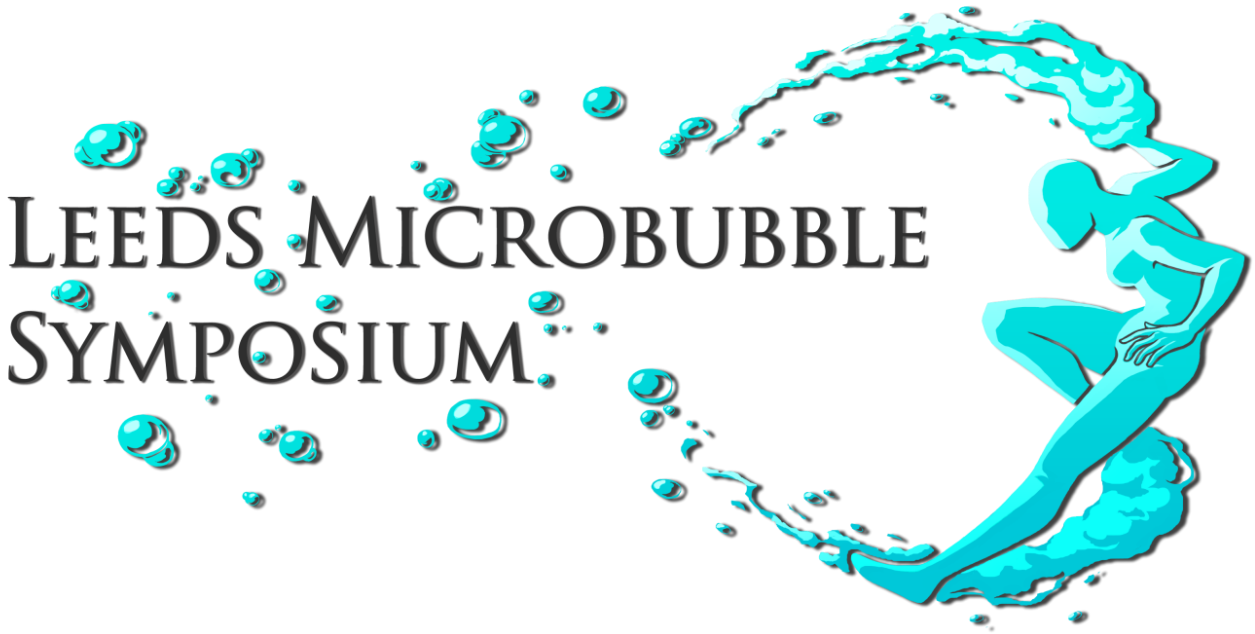
Leeds Microbubble Symposium



Fabrication, Characterisation and Translational Applications

16th & 17th July 2018

Weetwood Hall, Otley Road, Headingley, Leeds, LS16 5PS

Programme and Abstracts

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Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Michel Versluis,** University of Twente
Title	**Monodisperse microbubble ultrasound contrast agents: formulations, characterization, and advanced imaging potential**

Abstract

Monodisperse microbubble ultrasound contrast agents may dramatically increase the sensitivity and efficiency in ultrasound imaging and therapy. They can be produced in a microfluidic flow-focusing device, but questions remain as to the role of the device geometry, the liquid and gas flow, and the phospholipid formulation on bubble stability. We developed a model based on simple continuum mechanics equations that reveals the scaling of the coalescence probability with the key physical parameters. It is used to characterize short-term coalescence behavior and long-term size stability as a function of flow-focusing geometry, bulk viscosity, lipid co-solvent mass fraction, lipid concentration, lipopolymer molecular weight, and lipopolymer molar fraction. All collected data collapse on two master curves given by universal equations for the coalescence probability and size stability.

Resonant bubbles can also be separated from the polydisperse agent in an acoustic bubble sorting chip. Acoustically sorted bubbles are characterized by measuring scattering and attenuation simultaneously using narrowband acoustic pulses. For all driving pressures and frequencies employed, the scattering and attenuation coefficients can be modeled using a single and unique set of shell parameters confirming that acoustically sorted bubbles provide a uniform acoustic response. We also compare the sensitivity to a polydisperse pre-clinical agent and find that the sorted monodisperse agents had up to a two-orders-of-magnitude increase in sensitivity.

Finally, we present high-precision acoustic measurements of the nonlinear viscoelastic shell properties of phospholipid-coated microbubbles, data that we collected by tuning the surface dilatation of well-controlled monodisperse bubble suspensions through the ambient pressure. Upon compression, the shell elasticity was found to increase after which the monolayer collapses and the elasticity vanishes. During bubble expansion, the elasticity drops, first monotonically, then more rapidly to zero. Integration of the elasticity vs. surface area curves shows excellent agreement with the popular models for phospholipid-coated microbubble dynamics.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Judy Hadley,** Malvern Panalytical
Title	**Utilizing Resonant Mass Measurement to Characterize Ultrasound Contrast Agent Formulations**

Abstract

An accurate method of counting and sizing microbubble ultrasound contrast agents (UCA) is essential for quantitative microbubble enhanced ultrasound imaging. The performance of UCAs depends on the concentration of the preparation [1] and the resonance behavior of a microbubble, which in turn is inversely related to its diameter [2]. Measuring the size distribution of shell-stabilized perfluorocarbon gas bubbles however can be challenging. Formulation stability affects reproducibility and repeatability. Typically, preparation methods yield extremely polydisperse bubbles. Samples may also contain excess lipids resulting in difficulty in identifying the bubble component alone.

In this study we present the use of a novel particle characterization technique for measuring microbubbles, Resonant Mass Measurement, (RMM). RMM detects and counts particles in the size range around 50nm - 5µm, depending on particle density. The technology utilizes a Micro-Electo-Mechanical Systems (MEMS) sensor comprising a microfluidic channel embedded in a resonating cantilever. When individual particles in a fluid pass through the channel, a change in buoyant mass is detected and using material density, this mass is converted to size [3, 4]. The technique is highly accurate with sub femtogram sensitivity [5, 6].

For samples containing materials of two different densities, the technique enables differentiation and simultaneous sizing of the two populations. RMM has gained popularity for sizing subvisible particles in biopharmaceuticals owing to its unique ability to discriminate between protein aggregates and silicone oil in injectable products [7-12]. Recently, RMM principals have been used to carry out in-depth characterization of bubbles [13,14]. In this presentation we demonstrate the use of RMM for characterizing the size, concentration and buoyant mass of bubbles, which are parameters affecting ultrasound activity at clinically relevant frequencies (3-18 MHz), whilst simultaneously discriminating contaminants based on their buoyant mass.

1. Gorce J, Arditi M, Schneider M. "Influence of Bubble Size Distribution on the Echogenicity of Ultrasound Contrast Agents – A Study of SonoVue TM". Invest Radiol 2000;35:661-671. 2. Sprague M, Cherin E, Goertz D, Foster F. "Nonlinear Emission from Individual Bound Microbubbles at High Frequencies", Ultrasound Med. Biol., vol 36, pp313-324, Feb. 201 3.Burg, T., Godin. M., Knudsen, S., Shen, W., Carlson, G., Foster, J., Babcock, K., Manalis, S., Nature, 446, 1066-1069, (2007) 4.Godin, M., Bryan, A., Burg, T., Babcock, K., Manalis, S., Applied Physics Letters, 91, 123121 (2007) 5. Lee, J., Shen, W., Payer, K., Burg, T., Manalis, S., Nano Lett., 10(7), 2537-2542, (2010) 6.Olcum, S., Cermak, N., Wasserman, S., Christine, K., Atsumi, H., Payer, K., Shen, W., Lee, J., Belcher, A., Bhatia, S., Manalis, S., PNAS, vol111, no4, 1310-1315, (2014) 7.Patel, A., Lau, D., Liu, J., Anal. Chem 84, 6833-6840 (2012) 8. Weinbuch, D., Zolls, S., Wiggenhorn, M., Freiss, W., Winter, G., Jiskoot, W., Hawe,A., J Pharm. Sci 102:2152-2165, (2013) 9. Panchal J, Kotarek J, Marszal E, Topp, EM, AAPS J 16, 440-451, (2014) 10. Folzer, E., Khan, T., Schmidt, R., Finkler, C., Huwyler, J., Mahler, H., Koulov, A., J of Pharm Sci,104, 12, (2015) 11. Bai, S., Landsman, P., Spencer, A., DeCollibus, D., Vega, F., Temel, D., Houde, D., Henderson, O., Brader, M., 105, 50-63, (2016) 12. Teska. B., Brake, J., Tronto, G., Carpenter, J. Pharm Sci, 105,2053-2065, (2016) 13. Hernandez C, Gulati S, Exner AA. Cryo-EM Visualization of Lipid and Polymer-Stabilized Perfluorocarbon Gas Nanobubbles – A Step towards Nanobubble Mediated Drug Delivery. Nature Scientific Reports. Accepted 9/17/2017. In Press. 14. Nieves L, Hernandez C, Hadley J, Coyne B, Exner AA. Effect of the Surfactant Pluoronic on the Stability of Lipid-Stabilized Perfluorocarbon Nanobubbles. IEEE International Ultrasonics Symposium 2017 Annual meeting. In Press. 15. Hernandez C, Lilly J, Nittayacharn P, Hadley J, Coyne B, Kolios MC, Exner AA. Ultrasound Signal for Sub-Micron Lipid-Coated Bubbles. IEEE International Ultrasonics Symposium 2017 Annual meeting. In Press.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Eleanor Stride,** University of Oxford
Title	**Magneto-Acoustic Targeted Drug Delivery**

Abstract

Magnetically functionalised microbubbles have been investigated as multi-modality imaging agents and as carriers for magnetically targeted drug delivery. The latter application in particular requires simultaneous application of magnetic and acoustic fields to a target region. This can present a significant practical challenge, especially in vivo where access is typically limited. To address this, an integrated device capable of generating co-aligned magnetic and acoustic fields within a target tissue volume has been developed. A prototype system for pre-clinical experiments was designed to accumulate microbubbles at a distance of 10 mm from the probe's surface, commensurate with relevant tissue depths in small animal models. The ultrasound transducer was designed to maximise the acoustic pressure in the same region in order to induce cavitation. Previous studies have indicated that both microbubble concentration and duration of cavitation activity are positively correlated with therapeutic effect. The ability of the device to trap and activate microbubbles was therefore first assessed by a series of in vitro tests in a tissue mimicking phantom containing a single vessel of 1.2 mm diameter. At a flow rate of 4.2 mm/s magnetic trapping produced an increase in intensity under B-mode ultrasound imaging consistent with the predicted accumulation profile. When the microbubbles were exposed to the ultrasound field from the probe, the resulting cavitation activity was sustained for a period more than 4 times longer than that achieved with an identical acoustic field but in the absence of a magnet. Experiments in an in vivo pancreatic tumour model were then conducted and there was found to be a significant benefit in terms of tumour response to chemotherapy using the combined probe in comparison to the use of separate magnetic and ultrasonic devices. Finally a design for a device suitable for use on clinical lengthscales has been developed.

Estelle Beguin[1], Michael Gray[1], Lester C. Barnsley[1,2], Heather Nesbitt[3], Jason Sheng[3], Keiran Logan[3], Sukanta Kamila[3], Dario Carugo[1,4], Anthony McHale[3], John Callan[3], Eleanor Stride[1]
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Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Gabriela Calderó,** Institut de Química Avançada de Catalunya
Title	**Low-energy emulsification as a novel approach for microbubble preparation from perfluorocarbon nano-emulsions**

Abstract

Low-energy emulsification methods show interesting advantages over conventional ones, as they are simple, reproducible and cost-effective. They consist of using the chemical energy stored in the system to generate dispersed droplets. This allows achieving smaller and more homogeneous droplet sizes than those obtained using high-energy emulsification methods. The emulsification of perfluorocarbons is challenging due to their high density and both, their hydrophobicity and lipophobicity. The preparation of nano-emulsions with these components has been described so far using high-energy methods, such as sonication or high shear stirring. In this work, the preparation of perfluorocarbon nano-emulsions by low-energy methods has been studied in PBS / nonionic surfactant / [polymer solution + perfluorocarbon + apolar low density oil] systems at room temperature. Perfluorocarbon nano-emulsions have been obtained at a high oil-to-surfactant ratio with hydrodynamic droplet sizes typically below 300 nm and improved colloidal stability when the apolar low-density oil is present in the oil phase of the nano-emulsion. Further, globular-shaped perfluorocarbon-loaded polymeric nanocapsules have been obtained from the nano-emulsion templates with sizes below 250 nm as determined by dynamic light scattering. Perfluorocarbon encapsulation in the nanocapsules has been evidenced both, by spectral angle mapper classification of hyperspectral images and fluorine elemental microanalysis. Interestingly, cytotoxicity tests revealed improved cell viability of perfluorocarbon-loaded nanocapsules as compared to nanocapsules without the perfluorocarbon. Preliminary analyses suggest successful vapourization after mild heating and/or sonication of the perfluorocarbon-loaded nanocapsules, although the ultrasound attenuation in the frequency range between 0.4 and 6.6 MHz was low. This may be attributed to the high polymer content in the microbubble shell. Research is ongoing for ultrasound response optimization in these systems.

Gabriela Calderó, Carlos Rodríguez-Abreu, Albert González, Marta Monge, María José García-Celma, Conxita Solans

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Klazina Kooiman,** Erasmus MC
Title	**A new look at microbubble-mediated drug delivery using combined confocal microscopy and Brandaris 128 ultra-high speed imaging**

Abstract

Ultrasound-activated microbubbles can be used for drug delivery [1]. However, the mechanism is still unknown. To better predict and control the different drug delivery pathways, underlying biological and physical mechanisms of the microbubble-cell interaction need to be studied. Much insight into the cellular effects has been gained with confocal microscopy [2-4] and into the microbubble behavior with ultra-high speed camera studies [4,5]. However, to study all aspects of the microbubble-cell interaction simultaneously, a combined confocal microscope and ultra-high speed camera would be needed. A state-of-the-art optical imaging system was therefore developed by connecting an upright custom built Nikon A1R confocal microscope to the Brandaris 128 ultra-high speed camera [6], thereby achieving the nanometer and nanosecond resolution necessary to visualize cellular effects and resolve microbubble oscillation upon ultrasound insonification.

Confluent endothelial cells were evaluated for opening of cell-cell junctions with CellMask and for sonoporation with Propidium Iodide (PI). The cellular response of single aVβ3-targeted microbubbles (n=168) was monitored up to 4 min after ultrasound insonification (2MHz, 100-400kPa, 10-cycles). Cell-cell junctions opening occurred more often when cells were only partially attached to their neighbors (45%) than when fully attached (15%). Almost all fully attached cells showing cell-cell opening also showed PI uptake (92%). The mean microbubble excursion was larger when a cell was sonoporated (1.0μm) versus non-sonoporated (0.47μm). In conclusion, using the state-of-the-art imaging system we can now elucidate the relationship between microbubble oscillation behavior and drug delivery pathways.

References:
[1] Kooiman et al., Adv Drug Del Rev 2014, 72:p.28; [2] Hu et al, Ultrasound Med Biol 2013; 39:p. 2393; [3] De Cock et al, J Contr Rel 2015; 197:p. 20; [4] Helfield et al, Proc Natl Acad Sci USA 2016, 113:p.9983; [5] Kooiman et al, J Contr Rel 2011, 154:p.35; [7] Chin et al., Rev Sci Instru 2003, 74: p. 5026.

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Session 2	**Microbubble Ultrasound Techniques**
Oral Presentation	**John Hossack**, University of Virginia
Title	**Instrumented microfluidics device for fabricating, counting**

Abstract

Flow focusing microfluidic devices (FFMDs) have been explored over recent years as an approach for creating a stream of microbubbles. In certain modes of operation, they yield an exceptionally tight size distribution. However, devices operating in this mode of operation produce microbubbles that are: relatively large (typically > 8 □m), possess limited stability (several minutes) and have low production rates (10^5 to 10^6 /s). We previously determined that these qualities can used to advantage in the context of supplying a stream of large unstable microbubbles directly off the tip of a catheter for the purpose of accelerating sonothrombolysis. For this approach to translate from a laboratory bench setting, it is necessary to build instrumentation into the device to avoid the commonly used approach of placing the device within the field of view of a microscope. Thus, we developed a Micro Coulter Particle Counter (μCPC) as part of the microfluidic device to enable electrical impedance-based characterization (counting and sizing) of microbubbles passing through a sensing zone. We determined an optimal design of electrode layout taking account of fabrication considerations and anticipated range of microbubble dimensions. This involved an extensive FEA COMSOL simulation exercise accounting for device physical design and microbubble geometry / flow conditions. A total of 56 different permutations of electrode widths and spacings were modeled. Microbubbles considered ranged from 8 to 20 μm in diameter. Additionally, we determined that it was necessary to use a compensation factor to account for perturbed impedance change when microbubbles pass the sensing zone in close adjacency. The compensation factor takes account of detected count and a per design operating curve that accounts for liquid phase flow rate and microbubble count. Microbubble diameters determined using the μCPC were highly correlated with microscope-based sizing (R2 = 0.93). We could not detect errors in counting.

J. M. Robert Rickel and John A. Hossack

Session 2	**Microbubble Ultrasound Techniques**
Oral Presentation	**Hendrik Vos,** University Twente
Title	**High frame rate microbubble-enhanced velocimetry**

Abstract

In search of cardiac and vascular dysfunction, we aim at quantification of flow magnitudes and flow patterns in cavities and large vessels. Such quantification needs high frame rate (HFR) imaging, which we perform in 2D and 3D with contrast-enhanced ultrasonography (CEUS) using an open research scanner (Verasonics with ATL C5-2 and Oldelft 3D TEE propotype probes). In this presentation we will show an overview of the recent quantitative HFR CEUS studies within the consortium. These include:

- 2D in-vitro study of left-ventricular flow, and its optical PIV reference measurements;
- 2D study of the abdominal aorta in human volunteers and its phase contrast-MRI reference measurements;
- 3D in vitro study of left-ventricular flow and phase-contrast MRI with optical stereo PIV as ground truth.

In these studies, we have found practical rules of thumb that improve signal to noise ratio, contrast to tissue ratio, and accuracy. The findings can be summarized as:

1. To improve accuracy, coherent image compounding is avoided for fast flows; the compounding is rather applied in correlation / displacement domain.
2. Low contrast agent concentration (with bolus injection: 0.25 ml of SonoVue in humans) yielded some signal drop out but also non-saturated speckle which improved the correlation values for fast flows (>0.3 m/s);
3. Linear contrast imaging + SVD (Singular Value Decomposition) filtering provides higher contrast to tissue ratio than the amplitude modulation detection scheme;
4. Even with SVD filtering, aliasing is preferably avoided to separate tissue, contrast, and noise; we found that even with 1000 frames/s, the high rank SVD modes still had a substantial amount of bubble signal instead of the expected noise-only.
5. Ethical approval for human CEUS with the Verasonics research scanner is received increasingly easily with the increasing number of safely finished studies.

J. Voorneveld, S. Engelhard, H.J. Vos, H. Saaid, M. Reijnen, F. Gijsen, T. Claessens, M. Versluis, E. Groot Jebbink, S. Kenjeres, N. de Jong, and J.G. Bosch
Affiliations in consortium: Erasmus MC Rotterdam, TU Delft, University Twente, Leiden UMC, and Rijnstate Hospital Arnhem; the Netherlands. Ghent University; Belgium

Session 2	**Microbubble Ultrasound Techniques**
Oral Presentation	**Andrew Healey,** Phoenix Solutions AS
Title	**Acoustic cluster therapy for ultrasound mediated drug delivery: technology, proof of concept and clinical trial design**

Abstract

Dose limiting toxicities hamper the clinical efficacy of many drugs, particularly in oncology. One approach to overcome this issue is to use acoustically active particle formulations in combination with ultrasound, to spatially enhance drug delivery. Commercially available ultrasound contrast agents have been used, such as Optison, SonazoidTM and Sonovue, and several other technologies are being pursued such as acoustic droplet vaporisation (ADV), polymeric cups, various drug loaded particles, among others.

Acoustic cluster therapy (ACT) is in the ADV class. The ACT product is formed by reconstitution of commercially available, negatively charged microbubbles (Sonazoid™) with an emulsion of positively charged microdroplets of an inert, perfluorated oil. The active moiety comprises free flowing, microbubble/microdroplet clusters formed by electrostatic attraction. Two ultrasound exposure regimes are employed. Firstly, on exposure to diagnostic medical ultrasound fields of low power (mechanical index ~0.2 and above) the microbubble component acts as an evaporation 'seed' and transfers energy to the oil droplet resulting in vaporisation. The resulting bubble population produced has a mean diameter in-vivo of 22 microns with no bubbles larger than 44 microns. The size range has been engineered so that the microbubbles lodge at the micro vessel level in the vascular tree and remain for 5-10 minutes. The second ultrasound exposure utilises lower frequency ultrasound (500kHz) short low intensity pulses (MI ~0.2) which are closer to the resonance frequency of the larger ACT bubbles lodged in the microvasculature. This induces stable mechanical oscillations of the ACT bubbles, localized microstreaming, radiation and shear forces that increase the local permeability of the vasculature, increasing transport of the co-administered drug across the capillary barrier and through the extracellular matrix.

Details of the ACT technology are briefly reviewed along with pre-clinical proof of principle/concept studies, safety assessment, dose estimation and study outline for first in man clinical trials.

A J Healey, N Bush, A van Wamel, A Shah, P Prentice, S Sulheim, M Mühlenpfordt, G Box, V Kirkin, S Eccles, C de Lange Davies, J Bamber, S Kvåle, P Sontum.

Session 2	**Microbubble Ultrasound Techniques**
Oral Presentation	**Luke Richards,** University of Oxford
Title	**Patient specific ultrasound lenses for transcranial ultrasound**

Abstract

There has been recent interest in using patient specific ultrasound lenses to correct for the phase aberration caused by the skull in focused transcranial ultrasound. Here we apply acoustic lenses to the problem of creating a highly uniform field inside the skull, motivated by the requirements of targeted and ultrasound responsive nanodroplets being developed for the treatment of brain metastases. The phase and amplitude required for uniform sonication was determined from simulations of ultrasound propagation utilising CT data as a basis for the geometry of the patient's skull. From these simulations, silicone lenses were produced, capable of introducing phase inhomogeneity in the incident ultrasound beam to compensate for the effects of transmission through the skull. Experimental measurements with an ex vivo skull showed that phase uniformity could be at least partially restored within the skull cavity. Methods for modulating the amplitude of incident ultrasound have also been investigated. Stereolithography was used to directly print in highly attenuating (15dB/cm at 1MHz) materials, allowing rapid production of customised and selective acoustic absorbers. A 3D printed transducer backing was also studied, able to suppress transducer output in certain regions, and provide up to 15dB dynamic range across the surface of a planar ultrasound transducer."

Session 2	**Microbubble Ultrasound Techniques**
Oral Presentation	**Helen Mulvana**, University of Glasgow
Title	**An *in vitro* flow phantom for controlled investigation of TDD: development and validation**

Abstract

Ultrasound mediated targeted drug delivery (UmTDD) has been used to deliver drugs to cells in the lab and in animals. Microbubbles of gas are delivered into the blood stream alongside a drug and forced to oscillate using ultrasound. The oscillations create tiny pores in the cells targeted by ultrasound, increasing their drug uptake but, so far, delivery rates have remained low and unpredictable preventing the technology from being used in humans.

One aspect of the technique which has not received much attention is the impact that blood flow and blood vessel size and shape have on delivery rates. Using microCT to gather anatomically accurate data for 3D replication and theoretical analysis, we present an approach for the development, fabrication and validation of a blood vessel model for controlled investigation of UmTDD in vitro.

Session 2	**Microbubble Ultrasound Techniques**
Oral Presentation	**Elahe Moghimirad**, Institute of Cancer Research
Title	**Dynamic contrast enhanced ultrasound imaging; the effect of imaging modes and parameter settings on the time-intensity curve evaluated using a microvascular phantom**

Abstract

Dynamic contrast enhanced ultrasound (DCE-US) imaging has the potential to provide quantitative information which is sensitive to tumour perfusion, an indicator for tumor response to radiotherapy. To increase the reproducibility of time-intensity curve (TIC) characteristics, we are developing a 3D DCE-US imaging system. There are, however, many choices to be made in system design, such as whether to use plane wave (PW) or focused imaging (FI), and the values to use for parameters such as focal depth (FD), F-number (F#), mechanical index (MI) and number of angles (NA) (for PW imaging). We evaluated the effect of such choices on TICs using a microvascular flow phantom containing ~100,000 parallel microtubes, each 200 μm diameter. DCE-US 2D images were obtained using a Vantage™ (Verasonics Inc.) and a pulse-inversion algorithm. 800 frames were recorded at 10 Hz for PW and FI. All measurements were repeated 3 times, injecting 0.4 ml of contrast agent (Sonozoid™) and changing one parameter at a time, using the values: FD = 20, 40 mm; F# = 2, 4; MI = 0.11, 0.15, 0.25; NA = 3, 7, 11.

For a large region of interest which included the periphery of the phantom, TICs were sharp and single-peaked for F2 but broader and double-peaked for F4, consistent with F4 averaging over a greater focal volume than F2. Choosing a smaller more central ROI reduced the effect but did not eliminate it completely. Placing the focus deeper than the center reduced the TIC intensity due to attenuation but also resulted in a flatter TIC. PW intensity was greatest for 3 angles with evidence that this may be due to side lobe artefacts added to the contrast signal. TIC characteristics are thus expected to be highly sensitive to imaging parameters. This should be considered in longitudinal studies.

Elahe Moghimirad, Jeffrey Bamber and Emma Harris
Joint Department of Physics and CRUK Cancer Imaging Centre, The Institute of Cancer Research and Royal Marsden NHS Foundation Trust, Sutton, London, United Kingdom

Session 2	**Microbubble Ultrasound Techniques**
Oral Presentation	**Lori Bridal,** Laboratoire d'Imagerie Biomedicale
Title	**Quantification and Interpretation of Contrast-enhanced Ultrasound to Better Assess Tumor Status**

Abstract

Performant evaluation of the heterogeneity and inefficiency of the vascular network holds multiple interests for tumor assessment. Both structural and functional heterogeneity of tumor vascularization have been associated with disease progression and malignancy. Furthermore, therapeutic response and delivery are influenced by the molecular, structural and functional diversity within a tumor's vascularization. Zones with poor perfusion reduce efficiency of chemotherapy and radiotherapy and can contribute to the development of therapeutic resistance. Contrast-enhanced ultrasound offers a view of the tumor vascularization with strong capacities to assess dynamic flow and its spatial variability. Currently, therapeutic response is largely assessed based on overall, relative changes in contrast-enhanced intensity and flow parameters. We have worked to obtain more precise, quantitative mapping of parameters describing the tumor vasculature and its heterogeneity. Key steps toward this include machine-independent linearization of the data, choice of the kinetic models that are best adapted to the nature of the data and accounting for variability in the arterial input function. Recommended practices and current limitations for contrast-enhanced mapping of the heterogeneous tumor flow will be reviewed. Interpretation of this information and its integration into the clinical decision-making process pose additional challenges. Paths in this direction will be explored based on recent work to combine contrast-enhanced ultrasound data with other types of imaging parameters and use of mathematical models for tumor growth to better discriminate therapeutic response patterns. Contrast-enhanced ultrasound is well-positioned to provide valuable, additional information on the heterogeneous tumor's status for a more informed clinical decision.

Alain CORON1, Jérôme GRIFFON1, Delphine LE GUILLOU-BUFFELLO1, Michele LAMURAGLIA1,2, Lori BRIDAL1
1 Laboratoire d'Imagerie Biomedicale, France, 2 Medical Oncology, AP-HP, Beaujon Hospital, France

Session 2	**Microbubble Ultrasound Techniques**
Oral Presentation	**Meng-Xing Tang**, Imperial College London
Title	**High spatial and temporal resolution imaging with contrast enhanced ultrasound**

Abstract

The advent of microbubble contrast agents has inspired new methods for imaging blood flow, tissue perfusion and molecular targets using contrast enhanced ultrasound (CEUS) in cardiovascular and oncological applications. Recent advances have shown that it is possible to achieve CEUS imaging in very high temporal (high frame rate / ultrafast imaging, up to tens of thousands of fps at centimeter depth) and spatial resolution (super-resolution imaging, down to microns at centimeter depth). Such techniques open up new opportunities to image fast moving targets (e.g. a beating heart, arterial flow) and small targets with weak signals (microvascular flow).

In this talk I will introduce our recent work on the development and initial evaluation of high temporal and spatial resolution CEUS imaging and signal processing techniques. An ultrasound research platform was used to acquire data on phantoms, pre-clinical models and human volunteers. Results show that high frame rate CEUS enables the tracking of fast arterial and cardiac chamber flow, allowing quantification of flow vectors and flow volumes, as well as wall shear stress. Our Results also show that with advanced signal processing, 3D micro-vessels in normal and cancerous tissues can be visualized in vivo in high resolution and contrast.

Session 3	**Microbubble Plasmonic-based Photoacoustics**
Oral Presentation	**Paul Beard,** University College London
Title	**Photoacoustic imaging for the clinical and life sciences**

Abstract

Photoacoustic imaging is a new biomedical imaging modality based on the use of laser-generated ultrasound. It is a hybrid technique that combines the high contrast and spectroscopic-based specificity of optical imaging with the high spatial resolution available to ultrasound. As a consequence it overcomes the limited penetration depth/spatial resolution of purely optical imaging techniques such as multiphoton microscopy or diffuse optical tomography due to the overwhelming optical scattering exhibited by tissue. At the same time it retains their high contrast and spectral specificity enabling visualisation of anatomical features indistinguishable with other modalities such as ultrasound imaging. The technique has several specific features that make it a potentially powerful imaging tool. First, it can provide 3D images of vascular anatomy with high spatial resolution and contrast, the latter being a consequence of the strong absorption of haemoglobin at visible and near-infrared wavelengths. Second, it can reveal the distribution of blood oxygen saturation over the vasculature by obtaining images at multiple wavelengths and exploiting the spectral differences between oxy and deoxyhaemoglobin. Third, by extracting the Doppler shift from photoacoustic waves generated in red blood cells, measurements of blood flow can be acquired. As well as exploiting endogenous contrast provided by haemoglobin, there is also the potential, through the use of targeted contrast agents or genetic reporters to provide information at a cellular/molecular level. These attributes make the technique well suited to preclinical studies of a wide range of tissue abnormalities such as tumours and other pathologies characterised by changes in the structure, oxygenation and flow status of the vasculature. A high resolution small animal imaging system based on a novel interferometric ultrasound sensor has been developed. This has been used to obtain detailed non invasive 3D images of tumour vasculature and its response to anti-vascular therapy, mouse embryos and tumour cell distribution using genetically expressed reporters. The same technology has been further developed to realise a range of non invasive and endoscopic clinical imaging devices for the assessment of cancer, cardiovascular disease and microcirculatory abnormalities.

Session 3	**Microbubble Plasmonic-based Photoacoustics**
Oral Presentation	**Tim Devling,** iThera Medical
Title	**Raster-scanning optoacoustic mesoscopy for high resolution biomedical imaging**

Abstract

Photo- or optoacoustic imaging is a novel biomedical imaging technology relying on the detection of light generated ultrasound for image formation of biological tissue. Given the penetration and resolution issues inherent to diffuse optical imaging, the optoacoustic technique is generally seen as an advancement overcoming these limitations. The increasing use in both preclinical and clinical environments has demonstrated the value of optoacoustic imaging for molecular and bulk tissue functional imaging in a variety of pathological conditions.

Bridging the gap between macro- and microscopy, mesoscopic optoacoustic imaging is also seen as a practical alternative to optical based methods with the key strengths being capacity for non-invasive label free imaging (due to the use of endogenous tissue absorption to form the image) and potential for depth penetration up to several millimetres; compared to several hundred microns with optical based techniques. Here, we describe an innovative design that enables high resolution imaging (up to 10 microns) at depth (up to 3 mm) by means of raster-scanning optoacoustic mesoscopy (RSOM), implemented in an ultra-broadband (10-100 MHz) detection mode. Using tomographic reconstruction and frequency band separation to equally represent structures emitting low and high frequencies, we show the practical application of RSOM for imaging change in vascular structures associated with the angiogenic process in cancer, therapeutic vascular disruption, inflammation and cell tracking.

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iThera Medical GmbH, Munich, Germany.

Session 3	**Microbubble Plasmonic-based Photoacoustics**
Oral Presentation	**Jithin Jose**, FUJIFILM VisualSonics
Title	**'Graphene Meets Microbubbles': A Dual modality contrast agent for high resolution photoacoustic and ultrasound imaging**

Abstract

Photoacoustic (PA) imaging is a hybrid imaging modality, for non-invasive detection of tissue structural and functional anomalies. The approach is based on optical absorption, which uses pulsed laser-induced ultrasound from specific endogenous or the exogenous chromophores to map their distribution. The technique combines the advantageous properties of optical and ultrasound imaging. In contrast to purely optical imaging, PA imaging retains good spatial resolutions at higher imaging depths since ultrasound waves are not scattered as highly as photons inside biological tissue.
In the present work, we will discuss the design and implementation of a multi-modal imaging system where we integrate the PA imaging into a micro-ultrasound (US) platform. In addition, we will also discuss the development and validation of a "Graphene based microbubble"- a hybrid contrast agent for the PA and US imaging. The near-infrared (NIR) absorbing pristine graphene sheets are combined to poly(vinylalcohol), PVA, shelled microbubbles (MBs), leading to a significant photoacoustic signal enhancement and thus allow the deep tissue imaging. We will discuss the initial results of the full body photoacoustic imaging and the bio-distribution of graphene/PVA MBs in mice. We believe that this study has several elements of novelty in the field of photoacoustic imaging and represents a contribution to the understanding of biological impact of graphenic materials.

Reference

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Session 3	**Microbubble Plasmonic-based Photoacoustics**
Oral Presentation	**Jeff Bamber**, Institute of Cancer Research
Title	**Photoacoustic imaging and contrast agents in cancer research**

Abstract

This presentation provides an update on three areas of research that have been running at the Institute of Cancer Research, some in collaboration with groups at Imperial College London.
Photoacoustic imaging, microbubbles, and response to radiotherapy: Using our novel method of co-registering multispectral optoacoustic tomography (MSOT) and dynamic contrast-enhanced ultrasound (DCE-US) images, we have demonstrated in xenograft models in mice that while MSOT and DCE-US provide complementary information for improving our understanding of angiogenesis and hypoxia, microbubble peak contrast enhancement and arrival time have potential to act as clinical surrogates of total haemoglobin content and blood oxygen saturation respectively. We have also shown that blood oxygen saturation within the tumour is highly predictive of whether a tumour will respond to radiotherapy.
Gold nanorods (GNRs), imaging targeted radiotherapy dose enhancement and photothermal therapy: We have previously demonstrated that GNRs synthesised to have specific longitudinal plasmon resonance are able to provide multiplexed molecularly targeted photoacoustic imaging contrast that noninvasively classifies cancer cells according to cell surface receptor expression. We are now beginning to explore in vitro the value of such images for non-invasively predicting the local extent of radiotherapy dose enhancement produced by GNRs, and how photoacoustic imaging compares with multispectral x-ray imaging in this respect. In an entirely different application of GNRs, photothermal therapy, photoacoustic monitoring was shown to have potential for determining the time at which undesired GNR reshaping limits the value of continued therapeutic laser irradiation.
Sub-micron phase-change agents: Dye-coated perfluorocarbon droplets have been shown to provide three modes of imaging contrast, namely (i) photoacoustic, (ii) ultrasound after optical activation and (iii) ultrasound after ultrasound activation. For photoacoustic contrast, the increase in photoacoustic signal over dye or blood alone is particularly pronounced (> 56 dB) and offers a complement to strategies that we have been developing for improving photoacoustic penetration by clutter reduction. Since the droplets are of sub-micron size, all modes could potentially provide targeted extravascular contrast enhancement.

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Session 4	**Microbubble Translational Applications**
Oral Presentation	**Ali Sever,** Guy's and St Thomas' NHS Foundation Trust
Title	**Identification of Sentinel Lymph Nodes Using Microbubbles in Breast Cancer Patients**

Abstract

Sentinel lymph node biopsy (SLNB) in breast cancer patients has become an established procedure. Whilst there is no consensus, most breast units in the UK perform axillary clearance following a positive sentinel node biopsy. Grey-scale ultrasound of the axilla has limitations. A meta-analysis has shown that ultrasound and US-guided biopsy can identify the positive axilla in approximately 50% of the cases. Same study also showed that 25% of SLNB cases come as positive.

Ultrasound contrast material, microbubbles are proven to show the putative sentinel nodes accurately and also enables access for US-guided biopsy. It enables a better axillary staging in preoperative setting and has the potential to reduce two-stage surgeries (i.e. SLNB followed by axillary lymph node dissection). Indeed, a previous audit showed a statistically significant improvement by reducing two-stage surgery rate from 21% to 8%.

A recent multi-institutional study including four UK breast units has demonstrated comparable results confirming identification and biopsy of sentinel nodes using microbubbles is a reproducible technique.

Also a recent audit has shown that the combination of grey-scale and contrast enhanced ultrasound can filter out significant lymph node metastases and may guide the selection of patients who can safely avoid axillary surgery.

Session 4	**Microbubble Translational Applications**
Oral Presentation	Gareth LuTheryn, University of Southampton
Title	**Ultrasound responsive microbubbles enhance the activity of sub-inhibitory concentrations of gentamicin in the treatment of *Pseudomonas aeruginosa* biofilms**

Abstract

Introduction: The propensity of P. aeruginosa to both form a biofilm and readily select multi-drug resistant (MDR) mutants, augments its ability to survive conventional treatment1. Ultrasound (US) responsive gas microbubbles (MBs) provide a novel method of treating biofilms in chronic wound infections2,3. Microbubbles undergo volumetric oscillations or collapse upon exposure to an ultrasound field4; this makes them a powerful tool for the penetration of loose solids, such as a biofilm5.

Methods: Gas microbubbles with a mean diameter of 1.8 µm and stabilised by a phospholipid layer, (composed of DSPC:PEG40s, in a 9:1 molar ratio) were generated by sonication. An antibiotic-microbubble suspension (AMS) was applied to a P. aeruginosa biofilm, grown on a polypropylene coupon in a CDC bioreactor. In all experiments, the US frequency was 1 MHz and a sub-inhibitory concentration for biofilm eradiation (<10 µg/ml) of the aminoglycoside gentamicin was used.

Results: The application of gentamicin in combination with MBs and US achieved a 73% greater quantitative reduction in P. aeruginosa biofilm, than gentamicin administered alone. At a lower concentration of 1.2 ×107 MB/ml, a biofilm reduction of 99% was observed compared to gentamicin alone; this is significantly greater than the reduction achieved in treatments that used higher MB concentrations (6 ×107 MB/ml).

Discussion: This research has shown that the application of MB and US as adjuvants, allow ordinarily sub-inhibitory concentrations of antibiotic to kill P. aeruginosa biofilms. Building upon this study, ongoing research is focusing on understanding the mechanisms of the MB-biofilm interaction, investigating the effect of this treatment on polymicrobial biofilms, and developing alternative microbubble formulations.

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Session 4	Microbubble Translational Applications
Oral Presentation	**Maya Thanou,** Kings College London
Title	**Nanoparticles as functional tools in image guided Focused Ultrasound drug delivery**

Abstract

Localised drug delivery to tumours may be applied by the use of a blood compatible nanosized carrier agent able to respond to an external stimulus with a triggered drug release. Conventional thermosenstive liposomes (TSLs) or similar delivery systems lack the labels for in vivo tracking or tumour imaging and hence the ability to assess the optimal trigger time post administration. We developed a dual labelled thermosensitive liposomal (iTSL) delivery system for localised delivery by Focussed Ultrasound (FUS) triggered release. In addition to labelling for MRI, we introduced a Near-Infrared fluorescence (NIRF) label which greatly assisted in the near real time tracking of the carrier in our murine xenograft cancer model. This in turn allowed for optimisation of the FUS conditions and timings, allowing for triggered-release and functional delivery of the therapeutic drugs to the tumours. NIRF allowed for accurate imaging with little intrinsic tissue background fluorescence and good transmission & tissue scattering characteristics compared to visible region fluorophores. We synthesised these labels as lipid attached conjugates to ensure specific and lasting labelling of the carrier liposomes. MRI contrast enhancement ability and NIRF signals were assessed in vitro and in vivo. Nanoparticle (iTSLs) kinetics in murine tumours were assessed with optical imaging and at defined time intervals post i.v. injection, FUS was applied to induce a small increase in temperature to 42-43°C for 3-5 min. Imaging revealed both dramatic nanoparticles accumulation and drug release immediately after FUS treatment. Significant tumour growth inhibition was observed for the FUS treated tumours compared to those that were treated only with the drug nanoparticles. The applications of such multifunctional nanotheranostics with short and repeated FUS applications could have a transformative effect on cancer chemotherapy.

Session 4	**Microbubble Translational Applications**
Oral Presentation	**Sevan Harput,** Imperial College London
Title	**Super-Resolution Ultrasound Imaging with Microbubble Contrast Agents**

Abstract

Super-resolution ultrasound (SR-US) imaging can be achieved by localizing spatially isolated microbubble contrast agents over multiple imaging frames. This technique has the potential to revolutionize the way micro-circulation can be visualized and quantified in a wide range of clinical applications including cancer diagnosis, neuroimaging, and diabetes.

In this talk, we demonstrate 2D and 3D SR-US images in vitro, in an in vivo animal model and in human volunteers [1,2,3]. These SR-US images were generated by using clinical and research ultrasound systems with different imaging parameters to minimize the acquisition time and increase the image quality. A low concentration of microbubbles and a relatively low MI were used in all these acquisitions to ensure localization of spatially isolated microbubbles and to avoid microbubble destruction.

In a mouse model, the 2D SR-US method achieved 19 µm resolution, which was 6 times smaller than the ultrasound imaging wavelength. In human volunteers, 2D super-resolution images were generated using a clinical scanner with a handheld probe and diluted Sonovue microbubbles. Contrast enhanced ultrasound (CEUS) frames of a 50 second acquisition were combined after motion correction to achieve a resolution of 71 µm, which was 3.5 times smaller than the imaging wavelength. In an in vitro model with 200 µm vessels, 3D super-resolved volumetric images were generated using a high frame rate ultrasound research system.

Super-resolution imaging is still challenging, particularly given that the structures to be imaged can be on the order of 10 µm and that microbubble speeds can be <1mm/s. Motion correction error, low localization precision, long acquisition times and 3D imaging issues can induce limitations on super-resolution method and significantly reduce the achievable resolution [3,4]. After solving these problems, SR-US technique can visualize microvascular structures beyond the diffraction limit at depths which exceed those of existing optical or high-frequency ultrasound methods.

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Session 4	**Microbubble Translational Applications**
Oral Presentation	**Alexander Klibanov**, University of Virginia
Title	**Triggered drug delivery with microbubbles, nanodroplets and ultrasound.**

Abstract

Localized drug deposition in the area of disease may be useful in overcoming side effects of therapeutic interventions, especially in tumor treatment. Ability to enhance drug extravasation in the area of disease, as well as localized drug release from a carrier system by external stimuli may be useful. Acoustically active particles (gas-filled microbubbles and perfluorocarbon nanodroplets) may offer such capability, by localized deposition of ultrasound energy.

Non-destructive microbubble vibration in proximity to endothelium can make the vasculature more permeable, and drugs or can get from the bloodstream into the tissue (across blood-brain barrier, or blood-tumor barrier). Main advantage of this approach is the ability to use available clinical microbubbles, to ensure faster translation.

An alternative is to develop a dedicated carrier to sequester the drug and let it recirculate avoiding critical toxicity tissues (e.g., bone marrow and myocardium). Upon acoustic activation, carrier particles would release the drug locally within the ultrasound focal zone.

We have prepared doxorubicin-loaded liposome-microbubble complexes which carry over 1 pg drug per particle, sufficient to achieve administration of 6mg/kg doxorubicin in tumor-bearing mice. We have achieved successful suppression of tumor growth in these animals for two weeks by periodic (twice weekly) administration, in combination with 10 min tumor insonation (Birtcher Megason 1 MHz therapeutic ultrasound apparatus operated at ~200 KPa). Tumor growth was unrestricted in controls, when microbubbles were not loaded with drug, or when ultrasound was not applied.

We are now investigating red blood cells (RBC) as a triggered drug delivery system capable of extended (many hours and days) circulation time. A perfluorocarbon nanodroplet on RBC membrane allows carrier RBC contents release after a single pulse (10 cycles of 10 MHz ultrasound). RBCs can also be targeted magnetically or via peptides or other targeting ligands selective for tumor neovasculature, for further improvement of therapeutic delivery.

Z. Du, G. Diakova, A. Dixon, J. Chen, J. Farry, P. Zhang, J. Hossack, A. Klibanov

Session 4	**Microbubble Translational Applications**
Oral Presentation	**Silke Roovers,** University of Twente
Title	**Evaluation of microbubble-assisted ultrasound-triggered drug delivery in a 3d tumor model**

Abstract

Drug-loaded microbubbles have proven very promising for drug delivery purposes since they not only allow localized drug release but can also enhance the delivery of the drugs into the tissue. However, these encouraging results have been mostly based upon in vitro and animal models that do not match the human tumor environment. Therefore, we aim to bridge this gap by using 3D multicellular tumor models that mimic the in vivo situation more closely, while maintaining the controlled environment of an in vitro study. In this work, we evaluated the drug delivery potential of ultrasonic radiation in combination with microbubbles that have doxorubicin-loaded liposomes coupled onto their surface, in a 3D multicellular co-spheroid model consisting of both breast cancer cells and fibroblasts. We have found that compared to the control samples, there was a strong increase in liposomal delivery to the tumor spheroid cells, that seemed mostly present in the outer layers of the spheroid. However, when doxorubicin was released from the liposomes, the whole tumor spheroid could be penetrated by the small doxorubicin molecule and increased tumor killing was achieved in this way. In conclusion, although complete tissue penetration of the drug-carrying liposomes could not be achieved in this co-spheroid model, the results show that the this combination of liposome-loaded microbubbles and ultrasound causes an enhanced delivery of drug-loaded liposomes on the outer layers. This can provide a depot system in close proximity of the tumor tissue from which the drug can leak out, which could be a promising delivery strategy for chemotherapeutics such as doxorubicin, that have the correct biophysical characteristics to reach the target site when leaking out of the liposomes.

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Session 4	**Microbubble Translational Applications**
Oral Presentation	**Oliver Vince**, University of Oxford
Title	**Antibody targeted ultrasound-responsive nanodroplets for the therapy of brain metastases: a pre-clinical study**

Abstract

Metastatic tumors in the brain represent one of the leading causes of death from cancer with current treatments being largely ineffective and/or associated with significant side effects due to their lack of targeting. Conjugating MRI contrast agents with a monoclonal antibody for VCAM1 (anti-VCAM1) has previously allowed detection of brain tumor volumes two to three orders of magnitude smaller than those volumes currently detectable clinically.

In this study, a novel magnetic and acoustically responsive phospholipid-stabilised nanodroplet formulation has been conjugated with anti-VCAM-1. Preliminary in vivo tests have shown that these anti-VCAM-1 nanodroplets can be successfully targeted to both inflamed areas of the brain and brain metastases in a murine model. Acoustic droplet vaporisation of the anti-VCAM1 droplets, confirmed by passive cavitation detection, was also shown to cause blood brain barrier permeabilisation in vivo.

Extensive in vitro characterisation of the potential of these nanodroplets to target brain metastases using antibody and magnetic targeting, along with the ultrasound conditions required for various therapeutic effects has been completed and has been found to agree well with mathematical modelling.

The implications of these findings and the plans for future investigations into the therapeutic potential of these targeted and ultrasound responsive agents will be discussed.

Oliver Vince(a), Luca Bau (a), Sarah Peeters(b), Michael Gray(a), Alex Richards(a), Sean Smart(b),Nicola Sibson(b) and Eleanor Stride(a)

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Session 4	**Microbubble Translational Applications**
Oral Presentation	**Cyril Lafon,** INSERM
Title	**Opening the blood brain barrier with an implanted ultrasound device for increasing the penetration of Carboplatin into the brain: Preclinical and clinical results**

Abstract

The blood-brain barrier (BBB) limits the penetration of most drugs into the brain. Pulsed ultrasound in combination with injected microbubbles can transiently disrupt this BBB to increase the passage of drugs to brain tissue. An implantable unfocused ultrasound source operating at 1MHz, SonoCloud, was used to repeatedly disrupt the BBB in patients with recurrent glioblastoma (GBM) prior to carboplatin chemotherapy. The goal of the presentation will be to describe recent pre-clinical work on carboplatin activity in glioma models as well as an update on the clinical work.

Experiments were first performed in a primate model in order to assess the Carboplatin chemotherapy distribution after BBB disruption. Then, efficacy of combined carboplatin and BBB disruption was evaluated on mice bearing orthotopic human GBM xenografts. A first-in-man clinical trial at the University Hospital Pitié Salpêtrière, APHP, Paris, France was conducted. GBM patients with tumor recurrence had surgery to implant the SonoCloud device. It was then operated monthly in a <10-minute procedure in conjunction with IV administration of carboplatin and microbubbles. Patients were monitored clinically and T1w contrast-enhanced MR images were used to visualize BBB disruption.

BBB disruption resulted in a significant local increase of Carboplatin concentrations in the primate model and an increase in survival in GBM mouse models. Twenty-five patients were included in the study and 85 sonications were performed. BBB disruption was visible on MRI and depended on the applied ultrasound pressure. No carboplatin-related neurotoxicity was observed and only minor related adverse events were observed.

Pulsed ultrasound with the SonoCloud device was well-tolerated and may increase the effectiveness of drug therapies in the brain. Future work will aim at improving the efficacy of the treatment by sonicating larger volumes of brain. Clinical trial information: NCT02253212.

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Poster Presentations

Number	Name	Title
1.	Radwa Abou-Saleh	Hydrophobin Microbubbles: Microfluidic production, physical and mechanical characterisation
2.	Damien Batchelor	Microfluidic Microbubble Production in a Microspray Regime
3.	Ines Beekers	Cellular calcium during sonoporation revealed with combined confocal microscopy and Brandaris 128
4.	Matthew Bourn	Colorectal Cancer On-Chip: A Microfluidic Platform for the Testing of Microbubble Drug Delivery
5.	Jemma Brown	Development of Simultaneous Optical Imaging and Super-Resolution Ultrasound to Improve Microbubble Localisation
6.	Jack Cauldwell	Microbubbles: A new strategy for biofilm imaging and destruction
7.	Kevin Critchley	Morphological Control of Seedlessly-Synthesised Gold Nanorods using Binary Surfactants
8.	Fabio Domenici	Optically triggered phase-change contrast agents with polymer/surfactant hybrid shells
9.	Nicola Farrer	Platinum (IV) Liposome-Loaded Microbubbles as Theranostic Ultrasound-Responsive Contrast Agents
10.	Malcolm Haddrick	Transfer and Validation of a Targeted Microbubble Delivery Platform for Colorectal Cancer.
11.	Nicola Ingram	Towards clinically relevant therapeutic microbubbles
12.	Delanyo Kpeglo	On-chip Modelling of Pancreatic Cancer and its Stromal Environment
13.	Guillaume Lajoinie	A new microfluidic platform for real-time sizing and counting of microbubbles produced at MHz rates
14.	Simon Langeveld	Phospholipid-coated microbubbles: towards microbubble response on demand

15.	Sam Moorcroft	Liposomal encapsulation of anti-microbial peptides and gold nanorods
16.	Luzhen Nie	High Frame-Rate Echocardiography using Motion-Compensated Diverging Waves: in-vitro Evaluation
17.	Gaio Paradossi	A Tethering Study of RGD decorated Microbubbles on HUVEC Cells for Targeting of Tumor Brain Vasculature
18.	Sal Peyman	Improved microspray regime for microbubble formation
19.	Jordan Tinkler	Microbubble destruction for the treatment of in vitro S. aureus biofilms
20.	Mihnea Vlad Turcanu	Ultrasound and Microbubbles Promote the Retention of Fluorescent Compounds in the Small Intestine
21.	Papoole Valadbaigi	Generation of ultra stable microbubbles for industrial applications
22.	Liz Valleley	Combining epigenetic drug delivery with ultrasound-mediated microbubble destruction enhances tumour response in preclinical models of cancer
23.	Sunjie Ye	Au nanoplates for targeted PTT and PAI in NIR I and II

Poster Number: 1	**Radwa Abou-Saleh,** University of Leeds
Title	**Hydrophobin Microbubbles: Microfluidic production, physical and mechanical characterisation**

Abstract

Phospholipid microbubbles (MBs) with a perfluorocarbon gas core have been used as contrast agents for ultrasound (US) imaging, and as vehicles for targeted drug delivery where payloads are bound to the MBs. Different components of the shell architecture can change the physical and mechanical properties of the bubbles and therefore its US response.
Hydrophobins (HFBII) are amphiphilic proteins that are uniquely produced by filamentous fungi. They are primarily found coating the aerial hyphae. They are also found however within the cell walls of the hyphae forming insoluble structures that can only be dissociated with the use of specific acidic solvents. Although the most energetically favourable self-assembly of HFBII proteins is into needle-like shapes, we have optimised microfluidic production of circular MBs with a HFBII shell and perfluorocarbon gas core (HFBII-MBs). The properties and behaviour of PEGylated lipid, PEGylated HFBII and pure HFBII MBs in physiological conditions were compared. Lifetime and single bubble dissolution was determined through optical imaging. The mechanical properties of these bubbles were investigated for individual bubbles using atomic force microscopy (AFM), and preliminary US data collected to test the HFBII-MBs resonance frequency.
Results indicated that HFBII MBs are stable at physiological conditions over 3 hours, with PEGylated HFBII maintaining modal size, but decreasing in concentration. AFM results showed that the stiffness values for HFBII-MBs were higher across all forces, with a plasticity index of 0.20±0.10. Lipid coated MBs demonstrated a more plastic behaviour with a plasticity index 0.40±0.09. The response to US of the HFBII-MBs gave clear peaks in attenuation at fixed frequencies. The resonance peak of the PEGylated HFBII-MBs was at 3MHz, and for pure HFBII-MBs was 4MHz. The results demonstrate that HFBII-MBs are stable and have promising potential therapeutic applications.

Radwa H. Abou-Saleh, Jonathan Foster, Samuel Moorcroft, Neil H. Thomson, and Stephen D. Evans

Poster Number: 2	**Damien Batchelor,** University of Leeds
Title	**Microfluidic Microbubble Production in a Microspray Regime**

Abstract

Lipid stabilised gas core microbubbles are commonly used as Ultrasound Contrast Agents (UCAs) to improve the quality and contrast of ultrasound imaging with bubble sizes typically > 1µm. Image quality is increased due to the large change of acoustic impedance between microbubbles in the blood stream and surrounding tissue. Resonant frequency of these bubbles decreases with size, allowing for higher frequency ultrasound and therefore higher resolution images. The smaller size of these 'nanobubbles' allow deeper penetration into small capillaries in tumours, potentially, allowing imaging in areas previously unreachable by conventional microbubbles. Current research involves refinement of microfluidic production of perfluorocarbon filled microbubbles and nanoparticles. Bubbles are produced via flow-focusing microfluidic devices in a 'microspray regime', producing microbubbles of ~ 1.5µm diameter and concentrations of 10^9 particles/mL and nanoparticles of 100-200nm at concentrations of 10^11/mL. Robustness of the regime has been demonstrated using various outlet geometries, showing negligible change in bubble population. Further work will involve exploring on chip separation of microbubbles and nanobubbles via passive and active techniques such as pinched fractionation flow and acoustophoresis. Additionally, a single piece of "user-friendly" MATLAB based image analysis software has been developed to enable fast and reliable optical sizing and concentration measurements of microbubbles and other spherical particles.

Damien V. B. Batchelor, Louise Coletta, James R. McLaughlan, Sally A. Peyman, Stephen D. Evans

Poster Number: 3	**Ines Beekers,** Erasmus MC
Title	**Cellular calcium during sonoporation revealed with combined confocal microscopy and Brandaris 128**

Abstract

Ultrasound insonification of microbubbles can locally enhance vascular drug delivery by forming pores in the cell membrane (sonoporation), opening cell-cell junctions, and stimulating endocytosis. In order to enhance each of these pathways in a controlled and reversible manner, the underlying microbubble-cell interaction should be understood. This interaction can be studied with our recently developed state-of-the-art optical imaging system: the Brandaris 128 ultra-high speed camera, to visualize microbubble oscillation, coupled to a custom b! uilt Nikon A1R confocal microscope, to visualize the cellular response. To unravel the underlying drug delivery mechanisms we monitored intracellular calcium (Ca2+) fluctuations using Fluo-4, since Ca2+ plays a crucial role in membrane resealing, intercellular signaling, and opening of cell-cell junctions. At the same time, we evaluated for opening of cell-cell junctions with Cell Mask and for sonoporation with Propidium Iodide (PI). The response of confluent endothelial cells to single targeted microbubbles (n=138&! #x29; was monitored up to 4 min after ultrasound insonification (2MHz, 100-400kPa, 10 cycles). Sonoporated cells showed simultaneous PI uptake and increased Ca2+ levels. When the amount of PI uptake was low, the chance was higher for Ca2+ to return to its basal level before insonification, suggesting membrane resealing after sonoporation. When PI uptake was high, Ca2+ remained either elevated for >3 min or clustered into intracellular vesicles. The microbubble excursion amplitude was significantly smaller when Ca2+ returned to basal levels within&#x20! ;3 min ($p<0.01$). In addition, cell-cell junctions opened more often when Ca2+ remained elevated (52%) than when Ca2+ returned to basal levels (23%). Cells adjacent to a sonoporated cell showed a delayed Ca2+ increase, returning to basal levels within 3 min. In conclusion, by studying the interaction of microbubble behavior and both PI uptake and Ca2+ fluctuations with the state-of-the-art imaging system, we can further unravel ultrasound-mediated drug delivery and cellular recovery.

Beekers, Vegter, Tang, Mastik, Beurskens, van der Steen, Verweij, de Jong, Kooiman

Poster Number: 4	**Matthew Bourn, University of Leeds**
Title	Colorectal Cancer On-Chip: A Microfluidic Platform for the Testing of Microbubble Drug Delivery

Abstract

Colorectal cancer (CRC) is the fourth most common form of cancer in the UK and is responsible for the second highest number of cancer-related deaths. Current methods of testing novel cancer drugs on 2D and 3D cell cultures and animals often disregard a myriad of factors that may affect a drug's efficacy such as immune response and drug penetration. Development of a microfluidic platform which recreates the in vivo microenvironment of CRC would provide a system allowing for the testing and analysing of anti-cancer therapeutics, specifically those delivered using microbubbles.

Matthew D. Bourn, Delanyo Kpeglo, P. Louise Coletta, Stephen D. Evans

Poster Number: 5	**Jemma Brown,** King's College London
Title	**Development of Simultaneous Optical Imaging and Super-Resolution Ultrasound to Improve Microbubble Localisation**

Abstract

Super-resolution techniques which localise isolated microbubbles require a comprehensive understanding of the relationship between microbubble response and position. Boundaries, neighbouring bubbles and flow velocities can influence the bubble response and introduce localisation errors. Previous authors have acquired simultaneous optical and acoustic images of individual bubbles, either by entrapment or using very low concentrations. For flowing microbubble distributions appropriate to super-resolution, the relative depths of field of wide-field optical microscopy and! ultrasound imaging make it challenging to match corresponding optical and acoustic data. This work introduces the novel approach of using an adjustable optical aperture to extend the depth of field. An adjustable aperture (Thorlabs) was inserted as close to the back aperture of a water immersion x40 microscope (LUMPLANFLN, Olympus) as permitted by the optomechanics used, and the image focused on a CMOS sensor (Ximea). The resolution over the depth of field was characterised using a sector star re! solution target. A 200 µm cellulose tube was chosen as a vessel phantom. A dilute microbubble solution was introduced, and corresponding optical and plane wave acoustic data collected using an ULA-OP system (MSD Lab, University of Florence). The introduction of the aperture enabled the smallest structure in the resolution target, 4.4 µm, to be resolved at a distance of 100 µm from the optical focus. This corresponds to the depth of field required to detect microbubbles of radius 2.2 µm over the whole extent of&#x! 20;the tube phantom. Simple modelling showed that the required depth of field was reduced due to buoyancy. For example, bubbles of 1 µm radius will only be found in the top 50 µm of the tube after travelling 50 mm. Extending the depth of field will enable frames containing a number of microbubbles to be optically validified and matched to the corresponding acoustic acquisition.

J.Brown, K.Christensen-Jeffries, S.Harput, G.Zhang, J.Zhu, C.Dunsby, M.X.Tang, and R.J.Eckersley

Poster Number: 6	**Jack Cauldwell,** University of Leeds
Title	**Microbubbles: A new strategy for biofilm imaging and destruction**

Abstract

Staphylococcus aureus bacteria are the most common cause of infection in indwelling medical devices such as heart stents and catheters. S. aureus bacteria have the ability to form biofilms; surface bound communities of cells enveloped in an extracellular matrix composed of proteins, eDNA and polysaccharides. These biofilms make infections harder to treat as they ameliorate antimicrobial resistance and thus require longer treatment times. Therefore, there is a need for a clinical method of locating biofilm related infections in the body and developing a non-invasive treatment tailored to destroying the biofilm quickly and efficiently. This research seeks to utilise microbubbles as ultrasound contrast agents. These microbubbles will have their surfaces functionalised with proteins that will bind to receptors on the surface of the S. aureus biofilm and localise the microbubbles to the affected area. Using ultrasound we can then image the biofilm or use the microbubbles to deliver therapeutic payloads to the site of infection. These 'Seek and Destroy' bubbles may greatly decrease treatment times and eradicate the need to replace infected medical devices. In the battle of Bacteria vs. Bubbles, bubbles will prevail.

Poster Number: 7	**Kevin Critchley,** University of Leeds
Title	**Morphological Control of Seedlessly-Synthesised Gold Nanorods using Binary Surfactants**

Abstract

Our group has focussed in recent years on the development of novel nanomaterials and their surface functionalisation for use in cancer theranostics. The synthesis of metal nanoparticles with high absorption in the near-infrared and low quantum yield allows for their application in-vivo in photoacoustic imaging and plasmonic photothermal therapy. With recent work including the development of gold nanotubes [1] and multibranched gold nanoparticles [2] for these purposes.

We present here a well-characterised methodology to produce high purity gold nanorods (AuNRs) with tunable morphology, synthesized through a binary-surfactant seedless method [3]. The protocol yields monocrystalline AuNRs with diameters between 7 and 35 nm, with high shape yield and good monodispersity. The technique has demonstrated good reproducibility and scalability allowing synthesis of batches 500 ml in volume. Morphological control has been achieved through the adjustment of the molar concentrations of cetyltrimethylammonium bromide and sodium oleate in the growth solution, providing fine tuning of the optical scattering and absorbance properties of the AuNRs across the visible and NIR spectrum. Changes in the geometry of the end-caps were also observed as a result of manipulating the two surfactant concentrations.

L. Roach, S. Ye, S.C.T. Moorcroft, L. Coletta, K. Critchley, S.D. Evans

Poster Number: 8	**Fabio Domenici,** University of Rome Tor Vergata
Title	**Optically triggered phase-change contrast agents with polymer/surfactant hybrid shells**

Abstract

Phase-change contrast agents (PCCAs), based on the vaporization of perfluorocarbon liquid droplets, have been recently introduced as promising theranostic systems, combining significantly enhanced contrast in ultrasound imaging and ease of functionalization with targeting molecules and drugs [1]. In this context, our group defined a novel class of PCCAs characterized by a hybrid shell, consisting of a surfactant monolayer coated by biodegradable cross-linked dextran-methacrylate, which provides the system good echogenicity, stability and reversibility&! #x20;with respect to the acoustic vaporization of the decafluoropentane core [2]. Nevertheless, more efforts are needed to improve the control on the droplets size, directly related to the acoustic response after the transition to microbubbles, and to enhance the vaporization efficiency. With the aim to overcome such issues and to further upgrade the system to a dual-contrast agent for combined photoacoustic and ultrasound contrast-enhanced imaging, we optimized the PCCAs fabrication protocol, namely the emulsification process and the c! omposition of the surfactant layer, and integrated the shell with gold nanoparticles (AuNPs). The well-known ability of AuNPs to release near-field photothermal energy could in fact be exploited to improve the vaporization efficiency and to promote the photoacoustic effect [3]. A combined investigation will be presented, employing confocal fluorescence microscopy, UV-Vis absorption spectroscopy, dynamic light scattering, and acoustic spectroscopy. Our results point out the possibility to obtain PCCAs with sharp size distributions, tunable in the few micrometre range. The&! #x20;integration of AuNPs into the shell, both within the dextran-methacrylate matrix or onto the surfactant layer, was also demonstrated, enlightening a dramatic enhancement in the droplets vaporization efficiency upon combined excitation with proper ultrasound and laser light. [1] P.S. Sheeran and P.A. Dayton; Current Pharmaceutical Design 18:2152 (2012). [2] S. Capece et al.; Physical Chemistry Chemical Physics 18:8378 (2016). [3] K. Wilson et al.; Nature Communications ! 3(1):618 (2012).

F. Domenici, D. Palmieri, F. Brasili, I. Angelini, L. Oddo, B. Cerroni, S. Cerra, G. Paradossi

Poster Number: 9	**Nicola Farrer,** University of Oxford
Title	**Platinum (IV) Liposome-Loaded Microbubbles as Theranostic Ultrasound-Responsive Contrast Agents**

Abstract

Drug-loaded liposomes are a promising method of drug-delivery for targeted cancer therapy, with the aim of minimising toxic side-effects which result from off-target reactivity. Conjugation with microbubbles can enable ultrasound-triggered drug delivery. We demonstrate the liposomal encapsulation of the platinum(IV) drug iproplatin and the subsequent conjugation of drug-loaded liposomes to microbubbles. Liposomal encapsulation and release of iproplatin were monitored by RP-HPLC and ICP-MS. MCF-7 cells treated with iproplatin loaded liposome-micro! bubbles display a modest increase in uptake of platinum when exposed to ultrasound.

Nia Thomas, Richard Brownings, Eleanor Stride, Nicola J. Farrer*

Poster Number: 10	**Malcolm Haddrick,** Medicines Discovery Catapult
Title	**Transfer and Validation of a Targeted Microbubble Delivery Platform for Colorectal Cancer.**

Abstract

A challenge across the Pharmaceutical industry is to improve the rate of attrition in the delivery of new medicines to patients. Innovative drug delivery technologies have the potential to address this by overcoming cell permeability limitations or by 'rescuing' drugs with a low therapeutic index. Therapeutic delivery platforms may be impactful for new drug modalities across several diseases.

The Medicines Discovery Catapult (MDC), a not-for-profit research company supporting UK drug discovery, is collaborating with the University of Leeds to evaluate microbubble technology. Microbubbles (MBs) are safe and well tolerated, they are used clinically as a contrast reagent and have potentially useful properties as vectors for therapeutic agents. The aims of the collaboration are to assess Horizon microbubble production and UARP ultrasound application for first in man trials for colorectal cancer within two years.

Establishment of the Horizon, UARP and associated protocols at MDCs labs enabled the production of microbubbles (MB) and therapeutic complexes (ThMB). The ThMB complex consisted of MBs + SN38 (0.4mg/ml) containing targeted liposomes with a VEGFR2 antibody attached. In vitro characterisation for on-chip microbubbles production was 4.54E+08 ± 1.21E+08 ml-1 with an average size of 2.1±1.3µm and less than 1% ThMBs are greater than 8µm. Size and concentration was determined using Leica's 2D analysis software. To demonstrate in vivo efficacy, an SW480 xenograft tumour model in CD1 nude mice were dosed 5 times every 3 days with 100µL of freshly prepared ThMBs. Data showed tumour volume inhibition of 26%, with no observable toxicity issues, reproducible tumour accumulation and pharmacokinetic behaviour. In addition, ex vivo tissues and fluids were retained and are being profiled by mass spec imaging, Nanostring profiling and ddPCR detection strategies to provide foundational assays to further characterise the opportunity of microbubbles as a translational and effective pre-clinical technology for drug discovery applications.

Poster Number: 11	**Nicola Ingram,** University of Leeds
Title	**Towards clinically relevant therapeutic microbubbles**

Abstract

Current architecture for delivery of drugs using microbubbles has traditionally used biotin-avidin linkages to join the liposomally-encapsulated drug to the microbubble. In addition, antibody-targeted microbubbles have also used the biotin-avidin linkage due to the vast array of biotinylated antibodies that can be commercially supplied. However, the use of this linkage is not currently clinically approved and therefore may delay their use in clinical trials. Therefore, the Leeds Microbubble Consortium are creating therapeutic microbubbles using the FDA-approved maleimide-thiol linkage. Here we present very simple chemistry to reduce the amount of time and effort needed to generate targeted microbubbles that retain their functionality. Using Traut's reagent, we show how antibodies can be thiolated and that this thiolation does not disrupt their antigen recognition compared to native antibodies. We also show that these targeted microbubbles perform as well as biotin-avidin microbubbles in flow assays.

Nicola Ingram and P. Louise Coletta

Poster Number: 12	**Delanyo Kpeglo**, University of Leeds
Title	**On-chip Modelling of Pancreatic Cancer and its Stromal Environment**

Abstract

The malignant behaviour of pancreatic ductal adenocarcinoma (PDAC) is facilitated by its desmoplastic stromal environment resulting in biophysical rigidity, increased intra-tumoural pressure collapsing microvasculature and a physical barrier against the delivery of targeted therapeutics. A deeper understanding of the tumour microenvironment of PDAC will help provide therapy beneficial to patients.

2D cell culture systems and animal models significantly do not mimic exact in vivo physiology of diseases compared to 3D models. 2D models lack features such as the morphology and in vivo like responses to therapeutics, and animal models are limited by cost, time and the inability to correlate results to responses observed in humans. 3D models recapitulate the architecture and key features of tumour biology, and microfluidic technology provides a platform for developing promising in vitro 3D models. Microfluidics enable the efficient engineer of defined and controlled environments in comparison to static well formats and the efficient qualitative and quantitative assessments of cells and their environment for targeted therapeutics. Incorporating 3D organoid models with microfluidic technology will provide an efficient way of engineering tumour microenvironments and therefore the study of cellular features for targeted therapeutics with microbubbles.

Simple microfluidic chip devices have been fabricated to assess single and heterotypic cultures of pancreatic cancer cells and cells typical of its stromal environment. Off chip PANC-1 spheroids have been formed and cultured, and the spheroids were cultured for 10 days to assess the long term culture of spheroids. Also, PANC-1 cells have been co-cultured with fibroblast cells to assess the effect of fibroblast on the spheroid formation and culture of PANC-1 cells. On chip cells and spheroids have been seeded for culture.

Developing a 3D organotypic microfluidic chip model of PDAC and its stromal environment will provide an understanding of its tumour microenvironment for targeted therapeutic i.e. with nano- and microbubbles.

Delanyo Kpeglo, Margaret Knowles, Malcolm Haddrick, Stephen D. Evans and Sally A. Peyman

Poster Number: 13	**Guillaume Lajoinie,** University of Twente
Title	**A new microfluidic platform for real-time sizing and counting of microbubbles produced at MHz rates**

Abstract

In the last 30 years, microfluidics has become a key technology owing to its low volume requirements, its precision, and its compactness. A particular area of interest for microfluidics is the production of microscopic bubbles and droplets for biomedical applications. These particles can be produced in flow-focusing devices at rates exceeding a million bubbles per second, at high size monodispersity and with diameters down to 1 micrometer. Producing batches with a sufficiently high number of microbubbles for clinical use typically requires&#x! 20;operating these devices with high flow rates over an extended period of time. On the one hand, long-term operation challenges the production stability, owing to the inevitable variations in temperature, flow and pressure conditions. On the other hand, high-throughput operation of the devices at MHz production rates, complicates direct observation and control of the device behavior. Here, we present a new microfluidic machine based on flow-focusing chips that allows for a fully controlled production of phospholipid-coated m! icrobubbles at MHz rates. Using highly sensitive flow and pressure controllers coupled to an ultrafast optical detection technique, we are able to size and count in real time and in situ the freshly produced bubbles. Information on the production rate and bubble size further enables the use of a closed feedback loop to ensure a stable and easy operation. Access to quantities at the precise location where bubbles are produced will be instrumental to a better understanding and control of the production of stable monodisperse microbubbles using microfluidics.

Guillaume Lajoinie, Tim Segers, Gonzalo Collado Lara, Benjamin van Elburg and Michel Versluis

Poster Number: 14	**Simon Langeveld,** Erasmus MC
Title	**Phospholipid-coated microbubbles: towards microbubble response on demand**

Abstract

Microbubbles are used for ultrasound imaging and drug delivery. However, the microbubble s response to ultrasound is currently variable and unpredictable [1]. The variability in response is possibly due to lipid phase separation and immiscible phospholipid components in the coating. With the aim to develop a new coating so microbubbles will respond on demand, we investigated the lipid phase and ligand distribution in microbubble coatings and the influence of cholesterol as this affects phase distribution in lipid monolayers [2]. Phospholipid-coated microbubbles (composition in mol%: DSPC or DPPC 59.4; PEG-40 stearate 35.7; DSPE-PEG(2000) 4.1; DSPE-PEG(2000)-biotin 0.8) with a C4F10 gas core were made by sonication. DSPC-based microbubbles were also made with 10 mol% cholesterol (DSPC-Chol). Microbubble size distribution was studied using a Coulter Counter while lipid phase and ligand distribution was studied using high-axial-resolution 4Pi microscopy using Rhodamine-DHPE (0.01 mol%) and streptavidin-Oregon Green 488&#x2! 0;respectively. Microbubble behavior upon ultrasound insonification (20 to 50 kPa, 2 MHz, single 10-cycle burst) was studied using the Brandaris 128 ultra-high-speed camera. DSPC-Chol microbubbles were smaller in size (mean diameter 3.6 µm) than DSPC-based microbubbles (mean diameter 4.2 µm). Lipid phase separation was observed in both DPPC and DSPC-based microbubbles, but not in DSPC-Chol microbubbles. Heterogeneity of ligand distribution was the most for DSPC microbubbles (15%, n=38), less for DSPC-Chol microbubbles (12%! , n=25), and least for DPPC microbubbles (8.9%, n=49). Preliminary data show characteristic resonance behavior of DSPC-Chol microbubbles in response to ultrasound. These results show that cholesterol has an effect on lipid phase and ligand distribution of the microbubble coating, while retaining a functional response to ultrasound, demonstrating the potential of novel cholesterol-containing microbubbles as theranostic agents. [1] van Rooij et al, Ultrasound Med Biol 2015; [2] Yuan et al, J Micros! c 2002. Funding by the Phospholipid Research Center in Heidelberg, Germany, is gratefully acknowledged

S.A.G. Langeveld, I. Beekers, A.F.W. van der Steen, N. de Jong, K. Kooiman

Poster Number: 15	**Sam Moorcroft,** University of Leeds
Title	**Liposomal encapsulation of anti-microbial peptides and gold nanorods**

Abstract

Antimicrobial resistance has been labelled amongst the greatest threats to humanity by the World Health Organisation. In recent years, the development of stimuli-responsive drug delivery vehicles have emerged as a promising strategy to improve the administration of antimicrobial agents, offering significant advantages in improving anti-bacterial efficacy and overcoming antimicrobial resistance. Novel therapeutics have also been developed as antibiotics alternatives that can minimise the potential for drug-resistance development. For instance, certain antimicrobial peptides can exhibit bactericidal effects through the ability to physically lyse and disrupt the bacteria membrane and cell wall inducing cytoplasmic leakage. Such effects are believed to reduce the risk of drug resistance due to the higher metabolic cost of membrane repair than the rate at which the damage is inflicted.

Here, we present results demonstrating the liposomal encapsulation of the antimicrobial peptide IK8 and it's subsequent release in response to heating. The liposomes encapsulate 24 times the minimum inhibitory concentration of IK8 upon Staphylococcus aureus and exhibit leakage solely in the presence of bacteria, providing an initial release mechanism. Upon thermal disruption of the liposomes the IK8 is released providing complete inhibition of S. aureus proliferation. Ultimately, the IK8 loaded liposomes and light responsive gold nanorods will be incorporated into a hydrogel structure, creating a wound dressing that can provide release of therapeutics in response to laser irradiation. As such, this gel will overcome the issue of bacterial multidrug resistance through the delivery of the novel antimicrobial peptides at guaranteed lethal dosages.

Poster Number: 16	**Luzhen Nie,** University of Leeds
Title	**High Frame-Rate Echocardiography using Motion-Compensated Diverging Waves: in-vitro Evaluation**

Abstract

The combination of diverging waves and microbubbles could revolutionize the paradigm in contrast-enhanced echocardiography, by providing capabilities for high frame-rate vector flow mapping and improved image quality. However, the image formation by coherent summation of echoes from multiple steered diverging waves is subject to both tissue and microbubble motion, and thus a deterioration in the performance of spatial compounding. In this study, a method for 2-D motion estimation and motion compensation was proposed for diverging-wave echocardiography with in-vitro demonstration.

Luzhen Nie, Thomas Carpenter, David M. J. Cowell, James R. McLaughlan and Steven Freear

Poster Number: 17	**Gaio Paradossi,** University of Rome Tor Vergata
Title	**A Tethering Study of RGD decorated Microbubbles on HUVEC Cells for Targeting of Tumor Brain Vasculature**

Abstract

Malignant glioblastoma (MG) is a highly aggressive brain tumor representing one of the leading causes of death from cancer. Treatment of MG relies upon surgery; however complete tumor removal often fails, because of difficulties in identifying tumor borders during surgery. Intra-operative ultrasound-guided MG removal, supported by suitably engineered microbubbles (MBs), is a method to specifically visualize tumor borders for complete resection and focal delivery of therapeutic agents into the brain.

MBs were decorated with the peptide sequence arginyl-glycyl-aspartate (RGD) that finds its receptor in avβ3 integrins, overexpressed by endothelial cells during tumor progression.

In this contribution we report on an in vitro study of the bioadhesion mechanisms driving the interaction of the RGD-MBs with avβ3 integrins-rich human umbilical vein endothelial cells (HUVEC), under physiological flow conditions, cultured one side of a microchannel. RGD decorated MBs were fluxed in the microchannels, mimicking blood shear rate of vessels. Single-particle trajectories were classified in terms of dynamic parameters such as particle speed, acceleration and path length with respect to MB size and RGD surface density Duration of transient tethering, i.e. attachment – detachment events, were studied to determine a koff, treating the detachment step as a 1st order rate process 1. The dependence of the bioadhesion upon parameters such as the number of adhered MBs/unit area, RGD surface density on MBs, MBs size, shear stress values promoting MBs stripping was studied as well as the in vitro quantitative assessment of the efficacy of RGD-MBs tethering to cells. This study demonstrates that with RGD-decorated MBs the targeting of tumor vasculature and focal drug delivery can be achieved.

Barbara Cerroni, Flavia Righi Riva, Letizia Oddo, Fabio Domenici, Francesco Brasili, Gaio Paradossi

Poster Number: 18	**Sal Peyman,** University of Leeds
Title	**Improved microspray regime for microbubble formation**

Abstract

The use of microfluidics to fabricate microbubble contrast agents has been of interest for many years. The ability to control the diameter of the microbubbles, and produce monodisperse bubble populations allows bubbles to be tuned to have a resonant frequency to match the frequency of the transducer used for imaging, thus enhancing contrast imaging further. However, despite this elegant approach to fabricating microbubbles, microfluidic microbubble production inherently suffers from low bubble concentrations (~10^6 bubbles/mL), often falling below that required for imaging (~10^9 bubbles/mL).

At the University of Leeds we have developed an alternative microfluidic approach that produces a narrow size distribution of microbubbles at very high concentrations. This production regime, termed 'microspray' produces bubbles in an atomisation like manner at concentrations >10^8 bubbles / mL. It is believed bubbles are formed at the interface between the lipid and the gas at high velocity in 'pinch' areas through the nozzle.

Here we investigate a new chip design for improving microspray microbubble concentrations further. The new design features 'layered' liquid and gas streams to increase the proportion of liquid-gas interfaces in the production. In addition, a 'widget' designed into the nozzle increases the number of high velocity pinch areas in the nozzle. Bubble concentrations in this device are greater than 10^9 bubbles / mL and a promising improvement on the existing regime.

Poster Number: 19	**Jordan Tinkler,** University of Leeds
Title	**Microbubble destruction for the treatment of in vitro S. aureus biofilms**

Abstract

Biofilms are structured microbial communities embedded within a protective polysaccharide extracellular matrix (ECM), these colonies have the ability to form on organic and synthetic surfaces. Infections of this type are common and notoriously difficult to treat due to the presence of the ECM, enhanced antimicrobial resistance, and metabolic diversification within the biofilm. These infections commonly lead to inflammatory diseases and the failure of implanted medical devices, including catheters and pacemakers, replacing these devices can be both physically traumatic for the patient, and expensive to the healthcare provider. Microbubbles (MBs) are 1-10μm diameter lipid-stabilised bubbles of a heavy gas, they have seen extensive use since the late 1960s as ultrasound contrast agents due to their enhanced ability to scatter ultrasound waves compared to the surrounding blood medium. Since then, MBs have been investigated as methods of drug delivery through sonoporation and cavitation - a sudden and very energetic process. By targeting these microbubbles through the attachment of bacterial-specific proteins, it is thought that the ultrasound-induced bursting may provide a novel method of treating biofilms by breaking through the ECM and either killing cells outright, or by allowing drug penetration to deeper levels of the biofilm.

J Tinkler, Dr J Sandoe, Dr S Peyman, Prof S Freear, Prof S Evans

Poster Number: 20	**Mihnea Vlad Turcanu,** University of Glasgow
Title	**Ultrasound and Microbubbles Promote the Retention of Fluorescent Compounds in the Small Intestine**

Abstract

Background, Motivation and Objectives Focused ultrasound (US) can enhance tissue permeability and intestinal absorption of macromolecules. The motivation of this study was to determine the potential of US and microbubbles (MBs) to facilitate delivery of macromolecular therapeutic agents across the intestinal epithelium. The specific objectives of this study were to determine whether US and MBs facilitate delivery of fluorescent model drugs (1) through a cell monolayer in vitro and (2) to the porcine small intestine in vivo. M! ethods To investigate delivery across cell monolayers, Caco-2 cells (ATCC) seeded on ThinCert filters (Greiner Bio-One) were cultured for a minimum 3 weeks. A suspension of 200 µg/ml of 4 kDa FITC-dextran (Sigma UK) +/- 5% MBs (2 5 x 108 MBs/ml, SonoVue, Bracco) was introduced into an apical chamber through a channel in the centre of a miniature focused US transducer (4 MHz, 1 MPa PNP, 15 mm focal length, 1.79 mm2 focal area). The suspension! was delivered for 4 min at 0.05 ml/min, concomitantly with application of US. Fluorescent dextran in the basal chamber was measured with a plate reader. To investigate delivery in vivo, a tethered endoscopic capsule with an US transducer and a delivery channel was inserted through a stoma into the small intestine of terminally anaesthetised Landrace X pigs (PPL 70/8812). 5% CdSeS/ZnS quantum dots (QDs) (Sigma) were delivered under the protocol described above. Results and Discussion US with MBs led to higher delivery&#x20! ;of fluorescent dextran across Caco-2 cell monolayers than US alone. US and MBs also led to delivery of QDs to the wall of porcine small intestine in four out of five cases. These results suggest that focused US and MBs decrease epithelial barrier function enhancing the passage of macromolecules. Our work indicates potential applications in targeted treatment of gastrointestinal disease and oral drug delivery

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M Turcanu, F Stewart, B Cox, H Mulvana, D Vllasaliu, M Thanou, I Nathke, S Cochran

Poster Number: 21	**Papoole Valadbaigi,** University of Leeds
Title	**Generation of ultra stable microbubbles for industrial applications**

Abstract

Microbubbles have a variety of different applications but stabilizing them for sufficient duration remains a challenging problem. Air bubbles in water stabilized by particles ('Pickering' bubbles) can be indefinitely stable, but the slower mass transport of particles to interfaces compared to molecules means that the final stable bubble size is a fine balance between the rates of bubble formation, particle coverage and shrinkage. The aim of the current work is to see how to optimize this balance in two practical systems of interest: (a) class II hydrophobin (HFBII) as a novel food particle in combination with caseins and (b) using a synthetic (polybutylcyanoacrylate) particle system, formed in the presence of surfactant, as a comparable model system with non-food uses. HFBII acts like a very small Janus nanoparticle since it is believed not to unfold or denature at A/W interfaces and has a hydrophobic patch on one side. Cyanoacrylates are medical tissue adhesives, used for wound sealing and dressings, that when emulsion polymerized into particles appear to have unusually good bubble-stabilizing properties, though the reasons for this are unknown. In agreement with work elsewhere, we have shown that it is difficult to obtain a high volume fraction of microbubbles (size < 1 □m), mainly due to HFBII aggregation which slows down bubble coverage. Combining HFBII with other surface agents such as caseinate or low molecular weight surfactants (LMWS) increases the overrun considerably but there is an optimum surface activity of the second surfactant due to competition between the particle (HFBII) and the surfactant for the air/water (A/W) interface. The PBCA + surfactant system was studied in more detail, by controlling the rate of formation and initial size of the bubbles more exactly, so a balance was achieved whereby very efficient microbubble production took place (e.g., > 10 vol.%). However, our results also suggest the unique stabilizing properties of PBCA are connected with surfactant incorporation into their surface. Thus, both model systems are leading us to a more complete understanding of microbubble stabilization by the more real-world combination of proteins, low molecular surfactants and particles.

Papoole VALADBAIGI, Rammile ETTELAIE, Brent MURRAY

Poster Number: 22	**Liz Valleley,** University of Leeds
Title	**Combining epigenetic drug delivery with ultrasound-mediated microbubble destruction enhances tumour response in preclinical models of cancer**

Abstract

Cancer cells exhibit aberrant genetic and epigenetic changes that contribute to tumour development. Epigenetic drugs can reverse epigenetic chemical modifications in tumour cells, including DNA methylation, that silence key genes that would normally control cell growth. One such drug is the cytidine analogue decitabine (5-aza-2'-deoxycytidine or DAC) that inhibits DNA methylation, resulting in genome-wide hypomethylatiovn and gene re-activation. The resulting effects can include inhibition of tumour cell growth, cell death or re-sensitization of chemoresistant tumour cells to secondary anti-cancer agents. DAC is used currently to treat some haematological cancers, but it also has potential as a combination therapy for solid tumours. Our aim was to test the delivery of DAC to tumours in preclinical models of breast and colorectal cancer, in combination with ultrasound-mediated microbubble destruction for localized drug administration. By doing so, we hope to enhance therapeutic effects of DAC in vivo. Results from our pilot studies show that when DAC was delivered in combination with US destruction of microbubbles (either targeted to VEGFR2 or non-targeted), epigenetic effects were observed, indicating that drug delivery was successful. Treatment of tumours with low DAC doses revealed activation of tumour suppressor genes previously silenced by DNA methylation. A reduction of tumour volume was also observed. Our results show that delivery of DAC in this way could enhance therapeutic effects, prime tumours for secondary anti-cancer drugs and has the potential to reduce the required dose, thereby reducing off-target side effects in patients.

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Poster Number: 23	**Sunjie Ye,** University of Leeds
Title	**Au nanoplates for targeted PTT and PAI in NIR I and II**

Abstract

Photoacoustic (PA) imaging holds great potential for preclinical research and clinical practice. So far, most studies involve the laser wavelength in the first near infrared (NIR) window (NIR-I, 650-950 nm), while few studies have been performed in the second NIR window (NIR-II, 1000-1400 nm), mainly because of the lack of NIR-II absorbing contrast agents1. In comparison with the traditional NIR-I, the imaging resolution (signal-to-noise ratio) and the penetration depth of external light are remarkably increased in NIR-II window, owing to the reductions in the intensity of autofluorescence and the absorption/scattering of photons. Moreover, the NIR-II window has a higher value of maximum permissible exposure (MPE) to laser light compared to NIR-I (the MPEs of skin to laser are 1 and 0.33 W cm−2 for the NIR-II and NIR-I windows, respectively.) Thus the NIR-II window is a relatively safe spectral region for practical purposes1. Appropriate engineering of Au nanostructures, in terms of dimension, SPR absorption and surface properties, provides exciting opportunities for plasmonic nanostructures to be used for biomedical applications based on NIR-II window.

In this study, we fabricated Au nanoplates via a one-step surfactant-free approach. The as-prepared Au nanoplates have the following properties, which would be beneficial for the uses as agents for photoacoustic imaging and phototheraml therapy: Two dimensional shape with an average length of ~150 nm and a thickness of ~7 nm, leading to increased contact with cell surface (suggested by TEM image), which would contribute to improved targeting efficiency. SPR absorption in NIR I and NIR II window; (3) Low toxicity and easy surface-functionalization, owing to surfacant-free preparation. We have modified the Au nanoplates with VEGF2 antibody and demonstrated the in vitro targeting efficiency of VEGF2-Au nanoplates using dark-field microscopy imaging and ICP quantification. In addition, VEGF2-Au nanpoplates showed enhanced photothermal therapeutic effect in NIR 1 and II window, compared with as-synthesized Au nanoplates. In vivo MSOT imaging study has indicated the potential of VEGF2-Au nanoplates for targeted photoacoustic imaging (Further study are needed for validation.)

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