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4.3 Model of evBOW products toxicity

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GLOSSARY OF TERMS	
TERM	**DEFINITION**
BOW	Biogenic Organotropic Wetsuits
EVs	Extracellular Vesicles
MBDs	Magnetic Bead Devices
evMBDs	EV-membrane coated MBDs
MNPs	magnetic nanoparticles
SPION	superparamagnetic iron oxide nanoparticles
β	Clearance rate of toxic metabolite conversion
α	Base proliferation rate
k	Maximal viability of cells in experimental conditions
δ_D	Rate of cell death induced by nanoparticles
δ_M	Rate of cell death induced by toxic metabolite
C_o	Initial viability of cells
k_{2Co}	Rates of decrease in the concentration of monocytes
k_{2C2M2}	Concentration of deactivated macrophages
t_o	Initial phase of particles uptake by cells
μ	Rate of toxic metabolite production
D	Density of nanoparticles

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1 INTRODUCTION

BOW aims to produce nanodevices with a biomimetic surface by dressing Magnetic Bead Devices (MBDs) with one or more layers of Extracellular Vesicle (EV) lipid membrane, with designed abilities to produce theragnostic effects in the organism. This technology benefits from the complex biological function of the EV coating and the designed functions of the synthetic MBDs and aims to advance the possibility of recapitulating biomimetic functions on any synthetic nanodevice.

The present document represents **Deliverable 4.3 – Model of evBOW products toxicity**. It has been developed as part of WP4 "Biological performances of MBDs versus pristine EVs and MBDs".

We have developed mathematical models for predicting long-term adverse effects (e.g., cytotoxicity) in vitro. In our mathematical methodology, we take into consideration the kinetic processes unfolding in a cell culture exposed to varying concentrations of nanoparticles up to 54 hours. The models we present offer a more precise prediction of the delayed detrimental effects of nano-objects, in contrast to the frequently employed simplified approaches (Lin Z., et.al., 2022; Huang Y, et.al., 2021; Yuan D., et.al., 2019) for assessing biocompatibility of nanomaterials. At the same time, the organism's risk encompasses not only effects arising from "direct" cytotoxic processes but also effects linked to immune sensitization induced by introduced nanoparticles. Consequently, our second model is designed to forecast the probability of an exaggerated activation of the immune system, potentially leading to the initiation of an immune response. This approach can be further employed for comparative analysis to identify evMBDs with minimal impact on organisms. Superparamagnetic iron oxide nanoparticles (SPIONs) exert an influence on cell metabolism and subsequent cell death, characterized by nonlinearity due to the intricate processes of cellular uptake, intracellular trafficking, and clearance, leading to the release of toxic compounds. In silico modelling of the toxicological properties of nanoparticles can face challenges due to the limited availability of data and descriptors essential for capturing quantitatively relevant structure-activity relationships (QSAR) and validating corresponding mathematical models. The complexity of the structure and properties of nanomaterials necessitate ad hoc approaches. Recently, various methods have been proposed for simulations in the context of PBPK modelling of nanoparticles (Lamon L. et al., 2019). The heterogeneity of NPs in terms of structural and functional properties can challenge the study on their pharmacokinetics and intracellular localization (Yiye Li et.al. 2018) because affect the interaction of particles with cells and the biological system as a whole. Conversely, the formation of a biocorona, along with aggregation and degradation, markedly and dynamically alters the properties and disposition of particles, affecting the possibility to predict their efficiency. Moreover, a reliable analytical method to characterize and track nanoparticles in vitro and in vivo is lacking (F. Lotfipour, et.al., 2021).

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Despite this, certain innovative cell-based methods and computational approaches hold the potential to predominantly address this issue and reduce the reliance on experimental animals for assessing the toxicity of nanomaterials. Consequently, our efforts to characterize nanoparticle toxicity using conventional mathematical tools such as PK-Sim or IVIVC, which utilize a range of physicochemical properties, pharmacokinetic data, and tissue-specific information to simulate substance behaviour in the body, have proven unsuccessful. This is primarily because specific parameters of nanoparticles, such as size, shape, surface charge, and surface coating, are either not readily available or cannot be integrated into standard software. These nanoparticle-specific properties can profoundly influence their behaviour and toxicity, and their absence in PK-Sim restricts the scope of the simulations. Moreover, in vivo engineered nanomaterials undergo intricate interactions with biological systems, encompassing cellular uptake, intracellular transport, and accumulation in specific organs or tissues. The comprehension of nanoparticle behaviour in vivo is still evolving, and conventional approaches may not fully capture uncertainties or gaps in knowledge.

Examining the kinetic effects of nanoparticles in vitro may offer a solution to some of these limitations (Byrne, H.J. et al., 2019). The assessment of dynamic processes related to cytotoxicity can yield valuable insights into the direct interaction of nanoparticles with cells, including cellular uptake, intracellular localization, and potential toxicity pathways (as mentioned in our abstract). In vitro studies play a crucial role in unravelling the dose-response relationship, identifying concentration thresholds for toxicity, and understanding cellular responses to nanoparticles. Our mathematical model takes into account the phase of accumulation of nanoparticles in cells before the manifestation of toxic effects associated with active release of metabolites. We also account for the potential preservation of proliferation in the cell culture, a factor influencing simulation outcomes. In this context, for robust mathematical extrapolation, leveraging macro-organisms with similar biochemical pathways for utilizing released iron, a critical toxic factor of SPIONs, holds promise. There's a growing interest in using models like *C. elegans*, providing an alternative to more complex mammalian models for detecting various physical function parameters associated with both short- and long-term effects. This is particularly relevant for conservative metabolic pathways such as iron metabolism Beyond assessing nanoparticle safety in cell cultures, *C. elegans* toxicity grading assays offer a comprehensive view from a whole animal model with fully active digestive, endocrine, neuromuscular, and reproductive systems. Notably, reported results have demonstrated predictive capabilities comparable to rodent LD50 grading (Williams, P.L. et.al. 1988) that is why we use this animal for evaluation of studied nanoparticles using our model.

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2 MATHEMATICAL SIMULATION.

2.1 Integrated model of spion-derived nanoobjects toxicity using cell system in vitro

The initial phase of our simulation involves assessing toxicodynamic processes in vitro. Primarily, we consider the initial stage of particle penetration into the cells, where cytotoxic effects are not distinctly expressed. The duration of this phase is denoted by t_0. Subsequently, the explicit toxicity phase begins, and the density of the toxic metabolite M increases according to the following equation

$$\frac{dM}{dt} = \theta(t - t_0)(\mu D - \beta M)C, \quad (1)$$

where $\theta(t - t_0)$ represents step-function with the delay t_0, μ outlines a constant rate of toxic metabolite production, D is the density of nanoparticles, β is specific constant rate of toxic metabolite conversion into non-toxic substances. At both phases cell proliferation and death are governed by equation for cell viability C

$$\frac{dC}{dt} = \left(a\left[1 - \frac{C}{k}\right] - [\delta_D D + \delta_M M]\right)C. \quad (2)$$

Here the parameter a represents base proliferation rate, k is environmental carrying capacity (maximal viability of cells in experimental conditions), and δ_D and δ_M are specific constant rates of cell death induced by nanoparticles D and by toxic metabolite M, correspondingly (they can be negative for cell stimulating effects). Equation (2) includes logistic cell culture growth and adverse/stimulating effects of both initial agent – nanoparticles themselves, and secondary agent – subsequent iron release. The mathematical model consists of equations (1) and (2) and initial conditions $M(0) = 0$, $C(0) = C_0$. The mathematical model is defined by seven parameters t_0, μ, β, a, k, δ_D, δ_M.

Note: The model has some limitations:

1) *The model assumes that the nanoparticle density in the medium remains constant, which it could be true only in scenarios where there is a small amount of cell culture within the bioreactor;*
2) *The model considers nanoparticle density within the cells to be constant, assuming that the flux of nanoparticles into the cell is entirely compensated for by their destruction through metabolization;*
3) *The model suggests that metabolite diffusion from the cells and in the medium occurs rapidly enough for its concentration within each individual cell to depend on the total amount of metabolite produced. Alternatively, it suggests that the production of metabolite in each individual cell is proportional to viability.*

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Each of these limitations can be addressed by introducing corresponding rate equations for the amount of nanoparticles in the bioreactor and within a cell, as well as for metabolite distribution between the cell and the medium. However, it's worth noting that the model seems to be versatile enough to encompass all in vitro situations studied thus far. Indeed, the challenge arises when we cannot directly measure the density of the toxic metabolite. In this situation, there seems to be degeneracy in some parameters of equations (1) and (2): if we scale up μ and β by a common factor c and simultaneously scale down δ_M by the same factor c, $M(t)$ scales up by c, but measurable $C(t)$ does not change:

$$\mu \to c\mu, \;\; \beta \to c\mu, \;\; \delta_M \to \frac{\delta_M}{c} \qquad \Rightarrow \qquad M(t) \to cM(t), \;\; C(t) \to C(t).$$

Therefore, to fix the degeneracy we can take some prescribed value for one of the μ, β, δ_M parameters, the most natural choice being $\mu = \frac{0.1}{24}\ \mathrm{h}^{-1}/(\mu\mathrm{g/ml})$, according to what already described in D4.1. To estimate the other unknown parameters, we fitted $C(t)$ to the experiments by setting the experimental period and obtained all parameters of natural and induced cell death/proliferation. In cases where the density of free iron $M(t)$ was directly measured, we used this information fit also μ by incorporating known $M(t)$.

Then $IC50$ dosage at a given exposure time t_e can be calculated from the relation below with respect to the parameter D

$$C(t_e)|_{D=IC50} = 0.5C(0)|_{D=IC50},$$

where all parameters in the expression of $C(t)$, except the density of nanoparticles D, are fixed.

Example of calculations using data from experiments carried out with BOW MBDs-PDA and (see D4.1 for further experimental details): Time-dependent evaluation of in vitro experimental results concerning silica-embedded polydopamine-coated superparamagnetic iron oxide nanoparticles (MBDs-PDA) provided by partner HGMU are presented below. our model captures the substantial intracellular release of toxic metabolites, likely associated with iron, from nanoparticles, leading to observable cytotoxic effects. Specifically, we determined the median inhibition concentration (IC50 for 24h exposure) as 61.0 µg/ml under usual cultural conditions (S) and 12.44 µg/ml for serum free conditions (WS). Interestingly, the rate of metabolite formation μ in a serum-free medium at high initial concentrations (10; 50 µg/ml) was an order of magnitude higher compared to the corresponding rate of metabolite formation under conditions where serum was added at normal concentration (5% v/v).

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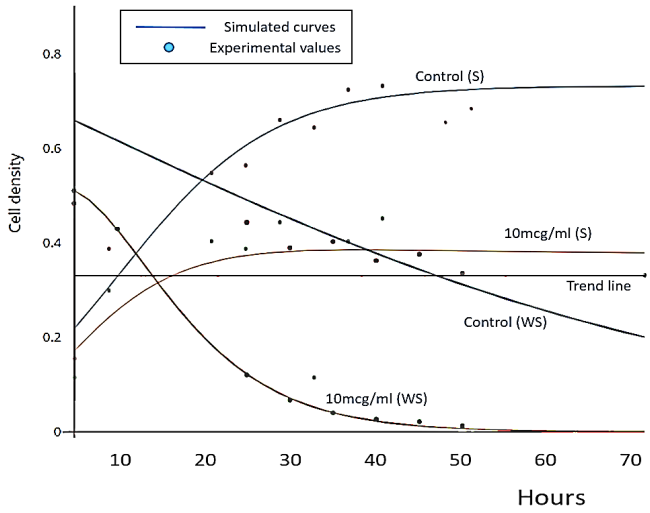

Figure 1. *Experimental data and fitting curves for multi-readout experiments with and without nanoparticles for different media (S; WS).*

The absence of serum predictably decreased the rate of proliferation processes in cell culture—by an average of 8-fold, as per our calculations. In the case of a high concentration of nanoparticles (50 μg/ml), the approximation of the kinetic curves eventually yields calculated values of a toxic metabolite at a level above 350 pg/cell/day (S), indicating obvious toxicity in both incubation modes (S and WS). The calculated metabolite formation rates μ are 0.00172 pg/s/cell (S) and 0.00478 pg/s/cell (WS). These rates can significantly stress intracellular detoxification systems. For a nanoparticle concentration of 10 μg/ml in the presence of serum, the resulting effect on the release of iron into the intracellular environment is 143 pg/cell/day (*D* = 10 μg/ml (S)). This value is almost 1.5 times higher than the threshold value of 100 pg/cell (Fe), considered a safe amount accumulated by cells treated with other magnetic NPs for further efficient development (Calero, et al. 2014; Goranov V. et al., 2020).

Subsequent experiment in vitro has been conducted by BIOS with MNPs (19nm SPIONs Iron core) covered with ampliphilic polymeric surfactant shell. Toxicity effects of these nanoparticles have been observed also in vivo for *C.Elegans* and used to validate toxicokinetic parameters of our model considering literature data (Amigoni L., et.al., 2021).

In this case preliminary analysis show that the duration of non-toxicity t_0, rate of clearance β, and base proliferation rate a are negligible, thus environmental carrying capacity k also cannot be defined meaningfully.

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This leads to a simplified mathematical model that is a direct consequence of the first model:

$$\frac{dM}{dt} = \mu DC,$$
$$\frac{dC}{dt} = -(\delta_D D + \delta_M M)C,$$
$$M(0) = 0, C(0) = C_0,$$

which, contrary to initial model, has an analytical solution for $C(t)$

$$C(t) = \frac{C_0}{\left(cosh\,(bt) + \left|\frac{\sqrt{D}\delta_D\, sinh\,(bt)}{2b}\right|\right)^2}, \quad b = \frac{\sqrt{D(2C_0\delta_M\mu + D\delta_D^2)}}{2}.$$

It allows much faster fitting and renders the values of parameters δ_D and δ_M together with standard errors as $\delta_M = (8.5 \pm 1.9) \cdot 10^{-3}\ h^{-1}/(\mu g/ml)$ $(P = 1.3 \cdot 10^{-4})$, $\delta_D = (1.34 \pm 0.24) \cdot 10^{-4}\ h^{-1}/(\mu g/ml)$ $(P < 10^{-5})$. Fit is robust, with $R^2 = 0.999$ and Bayesian information criterion $BIC = -118.7$ (Fig. 2). IC50 value for 72 h exposure $40 \pm 12\ \mu g/ml$ – quite close to defined in the previous experiment.

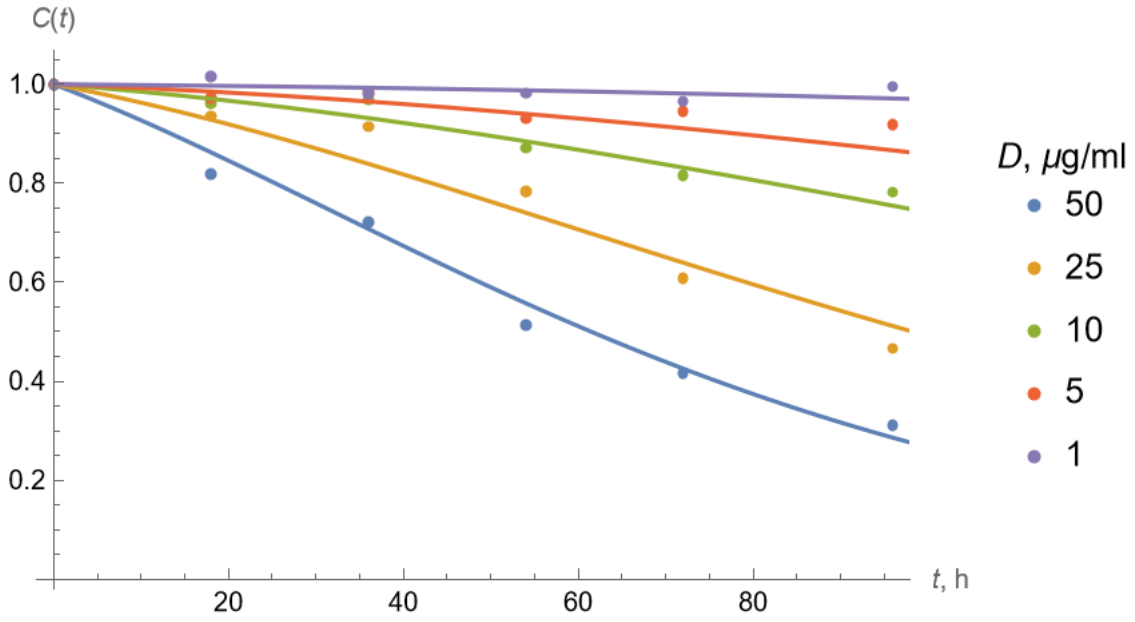

Figure 2. *Experimental data on cell viability $C(t)$ versus time and best fitting curves for human intestinal cells (CaCo2 cells)*

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In vitro-in vivo scaling can be achieved by additional modelling of the nanoparticle influence on animal model *Caenorhabditis elegans.*

We partially used the approaches proposed early for of one-compartment models of toxicokinetic (O'Flaherty E.J , 1981; Wambaugh JF et.al., 2018), considering that:

- Toxic substance is passed into the organism in mass quantity A into the "Gut Lumen" compartment that represents digestion system.
- There are two ways for the substance to get out of the "Gut Lumen" compartment: first it is going out or eliminated by chemical reactions etc. with the rate of k_{gutel}, second it is absorbed into the internal "Primary" compartment of the organism with the rate of k_{gutabs}.
- Besides a source of the substance by absorption from the "Gut Lumen" compartment into the "Primary" compartment there is a drain by clearance with the rate of k_{cl}.

The system of differential equations describing the model is:

$$\frac{dA}{dt} = -(k_{gutel} + k_{gutabs})A + f(t),$$

$$\frac{dD}{dt} = \frac{k_{gutabs}}{V_{dist}}A - k_{cl}D,$$

where $f(t)$ is the function that represents intake of toxic substance, in the case of one-time ingestion it is $f(t) = 0$, V_{dist} is the volume of distribution for the "Primary" compartment, D is the density of nanoparticles in the "Primary" compartment.

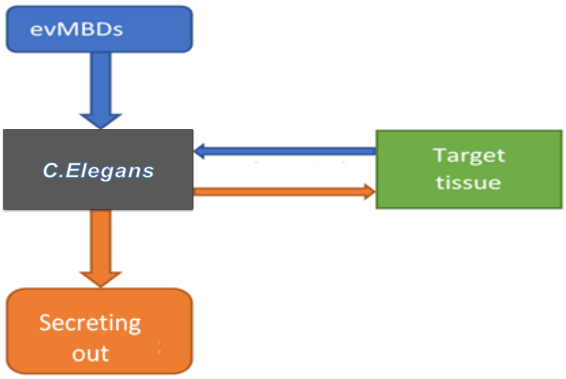

Figure 3. *Processing of nanoparticles in C. elegans.*

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Initial values for the case of one-time ingestion are $A(t=0)=A_0$, $D(t=0)=0$. The solution for the system is then (A is measured in mass units, C in mass per volume)

$$A(t) = A_0 e^{-(k_{gutel}+k_{gutabs})t},$$

$$D(t) = \frac{A_0}{V_{dist}} \frac{k_{gutabs}}{k_{cl}-k_{gutel}-k_{gutabs}} \left(e^{(-k_{gutel}-k_{gutabs})t} - e^{-k_{cl}t}\right).$$

Maximal concentration for the model is achieved at time:

$$t_{max} = \frac{1}{k_{cl}-k_{gutel}-k_{gutabs}} log\left(\frac{k_{cl}}{k_{gutel}+k_{gutabs}}\right)$$

and is equal to

$$D_{max} = \frac{A_0}{V_{dist}} \frac{k_{gutabs}}{k_{gutel}+k_{gutabs}} \left(\frac{k_{cl}}{k_{gutel}+k_{gutabs}}\right)^{\frac{k_{cl}}{k_{gutel}+k_{gutabs}-k_{cl}}}.$$

For the case of $k_{gutel}=0$ this simplifies to

$$D_{max} = \frac{A_0}{V_{dist}} \left(\frac{k_{cl}}{k_{gutabs}}\right)^{\frac{k_{cl}}{k_{gutabs}-k_{cl}}}.$$

Parameters A_0 that is mass of toxicant, and V_{dist} that is distribution volume of organism (can be changed to mass of organism without affecting the model structure) are obtainable immediately. The experiment can proceed in the next way: toxicant is given to organism in a known dose and then its content in the organism is traced in time. Then non-linear least-squares fitting can be used to obtain model parameters k_{gutel}, k_{gutabs}, k_{cl}.

Toxic influence is connected with the substance concentration $D(t)$ inside the "Primary" compartment that can be used as D input. For the case of continuous submersion of C.Elegans in the medium with constant concentration of nanoparticles after a short period of time fluxes into and out of the "Gut Lumen" compartment will compensate and then $A = const \times C_{NP}$, C_{NP} being the concentration of nanoparticles in the medium. It allows us to use simplified model:

$$\frac{dD}{dt} = k_u C_{NP} - k_c D,$$

where D is NP concentration in worms ($\mu g \cdot g^{-1}$ wet weight), C_{NP} is NP concentration in water ($\mu g \cdot ml^{-1}$), k_u is uptake rate ($ml \cdot g^{-1} \cdot h^{-1}$), k_c is clearance rate (h^{-1}).

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However, the absence of quantitative data on MNPs concentration in *C. elegans* prevents us from using this directly. The literature data (Yang Y.F. et.al., 2017) suggest the following parameters $k_u = 0.046 \pm 0.034\ \ h^{-1}/(g/ml)$, $k_c = 0.001 \pm 0.015\ \ h^{-1}$, and we use it as reference parameters for the model validation. The preliminary analysis shows that for worms the duration of non-toxicity t_0 is substantially different from zero. *C. elegans* does not reproduce during the experiments, so that $a < 0$ and environmental carrying capacity k is not constrained by the fitting. Moreover, both clearance rate β and specific toxicity of metabolite δ_M appears to be non-significant, so that the final mathematical model simplifies to

$$\frac{dC}{dt} = (a - \delta_D D)C, \qquad C(t_0) = C_0,$$

note that here D is nanoparticle concentration in medium. This model has an analytical solution for $C(t)$

$$C(t) = C_0\ exp\left(\theta(t - t_0)(a - \delta_D D)(t - t_0)\right).$$

The recovered parameters (according to literature data (Amigoni L., et.al., 2021)) with their standard errors are $t_0 = 26.6 \pm 9.5$ h, natural death rate $a = -(4.2 \pm 1.2) \cdot 10^{-3}\ h^{-1}$, specific toxicity $\delta_D = (1.2 \pm 0.4) \cdot 10^{-4}\ h^{-1}$/(μg/ml). LC50 for 72 h exposure is defined as $128 \pm 42\ \mu g/ml$ – substantially higher than for cell cultures studied.

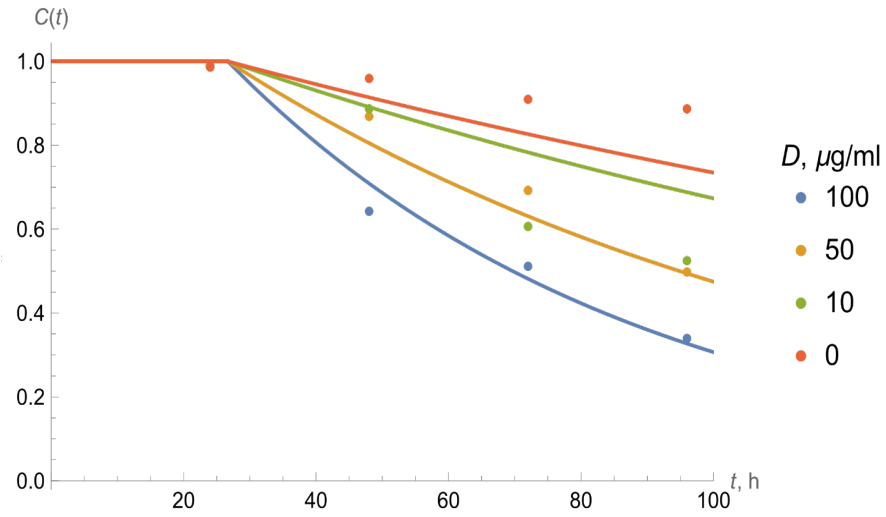

Figure 4. *Evaluation of experimental data cytotoxic effects of nanoparticles reveals toxicologic profile for C. elegans as a delayed fading exponent.*

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Further possible approaches for interspecies mathematical extrapolations are well-established in the literature (T. Vermeire et. al., 2001; D. Romeo et. al., 2022). Using interspecies extrapolation factor

$$k_i = 19$$

and LC50 to chronic IC50 factor

$$k_E = 26$$

EC50 extrapolation to human is then

$$EC50 = 0.26\ \mu g/ml$$

EC50 is the lifetime dose inducing non-cancer diseases in 50% of the population, considering 70 years of lifetime, a 70 kg body weight.

The results described above need to be extended by further experimentation, specifically the model should be supplemented to include other micro-environmental effects. The model's ability to predict higher toxicity levels compared to single-readout experiments underscores its potential in capturing complex micro-environmental effects and finite capacity within the bioreactor system. This suggests the importance of considering dynamic and time-dependent profiles in understanding the toxic effects of engineered nanomaterials. The conclusion drawn about the sufficient biocompatibility of the studied nano-objects, especially in the presence of serum, paves the way for their potential applications. Additionally, the developed model's capability to approximate time-dependent profiles of induced cell death and predict various features of toxic effects in a time- and concentration-dependent manner enhances its utility for further studies and applications.

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2.2 Model for evaluation of immune cell activation.

Our mathematical model is founded on assessing parameters that characterize the interaction of various immune factors within the M1/M2 paradigm. It considers the temporal dynamics of immune factor expression in vitro. The distinct responses of M1 and M2 factor expression to antigen influence have been documented in various literature sources, such as Martinez F.O. et al. (2014). Many studies explore specific forms of M1 or M2 activity, often manifested as soluble factor secretion. We adopt this manifestation as a marker and estimate it through mathematical analysis. We consider the plasticity of the M1/M2 state, acknowledging that macrophages can transition between pro-inflammatory M1 and anti-inflammatory M2 phenotypes, responding to the microenvironment's needs. Alternatively, they can remain in a naive state in the absence of external signals. The algorithm's foundation lies in the intricate details of immune cell interactions under antigen influence, as illustrated schematically in Fig.5.

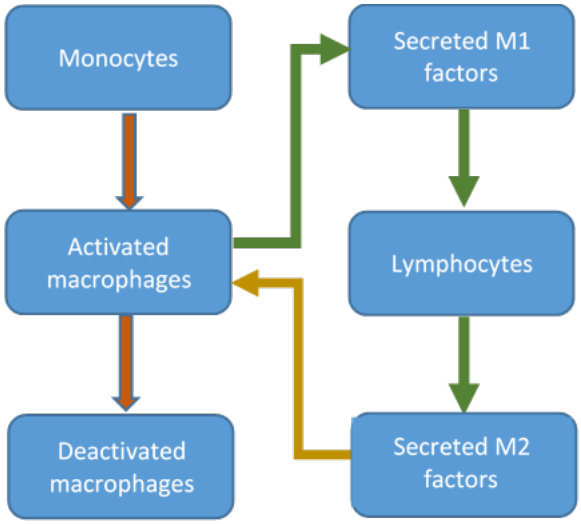

Figure 5. *Scheme of immune cell interaction (green arrows are activating influences and yellow are inhibitory once, brown arrows outline changes of mononuclear cell pools).*

The original algorithm is expressed as a system of four ordinary differential equations with constant coefficients. It accommodates changes in concentrations of non-activated (monocytes) and activated macrophages (C0, C1), as well as concentrations of pro- and anti-inflammatory factors (M1, M2) under experimental conditions. This system considers the exponential decrease of non-activated monocytes and is subsequently refined into a system of differential equations for activated macrophages and lymphocytes interacting through pro- and anti-inflammatory factors. The algorithm is based on the following equations:

$$C_0 = C_{00}\exp(-k_1 t)$$

$$\begin{cases} \dfrac{dC_1}{dt} = k_1 C_0 - k_2 C_2 M_2; \\ \dfrac{dM_1}{dt} = k_{M1}^{+} C_1 - k_{M1}^{-} M_1 \end{cases}$$

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According to our model, the rate of change in the concentration of activated macrophages is equal to the difference between the rates of decrease in the concentration of monocytes (k_1C_0) and deactivated macrophages ($k_2C_2M_2$). The latter is proportional to the concentration of lymphocytes (C2), where k2 is the coefficient of inhibition of monocyte transformation in activated macrophage under influence of anti-inflammatory factor M2, where k1 outline the transformation of C0 in activated macrophages.

The rate of change of the concentration of the anti-inflammatory factor M2 changes proportionally to the difference between the concentrations C2 and M1 and the anti-inflammatory factor M2 multiplied by the rate constants k^{+}_{M1} and k^{-}_{M2} respectively. Where k^{+}_{M1} is rate constant of M1 amplification under influence of activated macrophages and k^{-}_{M1} outlines the rate of released M1 auto-inhibition. Correspondingly rate coefficient of k^{+}_{M2} outlines amplification of M2 factor release by lymphocytes and activated macrophages, whereas coefficient k^{-}_{M2} outlines process of auto-inhibition of M2 factor release.

For the transient problem, initial conditions are defined as follows:

$$C_1(0)=0;\quad \left.\frac{dC_1}{dt}\right|_{t=0}=k_1C_{00};\quad \left.\frac{d^2C}{dt^2}\right|_{t=0}=-k_1^2C_{00};\quad \left.\frac{d^3C}{dt^3}\right|_{t=0}=k_1^3C_{00};\quad C_{00}=C_0(0);$$

$$M_2(0)=0;\quad \left.\frac{dM_2}{dt}\right|_{t=0}=0;\quad \left.\frac{d^2M_2}{dt^2}\right|_{t=0}=0;$$

$$M_1(0)=0;\quad \left.\frac{dM_1}{dt}\right|_{t=0}=0$$

Analytical solutions of these equations yield functions that approximate the experimental values. Model parameters are then fine-tuned to ensure that the model accurately represents the experimentally measured kinetics with the highest quality possible. To address the inverse coefficient problem through the least squares method, the Hook-Jeeves method is employed to find the global minimum of the objective function. The corresponding web tool using conventional approaches has also been developed. In particular, the service was implemented on the Apache web server platform using CGI applications written in the C++ programming language. WEB was programmed using HTML, CSS, PHP and JavaScript languages. The web server interacts with programs written in C++ using RabbitMQ queues.

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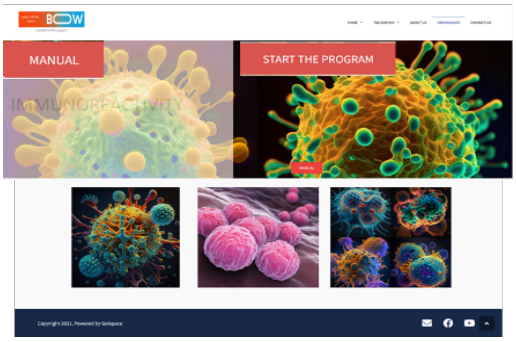

Figure 6. *Web-interface of the program.*

The website (fig.6) is designed to support various input and output formats, and it includes specially designed templates to process experimental values of different dimensions (https://biocompatibilitysystems.com/resources). Utilizing the approximation approach, we determine concentration values of the factors—Mph (activated macrophages), M1 (pro-inflammatory factor), and M2 (anti-inflammatory factor)—at maximum extremes and at characteristic times corresponding to peak values (Fig. 7).

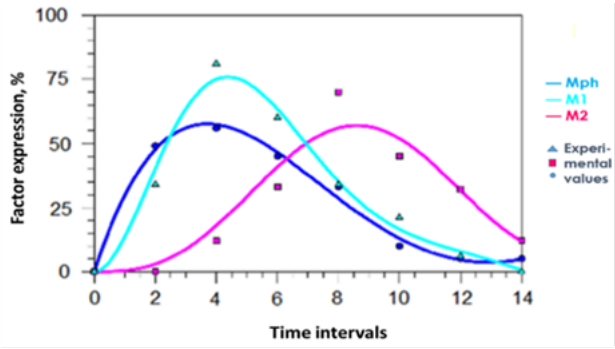

Figure 7. *Scheme of immune factor kinetic.*

We consider stages where macrophages play a pivotal role, initially acting as protagonists in the pro-inflammatory period, recruiting M1 macrophages to the site. This is followed by an anti-inflammatory phase where the M2 phenotype takes precedence. We calculate the ratio of maximum concentrations to concentration values at characteristic time points, which serves to characterize immune response features assessable under in vitro conditions. Subsequently, three parameters, expressed in relative units, are presented to the end user for evaluating the degree of immune cell activation. The data can either be stored in a database, accessible to any user, or stored individually after registering on the website. Thus, we selected a set of markers for the kinetics of M1/M2 immune cell activation.

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The initial model was developed using data generously provided by our partner IRIB-CNR, as outlined in D.4.2, as well as data from the literature (Alvarez M.M. et al., 2016). With this dataset, we fine-tuned the dynamic parameters associated with M1/M2 interactions within a mononuclear culture. Subsequently, BIOSYS conducted experiments using a mixed culture of mononuclear cells and lymphocytes, following a well-established methodology (Chang D.T. et al., 2008). These latest experiments served as the foundation for refining and validating this mathematical model. It is important to highlight that this mathematical model is well-suited as a screening tool for selecting compounds with minimal pro-inflammatory immune reactivity among similar compounds. The service is designed to cater to the biomedical community's needs, offering a straightforward and user-friendly tool for easy comparative analysis using various time-dependent experimental data obtained from immune cell culture.

3 CONCLUSION

Biological processes involving toxin metabolism and cell death often exhibit nonlinearity, owing to intricate interactions between cells and toxic compounds released from nanoparticles. This study focuses on developing a mathematical model aimed at predicting long-term adverse effects and assessing the biocompatibility of MNPs, employed in the manufacturing of evMBDs. The described mathematical model takes into account the temporal evolution of cytotoxicity/immunogenicity in vitro, considering kinetic processes such as the accumulation of nanoparticles in cells, release of metabolites, maintenance of proliferation potential, and kinetic processes of immune cell activation. The toxicodynamic model assesses kinetic cytotoxicity profiles, revealing higher toxicity values compared to single readout experiments for the investigated nanoparticles. This developed model enables the approximation of time-dependent cell death profiles and the prediction of various features of nanoparticle-induced toxic effects in a concentration- and time-dependent manner. Furthermore, the model, tailored for screening estimation of immune reactivity, is adapted for application using a special web tool and can contribute to further improving and disseminating project results. After further comparative studies, including the examination of a sufficient amount of evMBDs, the model enables the exclusion or indication of the need for modification of manufactured nano-objects. This facilitates the selection of optimal components for evBOW production, ultimately aiming for the most biocompatible outcome. Such an approach is intended to minimize the risk of potential adverse effects upon interaction with a microorganism in vivo.

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