ADDED FUNCTIONS OF LEATHER SURFACE BY Ag/TiO_2 NANOPARTICLES USE AND SOME CONSIDERATIONS ON THEIR CYTOTOXICITY

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Abstract. Nanoparticles showed a huge potential for new properties development in many economic sectors like electronics, medicine, textile, waste water treatment etc. The modification of surface functionality by using low concentrations of nanomaterials opens the possibility of lowering the ecological impact of chemical materials based on volatile organic compounds. The objectives of our research were related to the use of commercial nanoparticles based on Ag and TiO_2 with average particle size of 8 nm for leather surface functionalization and the investigation of the cytotoxicological impact of nanoparticle concentrations on human skin cells. The practical implications of the approach consist of multifunctional leather surface development, leather durability and comfort increase by generating antimicrobial and self-cleaning properties. The relation between leather functionality and the cytotoxicity concentration limit of nanomaterials was the hypothesis of our research. The main procedures for leather surface covering followed the classical recipes based on surface spraying with film forming composites with nanoparticle content. The optimized technology was evaluated by leather surface analyses regarding the antimicrobial (SR EN ISO 20645) and self-cleaning properties under visible light exposure as compared to leather surface covered without nanoparticles. The cytotoxicity tests were performed by incubation of keratinocytes (Human immortalized keratinocytes- HaCaT) with different concentrations of nanoparticles for 48 hours and measurement of cell viability by MTT (3-[4,5-dimethylthiazol- 2-yl]-2,5-diphenyltetrazolium bromide) assay protocol. Other tests were devoted to leather wearing simulation in order to estimate the potential transfer of nanoparticles on human skin and the health and safety impact. These simulations were based on rubbing test (SR EN ISO 11640) followed by scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX) analyses and by leachability tests (SR EN ISO 4098**)** performed in artificial perspiration solution followed by inductively coupled plasma -mass spectrometry (ICP-MS) according to SR EN ISO 17294-2 and SR EN ISO 16171. The main conclusions of our research showed that it is possible to add multifunctional value to leather surface by using Ag and TiO_2 nanoparticles with low impact on safety and health.

1 Introduction

Nanoparticles offer a great area for innovation because their properties differ significantly from ion or bulk materials through their unique chemical, electrical, optical, and biological activity which are mainly determined by the size, shape, composition, crystallinity or other structural properties [1]. The studies regarding the antimicrobial properties of nanoparticles [2] are the most numerous due to their efficiency on a large spectrum of bacteria and fungi. Silver, zinc oxide, titanium oxide, silica, carbon based nanoparticles or compounds of these showed promising functionalities regarding antimicrobial [3], photocatalytic [4, 5], conductive [6] properties or heat/fire resistance [5]. The potential of nanoparticles is still under research for leather industry and no commercial dedicated products are on the market.

The balance between efficiency and added cost can be improved by multifunctional properties generation under leather surface. Another limitation is the lack of information regarding the potential risk associated with specific nanomaterials and the need to evaluate every kind of treated

material [7]. Our previous studies showed that nanosilver doped nitrogen-titanium dioxide (Ag/N-TiO_2) has enhanced antimicrobial and photocatalytic properties and cytotoxicity tests on lung and skin human cells found that the concentration risk limits are of 500 µg/mL [8] which is very high as compared to other reference [9] with values of 10-25 µg/mL.

In the present research we have investigated the multifunctional properties of leather surface finished with commercial Ag/TiO_2 nanoparticles in solution, the scenarios regarding the potential nanoparticle releasing in wearing conditions and the potential nanoparticles cytotoxicity impact on human skin cells.

2 Experimental

2.1 Materials and Methods

2.1.1 Leather finishing with Ag/TiO_2 nanoparticles

Sheepskins were prepared by using an ecological commercial technology based on aldehydes and syntans in pilot plant station of Leather Research Department of ICPI.

Silver-doped titanium dioxide nanoparticles with average primary particle size <8nm [10], in solution (Ag425), were purchased from TiPE® AG, China. The composition of Ag425 solution was found to be 0.86% Ag and 0.72% Ti after the analyses performed by ICP-MS (Aurora M90, Bruker) according to SR EN ISO 17294-2:2017 and SR EN ISO 16171:2017.

The application of leather finishing (Figure 1) was performed by integration of Ag425 solution in base and top coat binders using classical finishing method by spraying [11].

Fig. 1. Leather finishing by spraying

2.1.2. Added functions and performance of new leather surface

Antibacterial properties of leather surfaces finished with Ag425 were assessed according to SR EN ISO 20645 against *Staphyloccocsus aureus* (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria). The photocatalytic (self-cleaning) properties were evaluated by simulation of leather surface staining with organic dyes, methylene blue (MB) and orange II (OII), known in literature as model stains for photocatalytic nanoparticles testing.

Leather sample and control surfaces were stained with the same quantity (0.5 µL) of dye solutions of 200 ppm (OII) and 1000 ppm (MB) concentration. The samples and control were exposed up to 5 hours to visible light (500W halogen lamp with irradiation between 400-700 nm) and were monitored in time by taking pictures and by measuring the stain discolouration with DATA Color Check Plus II portable device assisted by CIELab color management software.

2.1.3. Nanoparticles leachability

The leachablity test was performed with **artificial** perspiration solution **(SR** EN ISO 4098**) at pH=8, followed by Ag and Ti quantification with the i**nductively coupled plasma -mass spectrometer (**ICP-MS,** Aurora M90, Bruker**)** according to SR EN ISO 17294-2 and SR EN ISO 16171.

Other tests were performed by analyzing the rubbed and unrubbed areas of leather surfaces (SR EN 11640:2003) by using Scanning electron microscopy (FEI QUANTA 450 FEG) with energy dispersive X-ray spectroscopy (*SEM-EDX*).

2.1.4. Evaluation of nanoparticles cytotoxicity

The assessment of cell viability was based on induced cytotoxicity by Ag425 on keratinocyte (Human immortalized keratinocytes- HaCaT), using MTT (3-[4,5-dimethylthiazol- 2-yl]-2,5-diphenyltetrazolium bromide) technique described by Mosmann T in 1983 [12].

The viable cells with active metabolism have the ability to convert the tetrazolium salt MTT in formazan (which can be spectrophotometrically detected), by breaking the tetrazolium ring at the mitochondrial level by dehydrogenase enzymes. The cells which have not active metabolism, lose the ability to convert MTT in formazan, such that the color reaction serves as a marker for viable cells. The experimental protocol was the following: the cells were seeded at a density of 10,000 cells / well in a 96-well plate and incubated at 37 ° C in atmosphere of 5% CO_2 in order to be attached to the plate.

After 24 hours, the cells were treated with different concentrations of Ag425 (10, 50, 100, 200, 300, 400, 500, 750, 1000 µg/mL) and the cell viability was assessed after 24 and 48 hours of incubation. The nanoparticle medium was removed and 0.1 mL / well of MTT solution (Sigma Aldrich) was added, the concentration being 0.5 mg / mL. After 2 hours of incubation, the MTT solution was removed and the cells were lysed with 0.1 mL / lysis buffer (0.1 N HCl in isopropanol).

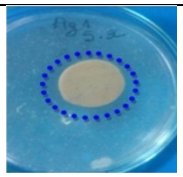
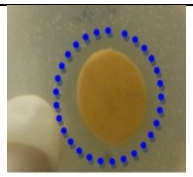
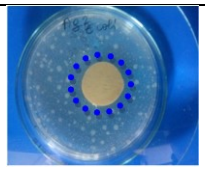
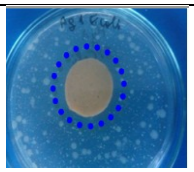
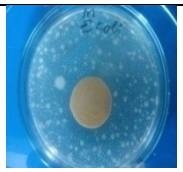
Absorbance was measured using a plate reader (Tecan GENios) at 570 nm wavelength with a reference wavelength of 690 nm. The results were expressed as percentages of control (cells incubated in the medium in the absence of Ag425).

3 Results and discussions

3.1 Added Value for New Leather Surfaces

The evaluation of antibacterial properties of leather surfaces was performed on different samples in different stages of research stages and showed that they have satisfactory level of protection with inhibitory area development as compared to the untreated surfaces (Table 1). According to the standard SR EN ISO 20645 the satisfactory level shows that the treatment has antibacterial effect.

Table 1. Antimicrobial properties of leather surfaces treated with Ag425

Test against *Staphylococcus aureus*		
Sample 1 with inhibitory area	Sample 2 with inhibitory area	Control sample without inhibitory area
		
Test against *Escherichia coli*		
		

The photocatalytic properties of new finished leathers were evaluated and showed that under the visible light exposure the rate of stain decomposition is higher for the treated surfaces as compared to untreated leathers (Figures 2 and 3). The discolouration of MB stains was more efficient as compared to OII stain, the effect was evaluated for many samples with reproducible results (Figures 4 and 5 show the results for two samples, Ag1 and Ag2).

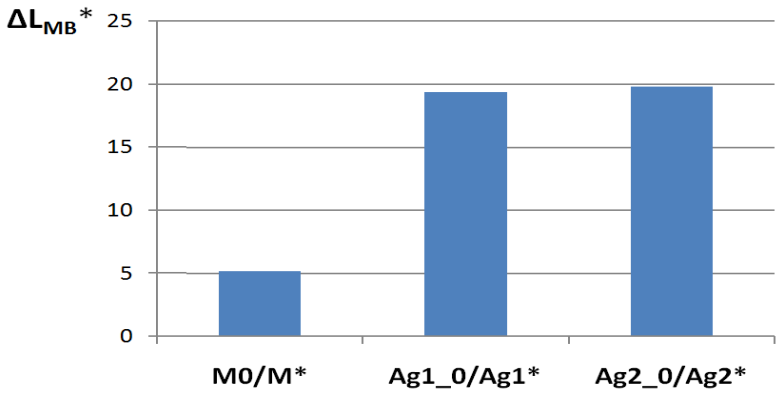


Fig. 2. Lightness difference of MB stain in initial state and after visible light exposure

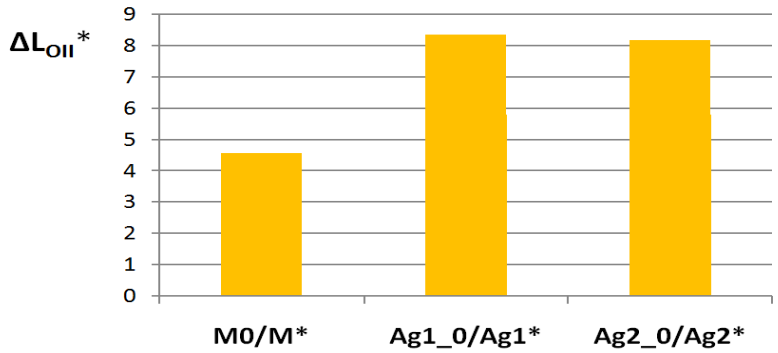


Fig. 3. Lightness difference of OII stain in initial state and after visible light exposure

Table 2. Self-cleaning effect after 30 minutes of visible light exposure

Control sample		Ag1 sample		Ag2 sample	
Initial stain	After 30' exposure to Vis light	Initial stain	After 30' exposure to Vis light	Initial stain	After 30' exposure to Vis light
					

3.2 Wearing Simulation Tests and Nanoparticle Leachability Evaluation

The aim of the tests was to set the concentration of nanoparticles which can detach from the leather surface in conditions of wearing. The evaluation of nanoparticles by washing the leather in perspiration solution [13] has taken into consideration the leather surface of 13.5 dm^2 which can be in contact with foot skin and a volume of 20-100 mL perspiration [14] which can be released in footwear wearing conditions. In Table 3 can be seen the average values of 3 analyses for Ag and Ti

which show that 8% of silver and 32% of titanium are released from the leather surface in perspiration solution.

Table 3. Leached nanoparticles and concentration in perspiration solution

Ag, µg/mL		Ti, µg/mL	
Initial concentration	Leached concentration	Initial concentration	Leached concentration
0.76-3.75	0.06-0.31	89-445	29-145

The other investigations were performed after the leathers were tested for rubbing fastness on rubbed surface and on unrubbed surface just next to the analysed area by mapping the elements with SEM-EDX such as semi quantitative results to offer a scenario for nanoparticles releasing and to supply reliable information regarding the potential impact on human skin. In this case the rubbing with perspiration solution leads to silver nanoparticles releasing by 33.3% and no releasing of titanium (Table 4). We can consider that the released concentration of nanosilver after the rubbing test with perspiration solution is still very low and according to the data of Table 3 can reach values of 0.25-1.25 µg/mL. In conditions of dry rubbing test, after 100 dry cycles, the nanoparticles were not released from the leather finishing (Table 5).

Table 4. SEM-EDX mapping of nanoparticles on rubbed surface of leather with perspiration solution in comparison with no rubbed surface

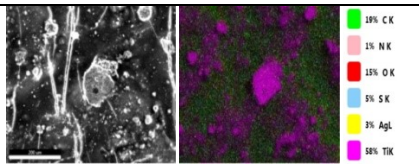
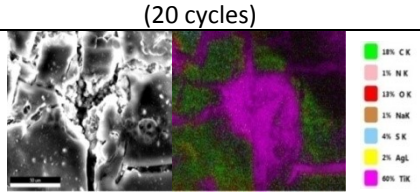
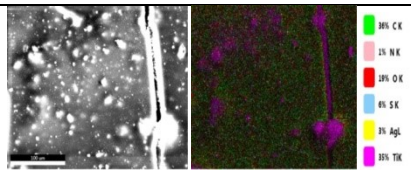
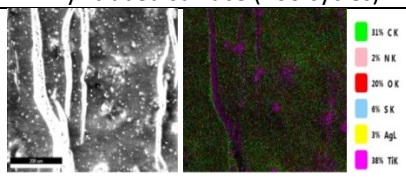
No rubbed surface	Rubbed surface with perspiration solution (20 cycles)
	
33.3% loss of Ag and no loss of Ti	

Table 5. SEM-EDX mapping of nanoparticles on dry rubbed surface of leather in comparison with no rubbed surface

No rubbed surface	Dry rubbed surface (100 cycles)
	
No loss of Ag or Ti	

3.3 Ag425K Nanoparticle Cytotoxicity

HaCaT cells have been treated with different concentrations of Ag425K for 48 hours, and the cellular viability was measured by MTT technique. The results of cellular viability test for nanoparticle concentrations of 10 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 750 µg/mL and 1000 µg/mL in comparison with the cells without nanoparticles (C) are presented in Figure 4.

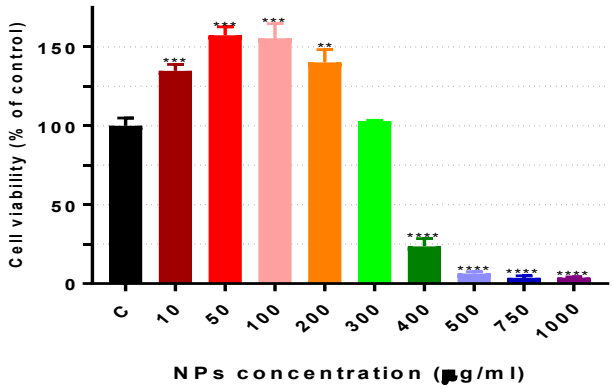


Fig. 4. Keratinocyte viability after 48 hours incubation with different concentrations of Ag425K in comparison with control sample. The average ± deviation of values are presented, the statistic was carried out with Anova test and the comparison was done with control sample and with every concentration of nanoparticles (* P<0.05, ** P<0.005, *** P<0.0008, **** P<0.0001).

In figure 4 it can be seen that Ag425K has an increased cytotoxic effect starting with the concentration of 400 µg/mL. Concentrations between 10 and 200 µg/mL of nanoparticles induce the proliferation of HaCaT cells by 134% at 10 µg/mL concentration and by 155% at 50 and 100 µg/ml respectively, whereupon the proliferation decreases at 140 and 200 µg/mL (relative to the control cells). At the concentration of 300 µg/mL no change was observed compared to control cells grown in the absence of nanoparticles. At concentrations greater than 300 µg /mL, there was a significant and dramatic decrease in the number of viable cells. Thus, keratinocytes exposed to Ag425K exhibit significantly reduced viability up to 23% at 400 µg/mL, 6% at 500 µg/mL and 3% at 750 and 1000 µg/mL. MTT results indicate that this type of nanoparticles stimulates the proliferation of HaCaT human keratinocytes at low concentrations (below 250 µg/mL) but becomes toxic to cells if they exceed 350 µg/mL.
The wearing simulation and tests showed that the concentration of leached nanoparticles are of maximum 145 µg/mL which is under the cytotoxicity limit of 350 µg/mL for human skin cells and the risk for consumer health can be avoided.

4 Conclusions

Many recent studies were devoted to smart leather processing with new nanomaterials with potential to be used in low concentrations for new properties development or as alternative to volatile organic compounds. The impact of new materials and processed products on human health and environment are of high interest the more they are at the nano scale. The use of commercial nanoparticles with particle size under 8 nm was found to be efficient in terms of antimicrobial properties and with photocatalytic potential for leather surface finishing. The wearing simulation tests and cytotoxicological evaluation on human skin cells showed that the released concentrations of nanoparticles can be managed by leather processing below cytotoxicological risk limits.

Acknowledgements

The works were funded by Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, project no 15/2015 under the frame of the ERA-NET for a Safe Implementation of Innovative Nanoscience and Nanotechnology program, project no 44 PHYSforTeL/2018 and project no 4PERFORM-TEX-PEL/2018 within PNCDI III.

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