

Spherical Individual Cell-Based Models

Limitations and Applications

Over the last decade a huge amount of experimental data on biological systems has been generated by modern high-throughput methods. Aided by bioinformatics, the '-omics' (genomics, transcriptomics, proteomics, metabolomics and interactomics) have listed, quantified and analyzed molecular components and interactions on all levels of cellular regulation. However, a comprehensive framework, that does not only list, but links all those components, is still largely missing. The biology-based but highly interdisciplinary field of *systems biology* aims at such a holistic understanding of complex biological systems covering the length scales from molecules to whole organisms. Spanning the length scales, it has to integrate the data from very different fields and to bring together scientists from those fields. For linking experiments and theory, hypothesis-driven research is an indispensable concept, formulating a cycle of experiment, modeling, model predictions for new experiments and, finally, their experimental validation as the start of the new iteration.

On the hierarchy of length scales certain unique entities can be identified. At the nanometer scale such functional entities are molecules and at the micrometer level these are the cells. Cells can be studied *in vitro* as independent individuals isolated from an organism, but their interplay and communication *in vivo* is crucial for tissue function. Control over such regulation mechanisms is therefore a main goal of medical research. The requirements for understanding cellular interplay also illustrate the interdisciplinarity of systems biology, because chemical, physical and biological knowledge is needed simultaneously.

Following the notion of cells as the basic units of life, the focus of this thesis are mathematical multi-scale models of multi-cellular systems employing the concept of individual (or agent) based modeling (IBM). This concept accounts for the entity cell and their individuality in function and space. Motivated by experimental observations, cells are represented as elastic and adhesive spheres. Their interaction is given by a model for elastic homogeneous spheres, which has been established for analysis of the elastic response of cells, plus an adhesion term. Cell movement is modeled by an equation of motion for each cell which is based on the balance of interaction, friction and active forces on the respective cell. As a first step the model was carefully examined with regard to the model assumptions, namely, spherical shape, homogeneous isotropic elastic body and a priori undirected movement.

The model examination included simulations of cell sorting and compression of multi-cellular spheroids. Cell sorting could not be achieved with only short range adhesion. However, it sorting completed with long range interactions for small cell numbers, but failed for larger aggregates. Compression dynamics of multi-cellular spheroids was apparently reproduced qualitatively by the model. But in a more detailed survey neither the time scales nor the rounding after compression could be reproduced. Based on these results, the applications consistent with the assumed simplifications are discussed. One yet established application is colony growth in two-dimensional cell cultures. In order to model cell growth and division, a two-phase model of the cell cycle was established. In a growth phase the cell doubles its volume by stochastic increments, and in a mitotic phase it divides into two daughter cells of equal volume. Additionally, control of the cell cycle by contact inhibition is included in the model.

After examination of its applicability, the presented model is used for simulations of *in vitro* growth of mesenchymal stem cells (MSC) and subsequent cartilage formation in multi-cellular spheroids. A main factor for both processes is the oxygen concentration. Experimental results have shown, that i) MSC grow much better *in vitro* at low than at high oxygen concentrations and ii) the MSC progeny harvested from low oxygen culture produce higher amounts of the cartilage components aggrecan and collagen II in multi-cellular spheroids than the ones from high oxygen culture.

In order to model these processes, IBM was extended by a stochastic model for cellular differentiation. In this model cellular differentiation is captured phenomenologically by two additional

individual properties, the degree of differentiation and the lineage or cell type, which are subject to fluctuations, that are state and environment dependent. After fitting the model parameters to the experimental results on MSC growth in monoclonal expansion cultures at low and high oxygen concentrations, the resulting simulated cell populations were used for initialization of the simulations of cartilage formation in multi-cellular spheroids. The model nicely reproduced the experimental results on growth dynamics and the observed number of functional cells in the spheroids and suggests the following explanation for the difference between the two expansion cultures: due to the stronger pre-differentiation found after expansion in high oxygen, the plasticity of these cells is smaller and less cell adopt the chondrogenic phenotype and start to produce cartilage. Moreover, the model predicts an optimal oxygen concentration for cartilage formation independent of expansion culture and a de-differentiating effect of low oxygen culture within 24h. Because all simulations comply with the concept of hypothesis-driven research and follow closely the experimental protocols, they can easily be tested and are currently used for optimization of a bioreactor for cartilage production.

Cell populations are composed of individual cells and regulation of population properties is performed by individual cell, but knowledge about individual cell fates is largely missing due to the problem of single cell tracking. The IBM modeling approach used for modeling MSC growth and differentiation generically includes information of each individual cell and is therefore perfectly suited for tackling this question. Based on the validated parameter set, the model was used to generate predictions on plasticity of single cells and related population dynamics. Single cell plasticity was quantified by calculating transition times into stem cell and differentiated cell states at high and low oxygen concentrations. At low oxygen the results predict a frequent exchange between all subpopulations, while at high oxygen a quasi-deterministic differentiation is found.

After quantifying the plasticity of single cells at low and high oxygen concentration, the plasticity of a cell population is addressed in a simulation closely following a regeneration experiment of populations of hematopoietic progenitor cells. In the simulation the regeneration of the distribution of differentiation states in the population is monitored after selection of subpopulations of stem cells and differentiated cells. Simulated regeneration occurs on the time scales estimated from the single cell transition times except the unexpectedly fast regeneration from differentiated cells in the high oxygen environment, which favors differentiation. The latter case emphasizes the importance of single outlier cells in such system, which in this case repopulate less differentiated states with their progeny.

In general, cell proliferation and regeneration behavior are influenced by biomechanical and geometrical properties of the environment e.g. matrix stiffness or cell density. Because in the model cells are represented as physical objects, a variation of friction is linked to cell motility. The cultures of less motile cells become denser at the same size and the effects of contact inhibition of growth more pronounced. This variation of friction coefficients allows the comparison of cultures with varying degrees of contact inhibition regarding their differentiation structure and the results suggest, that stalled proliferation is sufficient to explain the well-known differentiation effects in confluent colonies.

In addition, the composition of the simulated stem cell pool was analyzed regarding differentiation. In contrast to the established pedigree models, where stem cell can only be produced by asymmetric division, this model predicts that most of the cells in stem cell states descend from progenitor cells of intermediate differentiation states.

A more detailed analysis of single cell derived clones revealed properties that could not be described by the model so far. First, a differentiation gradient was observed in larger colonies, that was the opposite of the one predicted by the model. Second, the proliferative activity turned out to depend not only on oxygen, but also to be a property of individual clones persisting over many generations. Because the relation slow growth/pre-differentiation also holds for single cell derived clones, the general model of differentiation is extended by another heritable individual property. Motivated by the decline of proliferation and differentiation in culture and the high metabolic and epigenetic activity during cell division, each division event is assumed to de-stabilize stem cell states. Consequently, in the model the cells age in terms of cell divisions determines the

fluctuations in stem cell states and the environment the mean fluctuation strength.

Including this novel concept, that links aging to growth and differentiation dynamics, into the model reproduces the experimental results regarding differentiation gradient and persistent clonal heterogeneity. The spatial differentiation pattern can largely be explained by the spatio-temporal growth pattern of the mono-clonal cell assembly: cells close to the border of the cell assembly have undergone more cell divisions than those in the interior and therefore their stem cell states are less stable. Heterogeneity of single-cell derived clones depends on the age of the first cell in the clone. When the stem cell fluctuations equal the mean fluctuations strength, the proliferative activity passes a maximum at a certain age due to the destabilization of stem cell states. Thereafter the proliferative activity decreases, because more time is spent in non-proliferative differentiated states. Considering the number of divisions the cells have already undergone *in vivo* and after the initial expansion *in vitro*, it can be assumed that all cells have already passed this maximum. Interestingly, the model also predicts an optimal age for directed differentiation, when cells stably differentiate, but have not lost the required plasticity. According to the model, this clonal heterogeneity may be caused purely *in vitro*, but hypothetical simulation of *in vivo* aging yielded results consistent with experiments on MSC from rats of varying age.

Finally, the detailed molecular regulation mechanisms in a multi-scale tissue model of liver zonation was studied, in which the key molecular components were explicitly modeled. Hence, this model resolved the intracellular regulation in higher resolution than the above considered differentiation models which had summarized the intracellular control and differentiation mechanisms by a few phenomenological, dynamical variables. The metabolic zonation of the liver is essential for many of the complex liver functions. One of the vitally important enzymes, glutamine synthetase, (GS) is only synthesized in a strictly defined pattern. Experimental evidence has shown that a particular pathway, the canonical wnt pathway, controls expression of the gene for GS. A model for transport, receptor dynamics and intracellular regulation mechanism has been set up for modeling the spatio-temporal formation of this pattern. It includes membrane-bound transport of the morphogen and an enzyme kinetics approach to β -catenin-regulation in the interior of the cell. As an IBM this model reproduces the results of co-culture experiments in which two-dimensional arrangements of liver cells and an epithelial liver cell line give rise to different patterns of GS synthesis. The two main predictions of the model are: First, GS-synthesis requires a certain local cell number of wnt releasing cells. And second, a simple inversion of geometry explains the difference between the specific GS pattern found in the liver and in the co-culture experiments.

Summarizing the results presented in this thesis, it can be concluded that properties such as the occurrence of memory effects and single cells pursuing fates far off the population average could be essential for biological function. Considering the role of single cells in many tissues, the use of individual based methods, that are able to take such effects into account, can be expected to be a very valuable tool for the problems of systems biology.