Multifunctional nanoparticles and ultrasound to improve cancer therapy

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Background: 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.

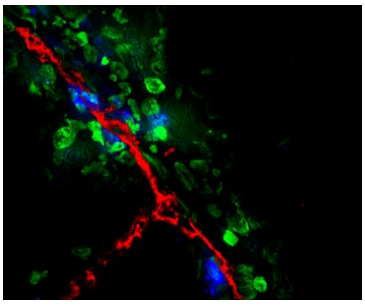

Figure 1Nanoparticles (blue) do not travel far from the blood vessels (red).The encapsulated drug is taken up by cells (green) close to blood

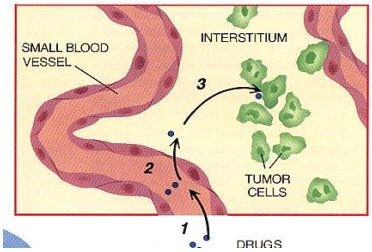

Figure 2The delivery of nanoparticles depends on 1) The blood vessel network 2) Transport across the capillary wall 3) Penetration through the ECM.

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 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 (US) has been reported to be able to improve drug delivery by different mechanical mechanisms, acoustic radiation force and cavitation. High frequency and highly focused US can induce acoustic radiation force pushing the NP across the capillary wall and through the ECM. Cavitation is the oscillation of gas filled microbubbles in the acoustic field. Such oscillations can be stable and generate mechanical shear stress on the capillary wall thereby increasing the vascular permeability or the microbubbles can collapse in a violent process generating jet streams that also increase the vascular permeability, improve the transport through the ECM and increase the cellular uptake of NP. The overall aim this project is to characterize NP and microbubbles to be used in therapy and study how ultrasound can be optimized to improve the delivery of distribution of NP in tumour tissue.

We provide 6 projects for the fall 2017 - spring2018:

1. **Mechanical properties of nanoparticles**
   Supervisors Catharina Davies Catharina.davies@ntnu.no, Astrid Bjørkøy astrid.bjorkoy@ntnu.no

   SINTEF material and Chemistry has developed a new polymeric nanoparticle which has the ability to form a shell around a gas bobble. We have successfully used this nanoparticle-microbubble to improve the delivery of nanoparticles to tumors in combination with focused ultrasound. The nanoparticles need to be further characterized to understand the effect of ultrasound on the nanoparticles. It is necessary to have knowledge about the mechanical properties of these nanoparticles, i.e, their elasticity/stiffness.
   Aim: Measure the elasticity/Young modulus of nanoparticles using atomic force microscopy.

2. **Distribution of nanoparticles in tumors exposed to focused ultrasound**
   Supervisors Catharina Davies Catharina.davies@ntnu.no, Andreas Åslund andreas.aaslund@ntnu.no, Einar Sulheim Einar.sulheim@ntnu.no, Marieke Olsman marieke.olsman@ntnu.no

   Ultrasound increases the uptake of nanoparticles in tumor tissue and improves the distribution of nanoparticles throughout the tumor. We are optimizing the delivery of various nanoparticles using different ultrasound parameters. Tumors growing in mice have been exposed to ultrasound after injection of nanoparticles.
   Aim: Characterize quantitatively the uptake and distribution of various nanoparticles in frozen sections from tumors exposed to ultrasound. Confocal laser scanning microscopy will be used to image the frozen sections, and image analysis to quantitate the uptake and displacement of nanoparticles from blood vessels. Typical confocal laser scanning image is shown in figure 1.

3. **Do ultrasound cause vascular shutdown?**
   Supervisors Catharina Davies Catharina.davies@ntnu.no, Annemieke van Wamel annemieke.wamel@ntnu.no, Sofie Snipstad sofie.snipstad@ntnu.no,

   We have indications to believe that high intensity ultrasound can destroy blood vessels, either by destroying the blood vessel wall or causing them to collapse.
   Aim: To study whether ultrasound can reduce the number of functional vessels, and at which ultrasound intensity vascular shutdown will take place. This will be done by imaging tumor sections of fluorescently labelled blood vessels using confocal laser scanning microscopy.

4. **Use the chicken chorioallantoic membrane (CAM) model to study effect of ultrasound on nanoparticle behaviour in tumor tissue**
   Supervisors Catharina Davies Catharina.davies@ntnu.no , Andreas Åslund andreas.aaslund@ntnu.no

   We have recently established the chicken embryo model to study tumor development and angiogenisis (vascularization). The model has the advantage of having functional blood vessels already 3 days after fertilization of the egg and the eggs can be grown in petri dish in an incubator (Figure 3). Tumor cells can be implanted into the CAM and used an ex vivo tumor model.

Aim: To design and establish an experimental set up to perform ultrasound treatment of the tumors growing on the CAM. Study the effect of ultrasound on delivery of drugs and nanoparticles in the CAM model.

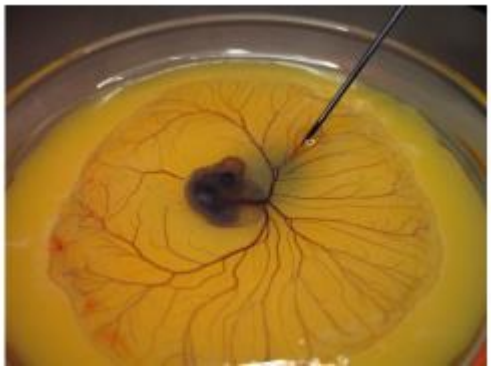

Fig. 3: The figure depicts injection of the dye Evans blue into a CAM.

5. **Study " Acoustic cluster therapy" using an in vitro system**
Supervisors Catharina Davies Catharina.davies@ntnu.no, Annemieke van Wamel Annemieke.wamel@ntnu.no

Acoustic Cluster Therapy (ACT) is a novel microtechnology drug delivery platform based on clusters of microbubbles and microdroplets. After intravenous injections, focused ultrasound is applied to the target tissue whereby the microbubbles transfer energy to the microdroplets, which undergo a gas-to-liquid phase shift. Growing in size, these large bubbles transiently block blood flow at the capillary level. Further exposure of ultrasound causes these large bubbles to oscillate and induce biomechanical effects enhancing the transport of drugs or nanoparticles across the capillary wall. We have successfully shown that this concept can be used to treat tumors growing in mice. To obtain more knowledge on the underlying mechanism, we want to establish an in vitro model to be used to study the behavior of the large microbubbles in 3 dimensional networks.
Aim: Establish a 3D microvascular network of endothelial cells and collagen fibers (Figure 4). Expose this microvascular network to ultrasound after injecting the microbubble-microdroplet clusters, and image by confocal laser scanning microscopy.

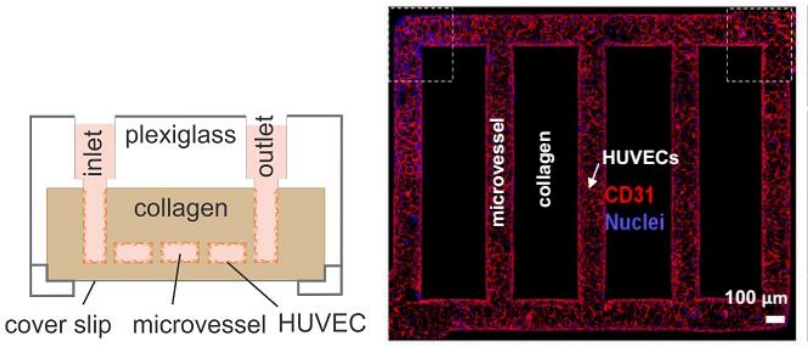

Fig 4: Proposed set up of a 3D vascular network

6. **Estimation for displacement of nanoparticles due to ultrasound radiation force using simulation and experimental methods.**

Supervisors Catharina Davies Catharina.davies@ntnu.no , Petros T. Yemane petros.t.yemane@ntnu.no

Acoustic radiation force is one of the mechanism for improved distribution of nanoparticles in tissue. We have recently worked on simulation of ultrasound radiation force and estimation of displacement of nanoparticles in tissue. Moreover, we are experimentally studying the displacement of nanoparticle in a collagen gel. Now we want to support the experimental method with new simulations.

Aim: First, we will simulate the ultrasound radiation force using **Wavesim and HIFU simulators** in Matlab. Second, estimate the displacement of nanoparticles due to the ultrasound radiation force and compare with experimentally results in collagen gel.