Nanoparticles in paints: A new strategy to protect façades and surfaces?

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Abstract. The paint and lacquer industries consider the use of nanosilver, photocatalytically active nanotitanium dioxide or nanosilica dioxide as additives for the protection of surfaces, against microbial, physical and chemical deterioration, as an alternative to conventional organic based additives. Nowadays it is not clear, if nanoparticles in paints will achieve the proposed effects, since there are no long time studies available. Another fact is that the potential risks of nanoparticles for the environment and the human health is still controversial discussed.

The most sensitive entry port for nanomaterials is the lung. However other human organs/systems may also be affected by nanoparticles. Therefore the aim of the study was to assess the potential hazard effects of the three most interesting particles for paints on the gastro-intestinal tract and the immune system in vitro.

In our study we could show that:

i) Nanosilver (TEM size 25 nm) was far less toxic than silver ions of comparable concentrations tested with cells representing the gastro-intestinal tract (CaCo-2) and immune cells (Jurkat, T-lymphocytes). A significant amount of necrotic cells could be observed after exposure of CaCo-2 cells to 27 µg/ml nanosilver for 48 h.

ii) Nanotitanium dioxide can adsorb UV-light and in the presence of water hydroxyl radicals are generated photocatalytically. The exposure of CaCo-2 cells and Jurkat cells to photocatalytically active nanotitanium dioxide (Hombikat UV 100, TEM-size 15 nm) under dark conditions, didn’t affected the cells significantly. However, the cells were able to incorporate nanotitanium dioxide, especially when cells were exposed to higher concentrations.

iii) Nanosilica dioxide improves the properties of the paints by increasing the water repellence. When cells were exposed to 243 µg/ml nanosilica dioxide (TEM-size 19 nm) for up to 48 h no cytotoxic effect could be observed.

1. Introduction

Microbial growth on façades is an aesthetical problem. Microorganisms are among others actively involved in the weathering and deterioration of façades and paints. In order to save energy more and more buildings have a thermal insulation. This increases the duration of wet periods due to dew condensation. This makes the system particularly vulnerable to biological attack, necessitating the use of biocides [1].

Since the ecotoxic biocides with a wide-protection range are forbidden by law (Biocidal Products Directive 98/8/EC), the paint industry is forced to use non-ecotoxic, non-persistent biocidal substances, e.g. organic based biocides [2]. Therefore the paint industry also considers using nanoparticles to increase the paint properties and to create new paint formulations, which fulfil the Biocidal Products Directive 98/8/EC regulations.

Paints containing biodegradable biocides might lose their protective impact, because the biocides could get washed out or could get inactivated by physical/chemical sources. Therefore the paint industry is focussing on different strategies to overcome this problem. The replacement of organic based, degradable biocides by nanomaterials is one of the different strategies, which the paint industry has taken in consideration. The paint industry considers therefore to use nanomaterials, such as nanosilver, photo-catalytically active nanotitanium dioxide or nanosilica dioxide to improve paint
properties, such as water repellence, scratch resistance, increase of the hydrophobicity and antimicrobial properties [3].

Silver nanoparticles incorporated into the paints act as depot of silver ions. The release of the ions is tuned by particle size and surface coating. How silver ions interact with microorganisms is not fully understood. However, silver ions are able to penetrate into bacteria and once in the cell they will interact with thiol groups of vital enzymes and inactivate them, which leads to dysfunction and cell death [4]. The biocidal potency of a silver additive is therefore directly related to the potential for releasing silver ions. In addition silver ions are interacting with DNA and can inhibit respiratory enzymes, as well as induce oxidative stress upon generation of reactive oxygen species (ROS) [4, 5]. Ionic silver is also able to bind to sulphur and phosphor containing molecules involved in the cell antioxidant defence [6].

The lacquer and paint industry uses photo-catalytically active nanotitanium dioxide as biocide. Photo-catalytically active nanotitanium dioxide absorbs UV-light and produces in the presence of water hydroxyl radicals. These hydroxyl radicals are strong oxidizers and can induce oxidative stress and inflammation leading to oxidative attacks of cellular constituents and by that protecting the painted surfaces from microbial growth [7, 8]. Cytotoxicity of photo-catalytically active nanotitanium dioxide is controversially discussed in literature. Size, chemistry, coating and functionalization determine the cytotoxicity of titanium dioxide. Especially the modification of the nanoparticles can change toxicological properties completely compared to the parent compound.

Nanosized silica dioxide is widely used in wood preservation (hydrophobation) and coating applications. Nanosilica dioxide in paints improves scratch resistance, increases the water repellence, protects the paint against corrosion and provides the products a high gloss [9]. Nanosilica dioxide does not agglomerate and is therefore very persistent in the water-phase. Exposure to high silica dioxide dust should be avoided. Dry blasting hazardous paints can still lead to an elevated release of silica dioxide and other metals. Exposure to high concentrations of respirable silica dioxide can lead to silicosis and cancer [10, 11]. Nanosilica dioxide can in addition induce necrosis at high concentration, when nanosilica dioxide doped paints are coming in contact with human tissue [12].

The most sensitive entry port for nanomaterials is the lung. The pulmonary uptake is the most probable scenario of exposure and therefore intensively studied [13]. However, other organs/systems may also be affected by nanoparticles. Nanoparticles might be released from the façade and transferred by run-off water into the surface water, and accidently consumed by animals and/or children. Therefore nanoparticles might come in contact with cells of the gastro intestinal tract and might affect also our immune system.

The aim of this study was therefore to focus on possible effects of nanomaterials present in surface water to cells of the gastro intestinal tract (CaCo-2 cells), as well as on our immune system (Jurkat cells). Reports about possible effects that can occur, when CaCo-2 cells or Jurkat cells are exposed to nanoparticles is sparse.

Metallic nanomaterials, which are releasing metallic ions, seemed to be the most toxic nanomaterials among all synthetic nanomaterials. The bioavailability and toxicity of these metallic nanoparticles (e.g. copper) is highly dependent on the amount of adsorbing particles in the water column [14]. Nanoparticles bound to other compounds forming larger complexes are less bioavailable and therefore less toxic. Nanoparticles, which are released and transferred into the surface water, can cause toxicological problems, as long as they are not adsorbed to other particles [15].

Fortunately the concentrations of nanometals (nanosilver, nanotitanium dioxide, nanozinc oxide) found in surface waters are in the range of ng/l, which is still low [16]. A goal of this study was to estimate the cytotoxic concentrations of different nanoparticles (nanosilver, nanotitanium dioxide and nanosilica dioxide), which might be used by the paint industry in the near future to improve the properties of the paints. The nanoparticles might be released from the paints and might be transferred into the surface water. Therefore CaCo-2 cells and Jurkat cells were exposed to different concentrations of nanoparticles and the cytotoxic concentrations of these particles were estimated.
2. Materials and Methods

2.1. Cell cultures
In this study the effects of three nanoparticles was evaluated on two different cell types (CaCo-2 cells and Jurkat cells). CaCo-2 cells were derived from the gastrointestinal tract and were used to investigate the cytotoxic effect of ingested nanoparticles via drinking water. CaCo-2 cells (order number 86010202) were obtained from Health Protection Agency Culture Collections (HPACC), Salisbury, UK. Jurkat cells are human T-lymphocytes and were used to investigate the immunotoxic effect of nanoparticles and paints. This cell line was provided by Dr. S. J. Korsmeyer, Harvard Medical School, Boston, USA.

2.2. Nanoparticles
Nanosilver, TEM-size 25 nm.
Nanotitanium dioxide, TEM-size 15 nm.
Nanosilica dioxide, TEM-size 19 nm.

Table 1. Nanoparticle characterization

| Material          | Nanosilver | Nanotitanium dioxide | Nanosilica dioxide |
|-------------------|------------|----------------------|--------------------|
| Function          | Biocide    | Biocide              | Scratch resistance |
| Endotoxin         | No         | No                   | No                 |
| contaminated      |            |                      |                    |
| Primary particle size and morphology (TEM) | 25 nm (spherical) to 80 – 90 nm (rods) | 15 nm | 19 nm |
| Hydrodynamic diameter (nm) | 91 nm | 386 nm | 195 nm |
| Zeta potential (mV) | -42 mV | -25 mV | -14 mV |
| Shape             | Some spherical, others rods | Spherical | Spherical |

The nanoparticles were examined by different physical-chemical methods, such as dynamic light scattering (DLS), zeta potential analysis, transmission electron microscope analysis (TEM), according to Smulders et al. [17].

2.3. Endotoxin measurement
The investigated nanoparticles were tested for potential endotoxin contaminations prior starting the cytotoxicity studies. In this study a kinetic chromogenic LAL assay (Limulus amebocyte lysate endosafe endochrome-K; US License 1197; Charles River Laboratories, Inc., Charleston, SC, USA) was used for testing the endotoxin content in the different nanoparticle-samples. The test procedure was performed according to the protocol of Charles River Laboratories, USA.

2.4. Apoptosis/necrosis
Cell viability was quantitatively examined by flow cytometry analysis (FACS) based on the binding of annexin V fluorescein and propidium iodide incorporation to distinguish between apoptotic and late apoptotic/necrotic cells. The staining procedure was performed according to the user protocol (PF032) of Calbiochem, D. The procedure was described in detail by Kaiser et al. [18].
2.5. Reactive oxygen species (ROS)
The release of reactive oxygen species (ROS) was investigated with the 2, 7-dichlorodihydro-
fluorescein reduction assay. The fluorescence intensity, which is a function of liberated reactive
oxygen species was analysed with a fluorescence plate reader at a wave length 485/528 nm. The
procedure was described in detail by Limbach et al. [19].

2.6. Release of cytokines
Nanoparticles may also activate stress-related kinases, which can induce the release of
proinflammatory cytokines, such as tumour necrosis factor α (TNF-α) and interleukin 8 (IL-8).
Interleukin 8 was shown to increase significantly in response to a variety of chemical irritants, following
the activation of primary proinflammatory cytokines, such as TNF-α. The release of proinflammatory
cytokines (TNF-α and IL-8) was analysed with an enzyme linked immunosorbent assay (Elisa),
(eBioscience, Inc. San Diego, CA, USA).

2.7. Cell morphology
Cellular uptake, respectively cell morphology was analysed by bright field microscopy; phase contrast
method at a magnification of 100 x (Nikon-Diaphot, Egg, CH). Cell morphology was in addition
investigated by fluorescence microscopy after staining the actin cytoskeleton with phalloidin-Alexa
Fluor 488 (Invitrogen, Lucerne, CH) and the nucleus with Drag5 (Biostatus, Leicestershire, UK).

2.8. Genotoxicity
Genotoxicity was measured by the single cell gel electrophoresis (comet) assay. The electrophoresis
takes place under alkaline conditions. The DNA fragments start to move in the agarose gel and were
forming, depending on the damage of the genome, a comet. The DNA is thereafter stained with
ethidium bromide and comets were analysed by fluorescence microscope. Quantification of the head
and tail intensities was done using the “Comet Assay IV” software. The procedure was described in
detail by Hirsch et al. [20].

3. Results and Discussion

3.1. Endotoxin measurement
Since the production and the storage of nanomaterials are not occurring under sterile conditions, it
cannot be excluded that nanomaterials got contaminated with endotoxins. Therefore, prior starting our
cytotoxicity studies, the nanomaterials (nanosilver, nanotitanium dioxide and nanosilica dioxide) were
analysed. The three tested nanomaterials were endotoxin free or the endotoxin level was not
detectable.

3.2. Apoptosis/necrosis
After treatment with the three well characterized nanoparticles (nanosilver, nanotitanium dioxide and
nanosilica dioxide) cell viability on epithelial cells (CaCo-2) and T-lymphocytes (Jurkat) was analysed
by life/dead measurements (apoptosis/necrosis). After the cells were exposed to nanotitanium dioxide
or nanosilica dioxide in different concentrations (1, 3, 9, 27, 81 and 243 µg/ml) for 24 h and 48 h no
significant effect was observed. However, a significant amount of necrotic CaCo-2 cells were observed
after exposure to 27 µg/ml nanosilver for 48 h (Figure 1). Jurkat cells were more robust against
nanosilver than CaCo-2 cells. The observed amount of dead Jurkat cells was not significant, when
these cells were exposed to the same nanosilver concentration for 48 h. However, after exposure of
Jurkat cells to a nanosilver concentration of 81 µg/ml for 48 h a significant amount of dead cells could
be observed.
Figure 1. Apoptosis/necrosis has been analysed quantitatively after CaCo-2 cells were exposed to nanosilver in different concentrations (1, 3, 9, 27, 81 and 243 µg/ml) for 48 h. CdSO₄ (25 µM) was used as positive control and the cells were exposed for 24 h. Data were represented as mean ± SEM, n = 3.

3.3. Release of reactive oxygen species (ROS)
CaCo-2-cells exposed to nanosilver and nanosilica dioxide in a concentration range of 1 to 243 µg/ml for 1 h induced only a low amount of reactive oxygen species (ROS). Jurkat cells exposed to nanosilver and nanosilica dioxide reacted in a similar way (data not shown). However, Jurkat cells showed a higher ROS release, when exposed to nanotitanium dioxide. An elevated ROS generation was already noticed, when cells were exposed to 81 µg/ml nanotitanium dioxide (Figure 2).

Figure 2. Jurkat cells exposed to nanotitanium dioxide incorporated a high amount of the nanotitanium dioxide, which resulted in a significant release of ROS. 3-morpholinosydnonimine hydrochloride (5 µM) was used as positive control. Data represented as mean ± SEM, n = 3.
3.4. Release of proinflammatory cytokines, e.g. interleukins
When CaCo-2 cells were exposed to nanotitanium dioxide and nanosilica dioxide no significant amount of IL-8 was released at all three investigated exposure times. However, it was different when cells were exposed to nanosilver. Higher concentrations of released IL-8 were found after cells had been exposed to 27 µg/ml nanosilver for 24 h and 48 h (Figure 3). This was the nanosilver concentration, where a significant amount of necrotic cells could be observed. Jurkat cells again behaved different. Jurkat cells exposed to nanosilver, nanotitanium dioxide or nanosilica dioxide released only minor amounts of IL-2.

![Figure 3. Release of interleukin 8 during exposure of CaCo-2 cells to different concentrations nanosilver. Tumor necrosis factor α (TNF-α, 50 ng/ml) was used as positive control; the cells were exposed to TNF-α for 24 h. Data represented as mean ± SEM, n = 3.]

3.5. Genotoxicity
Genotoxicity was investigated with the comet assay. When CaCo-2 cells were exposed to 80 µg/ml nanosilica dioxide no significant effect on cell viability could be observed. When cell cultures were exposed to the same concentration (80 µg/ml) nanotitanium dioxide for 48 h some DNA strand brakes could be observed, however the effect was not significant. When CaCo-2 cells were exposed to 5 µg/ml nanosilver for 48 h more DNA strand brakes occurred. The effect was however not significant. But cells exposed to 10 µg/ml nanosilver for 48 h showed a significant genotoxic effect. The tail intensities of the cells in the comet assay reached similar values as the positive control. This indicates that nanosilver at these concentrations leads to high DNA strand brakes (Figure 4).
When CaCo-2 cells were exposed to 9 µg/ml nanosilver for 48 h, less than 20 % of the cells died and cell death was not significant. Thus the results obtained in the genotoxicity test were in accordance with the results obtained with the life/dead measurements (apoptosis/necrosis). When CaCo-2 cells were exposed to lower nanosilver concentrations no significant cell death as well as no significant genotoxicity could be observed.
3.6. Cell morphology

Cell morphology was analyzed after exposure of CaCo-2 cells and Jurkat cells to these three nanoparticles (nanosilver, nanotitanium dioxide and nanosilica dioxide) at concentrations up to 243 µg/ml for 48 h. Nanosilica dioxide (up to 243 µg/ml) had no visible effect on cell morphology. This was also true for nanotitanium dioxide. However, bright field microscopic pictures showed that the nanotitanium dioxide got either adsorbed to the extracellular matrix of the CaCo-2 cells and Jurkat cells and/or got incorporated into the cells. Cell morphology was not notably affected by the high incorporation of nanotitanium dioxide (Figure 5A, 5B). These observations were not unusual. Thus it has been reported by other scientists that nanotitanium dioxide was taken up by various cell types without causing cytotoxic effects during the observation period [21]. CaCo-2 cells and Jurkat cells exposed to nanosilver at a concentration of 27 µg/ml for 48 h didn’t show any visible effects on cell morphology. At higher nanosilver concentrations cell growth decreased and/or the adherent cells started to detach from the surface.

Figure 5A. Bright field microscopic pictures. A: CaCo-2 cells grown in absence of nanotitanium dioxide, B: CaCo-2 cells exposed to 243 µg/ml nanotitanium dioxide for 48 h.
Figure 5B. Bright field microscopic pictures. A: Jurkat cells grown in absence of nanotitanium dioxide, B: Jurkat cells exposed to 243 µg/ml nanotitanium dioxide for 48 h.

Fluorescence microscopic pictures show that CaCo-2 cells exposed to 27 µg/ml nanosilver for 48 h started to detach from the underlying surface (Figure 6A). CaCo-2 cells, which were still alive after these 48 h of exposure, were not spread anymore. The actin cytoskeleton was disrupted and the remaining actin fibres were located in the near vicinity of the nucleus.

Figure 6A. Fluorescent microscopic pictures. A: CaCo-2 cells grown in absence of nanosilver, B: CaCo-2 cells exposed to 27 µg/ml nanosilver for 48 h. Staining: nucleus (blue), actin filaments (green).

During the exposure of Jurkat cells to 81 µg/ml nanosilver for 48 h the development of the actin cytoskeleton (green) of these cells were severely affected (Figure 6B).

Figure 6B. Fluorescent microscopic pictures. A: Jurkat cells grown in absence of nanosilver, B: Jurkat cells exposed to 27 µg/ml nanosilver for 48 h. Staining: nucleus (blue), actin filaments (green).
4. Summary / Conclusion

When CaCo-2 cells and Jurkat cells were exposed to nanotitanium dioxide or nanosilica dioxide (up to 243 µg/ml for 48 h) no severe effects on cell death could be observed. However, bright field microscopic pictures showed that the nanotitanium dioxide got either adsorbed to the extracellular matrix of the cells and/or incorporated into the cells. This might cause dysfunction after a prolonged exposure period. Severe effects were found, when CaCo-2 cells and Jurkat cells were exposed to nanosilver. When CaCo-2 cells were exposed to 27 µg/ml nanosilver for 48 h different cell parameters, such as cell death, cytokine release and cell morphology were affected. The same was true for Jurkat cells. When Jurkat cells were exposed to nanosilver (81 µg/ml) for 48 h a significant amount of necrotic cells was observed and cells showed a change in cell morphology.

Thus, at the time it is not clear, if the paint industry will replace the biodegradable, organic based biocides by nanoparticles. The efficiency of antimicrobial active nanoparticles in paints is still questionable. Nevertheless first preliminary studies show that the mechanical abrasion of nanoparticle-containing paints should not result in an increase of free nanoparticles in the environment, compared to the sanding of conventional paints without nanoparticles. The small amounts of nanoparticles, which may still be released during surface treatment do not induce any acute toxic response. Therefore we consider a rather low risk, however to support our findings further investigations on chronic or long term effects were needed.

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