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Cytotoxic effects of composite dust on human bronchial epithelial cells

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ABSTRACT

Introduction. Previous research revealed that during routine abrasive procedures like polishing, shaping or removing of composites, high amounts of respirable dust particles (<5 μm) including nano-sized particles (<100 nm) may be released.
Objective. To determine the cytotoxic potential of composite dust particles on bronchial epithelium cells.
Methods. Composite dust of five commercial composites (one nano-composite, two nano-hybrid and two hybrid composites) was generated following a clinically relevant protocol. Polymerized composite samples were cut with a rough diamond bur (grain size 100 μm, speed 200,000 rpm) and all composite dust was collected in a sterile chamber. Human bronchial epithelial cells (16HBE14o-) were exposed to serially diluted suspensions of composite dust in cell culture medium at concentrations between 1.1 and 3.3 mg/ml. After 24 h-exposure, cell viability and membrane integrity were assessed by the WST-1 and the LDH leakage assay, respectively. The release of IL-1β and IL-6 was evaluated. The composite dust particles were characterized by transmission electron microscopy and by dynamic and electrophoretic light scattering.
Results. Neither membrane damage nor release of IL-1β was detected over the complete concentration range. However, metabolic activity gradually declined for concentrations higher than 660 μg/ml and the release of IL-6 was reduced when cells were exposed to the highest concentrations of dust.

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Significance. Composite dust prepared by conventional dental abrasion methods only affected human bronchial epithelial cells in very high concentrations.

1. Introduction

During last decade, the use of engineered nanoparticles (NP), which are particles with a size smaller than 100 nm in all dimensions, has tremendously been rising. They are used in the production of electronics, tires, fuel cells, cosmetics, drugs and numerous other products. However, potential health effects during and after exposure to this particles have to date still not been sufficiently investigated and nanotoxicology has emerged as a new field within toxicology [1].

Also in dental materials engineered NP are used, in particular as filler particles in restorative composites. The addition of filler particles to composites is important to reduce polymerization shrinkage, to reduce thermal expansion and contraction stresses, to increase the mechanical strength, and to reduce water sorption [2]. Most used filler particles consist of silicon based materials under the form of pyrogenic silica or aluminum silicate glasses. These particles nowadays also contain some extra elements with high atomic masses, such as barium, strontium, zirconium or ytterbium to render composites radiopaque. Although the size of these amorphous glass particles is usually between 0.4 and 1 μm, they are usually combined with nano-particles (typically pyrogenic silica) to obtain optimal packing and filler load. To date, only one manufacturer succeeded in developing a composite material (Filtek Supreme XTE, 3M ESPE, St-Paul, MN, USA) consisting only of nano-sized filler particles while preserving the right viscosity necessary for manual manipulation.

Previous research showed that relatively high amounts of so-called 'respirable dust', which is the fraction of inhalable dust that may penetrate deeply into the lungs due to its small dimensions (<5 μm), can be released during routine procedures such as grinding and polishing new composites [3]. More in-depth research using state-of-the-art equipment revealed that most airborne composite dust was even nano-sized, irrespective of the type of composite [4]. Most composite dust consisted of small pieces of composite with the filler particles held together by resin, but single filler particles could also be observed by transmission electron microscopy (TEM) [4]. Considering the still increasing use of composites, not only as restorative material to restore decayed, traumatized or worn teeth, but also as pits and fissure sealant, and bracket adhesive in orthodontic dentistry, dentists and dental assistants may be exposed to this ultrafine (particulate matter with aerodynamic diameter <100 nm) composite dust on a daily basis.

Depending on the chemical composition, reactivity and size, respirable dust may induce adverse pulmonary and extrapulmonary health effects [1]. Most of the toxicological studies, with regard to composite materials, have focused on the toxicity of released components from bulk samples, while there is a lack of information regarding the toxicity of composite materials in the form of composite dust. There are, however, some reports that indicate that dental personnel is at higher risk for developing pulmonary symptoms, such as asthma [5,6].

The aim of this study was to examine different cell responses of human bronchial epithelial cells after exposure to composite dust particles. Viability, cytotoxicity, as well as the release of IL-1β and IL-6 were evaluated using a bronchial epithelial cell culture model. The null hypothesis of this study was that composite dust is not toxic for human bronchial epithelial cells.

2. Materials and methods

2.1. *Particulate matter sampling*

Five different commercial composites were used in this study (Table 1). They represent four different composite types according to filler size, including nano-composite, nano-hybrid, micro-filled hybrid and conventional hybrid composite. Samples in the form of composite sticks, with size 17.4 × 5.4 × 1.6 mm, were prepared in a metal mold. After application in the mold, the unpolymerized composite was covered with a glass plate in order to avoid the formation of an oxygen inhibition layer. Both sides of the samples were cured for 40 s according to the manufacturers' instructions, with a light-curing lamp (Bluephase 20i, Ivoclar-Vivadent, Schaan, Liechtenstein) with an output >1100 mW cm^{-2}. After quick contact decontamination (5 s in 70% ethanol (Hydral 70, VWR, Haasrode, Belgium)), composite samples were transferred to a sterile vial, which was sealed by an autoclavable sheet (Sterichamps® S4, Paul Hartman AG, Selestat Cedex, France) (Fig. 1). The vial was beforehand treated for 4 h at 200 °C to remove possible endotoxin contamination and subsequently autoclaved together with a counter-angle handpiece (Kavo Intracompact handpiece, Kavo, Biberach, Germany). Next, the handpiece was connected outside the vial to an electric micro-motor (EWL K9, Kavo) and the composite sample was ground with a rough diamond bur (842314014 Komet, Lemgo, Germany, grain size 100 μm). For each composite, another bur was used, which was cleaned ultrasonically in between. After maximum 5 times, the bur was replaced. All composite dust was collected in the vial. The whole procedure was performed in a laminar flow cabinet and composite dust was checked for bacterial contamination by incubation of the suspensions in bacterial medium (Brain heart infusion, BHI, Becton Dickinson and Company, Franklin Lakes, NJ, USA) for 24 h at 37 °C and 5% CO_2 [7]. For each composite, this procedure was carried out following the same standardized protocol.

Afterwards, composite dust particles were transferred to endotoxin free glass test tubes, suspended with cell culture