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MICROBUBBLES FOR ULTRASOUND IMAGING AND THERAPY

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Ultrasound is the most widely used medical imaging modality. The majority of ultrasound systems operate at frequencies in the 1 to 5 MHz range and form images using a hand-held transducer that is external to the body. It is capable of providing real-time information about tissue structure and blood flow in the heart and larger vessels. Unfortunately, in smaller vessels and capillaries blood detection is not possible due to the low signal strength of blood, tissue motion effects, and limited resolution (~0.5-1 mm). It is the blood volume in small capillaries, monitored as a function of time (and referred to as perfusion) of different organs such as liver, kidney and the heart muscle that is of high interest. Ultrasound contrast agents, which are administered intravenously, will increase the reflection of ultrasound by the blood pool and make it possible to provide perfusion images.

An ultrasound contrast agent is made of tiny microbubbles, which scatter ultrasound very efficiently. The bubbles consist of air or an inert gas and they are coated with a protein, lipid or polymer layer. This prevents the bubbles to either dissolve in the blood or to coalesce to form larger bubbles. The contrast agents are injected intravenously and they are so small that they are transported into the smallest capillaries. Using contrast enhanced echocardiography the cardiologist can see which part of the heart muscle is poorly perfused. The interaction of contrast agent bubbles and ultrasound is the focus of our current study.

When a gas bubble is hit by an ultrasound wave it is forced into volume pulsation. In the simplest situation, the size of the bubble decreases in the positive cycle of the ultrasound wave, and the bubble expands in the negative cycle. The volume pulsation of the bubble is frequency and amplitude dependent. The dependency on the acoustic pressure amplitude can be divided into three regimes. For small amplitudes of the ultrasound wave, the relative compression and expansion of the bubble is the same and, therefore, the bubble size is linearly related to the applied acoustic pressure. For higher amplitudes, however, compression generally retards relative to expansion and non-linearity occurs. Consequently, the bubble size is not linearly related to the applied acoustic pressure, and the bubble vibration contains second and higher multiples of the transmitted frequency. In this way, the backscatter signal from the bubble does not only contain the fundamental (transmitted) frequency, but also harmonic frequencies. As the echo properties of the

surrounding tissue is very different from that of the microbubbles, this allows for an efficient suppression of tissue echoes therefore increasing the quality of imaging and analysis for medical diagnosis. If the amplitude of the acoustic wave is increased more, the scattering level of most of the contrast agents increases abruptly for a short time. This has been associated with bubble rupture and release of free gas bubbles.

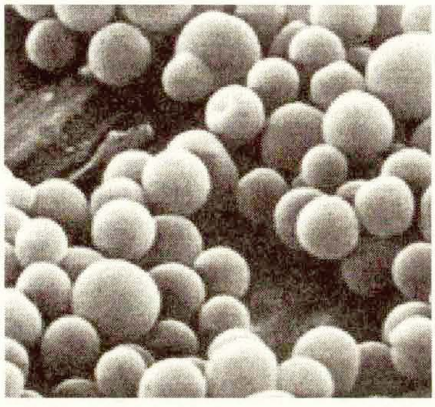

Fig.1. Scanning electron micrograph of coated microbubbles. The bubbles vary in size ranging from 1 to 10 micrometer.

To study the dynamics of the bubble upon insonification optically, e.g. under a microscope, it is necessary to use a high-speed camera. As the ultrasound frequencies used in medical diagnostic imaging are in the range of 1 to 10 MHz, a high-speed camera with a frame rate of 10 million frames per seconds (Mfps) or even faster is required to monitor the dynamics of these bubbles. We have constructed a digital ultra high-speed camera operating at up to 25 million frames per second based on the principle of a rotating mirror camera to optically study contrast agent behavior upon insonification down to a 40 nanosecond timescale. The results collected from these interesting experiments are tested and may be implemented in new diagnostic ultrasound applications in the near future.

There is a growing interest in the use of coated microbubbles for therapeutic applications. First, microbubbles can act as transport carriers for drug delivery. This can be done either by incorporating drugs in the shell material or by attaching drugs to the coating layer. By adding special targeting markers to the shell the transport can be directed and, in addition, with the use of ultrasound the targeted site can be acoustically imaged. The latter technique can be extended to a second application of therapeutically-designed microbubble systems, where bubbles are labeled with target antibodies, such as cancer cells and blood clots allowing an increased detection rate for tumors or thrombosis. A third application for therapy is known as sonoporation.

Here, the oscillations of ultrasound-driven microbubbles in close contact with a cell lead to an increased permeability of the cell to macromolecules, hence to an increased uptake of drugs or genes in close vicinity to the cell.

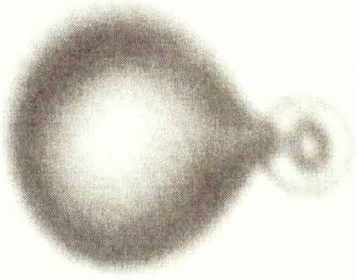

Fig. 2. Sonic cracking of a thick-shelled contrast microbubble. An expanding free gas bubble escapes through a small crack in the shell coating.

It was found that even small acoustic amplitudes lead to an intense microstreaming around microbubbles applying a high shear stress to the cell membrane. Also, the oscillating microbubbles can push against the cell membrane and thereby mechanically stimulate the cell. Finally, microbubbles driven at high acoustic pressures, i.e. in their non-linear regime, exhibit jetting phenomena which could potentially be exploited to locally (and temporarily) open pores in cell membranes to allow drug, radio nuclide or gene injection by the same jet. The ease of control of ultrasound will then allow for either a controlled way of drug delivery maintaining cell viability or, for high acoustic pressures, for a more radical way of cell destruction required to treat different pathologies. In all these cases the real-time optical observations with the Brandaris camera will allow for an improved understanding of bubble-cell interactions which opens new perspectives leading to optimized techniques for ultrasound therapy.

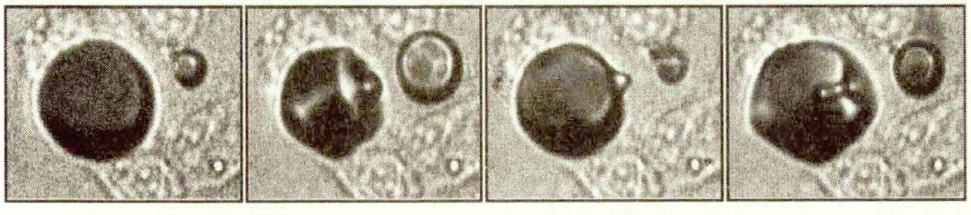

Fig.3. A microbubble driven into an unstable regime leading to jet formation. These so-colled nanosyringes can be use to increase molecular uptake for local drug and gene delivery or in more violent setting to kill cells.

The ultra high-speed camera is called Brandaris because it makes use of an optical principle similar to that of a light house. Research with the camera is performed in two labs in the Netherlands.

Dr. Michel Versluis is from the Physics of Fluids group at the University of Twente, headed by Prof. Detlef Lohse. The group works on a variety of topics such as multiphase flow, fully developed turbulence and thermal convection, granular matter, foams and slug flow and air entrainment. The group is specialized in the study of bubble dynamics with on-going research in sonoluminescence, lithotripsy and ultrasound contrast agents.

Prof. Nico de Jong is a senior scientist at the Experimental Echocardiography group of Erasmus Medical Center Rotterdam, The group is specialized in the technical application and fundamental understanding of emerging medical ultrasound techniques, such as contrast echography, superharmonic imaging, intravascular ultrasound imaging and 3D cardiac imaging.