Agent-based modeling of growing cell populations and the regenerating liver based on image processing

D I S S E R T A T I O N

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Summary

In the presented thesis we elaborated a general agent based model for multicellular populations. We used this model to shed light on the processes that determine the growth of avascular tumor spheroids and studied the key mechanisms of liver regeneration.

In order to make such analyses possible, we developed a comprehensive software tool that allowed us to effectively simulate, visualize and analyze the constructed computational model. We started with a minimal model for two-dimensional monolayers which are a common experimental technique for *in vitro* cell cultures. We successively advanced our model in order to reflect an *in vivo* situation more closely for example by simulating complex three-dimensional tumor spheroids embedded in granular medium and host tissue.

We proposed a biomechanical form of contact inhibition that was able to explain the experimentally observed linear growth of the diameter in monolayer cultures [Bru et al., 1998] [Bru et al., 2003] and their specific proliferation pattern where cells mainly proliferate at the monolayer border. Furthermore, our model could mimic the growth dynamics of monolayer cultures very precisely.

Subsequently, we considered three-dimensional cell aggregates by studying substrate detachment whereby normally two-dimensional monolayers due to the failure of certain control mechanisms expand perpendicular to the monolayer plane. Failure of growth control mechanisms is known to play an important role in the development of cancer [Hanahan & Weinberg, 2000]. By additionally introducing nutrient diffusion and consumption, we established a further extended model for three-dimensional tumor spheroids which are a common experimental model in therapeutically oriented cancer research. Surprisingly, we found that the proposed biomechanical form of contact inhibition also explains the growth of these tumor spheroids. Thereby, our model suggests in agreement with experimental data [Freyer & Sutherland, 1985] [Freyer & Sutherland, 1986] that the nutrient concentration in the environment of a growing tumor, which is widely

believed to control its growth, only determines the size of its necrotic core. Moreover, also in this three-dimensional situation our model precisely mimicked the growth dynamics and proliferation pattern of tumor spheroids *in vitro* where the necrotic core is enclosed by an intermediate layer of quiescent cells and an outer layer of proliferating cells [Kunz-Schughart, 1999].

We further advanced our model for the growth of three dimensional cell populations even closer towards *in vivo* tumors by including aspects from the surrounding tissue. We showed that the biomechanical properties of an embedding tissue have a major impact on the growth dynamics and morphology of growing cell populations by systematically varying the biophysical properties of the embedding tissue. Our model predicts Saffman-Taylor-like instabilities leading to fractal interfaces and an increased ability of cells to invade harsh environments if the motility of the embedding cells is small. We additionally observed large wavelength instabilities as a consequence of decreased density, increased elasticity, strong adhesion or increased cell size of the embedding tissue or granular medium. Interestingly, we found a nearly complete inhibition of tumor growth for specific properties of the embedding tissue which, if experimentally validated, could have direct therapeutical implications.

Furthermore, we achieved a remarkable agreement with experimental data on tumor growth dynamics by [Helmlinger et al., 1997] and [Galle et al., 2006]. However, the large variety of complex influences predicted by our model strongly indicates that the widespread experimental technique of embedding growing tumor spheroids in agarose gels [Helmlinger et al., 1997] [Galle et al., 2006] [Cheng et al., 2009] may not be sufficient to realistically capture all the biomechanical effects of an embedding tissue. Effects due to the granularity of the surrounding tissue, for example, are missing in experiments like those performed in [Helmlinger et al., 1997].

In contrast to chapter three where we mainly compared our model to published *in vitro* data, in chapter four we investigated a particular *in vivo* situation and studied the fascinating process of liver regeneration after intoxication with CCl_4, a prototypical substance for drugs inducing pericentral liver damage.

We established a procedure to use three-dimensional confocal laser scans to reconstruct *in vivo* tissues by image processing and image analysis. We then combined this very detailed and quantitative information with a further advanced version of our repeatedly experimentally validated model. We started with a minimal two-dimensional model for the regenerating liver lobule that nevertheless led to first impressions of the specific impact of the various factors that influence liver regeneration. On that basis we extended our model and created the first three-dimensional agent-based model of the regenerating liver lobule.

By capturing a 16 day regeneration process, our model underlined the importance of the complex columnar microarchitecture within the liver lobules, which is formed by hepatocytes and sinusoids. This microarchitecture ensures optimal exchange of metabolites between blood and hepatocytes. The model unambiguously predicted a so far unrecognized mechanism, the alignment of daughter hepatocytes along the orientation of the closest sinusoid, which we named hepatocyte-sinusoid alignment (HSA), as essential for liver regeneration. Only if HSA was included into the model the simulated tissue architecture was in agreement with the experimentally obtained data and no other likely mechanism could replace it. In order to experimentally validate the model prediction of HSA, we analyzed the orientation of daughter hepatocytes in relation to the sinusoids in three-dimensions. The results of this analysis clearly confirmed the model prediction and thus verified HSA as a yet unknown key mechanism of liver regeneration.
During this analysis we introduced novel techniques that made currently experimentally not accessible information available by image processing and analysis of volumetric datasets obtained by confocal laser scanning microscopy. In addition to the three-dimensional analysis of HSA, we used a similar approach to obtain further currently not experimentally available information on the average contact area between hepatocytes and sinusoids. Surprisingly, we found this parameter to allow for an automatic differentiation between normal liver tissue and hepatocellular carcinoma. The further pursuit of this finding will be interesting.

In summary, in this thesis we present an interdisciplinary approach to combine microscopic imaging, image processing and analysis and computational modeling - all in three dimensions. The integration of methods and results from different scientific fields like cell biology, physics and computer science enabled us to obtain new insights cancer research and hepatology.
We therefore consider the presented interdisciplinary approach and the corresponding procedures exemplary and widely applicable in the systems biology of tissues in general.

References

[Bru *et al.*, 1998] BRU, A., PASTOR, J. M ., FERNAUD, I., BRU, I., MAELLE, S., & BERENGUER., C. 1998. Super-rough dynamics on tumour growth. *Phys. rev. lett.*, **81**, 4008–4011.

[Bru *et al.*, 2003] BRU, A., ALBERTOS, S., SUBIZA, J. L., GARCIA-ASENSIO, J. LOPEZ, & BRU., I. 2003. The universal dynamics of tumour growth. *Biophys. jour.*, **85**, 2948–2961.

[Cheng *et al.*, 2009] CHENG, GANG, TSE, JANET, JAIN, RAKESH K, & MUNN, LANCE L. 2009. Micro-environmental mechanical stress controls tumor spheroid size and morphology by suppressing proliferation and inducing apoptosis in cancer cells. *Plos one*, **4**(2), e4632.

[Freyer & Sutherland, 1985] FREYER, J.P., & SUTHERLAND, R.M. 1985. A reduction in the in situ rates of oxygen and glucose consumption of cells in emt6 ro spheroids during growth. *J. cell. physiol.*, **124**, 516–524.

[Freyer & Sutherland, 1986] FREYER, J.P., & SUTHERLAND, R.M. 1986. Regulation of growth saturation and development of necrosis in emt6 ro multicellular spheroids by the glucose and oxygen supply. *Cancer res.*, **46**, 3504–3512.

[Galle *et al.*, 2006] GALLE, J., AUST, G., SCHALLER, G., BEYER, T., & DRASDO, D. 2006. Single-cell based mathematical models to the spatio-temporal pattern formation in multi-cellular systems. *Cytometry teil a*, 704–710.

[Hanahan & Weinberg, 2000] HANAHAN, D., & WEINBERG, R. A. 2000. The hallmarks of cancer. *Cell*, **100**(1), 57–70.

[Helmlinger *et al.*, 1997] HELMLINGER, G., NETTI, P.A., LICHTENFELD, H.C., MELDER, R.J., & JAIN, R.K. 1997. Solid stress inhibits the growth of multicellular tumor spheroids. *Nat biotech*, **15**(8), 778–783.

[Kunz-Schughart, 1999] KUNZ-SCHUGHART, L. A. 1999. Multicellular tumor spheroids: intermediates between monolayer culture and in vivo tumor. *Cell biol int*, **23**(3), 157–161.