scope of the thesis

Liver fibrosis is a chronic disease that results from hepatitis B and C infections, alcohol abuse or metabolic and genetic disorders. Ultimately progression of fibrosis leads to cirrhosis, a stage of the disease characterised by failure of the normal liver functions. Currently, there are no efficacious antifibrotic drugs available and the treatment of liver fibrosis is mainly based on the removal of the underlying cause of the disease, which will, however, not cure the disease or reverse it. Liver transplantation is the only treatment for patients with advanced fibrosis, but this is a highly complicated surgical operation, not even mentioning serious problems such as lack of donors and very high costs.

Hepatic stellate cells (HSC) are considered to be key players in the development of liver fibrosis. Chronically activated HSC produce large amounts of extracellular matrix which leads to impairment of the structure and function of the liver. In addition, HSC enhance fibrosis by secreting a broad spectrum of cytokines that exert pro-fibrotic action in other cells, and in an autocrine manner maintain their own activation. Therefore, therapeutic interventions which inhibit activation of HSC and HSC pro-fibrotic activity are currently under investigation world-wide. The ideal antifibrotic drug and/or drug delivery system should be well tolerated, accumulate preferentially in the liver and have limited side effects in other organs. Because of the complexity of the disease, the ideal therapy for liver fibrosis should simultaneously inhibit pro-fibrotic processes in HSC and pro-inflammatory actions of Kupffer cells and liver endothelial cells.

Liposomes are versatile drug carriers, that are already clinically applied for the delivery of cytostatic compounds to tumours as well as for the delivery of anti-fungal compounds. Liposomes are made of phospholipids and are considered non-immunogenic, non-toxic and biodegradable. In addition, liposomes can accommodate compounds with different chemical properties either in the aqueous interior or in the lipid bilayers.

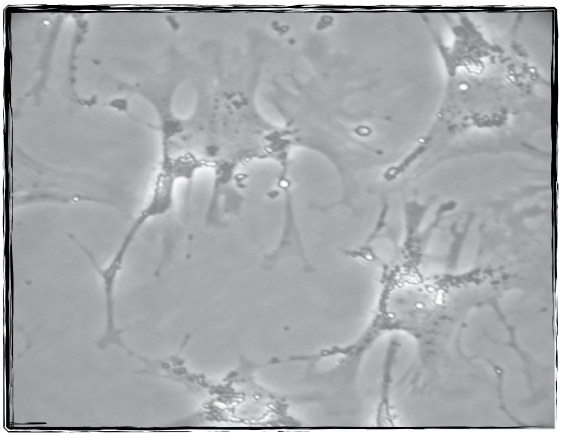
In this project we applied liposomes and explored their potential as drug carriers to HSC in the fibrotic liver. To specifically target them to HSC, liposomes were surface grafted with human serum albumin that, in turn, was derivatized with mannose 6-phosphate groups (M6P-HSA). The research described in this thesis focuses on the characterization of the interaction of M6P-HSA liposomes with HSC and other liver cells, such as Kupffer cells and liver endothelial cells, in vitro using cultured cells and in vivo in experimental model of liver fibrosis. Receptors involved in these processes were tentatively identified and features of M6P-HSA liposomes as drug carriers, including organ distribution and pharmacokinetic properties were studied.

The primary role of drug carriers is to deliver therapeutic compounds specifically to the diseased tissue. However, carriers, prepared from bioactive compounds may possess additional therapeutic properties against a disease. We prepared liposomes from dilinoleoylphosphatidylcholine (DLPC), which has been shown to have antifibrotic properties and studied the effects of DLPC containing liposomes on the activation of HSC and the progression of liver fibrosis in a rat model of this disease.

Changes in gene expression in HSC in fibrotic livers allow gene-therapeutic approaches to modulate HSC function and to inhibit the fibrotic process in the liver. Virus-mediated gene delivery of matrix metalloproteinases or proteins which interfere with signalling pathways of pro-fibrotic cytokines was shown to reduce experimental fibrosis. Specific transfection of HSC may improve the efficacy of delivered genes and minimize adverse effects caused by the expression of these genes in other cell types. In this thesis M6P-HSA

liposomes were used to create a targeted viral vector by fusing them with the Hemagglutinating Virus of Japan.

In summary, the aim of the research described in this thesis was to explore the potential of liposomes targeted to HSC as drug carriers in antifibrotic therapy and to investigate effects of bioactive compounds delivered by these liposomes on the progression of liver fibrosis. To our knowledge, this is the first study demonstrating that lipid-based drug carriers can be selectively delivered to HSC in the fibrotic liver.



HSC at day 3. The two Thursdays after a new isolation of stellate cells, being the 3rd and 10th day, are the standard moments to test their properties. Thursday quickly became the regular 'experiment day'.