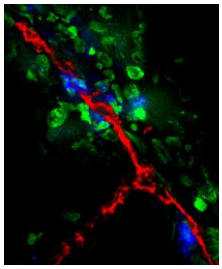
Multifunctional nanoparticles and ultrasound to penetrate the blood-brain-barrier and to improve cancer therapy

Ultrasound mediated delivery of NPs in tumour tissue

Nanotechnology has started a new era in engineering multifunctional nanoparticles (NPs) for improved cancer diagnosis and therapy, incorporating both contrast agents for imaging and therapeutics into so called theranostics NPs. Encapsulating the drugs into NPs improves the pharmacokinetics and reduces the systemic exposure due to the leaky capillaries in tumours. In most normal tissue the blood vessels are not leaky and the NPs are constrained to the blood, thereby reducing the toxicity to healthy tissue. Although the NP can extravasate from the blood to the extracellular matrix, the NPs do not to travel far away from the blood vessels. Thus, only a small population of cancer cells located close to the blood vessels will be exposed to the cytotoxic drugs as shown in the figure. A prerequisite for successful cancer therapy is that the therapeutic agents reach their targets and limit the exposure to normal tissue. To ensure high drug payload, the NPs have to be relatively large (100 -200 nm) and therefore the NPs face severe problems reaching the target cells. The delivery depends on the vasculature, the transport across the capillary wall, through the extracellular matrix (ECM), and if the final target is intracellular the NPs also have to cross the cell membrane.

Although the NPs may pass the tumour capillaries rather easily the, uptake and distribution of NPs and the released drugs are low and heterogeneously distributed in the tumour tissue. The drug has to penetrate the ECM which consists of a protein network of collagen embedded in a hydrophilic gel of glycosaminoglycans and proteo-glycans.



In order to improve the distribution of NPs the delivery should be combined with a treatment facilitating the delivery. Ultrasound has been reported to improve drug delivery in various ways: Increasing the permeability of the capillary wall, pushing the NPs through the ECM, enhancing the release of the drug from the NP and improving the cellular uptake.

The overall aim this project is to characterize NP to be used in therapy and study how ultrasound can be used to improve the delivery of distribution of NP in tumour cells and tissue.

Ultrasound mediated penetration of nanoparticles across the blood-brain barrier

One of the major challenges in treating diseases in the central nervous system (CNS) is the delivery of drugs to the brain. The access of molecules to the CNS is strictly controlled by the specialized and tight capillary endothelium that constitutes the blood-brain barrier (BBB). Furthermore, transport proteins in the endothelial cell membrane pump drugs back to the blood thereby reducing the efficiency of the therapeutic agent. Therefore, non-invasive approaches for controlled, temporary opening of the BBB and blocking the efflux, enabling the passage of therapeutics into the brain are needed.

Low-intensity focused ultrasound pulses in conjunction with intravenously administered gas microbubbles has been shown to non-invasively, transiently and selectively open the BBB in animals. The degree of BBB disruption is dependent on acoustic parameters such as frequency, acoustic pressure/intensity, pulse length and repetition frequency, and overall exposure time. The exact mechanism by which focused ultrasound causes BBB disruption is currently unknown. It is assumed that it is a combination of cavitation, i.e. oscillation of the gas bubbles, and radiation force, and the mechanism will depend on the frequency applied.

The overall aim of this project is to develop novel, integrative methodologies for drug delivery across BBB by using multifunctional NPs in combination with focused ultrasound and temporary knockdown of the efflux proteins.

Novel multifunctional nanoparticles and microbubbles

Both projects use new polymeric NPs which have the ability to stabilize gas bubbles, i.e. the NPs form a shell around the gas bubbles. The NPs-micro bubbles are made by SINTEF Material and Chemistry. The NPs can contain drugs, contrast agents for MRI and fluorescent probes for optical imaging. The gas microbubbles can be used both in ultrasound imaging and to improve ultrasound-mediated drug delivery.

Both projects are interdisciplinary research project between NTNU (Dept of physics- Catharina Davies, Dept of Chemical Engineering - Wihelm Glomm, Dept of Circulation and Medical Imaging - Bjørn Angelsen), SINTEF (Material and Chemistry - Yrr Mørch and Per Stenstad, Medical Technology - Rune Hansen) and St.Olavs Hospital (Anders Angelsen) studying how ultrasound can be used to improve the delivery of NP.

We provide 3 projects for the fall 2013:

1. **Cellular uptake and intracellular localization of nanoparticles**
   Supervisor Catharina Davies Catharina.davies@ntnu.no, Andreas Åslund Andreas@aslund@ntnu.no, Astrid Bjørkøy astrid.bjorkoy@ntnu.no ,

The interaction between the NP and cells depends on surface properties of the NP such as PEGylation and charge. The NP surface is coated with polyethyleneglycol (PEG) to increase the circulation time and improve the biodistribution.

Aim: Study the cellular uptake and intracellular localization of NP with various PEGylations on the surface.

Experimental outline: A human prostate cancer cell line will be used and the cells incubated with different NP. The uptake of fluorescently labelled NPs in cells will be studied by flow cytometry. The intracellular localization will be studied by confocal laser scanning microscopy. NPs will be co-localized with intracellular organelles such as endosomes, lysosomes and caveosomes. These organelles will be labelled with antibodies or pH sensitive dyes.

2. **Ultrasound enhanced uptake of nanoparticles**
   Supervisor Catharina Davies Catharina.davies@ntnu.no, Andreas Åslund Andreas@aslund@ntnu.no, Astrid Bjørkøy astrid.bjorkoy@ntnu.no ,

The effect of various ultrasound treatments on the cellular uptake will be studied. Ultrasound in the presence of gas bubbles is known to induce transient pores in the plasma membrane, so called sonoporation.

Aim: Determine optimal ultrasound treatment for sonoporation of the NPs

Experimental outline: A human prostate cancer cell line will be used and the cells incubated with NPs –micro bubbles and exposed to various ultrasound treatments. The uptake of fluorescently labelled NPs in cells will be measured by flow cytometry. The intracellular localization will be studied by confocal laser scanning microscopy.

3. **Ultrasound-induced penetration of nanoparticles across an artificial blood-brain barrier in vitro**
   Supervisor Catharina Davies Catharina.davies@ntnu.no, Sylvie Lelu Sylvie.lelu@ntnu.no

A layer of endothelial cells with tight junctions between the cells constitute an in vitro model for the BBB, and the effect of various ultrasound treatments on the flux of nanoparticles across the BBB will be studied

Aim: Determine optimal ultrasound treatment for inducing a flux of nanoparticles across the BBB

Experimental outline: An immortalized endothelial cell line RB4 will be grown in transwells and incubated with nanoparticle-microbubbles. This BBB will be exposed to various ultrasound exposures and the amount of nanoparticles on the opposite side of the endothelial cell layer measured by dynamic light scattering and spectroscopically.