246f Optimization and Control of Metabolic Activities in Hepatocytes

Marianthi G. Ierapetritou, Nripen S. Sharma, Hong Yang, Stephen A. Guzikowski, Martin L. Yarmush, and Charles M. Roth

Extracorporeal bioartificial liver (BAL) devices are perhaps among the most promising of technologies for the treatment of liver failure, but significant technical challenges remain in order to develop systems with sufficient processing capacity and of manageable size. One limitation is that during BAL operation, when the device is exposed to plasma from the patient, hepatocytes are prone to accumulate intracellular lipids and exhibit poor liver-specific functions. To ameliorate this downturn, it has been shown that pre-conditioning the hepatocytes in low insulin media followed by amino acid supplementation within the plasma dramatically increases hepatic function. This function, although higher, however, is not optimal, and thus we have explored the potential utility of metabolic engineering analysis to achieve optimal function. Based on hepatic intermediary metabolism, we have utilized mathematical programming techniques to predict the optimal biochemical environment of hepatocyte cultures towards the desired effect of increased albumin and urea synthesis.

In this regard, the concepts of linear programming are used to maximize function based on alterations in amino acid fluxes, with constraints on all other metabolites involved. These constraints are obtained from flux estimates using metabolic flux analysis. To investigate the feasible range of hepatic function, we have obtained a Pareto optimal set of solutions corresponding to liver-specific functions in the metabolic framework using multiobjective optimization. The proposed approaches have also been used to identify those amino acids and metabolic pathways that do not play an important role in maximizing hepatocyte function. An intriguing finding of this approach obviated the first step of the pentose phosphate pathway. Though this would be possible with a non-proliferating phenotype like hepatocytes, it implies inhibition of NADPH production, which would affect glutathione related metabolic reactions. The latter pathways mediate antioxidant defense mechanisms for hepatocyte survival and hence have to be incorporated to make the model more realistic and improve its predictive capability.

In addition, we have incorporated sensitivity analysis of urea synthesis based on variations in extracellular metabolite measurements. This is particularly useful since metabolite measurements are patient-specific and also because experimentally measured extracellular metabolite values are have a limited precision due to assay techniques and biological variability. The proposed model incorporates uncertainty in input metabolites utilizing a two stage stochastic model, where the first stage decisions correspond to the selection of amino acids in the supplementation, whereas the second stage represents the quantities that should be adjusted according to plasma variability. Thus, the proposed analysis demonstrates a novel analysis technique towards generating a systematic approach to investigate hepatocyte cultures and optimize different operating parameters for an extracorporeal device based on real-time conditions.

An extension of this work includes expanding the boundaries of the system to include transcription factor activities for the most important enzymes identified. That includes the consideration of flux distributions that are controlled by specific enzymes, the production of mRNA encoding these enzymes controlled by transcription factor activities, and the transcription factor activities controlled by extracellular mediators (growth factors and cytokines). We quantify these controls using metabolic control analysis, a novel extension of network control analysis, and flux control analysis, respectively. Ultimately, these tools will allow us to develop a quantitative understanding of the multi-layered regulatory networks controlling hepatocyte metabolism, thus enabling metabolic engineering analysis and molecular control of hepatocyte function in cell-based devices.