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Microbubbles and cell interactions.

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Ultrasonic imaging is a safe, cheap and reliable diagnostic technique widely used. In clinical ultrasound, blood cells cannot be differentiated from surrounding tissue, due to the low acoustic impedance difference between blood cells and their surrounding tissue. Thus, contrast agents have been developed to help to differentiate blood between other tissue types allowing perfusion diagnosis or tumour detection. These ultrasound contrast agents consist of micrometre-sized microbubbles of low-solubility gas encapsulated by a flexible shell.

Ultrasound is a mechanical wave alternating compression and expansion phases. Ultrasound contrast agents are capable to oscillate using specific ultrasound settings leading to a permeabilization of surrounding cells. This opens a window for ultrasound and microbubble targeted local delivery to enhance cellular uptake of therapeutic compounds. The increased

uptake of compounds by ultrasound in the presence of microbubbles is attributed to the formation of transient pores on the plasma membrane and this phenomenon is called sonoporation. It has been proposed that sonoporation is based on microbubble oscillations near a plasma membrane leading a cell “massage”, shear stress or microbubble jetting through the plasma membrane. The sonoporation technique has been used to deliver drugs in vitro and in vivo for gene or chemotherapeutic drug delivery applications. Most of studies show that microbubble presence is essential for the delivery. However the biological and physics mechanisms of sonoporation are still not understood.

The drug or gene delivery mechanism by sonoporation is dependant on the size of the molecules to transfer. The use of fluorescent dextran has shown a completely different localization profile depending of the molecular size of dextran after sonication. Small dextran (3kDa) are diffusly localised in the cytoplasm and in the nucleus, 70kDa dextran is localised only in the cytoplasm while dextrans over 150kDa are localised in patch structures suggesting endosomes. The drug transfer efficiency is dependant on the ultrasound parameters such as acoustic pressure, the duty cycle, and other parameters like the time of insonation and the concentration of microbubble. The use of luciferase reporter plasmid has permitted to identify the optimal parameters for an efficient drug delivery. The optimal parameters found were 1 MHz, 150kPa, 40% duty cycle, 60 sec of sonication time and 0.3% of microbubble. In these conditions less than 10% of cytotoxicity has been measured.

The intracellular kinetic of the drug delivery was observed by confocal microscopy using fluorescent drugs and cell markers. Two cellular markers were used, Rab7-eGFP which is a specific late endosome marker and Nup153-eGFP which is a nuclear marker. We used a fluorescent plasmid DNA as therapeutic drug and we have followed its location in the cell at several time points. The results showed that 3h after sonoporation most of plasmids were

located in the late endosomes and 6h after sonoporation plasmids reached the nucleus. To study the influence of the endocytosis process in sonoporation efficiency we have used inhibitors of the clathrin and non-clathrin endocytosis pathway. The results showed that the delivery was inhibited by 90% when depleting the cell DNA and by 95% in presence of chlorpromazine suggesting that the delivery of a plasmid DNA by sonoporation was an active mechanism mostly involving the clathrin pathway.

The interactions between microbubbles and cells have also been investigated by high-speed imaging during sonoporation at the optimal settings determined previously. Three acoustic pressures have been used 100, 150 and 200kPa. In function of the acoustic pressure, the interactions between microbubbles and the cell membrane observed were different. At 100kPa microbubbles were attracted to cells but stayed stuck to the membrane, at 150kPa microbubbles entry into cells have been observed, at 200kPa more intense interactions were seen. The results showed a phenomenon of microbubble entry to cell and this could be observed only at 150kPa. To prove this phenomenon we have used the fluorescence to label and to follow microbubbles during sonoporation. Confocal microscopy analysis has shown that after sonoporation fluorescent microbubbles were found inside cells. These results suggested that the microbubble entry could be closely correlated to the drug entry. The next step of this study will be to develop microbubbles that could encapsulate the drug in order to deliver it locally.

The applications of targeted drug delivery by sonoporation are numerous. The two main topics are the gene therapy and the drug delivery for cancer treatment. The main aim will be to develop original microbubbles for safe, non-invasive and target controlled drug delivery by sonoporation in order to enhance the delivery of their payload at a specific location. This

technique is currently evaluated in a pancreas cancer mouse model to enhance the delivery of therapeutics drugs for cancer treatment.

In conclusion, ultrasound and microbubbles offer by the same time the ability to be used in molecular imagery and therapy.