

246g Dynamic Metabolic Flux Analysis for Tissue Systems

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Many major advances in biotechnology are hindered by a lack of sufficient fundamental understanding of biological systems. In recent years, various quantitative approaches have been introduced for the study of microorganisms. In particular, cybernetic modeling is an interesting analysis method for biochemical systems based on the postulate that a biological organism is optimal with regard to certain evolutionary goals. Therefore, it is possible to predict the behavior of a biological system based on the objective function, provided that a cellular/metabolic objective can be formulated mathematically. However, the development in more complex multi-cellular systems, such as tissues, organs, or human metabolism, is still very limited, mainly due to the lack of knowledge about metabolic regulatory networks, which in essence are dynamic control phenomena.

In this work, a Dynamic Metabolic Flux Analysis (DMFA) method is presented for cybernetic modeling in tissue engineering problems. In this method, a metabolic flux model is combined with a cybernetically optimal regulatory network to predict the system transient responses to various stimuli. The regulatory network model is based on a modified Linear Quadratic Regulator (LQR) formulation, which assumes that the cells regulate their responses (i.e., fluxes) to optimize a homeostasis-type cybernetic objective, and results in an optimally tuned state-feedback proportional-integral control scheme. This model predicts how the net overall cell regulatory system behaves, or at least how it should behave in order to be optimal. While the problem can be formulated mathematically in different ways, the particular form employed enables the identification of a set of dynamics equations (linear differential equations) that predict the responses of a net overall cellular regulatory network. Further, this optimal network identified is a system property and not dependent on the type of stimuli applied or the experimental conditions, hence is a generalizable regulatory network model for the particular system studied. Thus, it can be used to predict system behavior under a variety of disturbances/manipulations.

The introduced modeling methodology is a hybrid of Metabolic Flux Analysis (MFA), which is an experimental support method, and dynamic Flux Balance Analysis (FBA), which is an *in silico* experiment method. Due to limited applicability of FBA and similar methods to mammal cells, the optimal control scheme employed requires pre- and post-stimuli MFA results, as well as a very limited number of transient data for determination of several parameters in the cybernetic objective. However, as a result of this limited data it enables modeling and prediction of transient responses of all metabolites and fluxes in the model, resulting in 10 fold or more reduction in the total number of analysis that needs to be performed; hence DMFA is very promising as an experimental support tool.

Further, based on the correlations between MFA and Metabolic Control Analysis (MCA), the results of DMFA can be used to perform an MCA study without additional experiments, with the capability of predicting time-dependent elasticity and coefficients. More significantly, it is demonstrated that combined MFA and MCA results for a particular system are sufficient for building DMFA models under some mild mathematical conditions.

While the proposed DMFA method is applicable to general metabolic systems, the homeostasis-type objective is more suitable for mammal cells and tissue systems. The DMFA methodology developed is applied to modeling of fatty acid metabolism in rat hepatocytes cultures, particularly time-response of lipid accumulation in hepatocyte cultures exposed to plasma with fatty acid. Stable cultures of rat hepatocytes maintained in the collagen sandwich configuration are used to measure the intracellular levels and fluxes of fatty acids and several related molecular species. Metabolic flux models obtained from literature are combined with literature and new experimental data. As a result, a cybernetic model that predicts qualitatively lipid accumulation in hepatocyte cultures is developed. The model is applied

to hepatic steatosis for predicting enzymes & genes that are critical for effective regulation of lipid accumulation. Optimal therapy schemes (determination of optimal dosage and combination of drugs) for treatment are also discussed.