Research article

N-Acetyl cysteine restores viability and function of rat odontoblast-like cells impaired by poly-methylmethacrylate dental resin extract

Masahiro Yamada, Norinaga Kojima, Wael Att, Norio Hori, Takeo Suzuki, Takahiro Ogawa

The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, Division of Advanced Prosthodontics, Biomaterials and Hospital Dentistry, UCLA School of Dentistry, Los Angeles, California, USA

There is concern that dental-resin materials directly loaded on a prepared tooth adversely affect dental pulp tissue by releasing the resin chemicals through dentinal tubes. This study determined whether self-curing polymethyl methacrylate (PMMA)-based dental resin extract adversely affected the viability and function of odontoblast-like cells and whether the cytotoxicity of this resin, if any, could be eliminated by *N*-acetyl cysteine, an antioxidant amino acid derivative. Odontoblast-like cells isolated from rat maxillary incisor dental pulp tissue were exposed to a PMMA resin extract with or without *N*-acetyl cysteine for 1 h and then cultured in osteoblastic media. The percentage of viable cells 24 h after seeding was 20% in cells exposed to the resin extract without *N*-acetyl cysteine, whereas 45% of cells were viable after exposure to the *N*-acetyl cysteine-supplemented extract. The cells that had been exposed to the extract showed a strong tendency for apoptosis associated with the increased reactive oxygen species production and decreased intracellular glutathione level, which was improved by the addition of *N*-acetyl cysteine. *N*-Acetyl cysteine supplementation almost completely restored the significantly reduced alkaline phosphatase activity and matrix mineralization by the resin extract. These results conclusively demonstrated that exposure of odontoblast-like cells to the resin extract impaired the cell viability and function and, more intriguingly, *N*-acetyl cysteine supplementation to the extract significantly prevented these toxic effects.

Keywords: cytotoxicity, antioxidant, PMMA resin, self-curing resin, reactive oxygen species

Introduction

Self-polymerizing poly-methylmethacrylate (PMMA)-based dental resin, which consists of a polymer powder and a liquid monomer, is one of the most frequently-used materials in dentistry. PMMA resin is often loaded directly onto prepared teeth for fabricating temporary tooth crowns. There have been concerns that chemical substances, released from the resin during and after polymerization, biologically affect dental pulp cells/tissue through exposed dentinal tubules.[1,2]

Previous studies have revealed biologically detrimental effects of dental resin products on dental pulp-derived cells. Treating human pulp fibroblasts with an extract of the resin-based pulp capping materials or the resin adhesive impaired cell viability in culture significantly.[3,4] In addition, 2-hydroxyethyl

Correspondence to: Masahiro Yamada, The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, Division of Advanced Prosthodontics, Biomaterials and Hospital Dentistry, UCLA School of Dentistry, 10833 Le Conte Ave. Box 951668, CHS, B3-068, Los Angeles, CA 90095-1668, USA Tel: +1 310 794 7652; Fax: +1 310 825 6345; E-mail: masa0316@ucla.edu
Received 29 May 2008, revised manuscript accepted 14 October 2008

DOI 10.1179/135100009X392430

methacrylate (HEMA) caused cell death and impaired the expression of cellular phenotype in human dental pulp stromal cell culture.[5] Oxidative stress caused by endogenous reactive oxygen species (ROS) generated by the chemical stimulation of released resin substances from a polymerized resin has been cited as an important mechanism underlying these biological adverse effects of resin materials.[2,6] Likewise, self-polymerizing PMMA-based dental resins have been recently found to show crucial toxic effects on dental pulp cells in association with a disturbance of intracellular redox systems.[7,8] In our previous study, culturing rat odontoblast-like cells directly on the commercial PMMA dental material resulted in the decrease of cell viability and the near complete suppression of odontoblast-like cell functions.[9] However, it is still unknown if PMMA dental resins exhibit their cytotoxicity on odontoblast-like cells under clinically relevant situations (*i.e.* on exposure to PMMA resin extract, but not in a direct contact with the polymerized resin). More importantly, the methodology to ameliorate the toxicity of PMMA resin has not been fully explored despite its clinical relevance.

N-Acetyl cysteine is an antioxidant cysteine derivative and is also metabolized into glutathione that, in turn, is a strong intracellular antioxidant.[2,10,11] Accordingly, *N*-acetyl cysteine not only acts as a direct oxidant scavenger,[12] but also improves intracellular glutathione systems compromised by oxidative stress.[12,13]

This study aimed to determine whether autopolymerizing PMMA resin extract adversely affected the viability and function of odontoblast-like cells derived from rat dental pulp tissue and, if so, whether supplementing PMMA extract with *N*-acetyl cysteine eliminates the extract-induced cytotoxicity and restores cell function.

Material and methods

Preparation of PMMA extract

A commercial self-cured PMMA-based dental resin (Unifast II; GC, Tokyo, Japan) was prepared by mixing the powder and liquid components on the bottom of a Falcon 50-ml conical tube (BD Biosciences, MD, USA) for 15 s according to the manufacturer's instructions (powder/liquid ratio of 0.6 g/0.4 g). After 3–5 min of polymerization, 5 ml of alpha-modified Eagle's medium (α-MEM; Gibco BRL Division of Invitrogen, Gaithersburg, MD, USA) without serum was added and incubated for 1 h at 37ºC. The area of resin surface exposed to the medium was estimated at 38.5 mm^2. *N*-Acetyl cysteine (Sigma; St Louis, MO, USA) was prepared as a 1 mol/ml stock solution by mixing a powder form of *N*-acetyl cysteine in HEPES buffer (Sigma). The pH of the *N*-acetyl cysteine-stock solution was adjusted to 7.2. Experimental groups in which different molar concentrations of *N*-acetyl cysteine were added to the extract (0 mM, 2.5 mM, 5.0 mM and 7.5 mM of extract volume) were examined. At 37ºC in 5% CO_2/95% air, the pH of the extract was within 7.1–7.2 regardless of the *N*-acetyl cysteine-concentration.

Cell culture

Odontoblast-like cells were isolated from dental pulp tissue of the upper-central incisors of 8-week-old male Sprague-Dawley rats and expanded in 100-mm diameter culture dishes as described previously,[14] and the cells were passed twice. At 80% confluence, the cells were trypsinized and centrifuged. The pelleted cells were incubated in a PMMA extract with or without *N*-acetyl cysteine for 1 h in 95% air and 5% CO_2 at 37ºC. To examine the effect of *N*-acetyl cysteine exposure on cell viability and function in the resin extract-free environment, we also prepared cells suspended in α-MEM containing *N*-acetyl cysteine at final concentrations of 2.5 mM, 5.0 mM, or 7.5 mM *N*-acetyl cysteine. After the 1-h incubation, the cells were centrifuged again and seeded (3×10^4 cells/cm^2) into 12-well culture-grade polystyrene plates containing α-MEM supplemented with 10% fetal bovine serum, 50 mg/ml ascorbic acid, 10 mM Na-β-glycerophosphate, 10^{-8} M dexamethasone, and an antibiotic/antimycotic solution. Cells incubated in medium without the extract were used as an untreated control. The culture medium was renewed every 3 days with the normal media not containing the PMMA extract or *N*-acetyl cysteine, *i.e.* cells were exposed to the PMMA extract or *N*-acetyl cysteine once. This study protocol was approved by the University of California at Los Angeles Chancellor's Animal Research Committee.

Cell viability analysis

The viability of the odontoblast-like cells was evaluated 24 h after seeding by flow cytometry using an apoptosis detection kit (Annexin V-FITC Kit; BD Bioscience, San Jose, CA, USA). The method is based on the binding of annexin V to phosphatidylserine and on the DNA-intercalating capability of propidium iodide. The percentages of viable cells (annexin V [–]/propidium iodide [–] cells), late apoptotic cells (annexin V [+]/propidium iodide [+] cells) and early apoptotic cells (annexin V [+]/propidium iodide [–] cells) were determined

according to the fluorescence pattern of the cell population. The cells that had been exposed to the resin extract with or without *N*-acetyl cysteine, with the various concentrations of *N*-acetyl cysteine without the resin extract, and the untreated cells were tested after 24-h incubation.

Detection of mitochondrial membrane potential collapse and caspase activity

The collapse of mitochondrial membrane potential ($\Delta\Psi m$) and activation of caspase 3 and caspase 7, typical apoptotic indicators, were determined by a simultaneous dual-staining kit (MitoCasp; Cell Technology, Mountain View, CA, USA) in combination with a flow cytometer. Active caspase staining is based on binding of carboxyfluorescein (FAM)-labeled fluoromethyl ketone (FMK) peptide and active caspase 3 and caspase 7. Mitochondrial membrane-potential collapse was detected by a cationic dye that accumulated in the mitochondria in proportion to the membrane potential. Caspase 3/7-activated cells or the mitochondrial membrane-potential ($\Delta\Psi m$) collapsed cells were defined as FAM-positive cells or cationic dye-negative cells, respectively. The cells that had been exposed to the resin extract with or without *N*-acetyl cysteine or the untreated cells were tested after 24-h incubation.

Quantification of intracellular ROS level and glutathione concentration

The total glutathione concentration in the cultures was quantified by 5,5′-2-nitrobenzoic acid colorimetry (DTNB; Dojindo Molecular Technologies, Gaithersburg, MD, USA). The intracellular ROS levels were assessed by 5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate fluorimetry (carboxy-DCF-DA; Invitrogen, Gaithersburg, MD, USA). The cells that had been exposed to the resin extract with or without *N*-acetyl cysteine and the untreated cells were tested after 24-h incubation.

Proliferation assay

Proliferative activity was measured in day-2 cultures by the colorimetric quantification of bromodeoxyuridine (BrdU) incorporation of DNA (Roche Applied Science; Indianapolis, IN, USA). The total number of cells in the same culture was also counted with a hematocytometer to calculate the proliferative activity per cell.

Quantification of alkaline phosphatase activity and matrix mineralization

To evaluate the alkaline phosphatase (ALP) activity of cells after exposure to the resin extract with or without *N*-acetyl cysteine or the various concentration of *N*-acetyl cysteine without the resin extract, ALP staining and the quantification of ALP enzymatic activity were performed on days 5 or 8 of culture. The cultures were stained using the azo-dyes method as previously described in detail.[14] The stained images were analyzed for their ALP positive area (%), which was defined as (stained area/total dish area × 100) using a digitized image analysis system (Image Pro-plus, Media Cybernetics, Bethesda, MD, USA). The cultures were subsequently nuclear stained with methylene blue to visualize microscopically the ALP activity in a cell colony unit. ALP enzymatic activity was determined by *p*-nitrophenyl-phosphate colorimetry (Wako Chemicals USA, Inc., Richmond, VA, USA).

To determine the mineralizing capability of the dental pulp cells after exposure to the resin extract, day-14 culture was subjected to von Kossa staining as described previously.[14] The von Kossa positive area was evaluated as described for ALP staining.

Statistical analysis

For all experiments, three independent cultures were evaluated in each group and there were at least three replicates in each experiment using different batch of the cells. All repeated measurements were expressed as mean ± SD and significant differences ($P < 0.05$) among the experimental groups were evaluated by one-way ANOVA and a *post hoc* Bonferroni comparison.

Results

Prevention of PMMA extract-induced apoptosis by N*-acetyl cysteine*

Flow cytometric analysis revealed that the percentage of viable cells and the percentage of late apoptotic cells after 24-h incubation were significantly affected among the experimental groups (one-way ANOVA, $P < 0.01$; $n = 3$). The percentage of viable cells decreased to 19.7 ± 2.51% in cells exposed to the resin extract in contrast to untreated controls (78.2 ± 6.44%; (Bonferroni, $P < 0.01$; $n = 3$; Fig. 1A). Adding *N*-acetyl cysteine to the PMMA extract increased the percentage of viable cells in the culture in a concentration-dependent manner (the PMMA extract with 5.0 mM *N*-acetyl cysteine, 39.4 ± 0.75%; with 7.5 mM *N*-acetyl cysteine, 46.6 ± 1.61%). Over 65% of the cells exposed to the resin extract showed late apoptosis (67.3 ± 5.12%) that was dose-dependently reduced to less than 40% by adding *N*-acetyl cysteine (the PMMA extract with 5.0 mM *N*-acetyl cysteine, 38.0 ± 7.86%;

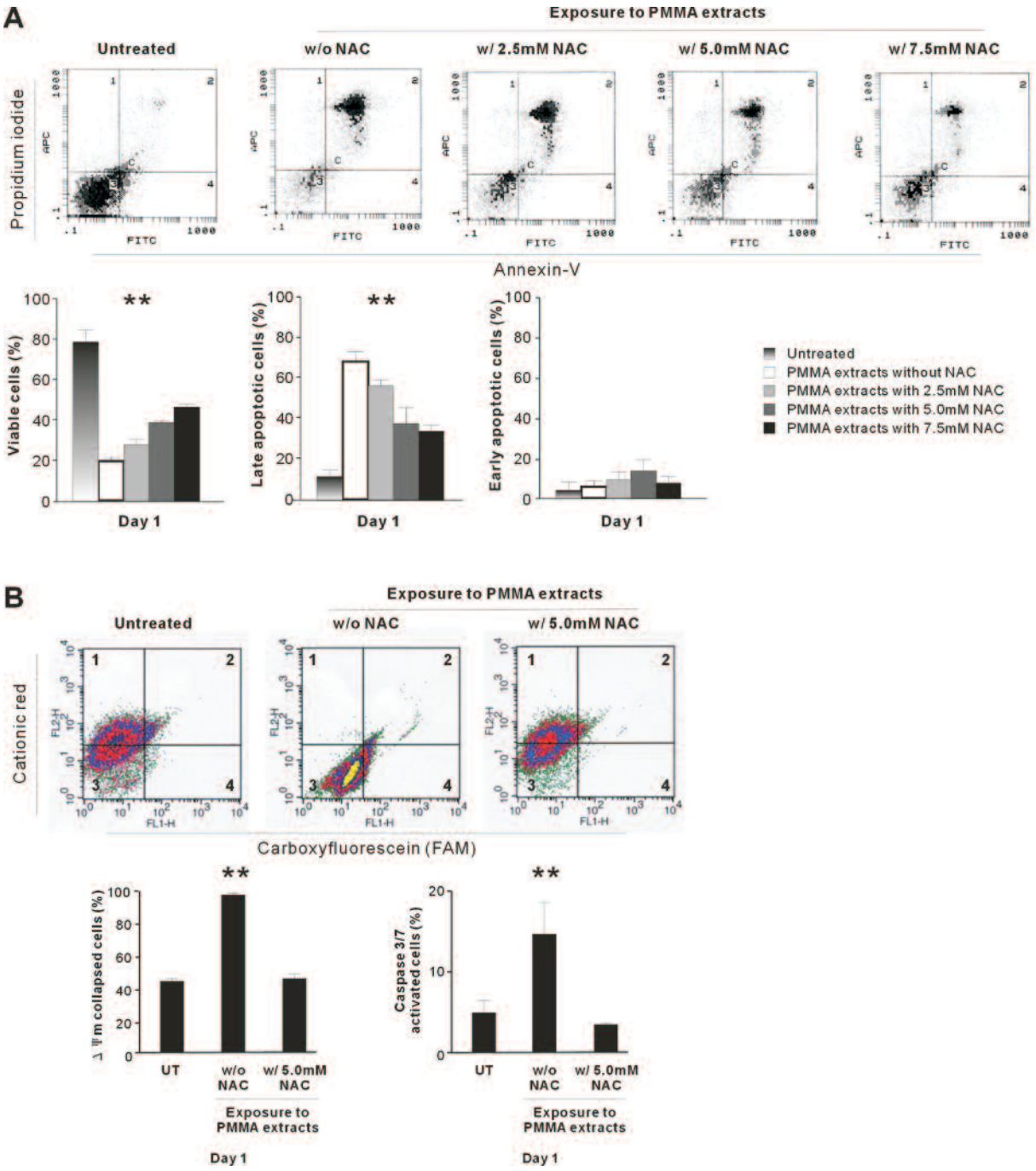

Figure 1 Flow cytometric detection of odontoblast-like cell viability (A) and the mitochondrial membrane potential (ΔΨm) collapse and the caspase 3/7 activity (B) in cell cultures 24 h after exposure to PMMA dental-resin extract with or without *N*-acetyl cysteine. Unexposed cells acted as the control. The flow cytometric images are shown at the top in each panel. The percentages of viable cells (quadrant 3), late-apoptotic cells (quadrant 2), and early-apoptotic cells (quadrant 4) are shown in the lower part (A). The percentages of ΔΨm collapsed cells (quadrants 3 and 4), caspase 3/7 activated cells (quadrants 2 and 4) are shown in the lower part of (B). Data are mean ± SD (*n* = 3). **Statistically significant (*P* < 0.01, one-way ANOVA)

with 7.5 mM *N*-acetyl cysteine, 34.1 ± 2.57%). There were no significant differences in the percentage of early apoptotic cells among groups (untreated, 4.67 ± 4.37%; the PMMA extract, 5.77 ± 2.96%; the PMMA extract with 5.0 mM *N*-acetyl cysteine, 13.8 ± 5.96%).

Exposure to the resin extract caused a collapse of ΔΨm and activated caspases 3 and 7 in the culture 24 h after seeding (Fig 1B). The percentage of ΔΨm-collapsed cells increased over 2-fold in cells exposed to the resin extract (97.3 ± 0.80%) compared to the

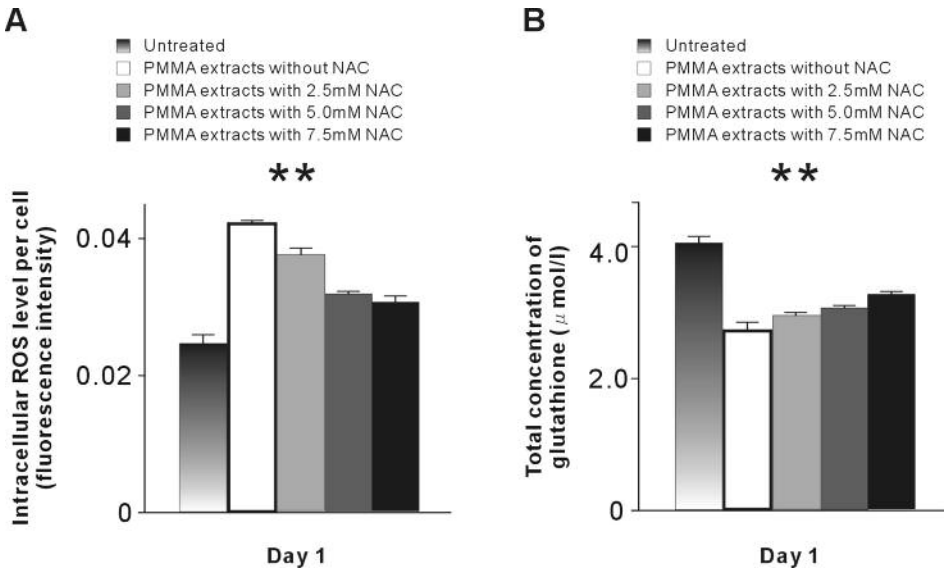

Figure 2 Intracellular ROS level (A) and total glutathione concentration (B) in odontoblast-like cell cultures 24 h after exposure to the PMMA dental-resin extract with or without *N*-acetyl cysteine. Unexposed cells acted as the control. Data are mean ± SD ($n = 3$). **Statistically significant ($P < 0.01$, one-way ANOVA)

untreated control (43.6 ± 2.89%; Bonferroni, $P < 0.01$; $n = 3$). Adding *N*-acetyl cysteine to the cultures restored viability to the levels observed in the untreated cultures (46.0 ± 2.82%). Likewise, the cells that had been exposed to the extract (14.5 ± 4.08%) showed a 3-fold increase in the percentage of caspase-activated cells in comparison with the untreated culture (4.95 ± 1.54%; Bonferroni, $P < 0.01$; $n = 3$). The addition of 5.0 mM *N*-acetyl cysteine prevented this resin extract-induced caspase activation (3.55 ± 0.17%).

Amelioration of PMMA-extract induced intracellular redox imbalance by N-*acetyl cysteine*

Intracellular ROS generation 24 h after seeding (Fig. 2A) was elevated by 70% in cells that had been exposed to the extract compared to the untreated culture (Bonferroni, $P < 0.01$; $n = 3$). However, *N*-acetyl cysteine decreased the intracellular ROS level dose-dependently to that seen in the untreated control.

Total glutathione 24 h after seeding was reduced by 65% compared to the untreated culture (Bonferroni, *P*

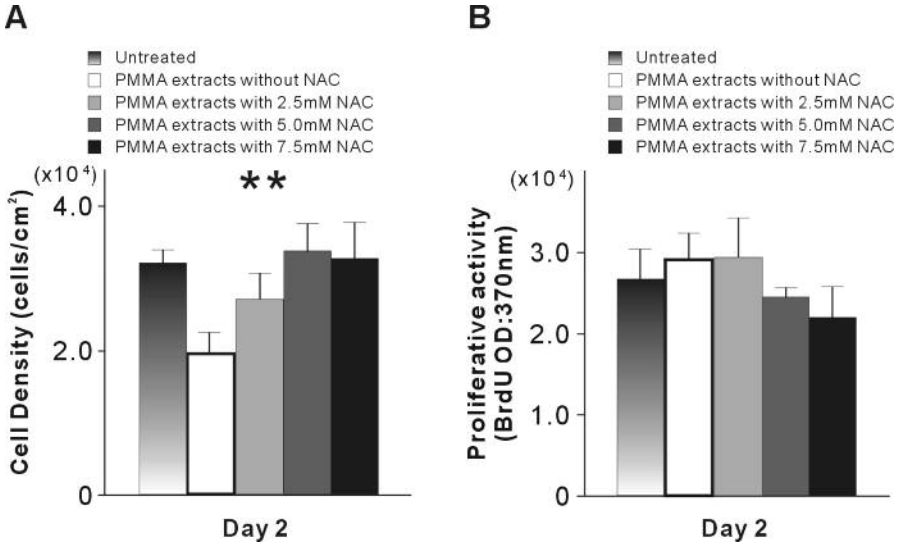

Figure 3 (A) Cell density and (B) proliferation (BrdU incorporation/the number of cells) on day 2 of odontoblast-like cell exposure to the PMMA dental resin extract before seeding with or without *N*-acetyl cysteine. Unexposed cells acted as the control. Data are mean ± SD ($n = 3$). **Statistically significant ($P < 0.01$, one-way ANOVA)

< 0.01; $n = 3$). *N*-Acetyl cysteine restored the glutathione levels in a dose-dependent manner to 80% of the untreated cultures (Fig. 2B).

Effects of exposure to PMMA extract on cell density and proliferation

The cell density was significantly affected among the experimental groups (one-way ANOVA, $P < 0.01$; $n = 3$). The cell density in the day-2 cultures exposed to the resin extract was 60% of the control culture (Bonferroni, $P < 0.01$; Fig. 3A). Cells supplemented with *N*-acetyl cysteine increased to numbers similar to the control culture. However, the cell-proliferating activity, the amount of BrdU incorporation divided by cell numbers, was not different among the groups (Fig. 3B).

Restoration of the PMMA extract-impaired cell function by N*-acetyl cysteine*

On day 5, the ALP-positive area in the cells exposed to the resin extract (5.24 ± 0.14%) was one-third of the untreated control (16.6 ± 0.88%; Bonferroni, $P < 0.01$; $n = 3$). *N*-Acetyl cysteine increased the ALP-positive area up to 15% in a dose-dependent manner (Fig. 4A,B). Microscopic images of the ALP-methylene blue dual-staining cultures revealed low ALP activity in the cells exposed to the resin extract despite dense cell aggregation. By contrast, cells exposed to *N*-acetyl cysteine-supplemented extract as well as the control cells exhibited strong and extensive ALP activity (Fig. 4A). The chemically quantified ALP activity in cells exposed to PMMA extract was one-half that of the control cells (Bonferroni, $P < 0.01$; $n = 3$). The ALP activity was dose-dependently restored by *N*-acetyl cysteine up to levels equivalent to control cells (Fig. 4C).

The von Kossa positive areas in the culture of cells that had been exposed to the extract (49.3 ± 0.94%) decreased by 40% compared to the cells that were not exposed to the resin extract (81.4 ± 0.62%; Bonferroni, $P < 0.01$; $n = 3$; Fig. 4D,E), which was microscopically confirmed by the limited von Kossa positive staining (Fig. 4D). The culture of cells exposed to *N*-acetyl cysteine-supplemented extract maintained von Kossa positive areas up to approximately 90% of the control (Fig. 4D,E) and the relatively intense von Kossa staining was detected under a light microscope (Fig. 4D).

Influence of N*-acetyl cysteine exposure on cell viability and function*

The majority of cells in the untreated cultures were viable 24 h after seeding (79.9 ± 4.77%). Exposure to the various concentrations of *N*-acetyl cysteine did not affect the percentage of viable cells (Fig. 5A). Similarly, there were no significant differences in the percentage of late or early apoptotic cells among the untreated culture and the cultures that had been exposed to the various concentrations of *N*-acetyl cysteine. On day 2, there was no significant difference in cell proliferation in the cultures exposed to *N*-acetyl cysteine (Fig. 5B). On day 8, exposure of the cells to *N*-acetyl cysteine before seeding did not result in a further increase of the ALP activity (Fig. 5C). The ALP enzymatic activity was not influenced by *N*-acetyl cysteine exposure (Fig. 5D).

Discussion

Dental pulp tissue contains odontoblasts, pre-odontoblasts, fibroblasts, immunocompetent cells, and dental-pulp stromal cells (DPSCs). DPSCs are derived from postnatal dental pulp tissue, and have the capacity to differentiate into odontoblast-like cells[16,17] that exhibit dentin-related gene expression and mineralized matrix production in osteoblastic media.[17–21] We have previously characterized the cells isolated from rat dental pulp tissue,[14] and most populations of the cells are positive for a DPSC membrane-protein marker. Cells cultured with dexamethasone-containing medium exhibited typical odontoblastic phenotypes including the ALP activity, mineralized nodule formation and dentin-related protein gene expression. This study was performed under similar culture conditions as that in the previous study. Therefore, we defined the rat dental pulp tissue-derived cells as odontoblast-like cells in this study.

This study demonstrated that both cell viability and odontoblast-like differentiation of the odontoblast-

Figure 4 (*see opposite page*) (A) Alkaline phosphatase (ALP) activity on day 5 of odontoblast-like cell exposure to the PMMA dental-resin extract before seeding with or without *N*-acetyl cysteine. Representative entire images of the ALP-stained cultures (A-I) and microscopic images of the ALP-methylene blue dual-stained cultures (A-II) are shown (bar = 50 μm). The percentage of ALP-positive area (B) and the ALP enzymatic activity quantification determined by the *p*-nitrophenyl-phosphate method (C) in the culture are shown as mean ± SD ($n = 3$). (D) The matrix mineralizing capability in the day 14 culture of odontoblast-like cells that had been exposed to the extract with or without *N*-acetyl cysteine before seeding evaluated by von Kossa staining. Representative entire images (D-I) and microscopic images (D-II) of the von Kossa-stained cultures. Bar = 250 μm. The percentage of von Kossa-positive area (E) in the culture is shown as the mean ± SD ($n = 3$). **Statistically significant ($P < 0.01$, one-way ANOVA)

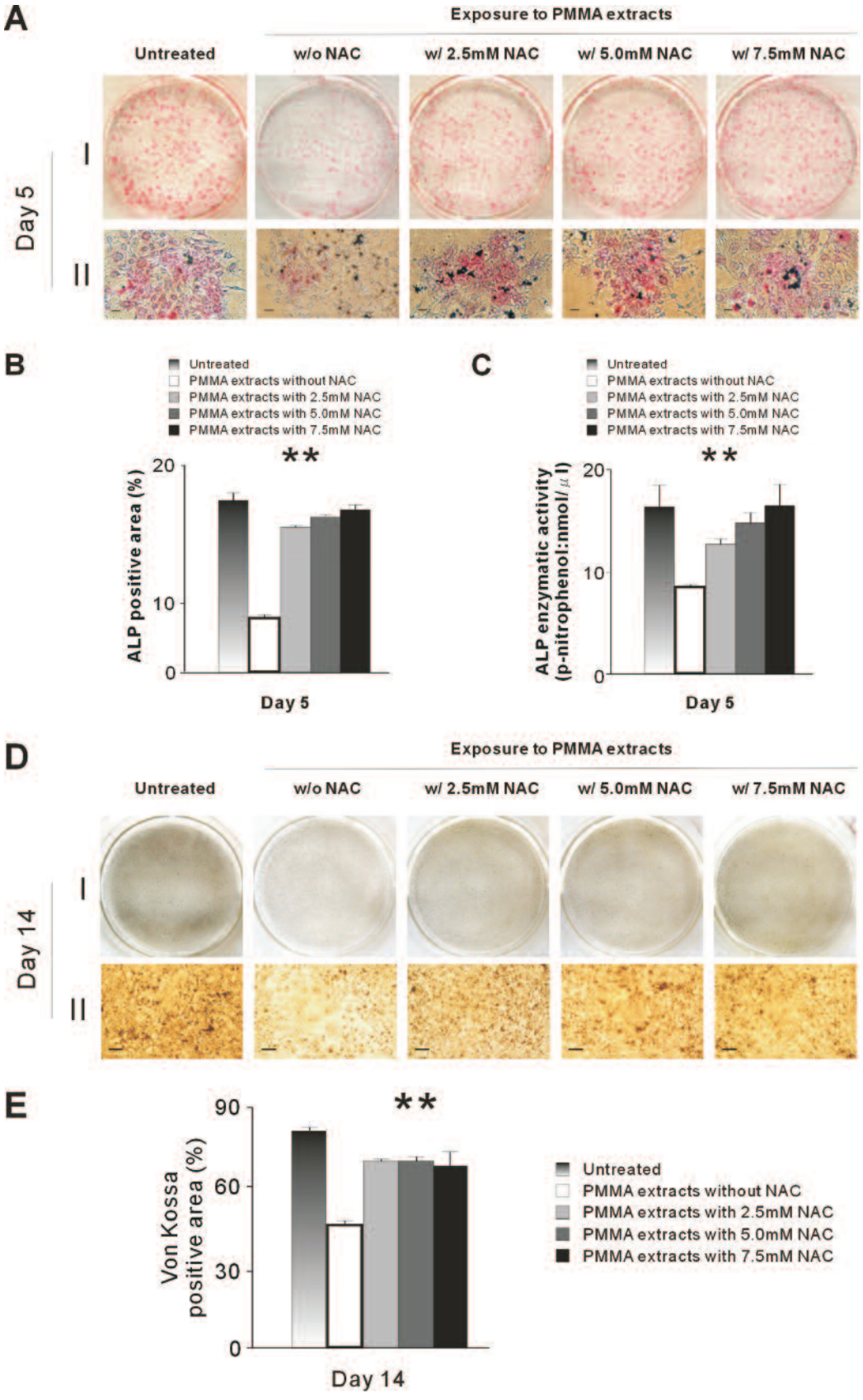

Figure 4 (*see opposite page for details*)

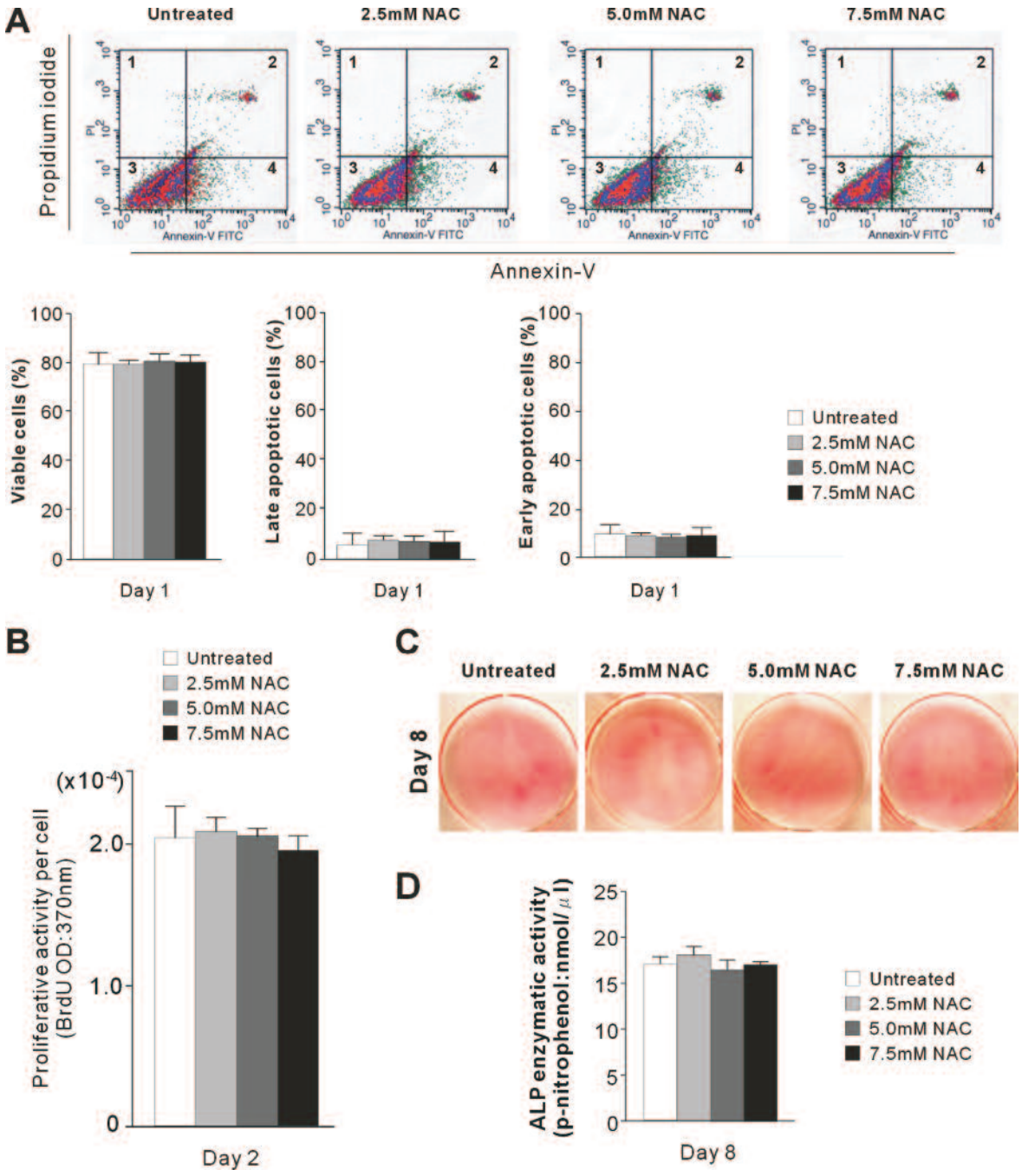

Figure 5 (A) Flow cytometric detection of cell viability at 24 h of culture after exposing the cells to α-medium before seeding with or without various concentrations of *N*-acetyl cysteine. The flow cytometric images are shown at the top of each panel. The percentages of viable cells (quadrant 3), late-apoptotic cells (quadrant 2), and early-apoptotic cells (quadrant 4) are shown in the lower part of (A). (B) Proliferation (BrdU incorporation/the number of cells) of day 2 odontoblast-like cells exposed to α-medium with or without the various concentrations of *N*-acetyl cysteine before the seeding. (C) Alkaline phosphatase (ALP) activity in the day 5 cultures with or without the various concentrations of *N*-acetyl cysteine before the seeding. Representative entire images of the ALP staining (D) and the ALP enzymatic activity quantification on day 8 of culture of odontoblast-like cells exposed to α-medium with or without the various concentrations of *N*-acetyl cysteine before seeding. Data are mean ± SD ($n = 3$)

like cells were impaired by a 1-h exposure to a PMMA resin extract. Exposure to the PMMA resin extract caused cell death and the appearance of cellular apoptotic indicators in most cell populations. Although the cells subsequently proliferated into dense colonies, the expression of ALP activity and matrix mineralization were notably restricted. This suggested that pre-exposure to the resin extract, even for a short period of time, influenced the culture from initial to the mature stages of odontoblast-like cell differentiation. More importantly, *N*-acetyl cysteine supplementation of the extract provided the cells with considerable protection, enabling the recovery of the cell viability and function to a level comparable to the control cultures.

The oxidative stress occurring from an imbalance between intracellular ROS and the antioxidant redox system is considered an important mechanism underlying the adverse effects of the resin extract on tissue and/or cells.[2,8,22–24] The intracellular level of ROS can be excessively increased by exogenous stimulation with UV light, ionizing radiation, or various chemicals including resin substances,[2,25] which deplete the intracellular level of the antioxidant glutathione, a non-enzymatic and a direct ROS scavenger. The generation of excessive amounts of ROS is considered to cause adverse biological effects not only by directly injuring cellular components[2,26–29] but also by indirectly regulating the signaling pathways inducing apoptosis.[8,22,24] For example, cell death caused by methacrylate is predominantly due to apoptosis supported by the activation of caspases.[30] In fact, the induction of apoptosis characterized by the caspase 3/7 activation was seen with the intracellular redox imbalance consisting of the increased ROS production and decreased intracellular glutathione. Recently, it has been reported that triethylene glycol dimethacrylate (TEGDMA) may disturb the protein kinase-B phosphorylation (PKB)/Akt,[24] which is a key element in cell survival, cell-cycle pathways, protein synthesis, and metabolism. The detailed cellular and molecular mechanisms underlying the cytotoxicity of PMMA resin will be of great interest to pursue in future studies.

We confirmed that *N*-acetyl cysteine can compensate for reduced glutathione levels in a dose-dependent manner. *N*-Acetyl cysteine is a membrane-permeable cysteine derivative and is metabolized into the glutathione precursor L-cyctein.[10,13] Therefore, *N*-acetyl cysteine can function as a glutathione supply source to compensate for the intracellular glutathione level depleted by excessive amounts of ROS and to maintain the balance of the antioxidant redox system.[13] *N*-Acetyl cysteine is also reported to improve methacrylate-induced effects on fibroblasts such as apoptosis, micronuclei generation, DNA base sequence defects, and cell-cycle arrest.[2,31,32] Moreover, it is hypothesized that *N*-acetyl cysteine affects the sulfhydryl residuals of intracellular redox-sensitive molecules such as activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB).[2,33] Interestingly, these transcriptional factors act in mitogen-activated protein kinase (MAPK) pathways, and are suggested to play a key role in mediating cell survival or differentiation.[34–36] In fact, a recent study demonstrated that *N*-acetyl cysteine inhibits 2-hydroxyethyl methacrylate (HEMA)-induced apoptotic cell death and restores the function of dental-pulp stromal and oral epithelial cells by increasing NF-κB activity. Although the one-time exposure of the cells to *N*-acetyl cysteine did not enhance odontoblast-like cell function in the present study, our previous study showed that *N*-acetyl cysteine treatment increased ALP positive area of the culture where *N*-acetyl cysteine was continuously applied every 3 days.[9] It will be interesting to explore the pharmacodynamic characterization of *N*-acetyl cysteine on these transcriptional factors and odontoblast-like cell phenotypes.

Autopolymerized PMMA dental resin is frequently loaded directly on prepared abutment teeth to fabricate temporary crowns despite the consistent release of chemicals from the resin during and after polymerization, which has led to the concern that pulp tissue may be exposed to the toxicity of resin chemicals through dentinal tubules.[1,2] From this perspective, the experimental design of this *in vitro* study, involving exposing odontoblast-like cells to resin extract for a certain time before seeding, was of clinical relevance. This study demonstrated that the adverse biological effects of PMMA resin on dental pulp cells observed under this experimental condition were improved by *N*-acetyl cysteine, which provides important information for development of more 'bio-friendly' resin products.

Acknowledgement

This work was partially supported by the Nissenken Institute.

References

1. Furuya K. [Electron microscopic study of canine dentin and odontoblast following the insertion of various composite resin monomers]. *Fukuoka Shika Daigaku Gakkai Zasshi* 1989; **16**: 572–599.
2. Schweikl H, Spagnuolo G, Schmalz G. Genetic and cellular toxicology of dental resin monomers. *J Dent Res* 2006; **85**: 870–877.
3. Cavalcanti BN, Rode SM, Marques MM. Cytotoxicity of substances leached or dissolved from pulp capping materials. *Int Endodont J* 2005; **38**: 505–509.

4. Annunziata M, Aversa R, Apicella A *et al*. *In vitro* biological response to a light-cured composite when used for cementation of composite inlays. *Dent Mater* 2006; **22**: 1081–1085.
5. Paranjpe A, Cacalano NA, Hume WR, Jewett A. N-Acetylcysteine protects dental pulp stromal cells from HEMA-induced apoptosis by inducing differentiation of the cells. *Free Radic Biol Med* 2007; **43**: 1394–1408.
6. Eropkina EM, Mamaeva EG, Eropkin M. [Effect of the bone cement on human fibroblast culture and possible ways of its correction by cytoprotector preparations]. *Eksp Klin Farmakol* 2003; **66**: 48–52.
7. Ciapetti G, Granchi D, Savarino L *et al*. *In vitro* testing of the potential for orthopedic bone cements to cause apoptosis of osteoblast-like cells. *Biomaterials* 2002; **23**: 617–627.
8. Vale FM, Castro M, Monteiro J *et al*. Acrylic bone cement induces the production of free radicals by cultured human fibroblasts. *Biomaterials* 1997; **18**: 1133–1135.
9. Yamada M, Kojima N, Paranjpe A *et al*. N-Acetyl cysteine (NAC)-assisted detoxification of PMMA resin. *J Dent Res* 2008; **87**: 372–377.
10. Lean JM, Davies JT, Fuller K *et al*. A crucial role for thiol antioxidants in estrogen-deficiency bone loss. *J Clin Invest* 2003; **112**: 915–923.
11. Meister A, Anderson ME. Glutathione. *Annu Rev Biochem* 1983; **52**: 711–760.
12. Gillissen A, Bartling A, Schoen S, Schultze-Werninghaus G. Antioxidant function of ambroxol in mononuclear and polymorphonuclear cells *in vitro*. *Lung* 1997; **175**: 235–242.
13. Gillissen A, Nowak D. Characterization of *N*-acetylcysteine and ambroxol in antioxidant therapy. *Respir Med* 1998; **92**: 609–623.
14. Nakamura H, Saruwatari L, Aita H, Takeuchi K, Ogawa T. Molecular and biomechanical characterization of mineralized tissue by dental pulp cells on titanium. *J Dent Res* 2005; **84**: 515–520.
15. Haque T, Uludag H, Zernicke RF, Winn SR, Sebald W. Bone marrow cells from normal and ovariectomized rats respond differently to basic fibroblast growth factor and bone morphogenetic protein 2 treatment *in vitro*. *Tissue Eng* 2005; **11**: 634–644.
16. Yamamura T. Differentiation of pulpal cells and inductive influences of various matrices with reference to pulpal wound healing. *J Dent Res* 1985; **64 (Special)**: 530–540.
17. Sloan AJ, Smith AJ. Stem cells and the dental pulp: potential roles in dentine regeneration and repair. *Oral Dis* 2007; **13**: 151–157.
18. About I, Bottero MJ, de Denato P, Camps J, Franquin JC, Mitsiadis TA. Human dentin production *in vitro*. *Exp Cell Res* 2000; **258**: 33–41.
19. Gronthos S, Brahim J, Li W *et al*. Stem cell properties of human dental pulp stem cells. *J Dent Res* 2002; **81**: 531–535.
20. Liu J, Jin T, Ritchie HH, Smith AJ, Clarkson BH. *In vitro* differentiation and mineralization of human dental pulp cells induced by dentin extract. *In Vitro Cell Dev Biol Anim* 2005; **41**: 232–238.
21. Yokose S, Kadokura H, Tajima Y *et al*. Establishment and characterization of a culture system for enzymatically released rat dental pulp cells. *Calcif Tissue Int* 2000; **66**: 139–144.
22. Ciapetti G, Granchi D, Cenni E, Savarino L, Cavedagna D, Pizzoferrato A. Cytotoxic effect of bone cements in HL60 cells: distinction between apoptosis and necrosis. *J Biomed Mater Res* 2000; **52**: 338–345.
23. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002; **82**: 47–95.
24. Spagnuolo G, Galler K, Schmalz G, Cosentino C, Rengo S, Schweikl H. Inhibition of phosphatidylinositol 3-kinase amplifies TEGDMA-induced apoptosis in primary human pulp cells. *J Dent Res* 2004; **83**: 703–707.
25. Achanta G, Huang P. Role of p53 in sensing oxidative DNA damage in response to reactive oxygen species-generating agents. *Cancer Res* 2004; **64**: 6233–6239.
26. Besaratinia A, Pfeifer GP. DNA adduction and mutagenic properties of acrylamide. *Mutat Res* 2005; **580**: 31–40.
27. Kleinsasser NH, Schmid K, Sassen AW *et al*. 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* 2006; **27**: 1762–1770.
28. Kleinsasser NH, Wallner BC, Harreus UA *et al*. Genotoxicity and cytotoxicity of dental materials in human lymphocytes as assessed by the single cell microgel electrophoresis (comet) assay. *J Dent* 2004; **32**: 229–234.
29. Yoshii E. Cytotoxic effects of acrylates and methacrylates: relationships of monomer structures and cytotoxicity. *J Biomed Mater Res* 1997; **37**: 517–524.
30. Spagnuolo G, Mauro C, Leonardi A *et al*. NF-kappaB protection against apoptosis induced by HEMA. *J Dent Res* 2004; **83**: 837–842.
31. Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A. 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* 2003; **66**: 476–42.
32. Lee DH, Lim BS, Lee YK, Ahn SJ, Yang HC. Involvement of oxidative stress in mutagenicity and apoptosis caused by dental resin monomers in cell cultures. *Dent Mater* 2006; **22**: 1086–1092.
33. Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of *N*-acetylcysteine actions. *Cell Mol Life Sci* 2003; **60**: 6–20.
34. Haddad JJ. Oxygen sensing and oxidant/redox-related pathways. *Biochem Biophys Res Commun* 2004; **316**: 969–977.
35. Wagner EF. Functions of AP1 (Fos/Jun) in bone development. *Ann Rheum Dis* 2002; **61 (Suppl 2)**: ii40–ii42.
36. Karin M, Lin A. NF-kappaB at the crossroads of life and death. *Nat Immunol* 2002; **3**: 221–227.