

# A novel *in silico* tool for dose assessment in cell culture nanotoxicology

Ermes Botte,<sup>1,2</sup> Pietro Vagaggini,<sup>1</sup>  
Joana Costa,<sup>1</sup> Lara Faccani,<sup>3</sup>  
Ilaria Zanoni,<sup>3</sup> Anna Costa,<sup>3</sup>  
Arti Ahluwalia<sup>1,2</sup>

<sup>1</sup>Research Centre “E. Piaggio”,  
University of Pisa; <sup>2</sup>Department of  
Information Engineering, University of  
Pisa; <sup>3</sup>Institute of Science and  
Technology for Ceramics, CNR, Faenza,  
Italy

## Abstract

Dose assessment is essential for understanding the mechanisms triggering nanomaterial toxicity *in vitro* and for meaningful translations to *in vivo*. We propose a novel computational approach for improving the accuracy of biological dose-response characterization, demonstrating its robustness for insoluble Engineered Nanomaterials (ENMs).

## Introduction

Although it is well-known that the cumulative dose of ENMs effectively delivered to cells (*i.e.* the so-called effective dose) significantly differs from the nominally administered one, *in vitro* dosimetry typically refers to nominal values.<sup>1</sup> Thus, toxicity rankings may be underestimated, making it difficult to properly assess ENM hazard. Due to their widespread use, ENM dose assessment is in fact considered a key point in nanotoxicology.<sup>2</sup>

In this context, *in silico* models provide a valuable tool for predicting the effective amount of ENMs reaching biological tissues in a given configuration.<sup>3</sup>

The best-known dosimetry models are the ISD3<sup>4</sup> and the DG.<sup>5</sup> These models implement the dynamics of nanoparticles through a culture medium with an adjustably sticky bottom and account for different phenomena (*e.g.*, diffusion, sedimentation, dissolution), all of which depend on the physicochemical characteristics of the material.

In this study, we integrated ISD3 and DG models within a ready-to-use Graphical User Interface (DosiGUI) enabling accurate prediction of the effective dose in a specific *in vitro* system. We validated this tool for insoluble ENMs by evaluating how the out-

put of each simulation fits experimental data and, as a proof-of-concept, we applied it to the estimation of the effective dose of ENMs perceived by HepG2 cells in a standard exposure scenario.

## Materials and Methods

DosiGUI was purposely developed as a standalone application, exploiting the App Designer Toolbox in Matlab (version R2019b, The MathWorks, Inc.).

Each model was firstly run for simulating the dynamics of insoluble ENMs (CeO<sub>2</sub>, TiO<sub>2</sub> and BaSO<sub>4</sub>) with a totally adsorptive bottom, starting from different administered doses (5, 25 and 50 µg/mL). In parallel, experimental tests were carried out in triplicate for validation. The set up consisted of a 50 mm high tube with a coating of gelatin at the bottom to mimic the sticky boundary condition implemented *in silico*. The concentration at the middle of the tube over time was measured using plasma emission spectrometry (5100 ICP-OES, Agilent Technologies). The goodness of fit for each model against the data was evaluated using correlation analysis (GraphPad Prism, v7).

We then employed DosiGUI to compute the effective dose perceived by HepG2 cell monolayers exposed to the same ENMs (nominal doses of 25, 50 and 250 µg/mL) for different times (4, 8, 24 and 72 h) in a 96-well plate (100 µL suspension volume, 37°C, 5% CO<sub>2</sub>, 95% RH). Since nanoparticle adsorption by cells (defined as the “stickiness” of the cell surface) is an ENM-specific feature, we first tuned the parameter describing “stickiness” for each ENM in both models. Plasma emission spectrometry was used for measuring the amount of ENM adsorbed by the HepG2 monolayer over time after exposure to different nominal doses. Correlation analysis between acquired and simulated data was performed in GraphPad Prism for identifying the most suitable values of cell stickiness, to be used thereafter for effective dose predictions.

## Results

DosiGUI was successfully validated for all the three ENMs. In particular, at least one of the models led to a robust fitting (*i.e.* R<sup>2</sup> > 0.75) for each configuration studied (Table 1).

As a proof-of-concept application, Figure 1 shows effective dose profiles over time for different nominal doses administered to HepG2 cells, predicted after having identified the stickiness parameter. Clearly,

Correspondence: Ermes Botte, Research Centre “E. Piaggio”, University of Pisa; Department of Information Engineering, University of Pisa, Italy  
E-mail: ermes.botte@phd.unipi.it

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cells do not uptake the entire amount of ENM administered, even for long times of exposure. On the contrary, we observed a “saturation” effect characterised by a reduction in the percentage of adsorbed ENM with increasing nominal dose.

The same result is highlighted in Figure 2, reporting the cumulative effective dose in the monolayer at specific time points as a function of the nominal dose.

## Discussion and Conclusions

The results demonstrate that our DosiGUI-based pipeline is a powerful and robust approach for ENM dose assessment

**Table 1. Results of the correlation analysis for validating DosiGUI predictions. The lowest R<sup>2</sup> value associated with each ENM among those obtained for the three nominal doses is reported.**

ENM	Goodness of fit (R <sup>2</sup> )
TiO <sub>2</sub>	0.83
BaSO <sub>4</sub>	0.76
CeO <sub>2</sub>	0.81

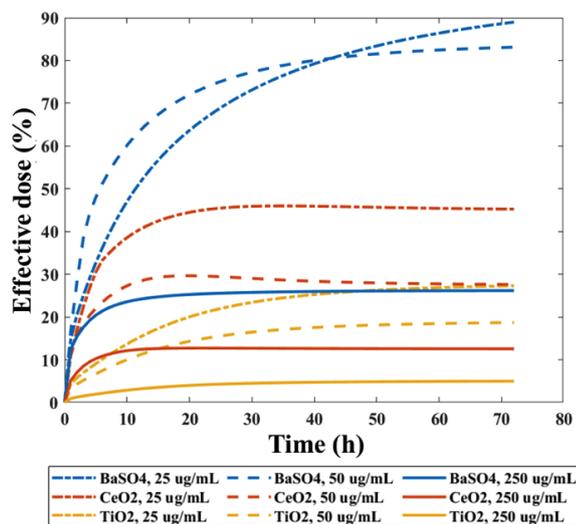


Figure 1. Effective dose profiles over time predicted by DosiGUI for each considered ENM, expressed as percentage of the nominally administered dose.

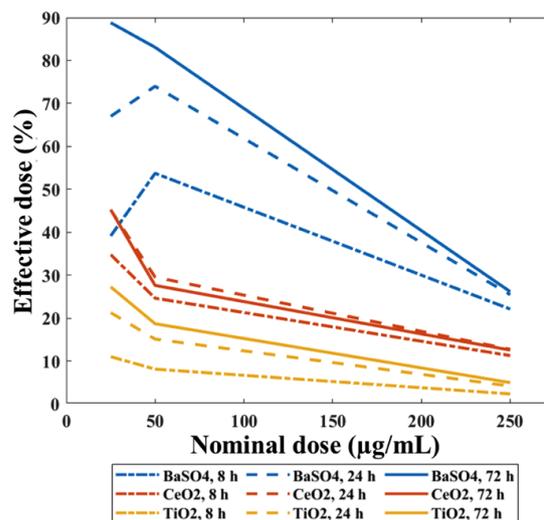


Figure 2. Effective dose profiles versus nominal dose predicted by DosiGUI for each ENM after different exposure times, expressed as percentage of the nominal dose.

*in vitro*. Future developments will focus on i) relating the predicted effective dose with its biological effects; ii) extending the study to other classes of ENMs, establishing a new reliable alternative to expensive and ethically sensitive animal tests in nanotoxicology; iii) extending the models, which only consider phenomena in the vertical direction, to 3-dimensional configurations.

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