Summary and conclusions

Achieving a localized drug delivery is key in the success of many therapeutic challenges nowadays. In tumor therapy, targeting chemotherapeutic drugs towards the cancer mass would not only improve their effectiveness, but also strongly reduce the detrimental side-effects associated with the treatment. It was envisioned that encapsulating chemotherapeutics in nanoparticles (termed nanomedicines) would improve their biodistribution and ensure a selective tumor uptake. However, reaching tumor site in sufficient amounts remained troublesome. Due to the limited therapeutic success of nanomedicines, microbubbles have been proposed as ultrasound-responsive drug carriers. It has been shown that nanoparticle-loaded microbubbles can locally release nanoparticles upon ultrasound exposure, enabling active delivery upon applying an external physical trigger. Moreover, their biophysical interactions with cells and tissues have demonstrated their ability to enhance nanoparticle and drug uptake. Notwithstanding the large amount of evidence for their therapeutic usefulness, the exact mechanisms behind this improved drug delivery have not yet been completely understood.

Recently, sonoprinting has been suggested as a mechanism through which nanoparticle-loaded microbubbles could elicit the delivery of large amounts of nanoparticles to the cells. To better understand this mechanism and evaluate its potential for tumor delivery in the clinic, we used a step-by-step approach with increasingly complex models (from 2D to 3D and finally to in vivo models), while maintaining a detailed analysis in a controlled manner.

As we aimed at closing the gap between fundamental in vitro studies and (pre-)clinical translation by using increasingly complex tumor models, we summarized crucial aspects of the tumor biology that are (in part) represented in these different models in Chapter 1. We furthermore introduced the use of nanomedicines in cancer treatment, and showed evidence that its limited clinical success is predominantly caused by the inability to reach their target site in sufficiently high doses. As a possible solution, we focused on ultrasound-triggered microbubbles, since they are not only useful for imaging applications, but also make excellent candidates for drug delivery.

Translating microbubble dynamics and therapeutic outcome from in-depth in vitro studies to in vivo results has proven challenging and has created almost separate research fields with little connection between them. In Chapter 2 we therefore sought to connect the knowledge from fundamental microbubble studies, to in vitro and in vivo preclinical and clinical reports. We provided a comprehensive overview on the unique properties of ultrasound-driven microbubbles and explained
that understanding the physics that governs microbubble behavior is crucial to achieve more controlled and efficient therapy. Additionally, we looked into recent work that has focused on microbubble-cell interactions on the single-cell level, with the aim to better understand drug delivery mechanisms and the impact of acoustic settings have on them. Finally, we explored how these delivery mechanisms apply to (pre-)clinical studies, and to which extent the results match the prior knowledge from in vitro studies. We concluded that acoustic settings and microbubble-related parameters play a key role in microbubble-cell interactions and in the associated therapeutic outcome. However, the exact mechanisms behind the improved drug delivery in a clinical situation have not yet been completely understood.

Considering our interest in sonoprinting as a novel ultrasound-based delivery mechanism, Chapter 3 aimed at a fundamental understanding of the sonoprinting phenomenon and the microbubble-cell interactions that lead to the observed effects. As we established that acoustic settings are of vital importance in microbubble-cell interactions, we quantified the impact of two acoustic settings (ultrasound pulse length and acoustic pressure) on the sonoprinting process. For this, we used a unique combination of 3 different advanced optical imaging techniques with frame rates ranging from 5 frames per second to 10 million frames per second, to reveal the biophysical interactions leading to the sonoprinting phenomenon. We found that acoustic pressures and pulse lengths need to be sufficiently high to allow the release of the nanoparticles, followed by their transport and deposition onto the cell surface caused by the movement of the microbubble cores under the influence of acoustic radiation forces. This forms an explanation to why higher acoustic pressures and pulse lengths are often required in microbubble-assisted therapeutic studies.

While in chapter 3, we have unraveled the biophysical microbubble-cell interactions leading to the sonoprinting phenomenon in a 2D setting, questions remained on the feasibility of this delivery mechanism in more relevant biological models. Hence, in Chapter 4, we made use of 3D multicellular mono- (tumor cells only) and cospheroids (tumor cells and fibroblasts), to study the sonoprinting mechanism in a more physiologically relevant model. The results revealed that ultrasound-triggered nanoparticle-loaded microbubbles could deliver large amounts of nanoparticles to the outer layers of 3D tumor spheroids, followed by a complete drug release into the deeper layers of the tumor spheroid. Using this approach, we could enhance the cytotoxicity of both Doxil®-like and ThermoDOX®-like liposomes. Furthermore, we prove that while the presence of stroma typically protects the tumor cells from liposomal treatments, the ultrasound-triggered nanoparticle-loaded microbubbles were equally effective in mono- and cospheroids.
Since microbubbles are too large to extravasate from the general circulation, the endothelial cell layer will be the first barrier drug-loaded microbubbles need to perturb in vivo. Yet, none of the in vitro models we employed included a functional microvascular system. For this reason, we subsequently studied the effect of nanoparticle-loaded microbubbles on the vasculature of mice in Chapter 5. We gathered preliminary data in both healthy mice, equipped with dorsal window chambers, as in tumor-bearing mice, and confirmed that microbubbles in general are able to open the vascular barrier, but that only nanoparticle-loaded microbubbles were able to locally deliver the nanoparticles to the vascular lining and the perivascular space, through sonoprinting. However, the local tumor delivery remained low compared to the total dose administered in the mice. Nevertheless, the data presented here could provide a base for future research directions, and help establish the requirements for successful translation into the clinic.

In the final chapter, Chapter 6, we discussed the relevance of this work and the future directions in the broader international context of microbubble-assisted drug delivery. Considering the limited successes of recent clinical studies involving microbubbles for drug delivery, it is clear that there is room for improvement in the microbubble formulations and ultrasound set-ups used. Firstly, there is a lot to be gained from accurately predicting and controlling the microbubble behavior. Fundamental research on microbubble dynamics and microbubble-tissue interactions will therefore remain the cornerstone in rational development of microbubble-based drug carriers and the selection of appropriate ultrasound conditions. Moreover, recent advances in microbubble formulations and ultrasound techniques can help harness the therapeutic potential of drug-loaded microbubbles in the future. Additionally, new insights into the effect of the microbubble treatment on the body can reveal new opportunities for tumor therapy. Overall, we concluded that clinical success of ultrasound-driven drug-loaded microbubbles will most likely be achieved through a well thought-out design of both the microbubbles and the ultrasound set-ups, combined with a thorough understanding of the underlying tumor biology. This highlights the need for an interdisciplinary cooperation between research fields with complementary expertise.