Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA20130

Grantee name: Svetlana Vihodceva

Details of the STSM

Title: Cytotoxicity Evaluation of Porous Pristine CeO_2 and Cu-doped CeO_2 Nano/microparticle Powder for Enhanced Microbicidal Applications with Minimal Human Hazard

Start and end date: 20/09/2023-20/10/2023

Description of the work carried out during the STSM

The aim of this Short-Term Scientific Mission (STSM) was to test the potential human hazard of materials that have thea potential to be used for antimicrobial applications. In this study, materials were selected based on their previously tested antimicrobial properties, this antimicrobial tests revealed that pure CeO_2 did not exhibit any antimicrobial activity. However, when CeO_2 was doped with copper (Cu), it displayed significantly enhanced and faster antimicrobial properties thancompared to CuO. ~~This improved antimicrobial effect can be attributed to the multifaceted action of Cu-CeO_2.~~ The antimicrobial properties and toxicity of porous Cu-CeO_2 powders against Gram-negative bacteria *Escherichia coli, Pseudomonas aeruginosa*, Gram-positive bacteria *Staphylococcus aureus*, and fungus *Candida albicans* were tested in water to mitigate the interference of the testing environment with powder physic-chemical properties, for 2, 4, and 24 h in 96-well plate at room temperature, covering concentrations from 1 to 1000 mg/L. The Cu-CeO_2 at concentrations from 100 mg/L upwards showed biocidal activity towards *E. coli* and *S. aureus* already after 2 h of contact and, towards *P. aeruginosa* after 4 h of contact, extending the exposure to 24 h caused total inhibition of growth at 10 mg/L, and at 100 mg/L in case of fungus. This improved antimicrobial effect can be attributed to the multimodal action of Cu-CeO_2. **The results of this STSM are helping in finding effective biocidal material with minimal human hazard. Such material could then be used for antimicrobial purposes on problematic surfaces.**

The study involved three groups of porous materials: pristine CeO_2 (as a negative control), CuO (as a positive control), and Cu-CeO_2. All these materials were synthesized using the same method and subjected to cytotoxicity analysis with a human cell line. The choice of the HaCaT keratinocytes cell line was motivated by its relevance to potential skin contact toxicity.

The STSM grantee underwent thorough training in cytotoxicity testing as an integral part of her research at the host institution. This training equipped her to assess the potential human cytotoxic effects of

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[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

antimicrobial materials and evaluate their practical applications. For the materials cytotoxicity evaluation Neutral Red Uptake (NRU) assay was used. NRU assay has been developed to quantify viable cells in monolayer cultures and is based on the binding ability of viable cells to the supravital dye neutral red in the lysosomes. The bound dye is then extracted from the viable cells using an acidified ethanol solution, and the absorbance of the solubilized dye is measured using a spectrophotometer.

The initial step involved exposing cells to various dilutions of the materials, ranging from 1000 mg/L to 0.1 mg/L, over a 24-hour period. After multiple repetitions, it was established that the materials did not exhibit any cytotoxic effects at concentrations of 100 mg/L and below. Based on this observation, additional concentrations ranging from 1000 mg/L to 100 mg/L, as well as a shorter 2-hour exposure period, were introduced. This shorter exposure time was chosen due to the rapid antibacterial activity of Cu-CeO_2 within just 2 hours. Inhibitory concentrations were calculated for all the tested materials and results of Cu-CeO_2 were compared against pristine CeO_2 and CuO to evaluate their potential hazards.

To demonstrate that these particles do not induce cytotoxic effects, we conducted microscopy visualization. To prepare the samples, all experiments were conducted in the same manner as in the cytotoxicity assay, with cells grown on glass slides suitable for microscopic analysis. Instead of using coloring agents, the cells were fixed with a solution of 4% formaldehyde in Dulbecco's Phosphate Buffered Saline (DPBS) and then stored in DPBS in a refrigerated environment. As a result, we were able to observe a fully intact and viable cell layer beneath the particles.

Description of the STSM main achievements and planned follow-up activities

The results obtained from this STSM significantly advanced the characterization of the materials synthesized by the grantee. These findings deepen our understanding of material properties and contribute to the collective knowledge in this field. The hypothesis that Cu-CeO_2 materials, with superior antimicrobial properties compared to CuO, would exhibit reduced toxicity~~,~~ was substantiated. The results provide valuable insights into their suitability for practical use. **The EURO-MIC Action acknowledges the importance of ensuring the safety and sustainability of antimicrobial materials as one of its goals: "RC6: Screen MIC mitigation strategies based on surface functionalisation and dosing of antifouling agent (e.g. biocides) in terms of their efficacy, efficiency, applicability and environmental impact."**

The results indicated that pristine CeO_2 did not demonstrate any cytotoxic effects even at the highest tested concentration of 1000 mg/L, aligning with its antimicrobial properties. On the other hand, CuO showed cytotoxic effects at a concentration of 250 mg/L already after 2 hours. Notably, Cu-CeO_2 only exhibited cytotoxic effects only at the 500 mg/L concentration after 24 h, with a minimum bactericidal concentration (MBC) of 10 mg/L against bacteria (*E. coli*, *S. aureus, P. aeruginosa*) and 100 mg/L against *Candida albicans*.

These results suggest that Cu-CeO_2 materials hold ~~a~~ significant promise for practical applications. The fact that they exhibit enhanced ~~antibacterial~~ antimicrobial activity and a limited cytotoxic effect at high concentrations positions them as potential candidates for various applications where ~~antibacterial~~ antimicrobial properties are desired. Moreover, their reduced toxicity compared to CuO highlights the possibility of using Cu-CeO_2 as a safer alternative in ~~antibacterial~~ antimicrobial formulations, which is essential for ~~public health and~~ safety. In summary, the study's findings underscore the potential of Cu-CeO_2 as a multifunctional material with exciting prospects for antimicrobial applications while minimizing adverse ~~health~~ toxic effects.

The grantee gained fresh insights and expertise in cytotoxicity assessment by participating in an interdisciplinary research group at the host institution. ~~By participating in an interdisciplinary research group at the host institution, the grantee gained fresh insights and expertise in cytotoxicity assessment.~~ This training ~~not only~~ enhanced her skill set ~~but also~~and contributed to her professional development. In addition, this STSM contributed to networking between researchers between Estonia and Latvia, developing up-to-date research skills, improving the quality of the research, ~~Additionally, the STSM~~and paved the way for future collaborations between the two institutions. Both parties ~~have expressed their~~

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~~willingness~~are willing to host reciprocal visits and conduct necessary analyses, fostering ongoing cooperation. **As a result, the STSM aligns with the following capacity building objective of the Action: "CB5: Recruit and train Early Career Investigators (ECI) to boost their career prospects during and after the Action by providing an interdisciplinary network platform."**

The research findings from the STSM were presented, and knowledge was shared during a seminar at the host institution on October 19, 2023. This event also served as an opportunity to promote the COST 20130 action. The results and insights acquired after the grantee's return will be disseminated at a seminar at Riga Technical University.

Furthermore, these findings will be incorporated into a collaborative publication. ~~.~~

In summary, the STSM facilitated the professional growth of grantee through opportunities for interdisciplinary collaboration and training. And made significant contributions to the development of safer and more sustainable antimicrobial materials. **Next step after STSM will be the evaluation of this material for antimicrobial purposes on problematic surfaces.** ~~. Furthermore, it facilitated the professional growth of grantee through opportunities for interdisciplinary collaboration and training.~~

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