The reproducibility of green synthesized nanomaterials: a study comparing the antimicrobial effectiveness of green-produced ZnO *vs.* fossil-based particles

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1. Abstract

The production of the annually required thousands, if not millions of tons of metallic nanoparticles typically involves the usage of fossil-based reagents and significant energy input. More and more different types of raw materials touch upon the planetary boundaries, and extensive energy consumption and high temperature requirements, as often applied during the production of nanomaterials, provide a long-lasting, negative influence on the environment. Driven by the goals of the European Green Deal, new concepts and approaches for green synthesis of nano- and advanced materials, therefore, attempt to combat these issues by reducing the generated environmental footprint, utilizing more sustainable and renewable products in the process. In this work, three different batches of zinc oxide (ZnO) nanoparticles were synthesized following the VERDEQUANT [1] green production process by Phornano. These batches were compared to fossil-based commercially available ZnO nanoparticles in respect to their antimicrobial effectiveness for generation of advanced surfaces, which has recently been defined as a priority research area in materials for the health and medical market by the Advanced Materials Initiative 2030 [2]. A number of physicochemical properties, like primary particle size, polydispersity and surface charge were investigated and compared. The dissolution rate & integrity of the nanoparticles and, thus, the potential toxic ion release was quantified by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) over time. Further, human safety concerns were taken into consideration by assessing the cytotoxic effects of the various ZnO nanoparticles on the identified two main areas of exposure, *i.e.* inhalation during the intended use as coating in face masks and dermal exposure during use on various surfaces, Hence, human lung epithelial cells (represented by A549 cell line) & primary human epidermal cells (keratinocytes) were used as models. In order to quantify functional performance, as ZnO nanomaterials have been well reported for their antimicrobial effectiveness, the different candidates (production batches) were assessed for several criteria and compared against bacterial, fungal and pseudoviral representatives. In line with the recently proposed holistic concept of evaluating safety and sustainability aspects well in relation with functional

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performance, we developed a novel antimicrobial scoring system to allow the direct comparison of results between unrelated assays and readout-methods and enable quantitative approaches that can be adopted by the Safety-and-Sustainability-by-Design (SSbD) [3] approaches. Results showed a comparable, if not better antimicrobial effectiveness, whilst posing minimized toxic effects to the investigated human cells. Similar and comparable results between green and fossil-based materials already represent vast improvements, since the significant reduction of energy and investment of renewable resources by green synthesized materials pose a tremendous advantage compared to conventionally produced materials.

2. References

[1] https://www.phornano.com/verdequant

[2] https://www.ami2030.eu/wp-content/uploads/2023/04/Ami2030-Dossier-2.pdf

[3] https://research-and-innovation.ec.europa.eu/system/files/2022-12/Commission%20recommendation%20-%20establishing%20a%20European%20assessment%20framework%20for%20safe%20and%20sustainable%20by%20design.PDF

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