THE STUDY OF Ag/TiO_2 NANOMATERIALS USE FOR LEATHER AND FOOTWEAR INDUSTRY

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Ecologic and health effects of applying materials with advanced functions for leather surface finishing are priorities for the European leather industry and contribute to the increase of added value and durability of leather and fur articles. The innovative properties of Ag/TiO_2 nanomaterials (NMs) on leather surface are due to their antimicrobial, self-cleaning and flame retardant characteristics. The NANO_SAFE_LEATHER project aims to contribute to the industrial development of safer and advanced leathers that make use of Ag/TiO_2NMs, which are ecological alternatives to volatile organic biocides, organic solvents and halogenated flame retardants. The cytotoxicity study of the Ag/TiO_2NM's efficiency for leather functionalization related to the dose response on human health is very important for their large scale application in footwear industry. The paper presents the strategy of research regarding the leather surface finishing by spraying and by physical deposition of Ag/TiO_2NMs, the impact of NMs use in industrial environment and in connection with wearing behavior. *In vitro* assay to study the cytotoxicity of the Ag/TiO_2NMs, leather functionalized with Ag/TiO_2NMs and leached Ag/TiO_2NMs on different human cells, the cell morphology and cell death mechanism as well as the exposure risk assessment on lung cells will allow to estimate for the first time the impact of NMs industrial application in leather and footwear industry and on consumer health. *In silico* study of human cells in vitro interacted with Ag/TiO_2NMs provides information on the potential changes induced by the NPs on cellular morphology and helps build a domain ontology.

Keywords: nanomaterials, Ag/TiO_2NMs, human health, leather surface, footwear industry.

INTRODUCTION

Human exposure to nanomaterials (NMs) is inevitable as NMs diversify and become more widely used and, as a result, NMs toxicology research is extremely important for health and the environment. Silver nanoparticles (AgNPs) based materials are already being used in wound dressings, catheters, cosmetics, textiles, plastic materials and household products due to their antimicrobial activity. There were already identified 260 products on the market with AgNPs and with antibacterial, antifungal, antiviral properties and other 400-500 products are estimated to be processed with NMs (www.foodandwatereurope.org).

Recent research demonstrated the efficiency of photocatalytical activity in the UV domain and particularly in the visible domain of nanomaterials based on titanium dioxide doped with nanosilver against a wide spectrum of microorganisms. Other properties of nanomaterials relate to photocatalytical decomposition of dirt on the treated surface or heat/fire resistance. The area of smart properties of nanomaterials includes photocatalytical decomposition of volatile organic compounds, which allows elimination of unpleasant odours or reduction of fogginess inside vehicles. These properties have been exploited to develop multifunctional textiles (https://www.ic.gc.ca/eic/site/textiles-textiles.nsf/eng) and leather with antimicrobial, self-cleaning properties and/or resistance to heat/fire (Lkhagvajav *et al*., 2015; Gaidau *et al*., 2011; Bitlisli and Yumurtas, 2008; Petica *et al*., 2015; Gaidau *et al*., 2014; Gaidau *et al*., 2013; Yang *et al*., 2012).

AgNPs and nano TiO_2, single or doped (Ag/TiO_2NPs), are the most efficient in terms of multifunctional properties: antimicrobial, self cleaning or flame retardant characteristics. The recent progresses (Bitlisli and Yumurtas, 2008; Petica *et al*., 2015; Yang *et al*., 2012) in leather surface functionalization with NPs open a huge area of application in leather industry for innovative footwear, smart upholstery and garment leathers. It is already known that 20–25% of the world's population has skin mycoses, making these one of the most frequent forms of infection. Increasingly frequent *tinea pedis* caused by *T. rubrum* and *T. interdigitale* infection seems to be a characteristic of highly developed countries with booming sport and fitness facilities, increasing prevalence of obesity and diabetes mellitus and an ageing population. The leather surface treated with AgNPs and doped TiO_2 has been proved to protect feet against bacteria and fungi and is an ecological alternative to volatile organic biocides. Leather surface functionalized with doped TiO_2 has flame retardant properties, self cleaning activity under UV-Vis light and allows replacing of organic halogenated materials, while improving leather durability.

Einaga (2006) showed that deposition of silver on the surface of titanium dioxide is effective for improving the activity of titanium dioxide, and the optimum concentration was determined for 2% silver by weight when photocatalytic activity increased four times. Increased activity of titanium dioxide nanoparticles doped with noble metals, particularly silver, was demonstrated for *Escherichia coli* (Jingbo *et al*., 2013).

Silver nanoparticles are predominantly prepared via chemical reduction, an industrially applicable method due to its simplicity and high efficiency (Cao *et al*., 2010; Nainani *et al*., 2012).

The methods of synthesizing NPs include redox, electrochemical, hydrothermal, photochemical, growth method, microemulsion synthesis, synthesis using microwave, methods of evaporation and condensation, etc. Silver nanoparticles of different shapes and sizes can be obtained by chemical methods, depending on the precursors and conditions of synthesis, influencing antimicrobial properties.

Obtaining Ag/TiO_2 doped nanomaterials by chemical methods is a more recent approach and involves modifying the known sol-gel methods by impregnation and chemical reduction of silver ions during the synthesis of titanium dioxide, followed by calcination at 400°C with its transformation from amorphous to anatase crystalline form (Kedziora *et al*., 2012). Another method of obtaining two- and three-component composites is based on the Stöber method (Stöber and Fink, 1968) by means of which core-shell structures of silicon and titanium dioxide-nanosilver are obtained. Electrochemical synthesis of Ag/TiO_2 nanoparticles, the method adopted in this paper, has the advantage of obtaining pure products without reaction intermediaries, compared with the chemical synthesis methods (Anicai *et al*., 2013). NPs deposition by physical methods (vacuum deposition) is another innovative method approached in the research project, intended for the leather substrate with improved environmental effects compared with conventional spraying treatments.

The paper presents the main strategies addressed in the NANO_SAFE_LEATHER project for synthesis and characterization of nanoparticles and leather treated therewith in order to evaluate the cytotoxicological potential of their use in the industry, thus enriching the European database on the toxicological impact of NPs application.

MATERIALS AND METHODS

Processing the Surface of Leather with Ag/TiO_2 Nanoparticles

The research strategy targets deposition of silver-based nanoparticles onto the surface of leather using conventional techniques of spraying film-forming polymers containing Ag/TiO_2NP and Ag/N-TiO_2 NP and physical deposition techniques based on DC Reactive Sputter Deposition.

Obtaining and Characterizing Ag/TiO_2 NPs and Ag/N-TiO_2 NPs and Leather Surfaces Coated therewith Using Conventional Techniques and Physical Deposition

Ag/TiO_2NP and Ag/N-TiO_2NPs were obtained by electrochemical deposition of silver on the surface of TiO_2NPs using the so-called “sacrificial anode method” (Stöber and Fink, 1968). For this purpose, TiO_2NPs powder of 20 nm average size (TitanPE Technologies, Inc., China) well dispersed with Na-PAA in deionized water and electrodes of 99.999% purity Ag plates (155 x 27 x 0.5 mm) were used. The electrochemical process was carried out under current densities between 0.01-0.06 mA.cm^{-2} for 2-8 hours involving a constant current pulse reversed generator with a mechanical stirrer. The resulted dispersion was washed, centrifuged, dried and ground in powder form.

The main assessed characteristics of the NPs were the concentration of silver deposited on TiO_2NP (ICP –OES, Agilent 725), the average particle size of water dispersion in Na-PAA and Zeta potential (Nanosizer, Nano ZS Malvern).

The crust leather surfaces for footwear lining were prepared (SC Taro Commimpex LTD) for finishing by spraying and by physical deposition through DC Reactive Sputtering method.

The classical finishing of leather surface was done by spraying of film forming polymers mixed by mechanical and ultrasound stirring (Petica *et al.*, 2015) of Ag/TiO_2NP and Ag/N-TiO_2NP powders. The leather surfaces were evaluated under UV/visible light irradiation (VL 204 lamp with irradiation at 365 nm and 500W halogen lamp) for model stains of Methylene Blue discoloration (self-cleaning effect) and contact angle modification (VGA Optima XE). The antibacterial sensitivity tests against *Escherichia coli* and *Staphylococcus aureus* were performed according to EN ISO 20645.

The main parameters of DC reactive sputtering method were set for specific characteristics of leather support and by using a pure target of silver and a pure target of titanium to achieve antimicrobial characteristics.

The resistance against *Staphylococcus epidermidis was* tested by agar diffusion method for the leathers treated by DC Reactive Sputtering.

The surface characteristics of leather samples uncoated and coated with Ag/TiO_2 by DC Reactive Sputtering were determined through the sessile drop contact angle technique. The photocatalytic activity of the leather samples covered with Ag/TiO_2NPs by DC Reactive Sputtering were evaluated by the decomposition of an organic dye (Methylene Blue) with combined UV/visible light irradiation.

The leather wearing behavior was preliminary tested by tribological method using 4 different counterpart materials that cover a wide range of frictions, namely PPH (Polypropylene), POM (Polyoxymethylene), PTFE (Polytetrafluoroethylene), PA 6.6 (Polyamide 6.6 or Nylon 6.6), PUR (Polyurethane) and NBR 70 (Nitrile rubber).

Cytotoxicity Test Strategy for Ag/TiO*2* NP Evaluation. *In Silico* Study of Human Cells

In vitro cytotoxicity studies of nanoparticles using different cell lines, incubation times, and colorimetric assays are increasingly being published. *In vitro* model systems provide a rapid and effective means to assess nanoparticles for a number of toxicological endpoints. They also allow development of mechanism-driven evaluations and provide refined information on how nanoparticles interact with human cells in many ways. Such studies can be used to establish concentration–effect relationships and the effect-specific thresholds in cells. The cytotoxicity study of the Ag/TiO_2NP's efficiency for leather functionalization related to the dose response on human health is very important for their large scale application and has not been done so far. *In vitro* assessment of the impact of Ag/TiO_2NPs and of the leather finished with Ag/TiO_2NPs, on different human cell lines (HaCaT keretinocytes, A549 cell line) using cytotoxicity analysis (such as MTT cell survival assay), cellular morphology, the investigation of cell death mechanisms and of the intracellular signaling pathways activated by manufactured Ag/TiO_2NPs will enrich the knowledge on NPs effects on human health at cellular level.

In silico approach, which tests experimental data by computer simulation analysis, will provide information on the morphology of human cells. Morphological data extracted from the cytotoxicity studies will be processed to obtain virtual representations of human cells.

DISCUSSIONS AND CONCLUSIONS

Ag/TiO_2 NPs and $Ag/N\text{-}TiO_2$ NPs were synthesized by loading concentrations of 0.5 to 1.6% silver on the surface of TiO_2NPs and $N\text{-}TiO_2$ NPs.

The average particle sizes of Ag/TiO_2 NPs and $Ag/N\text{-}TiO_2$ NPs in aqueous dispersions were of 45 nm and of 69 nm, with Zeta potentials of -40mV, and -47 mV, respectively, which means good stability.

The leather surface characterization under UV/Vis light exposure showed photocatalytic properties attributed to active species of O and OH- generation with effect on contact angle decreasing (ten times as compared to the unexposed surface) and hydrophilic characteristics. The treated leather surfaces with $Ag\text{-}TiO_2$NPs were sensitive to tested bacteria (*Escherichia coli* and *Staphylococcus aureus)* as compared to untreated leather surfaces.

Ag/TiO_2 NMs samples were deposited on leather surface by DC Reactive Magnetron Sputtering. Two targets were used (a pure target of silver and a pure target of titanium) and current density was altered in order to change the chemical composition of the films. Other parameters of deposition as work pressure, gas flux were varied in order to optimize the films deposition and to protect the organic support of leather. The method allows depositing alternative layers of silver (with concentration up to 10%) and/or titanium dioxide with antibacterial or photocatalytic properties.

The photocatalytic properties under UV/Vis irradiation were achieved only on titanium dioxide covered leather surfaces against Methylene blue simulated stains while the antibacterial properties were successfully achieved on leather surfaces covered by Ag/TiO_2 NPs.

The primary results of hydrophobic characterization showed that the introduction of silver promotes an increase of contact angle with values round to 110º.

The preliminary agar diffusion test held at some leather functionalized with Ag/TiO_2 NPs against *Staphylococcus epidermidis* showed a clear halo (absence of bacterial growth) around the samples, which may indicate antibacterial activity in the preliminary depositions, and these results will be verified with quantitative tests further on in the project.

The tribological tests intend to estimate the wearing behavior of leather treated with Ag/TiO_2 NPs. In this regard the coefficient of friction as a function of the sliding was recorded for 6 counterpart combinations from which 4 were selected (PTFE, PA 6.6, PUR and NBR 70). The tests were performed at different loads of 1, 5 and 10 N with radii of 5, 7 and 10 mm, respectively. The angular velocity was adjusted for each radius to keep the linear velocity constant at 10 cm/s. The sliding distance was set to 2000 laps. The experiments are in progress and will set the best parameter for leather surface wearing behavior evaluation by tribological tests.

Cytotoxicity studies were performed by exposing human keratinocytes (HaCaT cells) to various concentrations of different Ag/TiO_2 NPs for 48 hours and the cellular viability was determined by MTT assay. The organization of actin cytoskeleton was visualized by fluorescence microscopy after incubation of cells with different concentrations of Ag/TiO_2 NPs, by staining the cells with fluorescently-labeled Phalloidin. The intracellular reactive oxygen species (ROS) levels were quantified using the fluorogenic dye 2',7'-dichlorofluorescein diacetate (DCFH-DA), that measures hydroxyl, peroxyl and other ROS within the cell, using a TECAN spectrophotometer. The research is in progress and will allow establishing the dose response for Ag/TiO_2 NPs use in industrial environment.

In silico approach to study the influence of the MNMs on human cells provides information on the potential changes induced by the NPs on cellular morphology and help build a domain ontology.

The main conclusions of NANO_SAFE_LEATHER project are as follows:

► successful deposition of Ag/TiO_2 NPs and $Ag/N-TiO_2$ NPs on leather surface was carried out by classical technologies;

► leather surfaces treated with $Ag/N-TiO_2$ NPs showed photocatalytic properties with improved self-cleaning effect in visible domain and antimicrobial resistance;

► AgNP, TiO_2NPs and Ag/TiO_2NPs were successfully deposited on leather surface by DC Reactive Magnetron Sputtering method;

► the main parameters of DC Reactive Magnetron Sputtering method were set for soft structure of leather surface without damaging the material and using two targets (Ti and Ag);

► self-cleaning properties were developed on leather surface covered with TiO_2NPs by DC Reactive Magnetron Sputtering;

► antimicrobial properties on leather surface were tested successfully for leather surface covered by DC Reactive Magnetron Sputtering with Ag/TiO_2NPs;

► electrochemical NPs deposited on leather surface in film forming polymers by spraying showed photocatalytic properties under UV/Vis light exposure attributed to the active species generation (O and HO^-) with hydrophilic surface effect, proved by contact angle measurements;

► Ag/TiO_2NPs deposited on leather surface by DC Reactive Magnetron Sputtering generated hydrophobic structure of leather surface proved by increased contact angle at the value of 110º. This new characteristic opens the way to developing self-cleaning properties based on hydrophobic effect of NPs;

► the tribological test of leather surface is an innovative approach for wearing behavior evaluation of leather surface treated with NPs and setting the appropriate parameters (loads, angular velocity, sliding distance, counterpart material) was the first progress of the research;

► cytotoxicity studies were performed on human cells exposed to different concentration of Ag/TiO_2NPs according to different protocols for evaluation of cell growth, death mechanism, intracellular reactive oxygen species quantification with the final aim of establishing the dose response for working in industrial environment;

► *in silico* studies of human cells interacted with Ag/TiO_2NPs will allow to predict and to explain the NPs influence on cell life and risks on human health.

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