Discussion on "Endocytosis as a stabilizing mechanism for tissue homeostasis"

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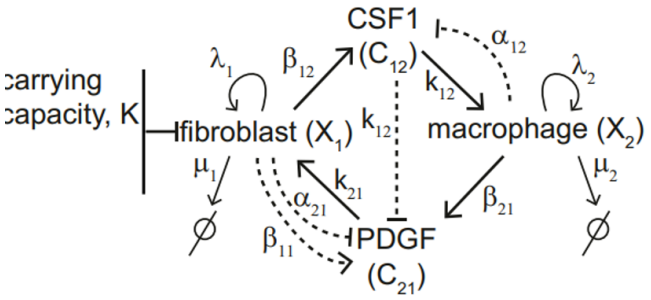

Figure 1. Circuit topology of the FB-MP model based on *Zhou et al.* experimental observations.

1. The paper uses only one model or compares alternative models for the same system? This paper first creates the model that replicates the cellular homeostasis of fibroblasts (FB) and macrophages (MP) based on the observations obtained in *Zhou et al.* experiences. The cell growth is signalled by growth factors (GF) and must be regulated through negative feedback to achieve homeostasis. The concentration of GFs depends on their rates of secretion into cells and the rates of removal, and cells are able to divide and are removed (apoptosis) at rates that are affected by the concentration of secretory factors.

The authors then search if there is different topologies that can achieve the steady state as well. This can be achieved by circuit inclusion or removal of interactions. In total, there are 144 different topologies when considering two cells. For this to be possible, they redefined the equations for the GFs. Later on, the last defined circuit was incorporated in circuit simulations of 3 and 4 distinct cell populations.

2. What type of rate expressions are used? The rate expressions of the two types of cells are considered, i.e fibroblasts (X_1) and macrophages (X_2) and of their respective growth factors, C_{21} and C_{12}. The expressions of the original FB-MP model are given by Equation 1, 2, 3 and 4.

$$\frac{dX_1}{dt} = X_1\left(\lambda_1 h\left(C_{21}\right)\left(1-\frac{X_1}{K}\right)-\mu_1\right) \tag{1}$$

$$\frac{dX_2}{dt} = X_2\left(\lambda_2 h\left(C_{12}\right)-\mu_2\right) \tag{2}$$

$$\frac{dC_{12}}{dt} = \beta_{12}X_1 - \alpha_{12}X_2 h\left(C_{12}\right) - \gamma C_{12} \tag{3}$$

$$\frac{dC_{21}}{dt} = \beta_{21}X_2\frac{k_{12}}{k_{12}+C_{12}} + \beta_{11}X_1 - \alpha_{21}X_1 h\left(C_{21}\right) - \gamma C_{21} \tag{4}$$

The cell equations of Equation 1 and Equation 2 take into account cell proliferation (minus) removal according to cell concentration. The "proliferation factor" considers proliferation rate, λ_i, the effect of each growth factor on its target cell, as described by a Michaelis–Menten-like function $h(C_{ij}) = C_{ij}/(C_{ij}+k_{ij})$ with halfway effect at k_{ij} or binding affinity, and, in the case of X_1, the carrying capacity by the function $1 - X_1/K$, with K as the carrying capacity, as seen in Figure 1.

The growth factors expressions of Equation 3 and Equation 4 consider secretion rates by cells and their removal by endocytosis rates (considering endocyting cell concentrations and effect of growth factor) and by degradation/diffusion rate. In Equation 4, the first term indicates the inhibition of the expression of C_{21} by C_{12}.

The expressions were then slighly altered to create the diferent circuit topologies. Using the $\theta, \omega = -1, 0, 1$ parameters to inhibit, shut down or activate gene expression of the other GF, $\beta_{11}, \beta_{22} \geq 0$ to secrete or not its own GF, and $\alpha 12$, $\alpha_{21} \geq 0$ to determine if endocytosis is the primarily mechanism of GF removal.

$$\frac{dC_{12}}{dt} = \beta_{12}X_1\left(1-\frac{1}{2}\theta(1+\theta)+\theta h\left(C_{21}\right)\right)+$$

$$+\beta_{22}X_2 - \alpha_{12}X_2 h\left(C_{12}\right) - \gamma C_{12} \tag{5}$$

$$\frac{dC_{21}}{dt} = \beta_{21}X_2\left(1-\frac{1}{2}\omega(1+\omega)+\omega h\left(C_{12}\right)\right)+$$

$$+\beta_{11}X_1 - \alpha_{21}X_1 h\left(C_{21}\right) - \gamma C_{21} \tag{6}$$

This way 144 different types of circuit topologies can be created ($3\times3\times2\times2\times2\times2$) and analysed. The authors also explored three and four cell circuits composed of the previous two-cell circuit modules.

3. How where the model parameters defined? Initially there was a total of 13 parameters, but after a dimension analysis, the equation was changed to a total of 8 parameters that were fixed with a reference set of biologically plausible values ($\tilde{\lambda}_1 = 3.7$, $\tilde{\lambda}_2 = 2.2$, $\tilde{\mu} = 1$, $\tilde{\alpha}_{12} = 10$, $\tilde{\alpha}_{21} = 1$, $\tilde{\beta}_{12} = 3.4$, $\tilde{\beta}_{11} = 6.8$). These parameters allowed a good description of the dynamics observed experimentally for the FB-MP circuit (*Zhou et al.*).

4. Does the model aim to make quantitatively accurate predictions? The model created only makes qualitative predictions since the objective of the authors is to analyse the dynamics of the system to find what type of

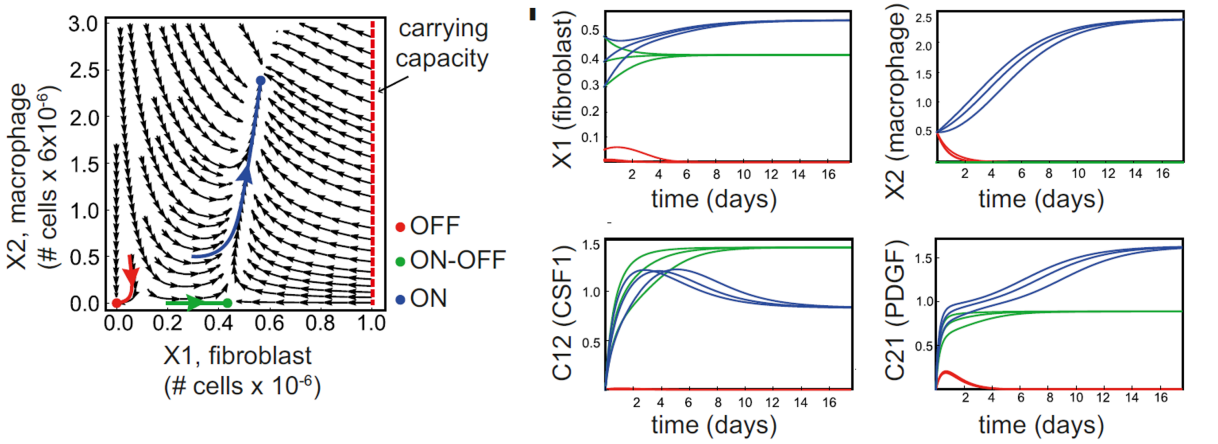

Figure 2. The phase portrait of the FB–MP circuit provided by the model of Equation 1, 2, 3 and 4, using biologically plausible parameter values and cell and GF concentrations dynamics for the trajectories highlighted.

interactions allows the system to achieve the stability of the two cells (steady state). The paper doesn't determine absolute values since that isn't the objective.

However, at the beginning of the paper, they recreated the quantitative results of the *Zhou et al.* experimental observations to determine if their system was reliable.

5. Is some bifurcation analysis or parameter scan study presented? If yes, why was it useful for the paper? The parameter space of the original FB-MP was explored and the existence of three fixed points occured for a wide range of model parameters, considering biologically plausible values. When these values were varied, e.g β and λ/μ by 10-fold and α by 100-fold, one or two of the fixed points were lost, leading to loss of one or both cell types regardless of initial conditions (these altered parameter sets provided phenotypes similar to degenerative diseases).

When analysing the different circuit topologies and, more specifically, comparing endocytosis vs cross-regulation as a stabilizing mechanism for cell homeostasis, the authors varied some parameters to analyse the parameters at which the models lose the ON state. With this, they could conclude that endocytosis was a much more robust mechanism for homeostasis than cross-inhibition, since it only lost the ON state for larger parameter variation.

For three and four cell configurations the parameters were also varied and a wide range of feasible parameters (variations up to 10-fold) showed stability. Note that with parameter variation, we can compare the robustness of each model and analyse if the models are viable for biological plausibel parameters.

6. Is sensitivity analysis presented? What conclusions could be obtained? Sensitivity analysis requires the computation of model values, to see how their alteration is *sensed* by the system. Therefore, no sensibility analysis was presented in the paper since the results are qualitative.

7. Are the attractors of the system characterized (number, type, stability, basins of attraction)? If yes, what are the implications of these findings for the study? The authors refer multiple types of attractors in the parameter space. A healthy ON, OFF bistable system with a ON/OFF unstable attractor. The ON steady state being a fixed point with homeostasis between the two types of cells, the OFF state a fixed point where neither type of cell survives and a ON/OFF unstable fixed point that depends on the circuit topology of the system. For example, in the original FB-MP system the fibroblasts secrete their own growth factor, so the unstable ON-OFF state has a concentration of fibroblasts and no macrophages since the fibroblasts do not depend on macrophages presence but the opposite happens, as seen in Figure 2.

Other circuit topologies presented ON, OFF bistability without the ON/OFF attractor (note that we consider always biologically plausible parameter intervals), and single fixed point attractor with loss of one type of cell or the two.

It's worth noting that the authors metion a necessary and suficient condition for a stable ON state, this being a downregulation of the growth factor of the macrophages, since its carrying capacity is not considered in the model[1]. They considered endocytosis and cross-regulation mechanisms and analysed the basins of attraction of each mechanism in the parameter space, concluding that the basin of atraction of the ON state was larger in endocytosis-based systems and OFF state basins smaller, indicating a two-cell homeostasis for a larger set of conditions (Figure 3). Also, endocytosis systems are 8-fold faster at reaching the homeostasis. We could interpret this saying that endocytosis is a good mechanism for fast repair of cell damage while cross-inhibition for preventing proliferation of cells at low concentrations.

[1]Due to the fact that the macrophages concentrations were always low in comparison to their carrying capacity in the *Zhou et al* observations.

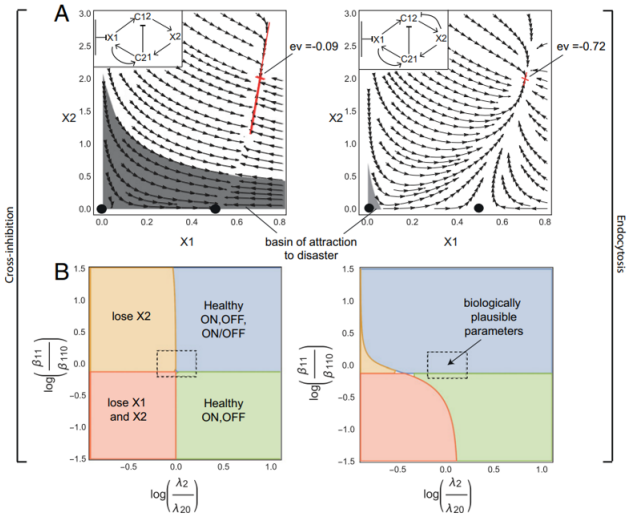

Figure 3. Comparison of endocytosis and cross-inhibition mechanisms. Endocytosis (right) provides a smaller basin of attraction for losing one or both cell types (gray region). The endocytosis circuit also reaches the stable ON state eightfold faster and is more robust with respect to parameter variation than the circuit with cross-inhibition.

8. Are model predictions validated? Bearing in mind that for the validation of predictions it would be necessary to compare the results/values obtained from the model with experimental values that could be available, this validation did not take place. As it was a qualitative model, there was no determination of exact values for the system, and there was no need for this validation. However, by the results visualized and by what is known to happen and previously published, the results and conclusions of this article seem to go accordingly with the information previously available.

9. Are in silico experiments performed? What types of perturbations are applied? What is the aim of those in silico experiments? In silico experiments are disturbances in the multiple values of the system that is being studied as a hypothesis, alterations that can be carried out in dependent variables' initial values or in parameter values. These perturbations are intended to carry out a quantitative comparison in relation to the experimental values available or obtained. However, in this work, no in silico experiment was performed.

10. Globally, why was the modeling approach useful to address the scientific question explored in the paper?

The main goal of this article was to understand the dynamics of homeostasis between two cell types, namely fibroblasts and macrophages, through the creation of a model that could recreate and predict this dynamics in a qualitative way. Based on the observations and experiments previously obtained by *Zhou et al.*, the authors wanted to try to understand how, despite the constant turnover of cells, they manage to maintain the appropriate ratios of the amount of these cell types, as well as trying to understand whether this model could be extrapolated to three and four cell types.

The co-culture circuit reproduced by the authors, as well as its use to define the essential parameters for the construction of the intended cell circuits, allowed the definition of essential criteria for the homeostasis that occurs between cell types. They were able to conclude that this homeostasis occurs through negative feedback mechanisms, essential to avoid uncontrolled proliferation of cells. Two types of downregulation mechanisms were proposed: cross-inhibition, where the growth factor of one cell inhibits the growth factor of the other, and endocytosis, where the growth factor is inhibited by the cell itself.

Endocytosis was identified as a more robust and faster mechanism for controlling cellular homeostasis, most likely due to the fact that there is no need to depend on other cells for the control of their own growth factors, as the cell itself controls them. Thus, endocytosis could be a essential mechanism for controlling the ratios of different cell types in cases of tissue damage or inflammation, while cross-inhibition would be a more effective mechanism in preventing cell proliferation from a less amount of cells. In addition, the authors also proved that the model developed in this article can be used in modular cell circuits to provide homeostasis to three and four cell types simultaneously.