

# Cytotoxicity of Dental Nanocomposite Particles

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## ABSTRACT

Safety assessment of nanocomposites needs to include the potential toxicity of nanoparticles. In this study we evaluated the cytotoxicity of a dental nanocomposite polishing dust, bis-GMA and Silica Aerosil fillers. L929 cells were incubated with test materials for 72h at 37°C/ 5%CO<sub>2</sub> and cell viability was detected using the MTT assay. Nanocomposite particles were mildly cytotoxic to L929 cells. SEM analysis showed NC particles adhered to cells in culture while TEM analysis showed cellular uptake of NC particles was restricted to the cytoplasm. Silica Aerosil OX50 was cytotoxic with an IC<sub>50</sub> value of 58 µg/ml. The IC<sub>50</sub> of bis-GMA was 15 µM. Addition of silica Aerosil OX50 worsened the dose-response of bis-GMA depending on the dose of silica. Our findings indicate that NC particles are taken up into cells and reside in the cytoplasm exhibiting some cytotoxicity to the cells, which could be due to the effects of the silica particles, unreacted methacrylates or both.

**Keywords:** nanocomposite, nanoparticles, cytotoxicity, silica Aerosil OX50, bis-GMA

## 1 INTRODUCTION

Current safety assessment of dental composites is focused on measuring the toxicity of leachable unreacted monomers [1]. With the advent of nanostructured materials for development of new dental composites, toxicity assessment needs to include the potential toxicity of nanoparticles [2]. Nanosized fillers have been used as one approach to improving the wear and esthetics of dental composites [3,4]. Some nanofill composites contain non-agglomerated particles of 20-75 nm and others contain nanocluster fillers that are loosely bound agglomerates of nanosized particles [5]. It has been reported that procedures such as polishing of the composite restoration can give off nanoparticles of <100 nm diameter [3,4]. Additionally, nanoparticles may be released during normal masticatory activities. Both sources of nanoparticles can alter the biocompatibility of dental nanocomposites. While silica fillers have been reported inert, bis-GMA has been shown cytotoxic in vitro and in vivo. Bis-GMA is not only cytotoxic [6], but it is also a skin sensitizer [7], DNA damaging [8], chromosome damaging [9] and apoptotic.

With these many activities, the interaction of dental monomers with nanoparticles becomes important for biocompatibility assessment of dental nanocomposites. The objective of this study was to evaluate the cytotoxicity of a nanocomposite dust and related components such as the methacrylate bis-GMA and silica fillers. It is thought that a nanocomposite dust particle could be a carrier of unreacted monomers and the toxicity measured can be the results of each leachable or a combination of both.

## 2 MATERIALS AND METHODS

### 2.1 Materials

The nanocomposite (NC) Filtek Supreme Plus, dental composite Z250, and the monomer resin bis-GMA were kindly donated by 3M-ESPE (Minnesota, US). Aerosil 200 and Aerosil OX50 (Degussa® Corporation, Germany) are amorphous silica powders containing silicon dioxide (SiO<sub>2</sub>) particles with mean particle size of 10 and 40 nm, respectively.

### 2.2 Characterization of Nanocomposite Dust

The NC was polymerized by light curing for 60 sec (3M curing light XL3000). Then, the polymer was polished using a finishing bur in a high-speed hand piece (Quite Air). In this procedure, generated dust was collected into a sterile glass container. This work was done in a bio-safety hood. Dust particles were analyzed by SEM in two ways: 1) dust particles were collected on a sticky surface for direct SEM reading and 2) dust particles were suspended in water, placed on thermanox plastic disks, solvent evaporated and prepared for analysis by SEM. This analysis was contrasted to the conventional dental composite Z250, which dust particles were prepared as for the NC. Elemental analysis was done by Energy Dispersive Spectrometry (EDS).

### 2.3 Cell Viability Assay

L929 mouse fibroblast cells, NCTC clone L929) were obtained from ATCC (Manassas, VA). Cells were grown in Eagle's MEM media (Sigma M4655), supplemented with 10% (v/v) fetal bovine serum, 100 units/ml penicillin, 0.1 mg/ml streptomycin. Cells (8000 cells/well) were incubated with test materials for 72h at 37°C, 5%CO<sub>2</sub> in a final

volume of 200  $\mu\text{L}$ . At end of incubation, the supernatant was removed and the monolayer of cells was assayed for cell viability using the methyl tetrazolium (MTT) salt. This assay detects the metabolic capacity of cells to biotransform the MTT reagent into formazan, which was measured at 570 nm in a microplate reader. Changes in cell viability were calculated as percent from the control cells.

### 3 RESULTS

Nanocomposite polishing dust contained clusters of “ready to give off” particles, with average diameter of  $29.9 \pm 2.9$  nm,  $n=10$  (Fig. 1). In contrast, Z250 composite dust shows average length of  $127 \pm 93$  nm for small particles,  $n=10$  (Fig. 2). Elemental analysis indicated particle composition as silica (Si) for the nanocomposite (Fig. 3) and the conventional composite.

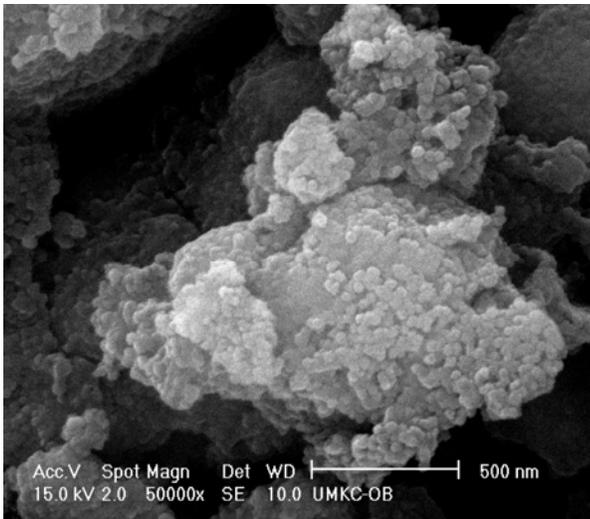


Figure 1. SEM picture of nanocomposite cluster

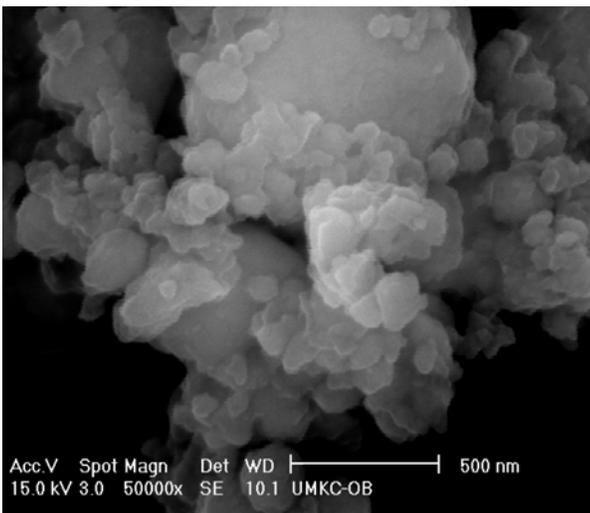


Figure 2. SEM pictures of Z250 composite dust

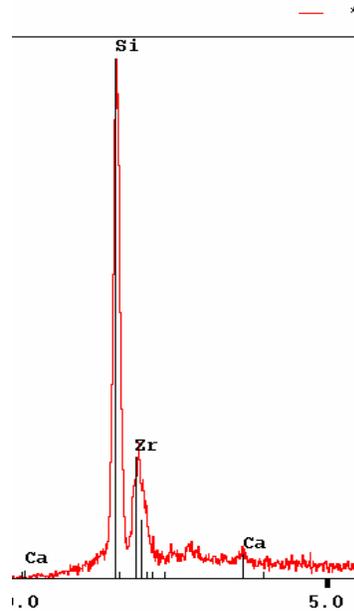


Figure 3. Elemental analysis with EDS indicated the major element composition in the nanocomposite was Si

Nanocomposite dust particles dispersed in water settled to the bottom of containers as agglomerates of different sizes including those of  $<100$  nm agglomerates (Fig. 4, SEM).

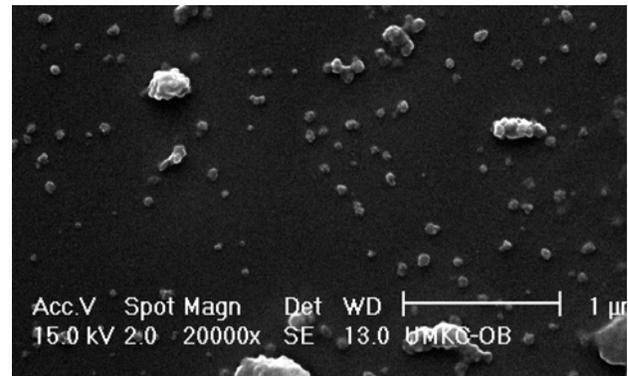


Figure 4. Settling Nanocomposite particles

#### 3.1 Cytotoxicity of Nanocomposite Dust

Nanocomposite particles were mildly cytotoxic to cells with percent of viable cells different from the control ( $p < 0.05$ ) (Fig. 5). The cytotoxicity of particles suspended in water (NC1) were not different ( $p > 0.05$ ) from particles washed with ethanol for 4 hours, dried and re-suspended in culture media (NC2) for viability testing. This treatment was done to get rid off un-reacted methacrylate monomers. SEM analysis of cells during incubation period showed adherence of NC particles to the cell surface (Fig. 6). TEM photographs of L929 cells indicated uptake of nanocomposite particles into the cell, however, particle uptake was restricted to the cytoplasm and did not enter the nucleus (Fig. 7).

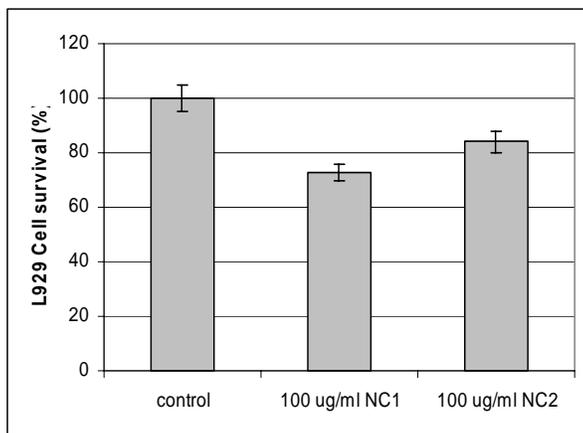


Figure 5. Mild cytotoxicity of nanocomposite dust before (NC1) and after (NC2) washing with ethanol

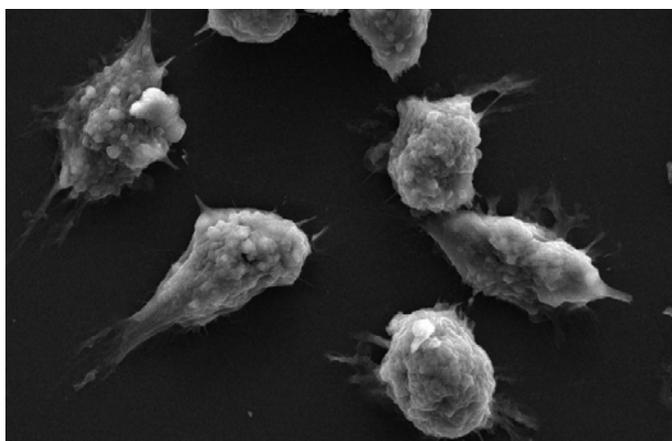


Figure 6. Adherence of Nanocomposite dust particles to surface of cells (SEM picture)

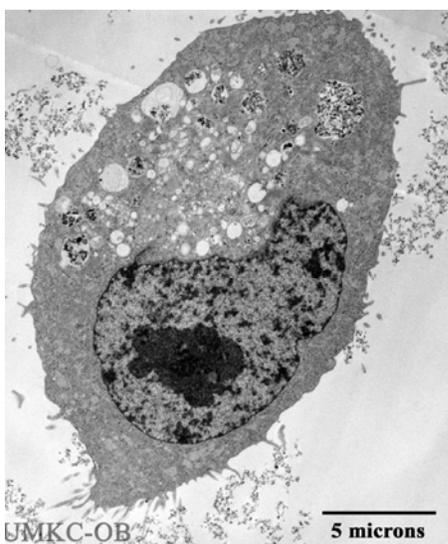


Figure 7. L929 cell uptake of nanocomposite particles

### 3.2 Cytotoxicity of Components

Silica fillers and bis-GMA were evaluated for cell viability effects under similar conditions as for NC studies. Several doses ( $\mu\text{g}/\text{ml}$ ) of silica Aerosil OX50 were incubated with 8000 cells per well and after 72 hour incubation, cell viability was measured with the MTT reagent. The silica Aerosil OX50 was cytotoxic to L929 cells (Fig. 7). 58  $\mu\text{g}/\text{ml}$  of silica Aerosil OX50 inhibited the normal cell proliferation of cells by 50% ( $\text{IC}_{50}$ ). Under similar conditions, the  $\text{IC}_{50}$  of BisGMA in DMSO 0.4% was 15  $\mu\text{M}$ . The cell viability exhibited by 3.1 and 12.5  $\mu\text{M}$  bis-GMA was decreased upon addition of a subtoxic dose of silica OX50 (40  $\mu\text{g}/\text{ml}$ ) particles (Fig. 8). However, upon addition of a toxic dose of silica Aerosil OX50 (75  $\mu\text{g}/\text{ml}$ ), the dose-response of bis-GMA was more deadly to the L929 cells. In contrast to silica Aerosil OX50, silica Aerosil 200 was non-cytotoxic up to 1mg/ml even though cellular uptake of Silica occurred as in Figure 6.

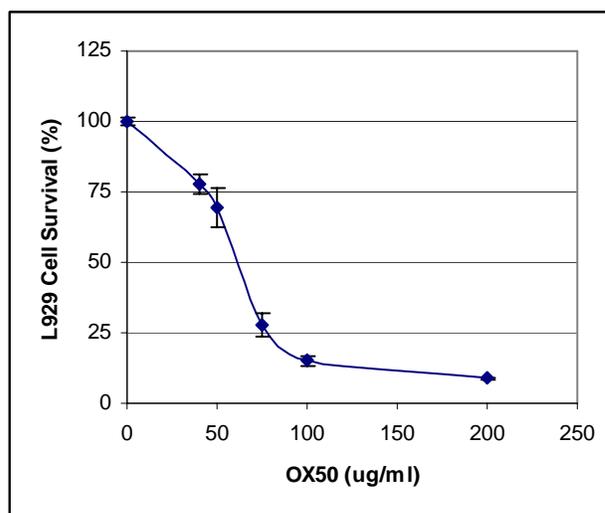


Figure 7. Cytotoxic dose-response of silica Aerosil OX50

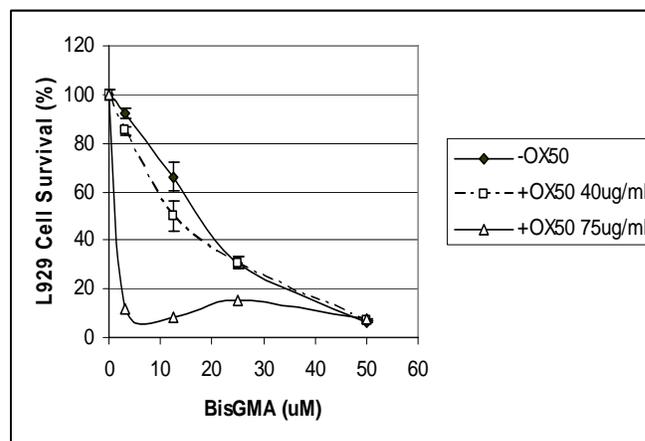


Figure 8. Cytotoxicity dose-response of bis-GMA alone and in mixtures with silica Aerosil OX50 particles

## 4 DISCUSSION

In the process of nanocomposite polishing, dust with large clusters of nanoparticles (Fig. 1) made of silica element (Fig. 3) were obtained. Large agglomerates (~1  $\mu\text{m}$ ) of nanocomposite dust and silicon dioxide particles were found in the cytoplasm of cells (Fig. 6) suggesting that re-agglomeration of nanoparticles may have occurred.

However, cellular uptake of particles was restricted to the cytoplasm and their absence in the nucleus may favor lack of interaction with genetic material making these devoid of mutagenic effects as for most particulate matter [10, 11].

A property of the nanocomposite particles was their adherence to the cell surface while in culture conditions. This property has been reported previously for Minusil silica particles, which was associated with binding of silica to cell membrane phospholipids, formation of free radicals and increases in paracellular permeability of lung alveolar epithelial cells [12]. In our study with L929 fibroblast cells, the adherence property may have facilitated cellular uptake of silica particles (Fig.6), and in turn this may have caused the cellular damage exhibited by nanocomposite particles and silica Aerosil OX50. A previous study with A549 epithelial cells demonstrated the cytotoxicity of amorphous silica was in part related to their cellular uptake also [13].

Our cell viability studies indicated that nanocomposite particles are taken up into cells; reside in the cytoplasm and exhibit mild cytotoxicity to the cell monolayer (Fig.6). This cytotoxicity was measured as a loss of 20% of viable cells. This level of loss in cell viability was observed also with some concentrations of silica Aerosil OX50 (Fig. 7) and bis-GMA (Fig. 8) but not with Silica Aerosil 200 (data not shown).

We postulated that the level of toxicity exhibited by nanocomposite dust particles must account for the effect of their methacrylate resin component. Accordingly, nanocomposite dust particles could be seen as carriers of unreacted monomers and the toxicity measured could be the result of the methacrylate monomers, the silicon dioxide or a combination of both. It is well known that the unreacted dimethacrylate component, bis-GMA, is cytotoxic to many types of cell monolayers at low and high cell densities [6, 8]. With low cell density, cell proliferation was inhibited in the presence of bis-GMA, which required a concentration of 15  $\mu\text{M}$  to inhibit 50% ( $\text{IC}_{50}$ ) of cell growth.

Studies with mixtures of bis-GMA and silica Aerosil OX50 exhibited different responses. At sub-toxic concentrations of bis-GMA and silica Aerosil OX50 there was a synergistic effect towards increased cytotoxicity. However, when bis-GMA was evaluated in the presence of a toxic concentration of silica Aerosil OX50, (75  $\mu\text{g}/\text{ml}$ ), the dose-response of bis-GMA was more deadly to cells (Fig. 8), the toxic dose of silica predominated. However, these results can not be directly related to the cellular effects exhibited by the NC particles in this study because the filler components of the dental nanocomposite were proprietary.

Future studies must undertake the evaluation of known nanocomposite formulations such that the cellular effect of the mixtures of monomers and nanofillers can be evaluated in a systematic way. We also recommend studies that include the exposure of cells to nanocomposite dust particles for longer than 72h incubation periods. This may be more relevant to the biocompatibility of dental nanocomposites in view of their advantageous longevity and polish retention [11]. Further studies are also needed to investigate the effects of nanocomposite particles on paracellular permeability and their relevance to inhalation of nanocomposite dust particles during polishing procedures.

## KNOWLEDGMENT

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