Executive Summary

Prof. Alke Fink, Université de Fribourg

Project "Smart in-vivo drug delivery"

Stimuli-responsive drug carriers are promising runners in the quest to overcome poor bioavailability and to reduce side effects. In this context, implementing triggers to remotely release encapsulated drugs from their encasement has become a more and more prominent vision to control the spatial and temporal delivery of active substances, enabling the physician to release the drug precisely where it is needed. This approach would consequently reduce systemic toxicity and – more importantly - unnecessary discomfort for the patient.

To date, liposomes have proven themselves as steadfast drug carriers and have found their way from the bench to the bedside. Their biocompatibility make them model structures in developing nanocapsules for drug delivery. Moreover, liposomes are known to favorably accumulate around tumors as a consequence of the enhanced permeability and retention effect (EPR). Nonetheless, releasing the drug from its encapsulation remains challenging: Trapped active substances usually leak over time by passive diffusion, which is relatively slow and in turn limits bioavailability and effectiveness. Lowering the crystalline-to-fluid phase transition temperature of the vesicle membrane (i.e., by varying the lipid composition) is a way to accelerate this process, yet increases liposome leakiness within the blood stream over time. Nonetheless, this constraint can be overcome by implementing active triggers within the system to instantly provide the required energy, consequently rendering the membrane permeable when needed. Light, temperature and pH are common examples to attain this goal, and either relies on directly modifying the lipids or enhancing them with inorganic components.

In this context, nanoparticles have become common candidates for this task, as they exhibit unique material- and size-dependent properties (i.e., thermal, optical or magnetic). We have focused on applying two different types of nanoparticles; i.e., superparamagnetic iron oxide nanoparticles (SPIONs) and gold nanorods. To associate them with the liposomes, we followed two fundamentally different approaches: Either to incorporate the materials directly in between the lipid bilayer or to functionalize them via chemical coupling to the liposome surface. High resolution microscopy techniques expose the samples either to aggressive embedding/preparation steps and high vacuum environments, which inevitably alter the specimens. To avoid these difficulties, we relied on cryo-transmission electron microscopy (cryo-TEM) and cryo-electron tomography to visualize them under native conditions.

In regard to embedding the nanoparticles in between the lipid bilayer, our experiments have yielded interesting findings: Incorporated nanoparticles were not evenly distributed around the liposome circumference but clustered at one axis, an observation which has not been previously described. In short, we have developed and optimized an approach to overcome the physical constraints of lipid bilayers by implementing flexible clusters of individual nanoparticles in the lipid membrane. By forming our liposomes by surfactant dialysis, and consequently enabling the liposomes to grow around already-formed clusters, we were capable of including structures of more than 60 nm in size in between the lipid sheets. The resulting hybrids were named Janus magnetic liposomes. However, this self-assembly procedure is only successful when the ideal experimental parameters are precisely set: any other condition either results in individual nanoparticle-lipid micelles or irreversible nanoparticle aggregates. After having elaborated these parameters and the self-assembly

pathway, we applied this knowledge to incorporate larger SPI-ONs in between the bilayer. Our results prove that contemporary notions on the flexibility of lipid bilayers do not cover the entire story and show that this approach is not as limited as assumed. In the meantime, we have successfully released a hydrophilic dye acting as a model drug from these hybrids. Small nanoparticles (i.e., d = 5.4 ± 0.7 nm) were sufficient for this task.

We also investigated an alternative to this setup; we pursued the application of gold nanorods, which adsorb and convert near-infrared light (NIR) to heat to an extremely effective degree, as triggers for drug release in parallel. NIR is a very suitable trigger, as it penetrates to a certain degree through human tissue and is already found in the clinics for photothermal therapy.

For preliminary feasibility trials, we actively loaded these hybrids with doxorubicin, a model cancer drug commonly used in liposome research. A successful remotely controlled drug release was demonstrated and quantified by UV-Vis spectroscopy after exposing these hybrids to an NIR laser. These observations were repeated in vitro with a murine breast cancer model cell (4T1). Liposomes loaded with doxorubicin were exposed to NIR light before being added to the cell culture in order to avoid thermal damage to the cells themselves (i.e., in order to quantify the chemical effect alone). Released doxorubicin decimated the cells, an indication that the drug was not altered by the thermal release stimulus. On the other hand, doxorubicin, which remained encapsulated, did not exhibit a significant effect on the cell cultures, despite possible uptake of some vehicles through cells or leakage. Further feasibility trials in regard to release in tissue were successful. We used porcine skin as a model tissue to judge whether a triggered release could be achieved within a tissue.

To validate our systems for any future development, we have established the machinery to controllably trigger the release from these vesicles. This includes the setup of an alternating magnetic field generator and a NIR laser. These setups enabled us to perform feasibility trials and to judge whether the pursued paths were realistic for any kind of future development. In this regard, we optimized our setups to suit established norms in the fields of hyperthermia and photothermal therapy to assure that the hybrids we designed would suit clinically relevant requirements.