Program “Immune” (Immunoreactivity) (adapted for soluble antigens and nanoparticles).

Approach (background, methodology and mathematical simulation): The M1/M2 paradigm proposed at the beginning of the 21st century states that macrophages could switch their phenotype from pro-inflammatory (M1) to anti-inflammatory (M2) and vice versa depending on the microenvironment, or maintain the naïve M0 state in the absence of external signals. Numerous studies address specific expressions of M1 or M2 activity in the form of soluble factor secretion by macrophages in extracellular space (Poltavets et.al. 2020). Alternatively, activated M2 macrophages have pronounced anti-inflammatory properties. E.g., these cells are active phagocytes involved in extracellular matrix remodelling and angiogenesis. M2 macrophages can release factors that usually modulate/suppress the immune response and inhibit an inflammation.

Macrophages play a dual role, promoting inflammatory responses on the one hand and supporting tissue regeneration on the other. In patients with genetic syndromes (e.g., muscular dystrophies) or systemic diseases (obesity, autoimmune responses, some kind of tumours), macrophages are involved in exacerbating fibrosis, atherosclerosis, tumor growth, etc. In the acute phase of inflammation, macrophages are classically activated as M1. Together with neutrophil granulocytes, they collect dead cells and tissue debris at the site of injury and release various proinflammatory cytokines that stimulate exudation. At the end of the acute phase, the number of alternatively activated (M2) macrophages at the site of inflammation increases, while the number of M1 cells decreases, indicating the involvement of M2 cells in the transition to the productive phase of inflammation and regeneration (Jiang, H.R.et.al 2012).

M1 macrophages are very important in the initial phase of inflammation: they provide site debridement by phagocytosis and promote chemotaxis of other immune cells by expressing proinflammatory cytokines, especially IL-1, IL-6, IL-8, TNF-α and IFN-γ. The outcome of the inflammatory response (regeneration or fibrosis) correlates with the timely switch of macrophage polarization from M1 to M2 (Chung, L., et.al. 2017). The switch to the M2 phenotype occurs on days 3-7: macrophages acquire anti-inflammatory properties and begin to secrete IL-10 and TGF to promote regeneration and angiogenesis (Martinez, F.O. et.al. 2017). Imbalanced M1 and M2 macrophage polarization in the human body can cause chronic diseases. With the advent of the M1/M2 paradigm, the course of typical pathological processes can be reevaluated considering macrophage polarization.

In the context of bio(nano)material implantation, although the initial presence of M1 macrophages promotes a necessary inflammatory response, otherwise prolonged persistence of M1 macrophages leads to edema, granulomas and fibrous encapsulation, resulting in chronic inflammatory events and failure of biomaterial integration (Rukmani Sridharan et.al. 2017). This is particularly detrimental for regenerative biomaterials, which aim to replace lost tissue and avoid the formation of scar tissue.

M2 macrophages consistently express scavenger and mannose receptors (CD206), release anti-inflammatory cytokines such as IL -10 within the M2 subsets, m2a (induced by IL -4 and IL -13) and M2b (induced by immune complexes and agonists of Toll-like receptor subsets immunoregulatory functions by triggering anti-inflammatory Th2 lymphocyte responses (through secretion of IL -10, IL -1ra and IL -6).

The presence of such anti-inflammatory cytokines and tissue remodelling responses can support the vascularization of implanted biomaterials by inhibiting the formation of fibrous tissue, thereby significantly improving the integration of the biomaterial and allowing it to perform its intended function. Following surgical implantation, the initial M1 response has been shown to be responsible for the recruitment of inflammatory cells to the site of injury and initiates the foreign body response necessary for wound healing.

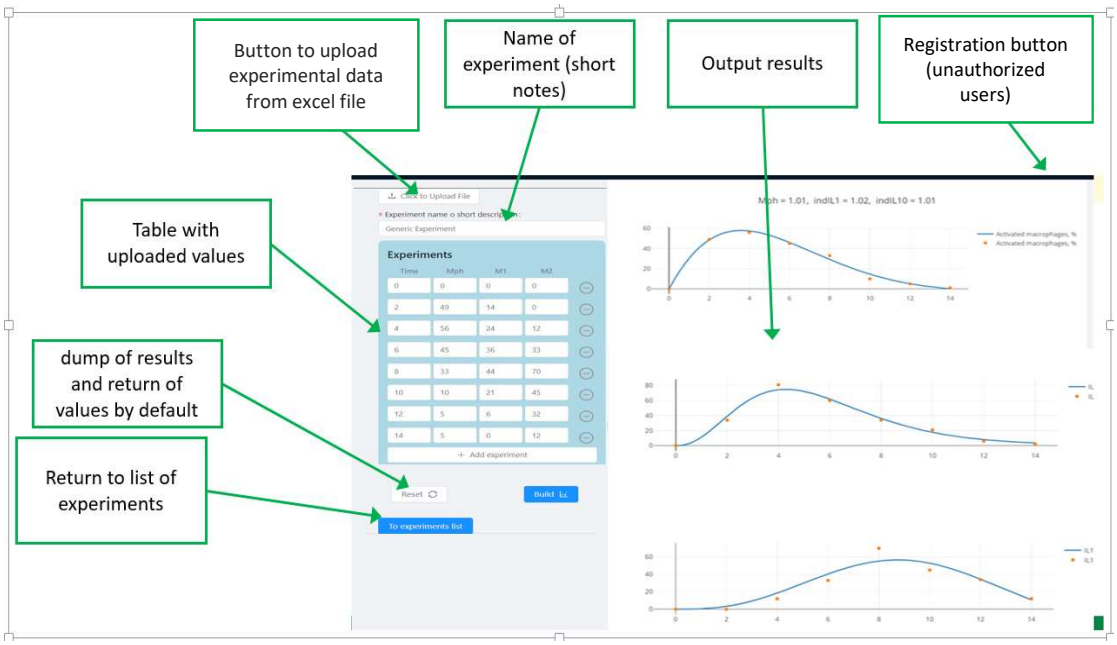
However, after this initial response, continued activation of M1 cells leads to the production of toxic reactive oxygen intermediates that result in excessive oxidative damage to the biomaterial. In addition, the formation of a fibrous capsule as a result of the ongoing inflammation could impair the ability of regenerative biomaterials to promote tissue formation or degrade in the intended manner (Yanez M, et.al 2017). Therefore, a subsequent transition to the M2 phenotype, which promotes tissue remodelling and repair, is generally considered a favourable adaptation.

According to various literature sources, the factors induced when immune cells come into contact with antigen in vitro may have specific kinetics (some publications also suggest lymphocytes coculture as effectors in combination with macrophages) (Chung, L., et.al. 2017; Xiaoyuan Miao et.al. 2017). This opens the possibility to estimate, at least roughly, the activity of the immune response to an antigen. In the context of our model, if we need to assess the immunoreactivity of nanoagents, the initial presence of M1 macrophages will promote the inflammatory response, a prolonged presence of M1 will lead to severe outcomes that can cause chronic inflammatory events and failure of cellular activity (Jinhua Li ., et.al 2021; Shanze Chen et.al 2023).

This is particularly detrimental to regenerative influences, where the goal is to replace lost tissue and avoid the formation of scar tissue or inflammation in site of an introduction. Thus, the model includes four cell components and two parameters that characterize M1 and M2 activities.The model describes the immunological response over time. The typical evolution involves first an increase and then a decrease in the number of activated macrophages over time, followed by the same behaviour of M1 and then M2 concentrations.

The original algorithm is represented by a system of four ordinary differential equations with constant coefficients. It takes into account the change in the concentrations of non-activated (monocytes) and activated macrophages (C0, C1) and the concentrations of pro- and anti-inflammatory factors (M1, M2) under experimental conditions, mainly described by T. Chang (2008). This system takes into account the exponential nature of the decline in non-activated monocytes and is successively reduced to three third-order differential equations for activated macrophages and lymphocytes interacting by pro- and anti-inflammatory factors released in cell culture.

WEB interface: The pro-inflammatory phase is characterized by M1 factors (e.g. IL -1) and macrophage activation, while the anti-inflammatory phase with reciprocal action and secretion of M2 factors (e.g. IL -10) is accompanied by an attenuation of the immune response.



Input page of “Immunoreactivity”

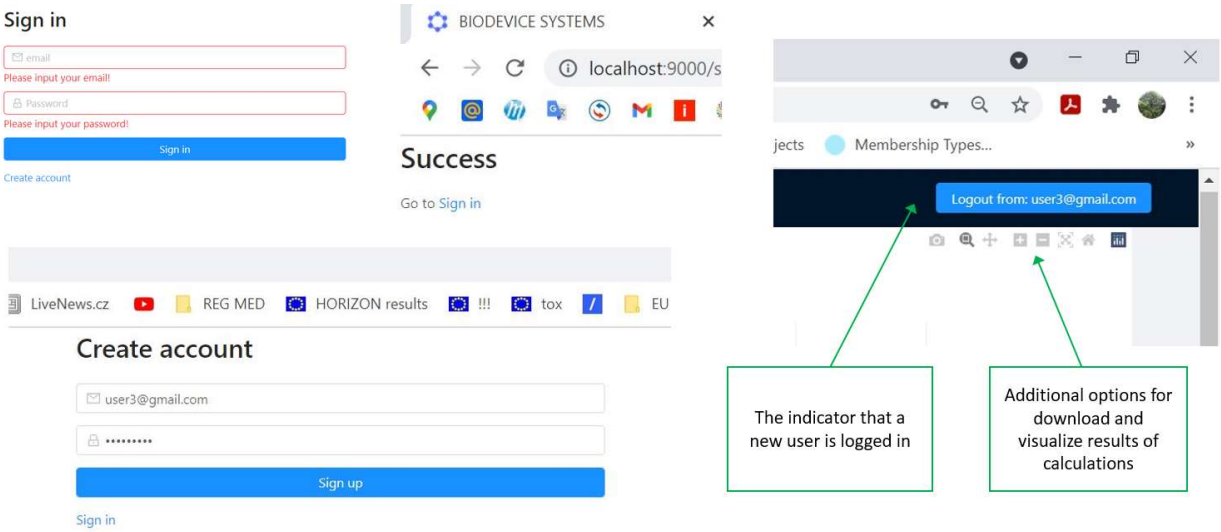
There is a button to upload the file in the form of a special Excel file with experimental values that can also be used for data entry input. The end user has an option to select three sets of kinetic values depending on time - kinetic parameters of macrophage activation, M1 - kinetic parameters and M2 - kinetic parameters.

Once the parameters are set (end user input or selection of default values), the calculation can be performed. The finished calculation provides the results, which can be downloaded.

Each result is stored in the database and can be retrieved when required. The results are displayed as images showing the toxicodynamic process in the cell culture over time. Once the calculations have been performed, the results can be downloaded as a *.png image to a local computer or in the form of an Excel file.

The individual modeling sets can be marked and downloaded in the "List of experiments" with a special short note or according to the exact time of the simulation process. Below you will find an example of the simulation of a biomaterial that did not trigger an excessive immune response (name of the experiment: "Normal immune response").

Registration of a new user is possible via the top button at the bottom right. If you click on the blue button in the upper right axis of the desktop, a registration page opens where you can set a password and use the user's e-mail address as login.



Authorization form and plot-panel.

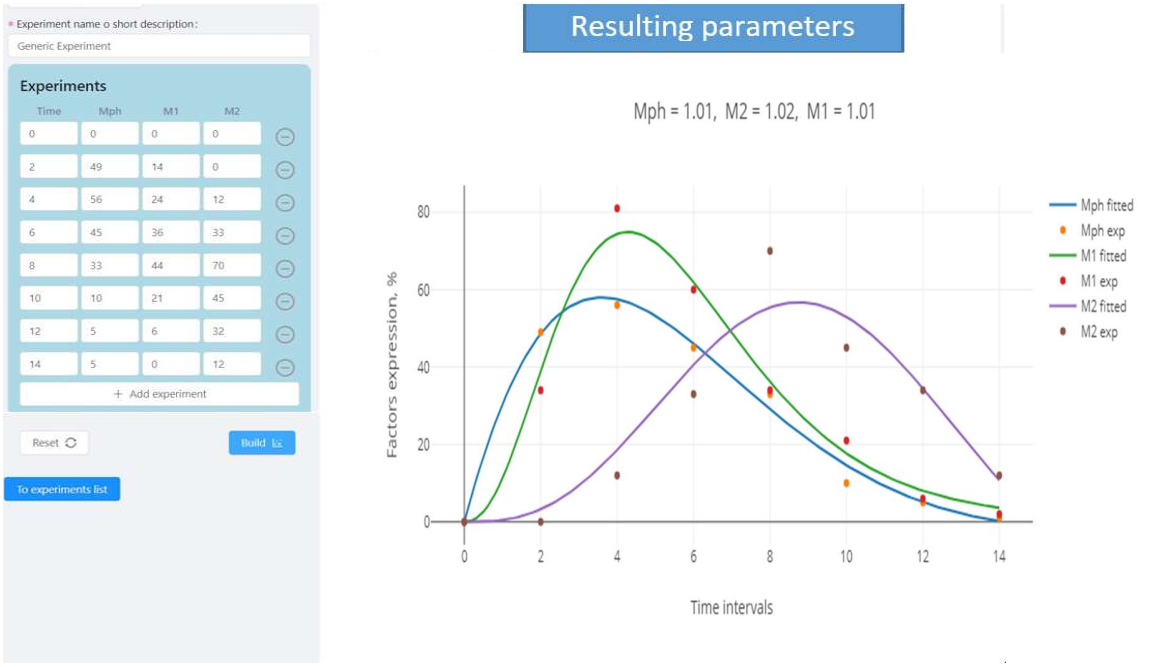
The specific behavior of the expression (secretion) of M1 and M2 factors under the influence of an antigen is mentioned in a variety of literature sources (Kumolosasi et. al, 2014., Tedesco et. al, 2018.) and shown in figures below. The simulation is based on the (in vitro) evaluation of parameters describing the interaction of cells as targets of an antigen using released immune factors and takes into account time-dependent kinetic processes of the expression of these factors.
Numerous studies work with specific forms of expression of M1 or M2 activity in the form of soluble factor secretion, which we also use as a kind of marker estimated by mathematical analysis.

Analytical solutions of these equations yield functions that approximate the experimental values. The model parameters are then adjusted so that the model describes the experimentally measured kinetics with the best possible quality.
As a result, three parameters, expressed in relative units, are proposed to the end user to evaluate the degree of immune cell activation. The data can either be stored in a database and be available to any user, or it is possible to save individual data/results after registration on the website.

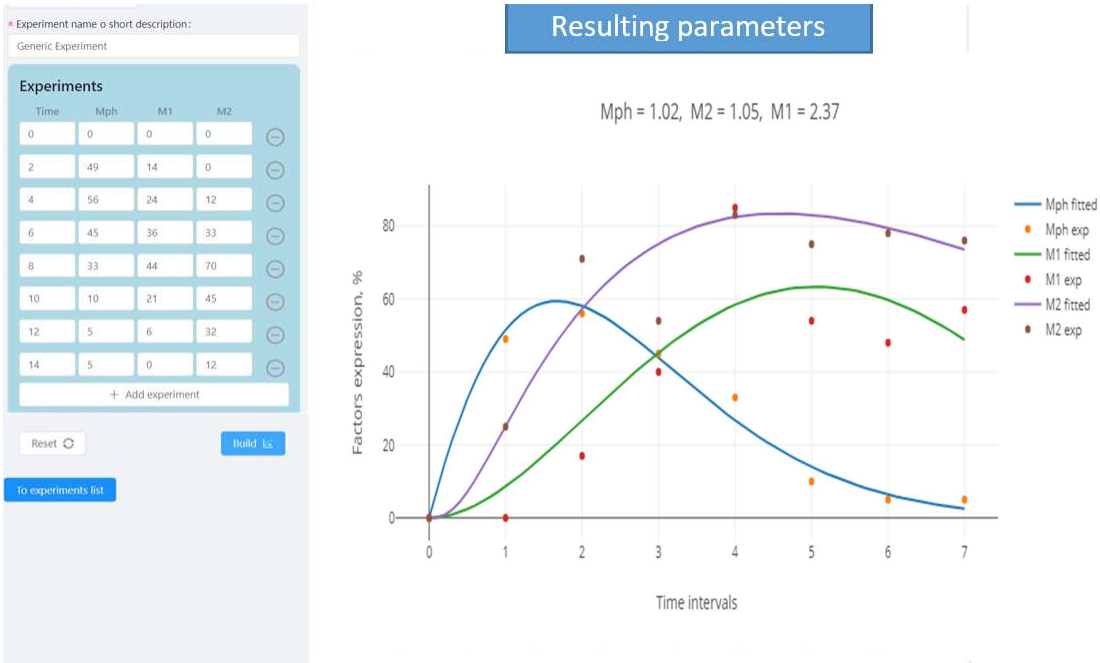
Using the model, we find the specific indices of the factors: Mph (activated macrophages), M1 (pro-inflammatory factor) and M2 (anti-inflammatory factor) at the maximum extreme values and at characteristic times for peak values. The resulting indices not exceeding 1.1 indicate the absence of exaggerated immunoreactivity to the antigen, while a value above 1.1 indicates a hyperergic behavior of immune cell activation in vitro.

The figure below illustrates the "normoergic" and "hyperergic" activation of immune cells in the following figures.

The separate modeling set can be marked and downloaded with a special short note or after the exact time of the simulation procedure in the “List of experiments”. The resulting values that do not exceed the ratio 1.1 (Mph=1.01; M1(IL-1) =1.01;M2 (IL-10)) are considered “normoergic” immune cell activation, while exceeding these values in at least one of the indices (more than 0,2) indicates excessive/unbalanced immune cell activation.



Result of simulation #1. "Normoergic immune response; according to E. Kumolosasi (2014) experimental data"



Result of simulation #2. "Pathological immune response; according to E. Kumolosasi (2014) experimental data"