents and acrylic compounds. *IARC Sci Publ* 125:179-198.

Spagnuolo G, Galler K, Schmalz G, Cosentino C, Rengo S, Schweikl H (2004a). Inhibition of phosphatidylinositol 3-kinase amplifies TEGDMA-induced apoptosis in primary human pulp cells. *J Dent Res* 83:703-707.

Spagnuolo G, Mauro C, Leonardi A, Santillo M, Paterno R, Schweikl H, *et al.* (2004b). NF-kappaB protection against apoptosis induced by HEMA. *J Dent Res* 83:837-842.

Spagnuolo G, D'Antò V, Cosentino C, Schmalz G, Schweikl H, Rengo S (2006). Effect of N-acetyl-L-cysteine on ROS production and cell death caused by HEMA in human primary gingival fibroblasts. *Biomaterials* 27:1803-1809.

Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A (2003). TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. *J Biomed Mater Res A* 66:476-482.

Tanoue T, Nishida E (2003). Molecular recognitions in the MAP kinase cascades. *Cell Signal* 15:455-462.

Thorburn A (2004). Death receptor-induced cell killing. *Cell Signal* 16:139-144.

Torres M, Forman HJ (2003). Redox signaling and the MAP kinase pathways. *Biofactors* 17:287-296.

Volk J, Engelmann J, Leyhausen G, Geurtsen W (2006). Effects of three resin monomers on the cellular glutathione concentration of cultured human gingival fibroblasts. *Dent Mater* 22:499-505.

Volkel W, Colnot T, Csanady GA, Filser JG, Dekant W (2002). Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chem Res Toxicol* 15:1281-1287.

Wada H, Tarumi H, Imazato S, Narimatsu M, Ebisu S (2004). In vitro estrogenicity of resin composites. *J Dent Res* 83:222-226.

Wataha JC, Rueggeberg FA, Lapp CA, Lewis JB, Lockwood PE, Ergle JW, *et al.* (1999). In vitro cytotoxicity of resin-containing restorative materials after aging in artificial saliva. *Clin Oral Investig* 3:144-149.

Walther UI, Siagian II, Walther SC, Reichl FX, Hickel R (2004). Antioxidative vitamins decrease cytotoxicity of HEMA and TEGDMA in cultured cell lines. *Arch Oral Biol* 49:125-131.

Watson WH, Yang X, Choi YE, Jones DP, Kehrer JP (2004). Thioredoxin and its role in toxicology. *Toxicol Sci* 78:3-14.

Wenger RH (2000). Mammalian oxygen sensing, signalling and gene regulation. *J Exp Biol* 203(Pt 8):1253-1263.

West KA, Castillo SS, Dennis PA (2002). Activation of the PI3K/Akt pathway and chemotherapeutic resistance. *Drug Resist Updat* 5:234-248.

Winter K, Pagoria D, Geurtsen W (2005). The effect of antioxidants on oxidative DNA damage induced by visible-light-irradiated camphorquinone/N,N-dimethyl-p-toluidine. *Biomaterials* 26:5321-5329.

Wu G, Fang YZ, Yang S, Lupton JR, Turner ND (2004). Glutathione metabolism and its implications for health. *J Nutr* 134:489-492.

Yoshii E (1997). Cytotoxic effects of acrylates and methacrylates: relationships of monomer structures and cytotoxicity. *J Biomed Mater Res* 37:517-524.

Yu C, Rahmani M, Almenara J, Sausville EA, Dent P, Grant S (2004). Induction of apoptosis in human leukemia cells by the tyrosine kinase inhibitor adaphostin proceeds through a RAF-1/MEK/ERK- and AKT-dependent process. *Oncogene* 23:1364-1376.

Zafarullah M, Li WQ, Sylvester J, Ahmad M (2003). Molecular mechanisms of N-acetylcysteine actions. *Cell Mol Life Sci* 60:6-20.

Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR (2004). Iron, brain ageing and neurodegenerative disorders. *Nat Rev Neurosci* 5:863-873.

Zhivotovsky B (2004). Apoptosis, necrosis and between. *Cell Cycle* 3:64-66.