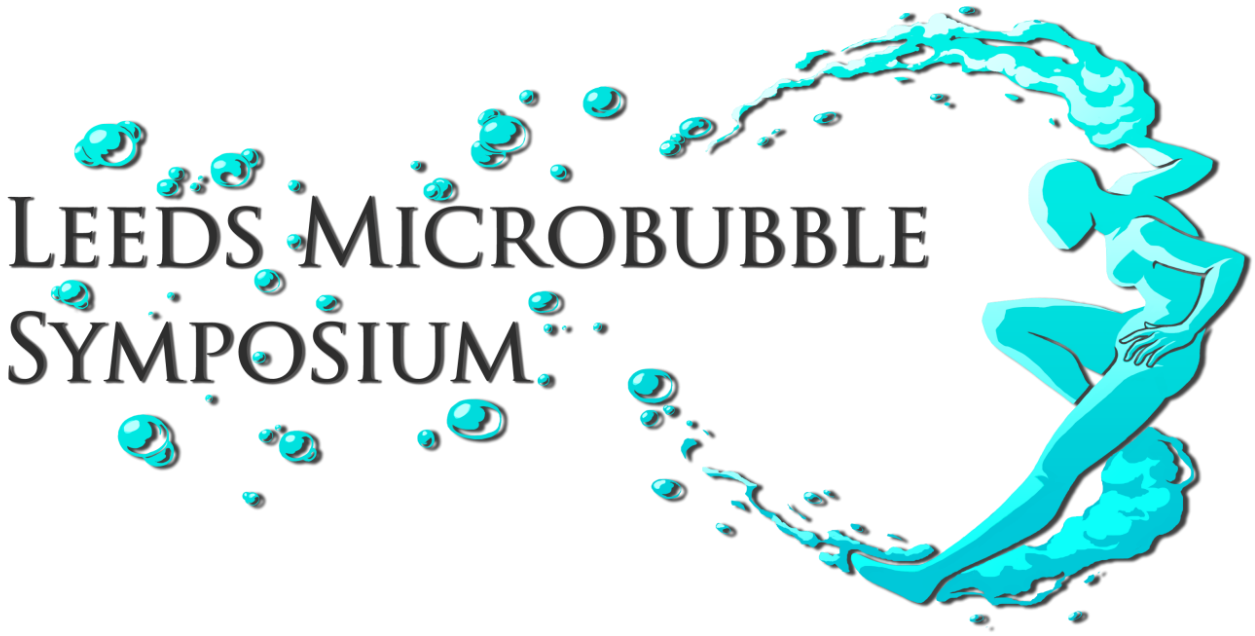
Leeds Microbubble Symposium



Fabrication, Characterisation and Translational Applications

8th & 9th July 2019

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

Programme and Abstracts

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Programme: Monday 8th July 2019

12:30 – 13:00	**Registration**	
13:00 – 13:10	Welcome	Professor Stephen Evans
SESSION 1	**Microfluidics/Bubble Architecture**	**CHAIR: Stephen Evans**
13:15 – 13:50	Tunable microbubble shell stiffness for non-invasive blood pressure measurement	Michel Versluis
13:50 – 14:25	Efficient trapping of fluorinated therapeutics in phospholipid monolayers using fluorous interactions. Implications for microbubble design	Marie Pierre Krafft
14:25 – 14:45	Therapeutic Microbubble Production, Stability and Long-term storage	Radwa Abou-Saleh
14:45 – 15:15	**REFRESHMENTS**	
		CHAIR: Neil Thomson
15:15– 15:50	Sonoprinting with nanoparticle-loaded microbubbles from 2 and 3D to in vivo	Ine Lentacker
15:50 – 16:10	New microfluidic models for therapeutic evaluation	Sal Peyman
16:10 – 17:00	Poster blitz session	
	BREAK FOR EVENING	
17:45 – 18:45	Drinks and Posters	
19:30 – 21:30	Symposium Dinner – Headingley Cricket Ground	
21:30 onwards	Informal networking	

Programme: Tuesday 9th July 2019

SESSION 1 cont'd	Microfluidics/Bubble Architecture	CHAIR: Steve Freear
09:00 – 09:35	Microbubble-Mediated Intracellular Drug Delivery for Treatment of Chronic Infections	Eleanor Stride
09:35 – 09:55	Improved coalescence stability of monodisperse phospholipid-coated microbubbles formed by flow-focusing at elevated temperatures	Tim Segers
09:55 – 10:30	The microbubble–endothelial cell–drug interaction revealed at nanosecond and nanometer resolution	Klazina Kooiman
10:30 – 10:50	Contrast agent microbubble-jetting during initial interaction with sub-MHz focused ultrasound	Paul Prentice
10:50 – 11:20	**REFRESHMENTS**	
		CHAIR: Richard Bushby
11:20 – 12:00	Big Insights from Small Bubbles: Lessons from Headspace GC/MS	Agata Exner
12:00 – 12:20	Characterizing bubbles: challenges and best practices	Judith Hadley
12:20 - 13:30	**LUNCH**	
SESSION 2	**Microbubble Translational Applications**	**CHAIR: Louise Coletta**
13:30 – 14:20	Targeting microbubbles to the tumor vasculature, not via selective molecular biomarkers	Alexander Klibanov
14:20 – 14:55	Observations on microbubble-based targeting of chemo- and sonodynamic therapy in the treatment of pancreatic cancer	Anthony McHale
14:55 – 15:30	**REFRESHMENTS**	
		CHAIR: Nicola Ingram
15:30 – 15:50	Multi-modal contrast agents: Photoacoustic imaging and therapy	Jithin Jose
15:50 – 16:40	Ultrasound sonoporation and drug delivery in pancreatic adenocarcinoma	Odd Helge Gilja
16:40 – 17:10	**Closing remarks & Poster Prizes**	**Sir Alex Markham**

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Michel Versluis,** University of Twente
Title	**Tunable microbubble shell stiffness for non-invasive blood pressure measurement**

Abstract

Invasive catheter-based methods are presently the only routine solution for in-vivo pressure measurements, e.g. to diagnose portal hypertension. Bubbles are ultimately the most sensitive pressure sensors around and it has been proposed to utilize phospholipid-coated microbubbles for non-invasive blood pressure measurement. Hydrostatic pressure sensing using lipid-coated microbubbles relies on the change in the microbubble resonance behavior with ambient pressure. An increased ambient pressure decreases the bubble size, which leads to an increased lipid packing density, which in turn increases the shell stiffness until the buckling radius is reached. When the microbubble shell buckles, it reaches a tensionless state with a lower resonance frequency. Thus, the effective shell stiffness, and the corresponding resonance frequency, decreases with increasing ambient pressure. However, the use of this unique non-linear feature for blood pressure measurements is limited by the acoustic inhomogeneity of commercially available UCAs resulting from their polydispersity in size and in their shell properties. We recently fully characterized the parameter space for stable monodisperse microbubble synthesis by flow-focusing. We identified the optimal settings for the production of coalescence-free microbubble suspensions, including a more efficient route at elevated temperatures. However, optimal microbubble design for non-invasive blood pressure measurements requires more than control over microbubble size and stability alone, as shell stiffness governs the resonance frequency shift upon bubble compression. Here, we show that the shell stiffness of microbubbles formed by flow-focusing can be precisely tuned over one order of magnitude with values between 0.5 N/m to 5.0 N/m. We also show that the subharmonic response of these monodisperse microbubbles is strongly dependent on the ambient pressure. This work is therefore a route to the design of a novel class of microbubble contrast agents tailored for non-invasive blood pressure measurements.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Marie Pierre Krafft,** University of Strasbourg (CNRS)
Title	**Efficient trapping of fluorinated therapeutics in phospholipid monolayers using fluorous interactions. Implications for microbubble design**

Abstract

Fluorinated therapeutics are key for the diagnosis of neurodegenerative diseases (e.g. amyloid imaging) and treatment of cancers, calling for efficient carriers. Microbubbles show definite potential to deliver active compounds and are intensely investigated for their ability to cross the brain blood barrier, facilitating access to the nervous central system. However, their development is hindered by their limited pay-load as compared to emulsion droplets, liposomes, or nanoparticles. We found that fluorocarbon gases can be extremely effective for promoting adsorption at the air/water interface of a wide range of molecules, including surfactants, phospholipids, proteins, biomarkers and diblock copolymers [1]. As an important consequence, the encapsulation of such compounds in the shell of microbubbles is enabled or strongly facilitated, opening a new approach to drug delivery driven by hydrophobic interactions [2]. We will focus on a series of F-nitrosoimidazoles taken as a model of a fluorinated bioactive agent, EF5, which is investigated in the clinic as a cell hypoxia biomarker and a tracer for PET when radiolabeled with 18F. Using neutron reflectometry, we were able to demonstrate that substantial concentrations of these F-biomarkers (up to 1:1 molar ratio with respect to the phospholipid) can be trapped in the interfacial film. Interestingly, nanoparticles, including of iron oxide, carbon and cerium oxide, can also be recruited at a gas/water interface by fluorocarbons and form stable microbubbles [3]. These new phenomena will be presented along with some of their perspectives of applications.

[1]. C. Counil, M. P. Krafft, in Frontiers of Organofluorine Chem. Ed. I. Ojima, World Scientific, Singapore (2019) doi.org/10.1142/q0217.
[2]. G. Yang, M. O'Duill, V. Gouverneur, M. P. Krafft, Angew. Chem. Int. Ed., 54 (2015) 8402; L. Gazzera et al. Angew. Chem. Int. Ed., 55 (2016) 10263.
[3]. C. Justeau, A.V. Vela-Gonzalez, J. R. Riess, M.P. Krafft, ACS Sustain. Chem. Eng. 6 (2018) 11450.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Radwa Abou-Saleh,** University of Leeds
Title	**Therapeutic Microbubble Production, Stability and Long-term storage**

Abstract

Radwa H. Abou-Saleh, Aileen Delaney, Benjamin Johnson, Nichola Ingram, Sally Peyman, Louise Coletta, Neil H. Thomson, Stephen D. Evans

We have previously described a MF- spray regime as a method for the production of diagnostic and therapeutic MBs, with an average bubble diameter of ~2 μm, at concentrations of up to 109 MBs/ml[1]. This has been used for the production of therapeutic MBs (Th-MBs) with attached payloads, of liposomes for hydrophilic drug delivery[2] or Lipid-Oil-Nanodroplets (LONDs) in case of hydrophobic drug delivery[3]. Here, we present recent developments for the translation of the production of therapeutic MBs focusing on: i) Improved MB lifetime – role of surface tension in controlling lifetime ii) Role of glycerol in interactions within lipid shells – shell stiffening iii) liposomal/MB coupling chemistry - moving away from neutravidin/biotin linkers; iv) Freeze-drying Th-MB for long-term storage and reconstitution at point-of-care.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Ine Lentacker,** UGhent
Title	**Sonoprinting with nanoparticle-loaded microbubbles from 2 and 3D to in vivo**

Abstract

Ultrasound-triggered drug-loaded microbubbles have great potential for drug delivery, owing to their ability to release drugs locally while simultaneously enhancing their delivery into the target tissue. We have recently proposed "sonoprinting" as a key mechanism for ultrasound-triggered delivery of nanoparticles from nanoparticle-loaded microbubbles. With high-speed imaging we visualized the biophysical process underlying sonoprinting. It was shown that sonoprinting leads to the local deposition of the microbubble payload onto cells grown in 2D monolayers. However, the rigid membranes supporting 2D cell cultures are a source of acoustic reflections and aspherical microbubble oscillations, which can influence microbubble-cell interactions. Therefore we investigated the ultrasound-triggered nanoparticle delivery from nanoparticle-loaded microbubbles on free-floating 3D multicellular mono- (tumor cells only) and cospheroids (tumor cells and fibroblasts), to verify whether sonoprinting also occurs in more complex and physiologically relevant tissues. Our results show that sonoprinting can be a very powerful tool to deliver large amounts of nanoparticles to the outer layers of 3D tumor spheroids, followed by a complete drug release into the deeper layers of the tumor spheroid. Sonoprinting significantly enhanced the cytotoxicity of both Doxil®-like and ThermoDOX®-like liposomes. Finally we performed a preliminary experiment in a window chamber model to verify if sonoprinting could also stimulate to the local deposition of liposomes in vivo.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Sally Peyman,** University of Leeds
Title	**Organ on chip: New microfluidic models for therapeutic evaluation**

Abstract

The use of 2D monolayers of cancer cells for drug evaluation has been used for many years, however these models lack the complexity of 3D tissues found in the body and do not show many of the features of in vivo cells that lead to drug resistance, such as drug penetration beyond a monolayer and interstitial pressures. The use of 3D cell culture, such as spheroids, has recently addressed some of these issues as cells are encouraged to grow and interact in a way much more representative of cell growth in the body and indeed, these models show greater drug resistance than their monolayer counterparts. However, there are still may aspects of the physical microenvironment of in vivo tumours that are not accurately represented in these static 3D models, such as fluid flow, shear stress and the mechanical properties of the surrounding stroma, an aspect suspected of playing a vital role in drug resistance. Organ on chip is an area of microfluidics that provides a platform through which cells can be cultured and subjected to both chemical and physical parameters that closely mimic in vivo tissues. Here we present two organ on chip approaches for the evaluation of therapeutics. The first, we trap co-culture spheroids of colorectal cancer and fibroblasts (HT116 and HFF) on-chip and subject them to continuous flow and exposure to Doxorubicin for assessment. Secondly, a co-culture model of pancreatic cancer (PANC1 and stellate cells) in which the mechanical properties of the stroma are recapitulated by on-chip culture in ECM. Microfluidic approaches provide excellent control over the physical and chemical microenvironments of 3D cultures so that they are more representable of in vivo conditions than traditional in vitro cell models. In addition, microfluidic devices provide a robust platform for live cell imaging and rapid evaluation of potential therapeutics.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Eleanor Stride**, University of Oxford
Title	**Microbubble-Mediated Intracellular Drug Delivery for Treatment of Chronic Infections**

Abstract

Urinary tract infections (UTI), represent one of the most common infectious diseases globally and impose a significant economic and healthcare burden. Common uropathogenic bacteria have been shown to invade the urothelial wall during acute UTI, forming latent intracellular reservoirs that can evade antimicrobial agents and the immune system. This behaviour facilitates the high recurrence rates seen after oral antibiotic treatments, which are not able to penetrate the bladder wall and accumulate to an effective concentration. Meanwhile, oral antibiotics may also exacerbate antimicrobial resistance and cause systemic side effects. Using a human urothelial organoid model, the ability of phospholipid microbubbles to deliver drugs into the cytoplasm of apical cells under ultrasound exposure was evaluated. The microbubbles were decorated with liposomes containing the non-cell-permeant antibiotic gentamicin and a fluorescent marker. The microbubble suspension was added to buffer at the apical surface of the bladder model before being exposed to ultrasound (1.1 MHz, 2.5 Mpa, 5500 cycles at 20 ms pulse duration) for 20 seconds. The results show that intracellular delivery with microbubbles and ultrasound was over 16 times greater than the control group and twice that achieved by liposomes that were not associated with microbubbles. Moreover, no cell damage was detected. Together, the data indicate that ultrasound-activated microbubbles can safely deliver high concentrations of drugs into urothelial cells, and have the potential to be a more efficacious alternative to traditional oral antibiotic regimes for UTI and other chronic infections.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Tim Segers,** University of Twente
Title	**Improved coalescence stability of monodisperse phospholipid-coated microbubbles formed by flow-focusing at elevated temperatures**

Abstract

Monodisperse phospholipid-coated ultrasound contrast agent (UCA) microbubbles can be directly synthe- sized in a lab-on-a-chip flow-focusing device. However, high total lipid concentrations are required to minimize on-chip bubble coalescence. Here, we characterize the coalescence probability and the long- term size stability of microbubbles formed using DPPC and DSPC based lipid mixtures as a function of temperature. We show that the coalescence probability can be dramatically reduced by increasing the temperature during bubble formation. Moreover, it is shown that the increased coalescence stability can be explained from an exponential increase of the relative viscosity in the thin liquid film between the collid- ing bubbles. Furthermore, it was found that the relative viscosity of a DPPC lipid mixture is 7.6 times higher than that of a DSPC mixture and that it can be explained solely from the higher DPPC liposome concentra- tion. Regarding long-term bubble stability, the ratio of the initial on-chip bubble size to the final stable bubble size was always found to be 2.2 for DPPC and DSPC coated bubbles with 10 mol% DPPE- PEG5000, independent of the temperature. Moreover, it was demonstrated that the microbubble suspen-sions formed at elevated temperatures are highly stable over a time window of 2 to 4 days when collected in a vial. All in all, this work shows that, by increasing the temperature during bubble formation from room temperature to 70 °C, the efficiency of the use of phospholipids in microbubble formation by flow- focusing can be increased by 5 times.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Klazina Kooiman,** Erasmus MC
Title	**The microbubble–endothelial cell–drug interaction revealed at nanosecond and nanometer resolution**

Abstract

Ultrasound-activated microbubbles can locally increase vascular permeability to enhance drug delivery [1]. To control and optimize the therapeutic potential, the microbubble–endothelial cell–drug interaction needs to be elucidated. This interaction remains largely unexplored due to the huge range in time scales involved, namely microseconds for the microbubble oscillation and seconds to minutes for the cellular response. This led us to create unique technology [3] by coupling the Brandaris 128 ultra-high-speed camera [2] to a custom-built Nikon A1R+ confocal microscope. With this optical imaging system we can now achieve the nanosecond and nanometer resolution necessary to resolve the microbubble oscillation and the cellular response concurrently. Confluent endothelial cells were evaluated for sonoporation with propidium iodide (PI), opening of cell-cell contacts with CellMask, and intracellular calcium fluctuations (Cai2+) with Fluo-4. The cellular response to insonification of single aVβ3-targeted microbubbles (n=206; 2MHz, 100-400kPa, 10-cycles) was monitored up to 4 min during which microbubble oscillations were recorded with the Brandaris 128 (~17 Mfps). Sonoporation occurred when microbubble excursion amplitudes exceeded 0.7 μm. For larger microbubble excursion amplitudes, the size of the created pore increased, as determined from PI influx quantification, and the severity of Cai2+ uptake and waves to adjacent cells also increased. Microbubble-mediated opening of cell-cell contacts occurred as a cellular response upon sonoporation and did not correlate with microbubble excursion amplitude itself, but did correlate with more severe Cai2+ uptake. The drug delivery outcomes were independent of nonlinear microbubble behavior, microbubble location, and cell size. In conclusion, by studying the microbubble–cell–drug interaction at nanosecond and nanometer resolution the relationship between drug delivery pathways and their underlying mechanisms was further unraveled.

[1]. Kooiman et al., Adv Drug Del Rev 2014, 72:p.28;
[2]. Chin et al., Rev Sci Instru 2003, 74:p.5026;
[3]. Beekers et al., Ultrasound Med Biol 2019, accepted.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Paul Prentice,** University of Glasgow
Title	**Contrast agent microbubble-jetting during initial interaction with sub-MHz focused ultrasound**

Abstract

Background: Microbubble-cavitation in response to focused ultrasound exposure at sub-MHz frequencies is of significant interest for emerging applications in transcranial therapy of the brain, for which frequencies of several 100 kHz are required to obtain sufficient transmission across the skull. Although a sizeable volume of literature exists on high-speed imaging of microbubbles under conventional medical ultrasound frequencies, response to such sub-resonant driving at relevant pressure amplitudes is less well studied. Methods: Dilute samples of SonoVue contrast agent were pumped through a 500 µm polycarbonate capillary, for exposure to focused ultrasound at f0 = 200 kHz. High-speed imaging at 10 million frames per second was used to observe the initial microbubble response to peak-negative pressure (PNP) amplitudes from 0.5 – 1.5 MPa. Results: Microbubble-cavitation jetting behaviour is consistently observed. At all pressure amplitudes, jets initially form due to asymmetric collapse under the action of the local pressure gradient over the first (significant) inflation phase. At higher PNPs, inertia sustains the subsequent re-inflation, such that a counter-jet in the opposite direction can also form, before the disintegration to a cavitation bubble cloud. At the lower PNPs, repeated bubble-jetting is accompanied by intermittent translation. The observations demonstrate that microbubbles are predisposed to jetting under the focused ultrasound parameters typically used for therapy of the brain.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Agata Exner,** Case Western Reserve University
Title	**Big Insights from Small Bubbles: Lessons from Headspace GC/MS**

Abstract

Gas volume of ultrasound contrast agents is related to their acoustic activity. The amount of gas in a bubble sample is typically calculated from a measured bubble diameter, bubble concentration and gas density. The estimated gas volume is then used to normalize bubble doses in experiments comparing acoustic activity for bubbles of different sizes or shell contents, both in vitro and in vivo. Because there are numerous assumptions made in these calculations, there may be errors introduced in the final gas volume prediction, thus making the comparisons between formulations difficult. For example, bubble populations measured using a Coulter Counter have a typical detection limit of ~500 nm. Since there can be a significant number of bubbles of smaller size, the particle count can be underestimated. The coincidence error can also lead to overestimation of particle diameter. Likewise, techniques for obtaining the size and concentration of nanobubbles are challenging with limitations that are particular to each technique. Headspace gas chromatography/mass spectrometry (GC/MS) is a well-validated method to accurately measure the specific gas content of any sample. In recent experiments we have used the GC/MS method to measure the total gas concentration contained in a solution of lipid shell-stabilized bubbles containing C3F8. Coupling this analysis with resonant mass measurement (RMM) of bubble concentration and buoyant mass can provide significant insight into the size and gas volume distribution of nanobubble and microbubble formulations. The superb sensitivity of perfluorocarbon measurement via GC/MS also enables unique applications such as detection of the gas in tissues and cells, which can be used to explore biodistribution and cell uptake of bubble-based agents. This presentation will summarize our recent findings using GC/MS and RMM to: 1) probe the gas volume of microbubbles, 2) provide evidence for the presence of nanobubbles, and 3) characterize nanobubble activity in cancer cells.

Session 1	**Microfluidics/Bubble Architecture**
Oral Presentation	**Judith Hadley,** Malvern Instruments
Title	**Characterizing bubbles: challenges and best practices**

Abstract

The utility of nano and microbubble-enhanced ultrasound imaging depends not only on the bubble size distributions but also on their number concentrations. A precise, accurate and documented method for sizing and counting of nano and microbubbles is essential. However, for a variety of reasons repeatable and reproducible measurements of bubbles are often not easy to attain. Additionally, different technologies may at times provide differing results. Today a range of automated technologies are available to measure and characterize ultrasound contrast agents. The more traditional technologies such as electro-impedance volumetric sensing, optical microscopy, laser diffraction (LD) and dynamic light scattering (DLS) have been used for many years to characterize bubble dispersions and are now joined by some newer options such as nanoparticle tracking analysis (NTA) and resonant mass measurement (RMM). However, few studies exist comparing measurement results from complementary or orthogonal technologies for the same sample. This study discusses the challenges of obtaining repeatable and reproducible measurements. We focus on four orthogonal measurement technologies and discuss the intra-method variability and how it impacts results: DLS, LD, RMM and NTA. The model bubble system used in this study consisted of submicron bubbles with a lipid stabilized octafluoropropane gas core formulated by mechanical agitation followed by nanobubble isolation using centrifugation and filtration. All measurements across these technologies were performed on the same nano or micro bubble stock solutions to minimize potential errors from individual sample preparation. The challenges of accurately and repeatably measuring bubbles are discussed as well as guidelines for optimal measurements. The advantages and disadvantages of each technique and their impact on results are discussed.

Session 2	**Microbubble Translational Applications**
Oral Presentation	**Alexander Klibanov,** University of Virginia
Title	**Targeting microbubbles to the tumor vasculature, not via selective molecular biomarkers**

Abstract

Molecular ultrasound imaging has moved from early-stage idea towards clinical trials. The general approach uses molecular biomarkers, selectively overexpressed on endothelium in tumor vasculature, e.g., VEGFR2, alphaVbeta3, VCAM-1, or neuropilin, as targets for microbubbles that carry antibodies or target-specific peptides. However, there is an alternative for selective imaging of tumor vasculature, based on vascular physiology. Tumor neovasculature is abnormal, irregular and tortuous, unlike the organized and regular fractal normal tissue vasculature. We hypothesized that the areas of the tumor vasculature exist, where blood flow and shear are much lower than in the norm. We describe the microbubbles that target slow-flow areas: they have some level of affinity to vascular endothelium in all vessels, but they will not be retained adherent to endothelium in the conditions of high-shear flow in normal vasculature, so adhesion will happen exclusively in low shear flow. We have successfully tested two slow-flow-targeted bubble designs. First is targeting through electrostatic interaction, where microbubbles carry a net positive surface charge. This allows microbubble adhesion to negatively charged glycocalyx coat of the surface of vascular endothelium. Adjustment of charge surface density results in specific adhesion and imaging of the tumor in a murine model following intravenous administration of microbubbles. Second technique is based on lectin-decorated microbubbles (lectins are known as endothelium stains). Lectin-microbubbles selectively adhere to tumor vasculature via an interaction between the lectin and carbohydrate residues of endothelial glycocalyx. Adjustment of lectin surface density on the bubble shell allows preparation of targeted microbubbles that adhere in low-shear flow in the tumors, but are not retained in normal tissue vasculature with fast high-shear flow, as demonstrated by contrast ultrasound imaging in a murine model. Overall, nonspecific controlled adhesion to vascular endothelium allows contrast ultrasound imaging of tumors, based on tumor blood flow, without targeting specific molecular biomarkers

Session 2	**Microbubble Translational Applications**
Oral Presentation	**Anthony McHale,** Ulster University
Title	**Observations on microbubble-based targeting of chemo- and sonodynamic therapy in the treatment of pancreatic cancer.**

Abstract

Pancreatic cancer remains one of the most recalcitrant forms of the disease with a five-year survival rate of less than 5%. Whilst a number of novel therapeutic approaches that target signal transduction pathways have emerged over the past number of years, none of these has significantly influenced this negative prognosis and both surgery and chemotherapy still offer the best outcome. Cognisant of the latter we have sought to develop ultrasound-targeted systems that would enable treatment of lesions in a site-specific manner that would overcome the particular challenges presented by pancreatic cancer. To this end, we have developed a highly adaptable microbubble-based vehicle bearing both chemotherapeutic drugs together with a sonosensitiser in order to exploit the combined therapeutic capabilities of chemotherapy and sonodynamic therapy in a site-specific manner. In the presence of ultrasound, both payloads are deposited in a site-specific manner and the stimulus also serves to activate the sonosensitiser with the resultant production of cytotoxic reactive oxygen species. Using this targeted approach enables administration of sub-therapeutic doses of the cancer chemotherapeutic drug together with the harmless sonosensitiser. Since the generation of cytotoxic reactive oxygen species during sonodynamic activation is 'fuelled' by the availability of oxygen and one of the major characteristics of pancreatic cancer is hypoxia, we have also enabled the microbubble-based vehicle to carry oxygen. Using a number of murine models of pancreatic cancer to test our microbubble-based system, we have demonstrated enhanced efficacy in controlling tumour growth at chosen target sites. Here we will present some of our preclinical data together with observations on positive collateral effects of this targeted approach.

Session 2	**Microbubble Translational Applications**
Oral Presentation	**Jithin Jose**, FUJIFILM Visualsonics
Title	**Multi-modal contrast agents: Photoacoustic imaging and therapy**

Abstract

Photoacoustic 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 photoacoustic (PA) imaging into a micro-ultrasound (US) platform. In addition, we will also discuss the development and validation of a 'Multi-modal Microbubbles' (MB) - a hybrid contrast agent for the PA and US imaging. We will discuss the initial results of the full body photoacoustic imaging and the bio-distribution of MBs in mice.

Session 2	**Microbubble Translational Applications**
Oral Presentation	**Odd Helge Gilja,** National Centre for Ultrasound in Gastroenterology
Title	**Ultrasound sonoporation and drug delivery in pancreatic adenocarcinoma**

Abstract

Pancreatic adenocarcinoma (PDAC) is one of the greatest challenges of cancers to treat due to aggressive biology, late diagnosis, encasement of large blood vessels and often presence of metastasis; hence surgery is rarely an option. Adding chemotherapy generates only modest responses but is not curative in this setting, mainly because its use is severely hampered by toxic effects to vital organs. As a result, the survival is very low. The mortality of the inoperable patients is 50% within 3 months and 90% within 12 months. Therefore, we aimed to develop an orthotopic PDAC mouse model and to test the concept of sonoporation and its safety. Furthermore, we conducted a Phase I study were we investigated the safety and the ability of inducing sonoporation in a clinical setting, using commercially available technology, to increase the patients' treatment cycles and to possibly increase the overall survival in patients with pancreatic adenocarcinoma. For the clinical study, 10 Patients were treated using a customized configuration of a commercial clinical ultrasound scanner (GE LOGIQ 9) over a time period of 31.5 min following standard chemotherapy treatment with gemcitabine. We found in mice very good safety and reduced tumor growth in the sonoporation group. The 10 patients were able to undergo an increased number of treatment cycles. Compared to historical data, there was increased survival with 60% of patients surviving 12 months. In conclusion, it is feasible to safely combine ultrasound, microbubbles, and chemotherapy both in an experimental and clinical setting. Our study also indicates increased survival in patients with inoperable, locally advanced PDAC.

[1]. Postema M, Gilja OH. Ultrasound-directed drug delivery. Curr Pharm Biotechnol 2007;8(6):355-361.
[2]. Kotopoulis S, Delalande A, Popa M, Mamaeva V, Dimcevski G, Gilja OH, Postema M, Gjertsen BT, McCormack E. Sonoporation-Enhanced Chemotherapy Significantly Reduces Primary Tumour Burden in an Orthotopic Pancreatic Cancer Xenograft. Mol Imaging Biol. 2013 Jul 23. [Epub ahead of print] PubMed PMID: 23877869.
[3]. Kotopoulis S, Dimcevski G, Gilja OH, Hoem D, Postema M. Treatment of human pancreatic cancer using combined ultrasound, microbubbles, and gemcitabine: A clinical case study. Med Phys. 2013 Jul;40(7):072902. doi: 10.1118/1.4808149. PubMed PMID: 23822453.
[4]. Postema M, Gilja OH. Contrast-enhanced and targeted ultrasound. WJG 2011;7,17(1):28-41.
[5]. Dimcevski G, Kotopoulis S, Bjånes T, Hoem D, Schjøtt J, Gjertsen BT, Biermann M, Molven A, Sorbye H, McCormack E, Postema M, Gilja OH. A human clinical trial using ultrasound and microbubbles to enhance gemcitabine treatment of inoperable pancreatic cancer. J Control Release. 2016 Oct 12;243:172-181. PubMed PMID: 27744037.

Poster Presentations

Number	Name	Title
1.	Tahani Albogami	Optically Mapping Temperature in the Vicinity of Gold Nanorods
2.	Amjad Aljaloud	Sympathetic laser cooling of liquids with cavitating bubbles
3.	Damien Batchelor	Nanobubbles for Ultrasound-Triggered Drug Delivery
4.	Matthew Bourn	Colorectal Cancer On-Chip: Microfluidic Platforms for the Testing of Microbubble Mediated Drug Delivery
5.	Veerle Brans	Enhancement of ultrasound-mediated cellular drug delivery via cell membrane modulation with lysophosphatidylcholine microbubbles
6.	Christa Brown	Controlled liposomal drug release with hydrogel encapsulation
7.	Nigel Bush	The effect of anaesthetic choice on efficiency of acoustic cluster therapy (ACT) bubble activation - preclinical studies in mice
8.	Gonzalo Collado Lara	The Effect of Radiotherapeutic Radiation on the Properties of Clinically Approved Microbubbles
9.	Joke Deprez	Evaluation of liposome loaded microbubbles as theranostic tool for rheumatoid arthritis.
10.	Fabio Domenici	DODAB-Shelled Microdroplets For Antitumour Drug Delivery
11.	Georg Feichtinger	Intra-articular in vivo gene delivery for regenerative medicine applications
12.	Nicola Ingram	Towards clinically relevant therapeutic microbubbles

13.	Joop Kouijzer	Vancomycin-targeted microbubbles as a novel treatment of Staphylococcus aureus biofilms
14.	Delanyo Kpeglo	On-chip Modelling of Pancreatic Cancer and its Stromal Environment
15.	Maia Munteanu	Dose response effect of liposome encapsulated SN38 therapeutic microbubbles in a colorectal cancer xenograft mouse model
16.	Luzhen Nie	Motion Compensation for High-Frame-Rate Contrast-Enhanced Echocardiography Using Diverging Waves
17.	Neil Thomson	Molecular effects of glycerol on lipid monolayers: Impact on microbubble physical and mechanical properties
18.	Jordan Tinkler	Targeted microbubbles for S.aureus biofilm treatment

Poster Number: 1	**Tahani Albogami,** School of Physics & Astronomy, University of Leeds
Title	**Optically Mapping Temperature in the Vicinity of Gold Nanorods**

Abstract

When gold nanorods (AuNRs) are irradiated with light of a frequency that matches their resonance frequency, the light is efficiently absorbed. Almost all of this energy is converted into heat by non-radiative processes and is subsequently locally dissipated [1]. This is called the photothermal effect. For gold nanorods the wavelength at which this occurs is in the near infrared region of the spectrum. Near infrared light can penetrate through soft tissue and thus this photothermal effect could be adopted for future cancer treatments, where the local heating from the irradiated gold nanorods results in cancer cell death. Accurate temperature measurement in this regime is difficult, since we are dealing with the critical dimension such as single cell. Few studies have been conducted on direct measurements of local temperature associated with irradiated AuNRs in a biologically relevant context. Luminescence thermometry is a promising technique for measuring the temperature in tissues. Recent studies have shown quantum dots (QDs) to be sensitive to temperature and able to report temperatures variation. Here we present AuNRs that are coated with silica shell to resist shape change under laser irradiation, decrease toxicity of CTAB, protect AuNRs from aggregation and make them thermodynamically stable [2]. The AuNRs/silica will be incorporated with QDs which will provide stable fluorescence tracking and temperature measurement capability to the AuNRs in the cells.

[1]. HUANG, X., KANG, B., QIAN, W., MACKEY, M. A., CHEN, P. C., OYELERE, A. K., EL-SAYED, I. H. & EL-SAYED, M. A. 2010. Comparative study of photothermolysis of cancer cells with nuclear-targeted or cytoplasm-targeted gold nanospheres: continuous wave or pulsed lasers. Journal of biomedical optics, 15, 058002.

[2]. ASHRAFI, S. J., YAZDIAN, F., ZAREMI, A. S. H., MOHHAMADNEJAD, J., DINARVAND, R. J. B. & JOURNAL, P. 2016. Thermal Distribution of Silica Coated Gold Nano Rods in Tissue-Like Phantom as In Vitro Model for Plasmonic Photo Thermal Therapy. 9, 1189-1201.

Tahani Albogami, Kevin Critchley, Zhan Y Ong, and Stephen D Evans

Poster Number: 2	**Amjad Aljaloud,** School of Physics & Astronomy, University of Leeds
Title	**Sympathetic laser cooling of liquids with cavitating bubbles**

Abstract

Laser cooling enables us to routinely transfer strongly confined particles, like single trapped ions, to nano-Kelvin temperatures. Here we propose to use this powerful technique in an analogous but less ideal situation and to laser cool an atomic gas inside cavitating bubbles to very low temperatures. Once the bubbles have been cooled, they can be used to lower the temperature of a surrounding liquid via sympathetic cooling. Our laser cooling scheme consists of two stages, thermalisation and cooling stages. During cooling stages, laser driving reduces the number of phonons in a collective vibrational mode of the atomic gas very rapidly. Thermalisation stages re-distribute the energy of all vibrational modes, until all of them reach a very low temperature.

Amjad Aljaloud and Almut Beige

Poster Number: 3	**Damien Batchelor,** School of Physics & Astronomy, University of Leeds
Title	**Nanobubbles for Ultrasound-Triggered Drug Delivery**

Abstract

The use of microbubbles for delivery of chemotherapeutics is an already well established research area. Microbubbles in combination with an ultrasound pulse can be used for triggered release, reducing toxicity and increasing efficacy via sonoporation. However due to microbubble size, they are confined to the vasculature and as such can limit drug penetration to the tumour site. As such, the possibility of enhanced tumour uptake and increased stability of nanobubbles, compared to traditional ultrasound contrast agents, makes them an attractive prospect for triggered drug delivery. Here we have developed a novel nanobubble based system for ultrasound mediated drug delivery. Nanobubbles have been passively encapsulated inside liposome alongside the model drug Calcein. Nanobubble encapsulation efficiency was calculated to be 21.8 % and with an average final size of 272 ± 84.9 nm. Clinical frequency B-mode imaging has been used to demonstrate echogenicity of the nanobubble-liposome complex. High intensity focused ultrasound has been used to achieve drug release of 36.3 % using high duty cycle exposure while lower duty cycle exposures have demonstrated significant decreases in echogenicity. Excellent drug loading stability has been demonstrated at physiological temperature, with drug leakage of 0.04 % after incubation for 30 minutes. Liposomal-nanobubbles show great promise for triggered drug release and advances in the field with potential to increase drug efficacy and enhance tumour penetration whilst reducing toxicity. Future work will consist of developing the nanobubble-liposome complex for delivery of chemotherapeutic agents to in vitro and in vivo models. Cytotoxicity and drug distribution will be investigated via confocal fluorescence and Raman microscopy.

D. V. B. Batchelor, R. H. Abou-Saleh, S. A. Peyman, J. R. McLaughlan, P. L. Coletta, S. D. Evans

Poster Number: 4	**Matthew Bourn,** School of Physics & Astronomy, University of Leeds
Title	**Colorectal Cancer On-Chip: Microfluidic Platforms for the Testing of Microbubble Mediated 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 1. Such a high mortality rate arises from difficulty detecting early stage CRC along with its tendency to metastasize to the liver. It is evident that improvements in both drug delivery and effectiveness are necessary to improve the treatment of CRC. Current pre-clinical methods of testing novel anticancer therapeutics disregard a myriad of factors that may affect a drug's efficacy. 2D monolayer cell cultures, used to test potential drugs, lack cell-cell connections, multicellular composition and surrounding extracellular matrix (ECM), all of which are present in in vivo tumours. Neglecting to recreate these physiological features results in these models failing to produce results observed in the clinic 2. Microfluidic multicellular 3D cultures supplied by a constant flow of nutrients significantly improve on 2D models. These models have further increased in complexity with the emergence of organ-on-chip technology, which incorporates multiple cell types and ECM components into microfluidic chambers. In particular, the production of fully perfusable vasculature on-chip has allowed for the study of drug penetration through the endothelium into the tumour tissue 3. Here we present two microfluidic systems developed for the study of microbubble mediated drug delivery to 3D tumour culture models. The first, is a microfluidic trap array used to trap tumour spheroids which can then be exposed to therapeutics under flow. Initial results have observed the response of spheroids to 10μM Doxorubicin. Second, is a system which aims to produce healthy vasculature growing alongside a tumour. This aims to observe the invasion of tumour cells into healthy tissues and the formation of leaky tumour vasculature, which will then be treated with targeted microbubbles.

[1]. Cancer Research UK, www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer, Accessed 06/2019.
[2]. Sant, S. & Johnston, P. A. The production of 3D tumor spheroids for cancer drug discovery. Drug Discov. Today Technol. 23, 27–36 (2017).
[3]. Nashimoto, Y. et al. Integrating perfusable vascular networks with a three-dimensional tissue in a microfluidic device. Integr. Biol. (United Kingdom) 9, 506–518 (2017).

Bourn M. D., Ingram N., Coletta P. L., Evans S. D., Peyman S. A.

Poster Number: 5	**Veerle Brans,** University of Oxford
Title	**Enhancement of ultrasound-mediated cellular drug delivery via cell membrane modulation with lysophosphatidylcholine microbubbles**

Abstract

Ultrasound exposure of circulating cavitation agents, such as phospholipid-shelled microbubbles, and/or drug-carrying vehicles, such as liposomes, has been shown to enable the delivery of a therapeutic payload to target locations within the human body. The destruction of these agents also leads to localised deposition of their various constituent parts. Initial observations demonstrate that transfer of these bubble components, such as phospholipids, between microbubbles and cells can alter cell membrane hydration and lipid order. Microbubbles incorporating conical lipids, such as lysophatidylcholine or Lyso-PC, were developed. The impact of lipid transfer from these microbubbles to cell membranes was investigated. The ultrasound-mediated triggered release of Lyso-PC from microbubbles was found to significantly increase sonoporation as measured by the uptake of a fluorescent model drug compared to controls. Lyso-PC-incorporating microbubbles are also an interesting candidate for ultrasound-mediated blood-brain barrier opening, as evidenced by the reversible disruption of tight junctions by Lyso-PC exposure and increased blood-brain-barrier disruption in vivo. However, the underlying mechanisms behind this increase in therapeutic delivery potential of the microbubbles are currently unknown. Elucidating these mechanisms requires quantitative investigation of microbubble-cell interactions. This work employs a new strategy using advanced fluorescence microscopy and ultrasound to investigate microbubble-cell membrane interactions to exploit Lyso-PC-incorporating microbubbles to further enhance cellular drug delivery and ultrasound-mediated permeabilization of the blood-brain barrier.

Veerle Brans, Oliver Vince, Miles Aron, Michael Gray, Erdinc Sezgin, Eleanor Stride

Poster Number: 6	**Christa Brown,** School of Physics & Astronomy, University of Leeds
Title	**Controlled liposomal drug release with hydrogel encapsulation**

Abstract

Prolonged exposure of chemotherapeutic agents to tumour cells over several cell cycles has been found to be more effective for the treatment of cancer than a short burst of chemotherapeutic agents [1]. Currently, stimuli sensitive liposomes lack the ability to control the release profile of encapsulated drug to control the release profile, and therefore the timescale over which tumour cells are exposed to therapeutic agents [2]. Hydrogels are highly porous, 3D networks that are swollen with water, creating opportunities to design biocompatible, responsive materials for triggered drug delivery. A composite liposome hydrogel system would provide a unique method of prolonged targeted drug release at the tumour site. We are investigating such as system involving liposomes encapsulating nano-hydrogel embedded with nanobubbles termed 'lipogels'. This system mimics the ability of natural cells to take advantage of properties from both the lipid bilayer and nano-hydrogel to create a multifunctional system. The lipid bilayer aims to protect the hydrogel from degradation in the bloodstream, as well as reducing the side effects of toxic drugs on healthy cells by targeting to the region of interest. The presence of a drug loaded gel within the liposome core aims to further control the speed that the drug molecules are released. Additionally, by microfluidically producing and manipulating GUVs with encapsulated micro-hydrogel (10 – 20 μm) will provide an attractive model system translation of mechanical properties from the cross-linked network to the elasticity of the lipogel can be accurately determined.

Christa P. Brown, Louise Coletta, Sal Peyman, Stephen D. Evans, Lorna Dougan.

Poster Number: 7	**Nigel Bush,** The Institute of Cancer Research
Title	**The effect of anaesthetic choice on efficiency of acoustic cluster therapy (ACT) bubble activation - preclinical studies in mice**

Abstract

The choice of anaesthesia has been shown to significantly affect microbubbles used for ultrasound (US) contrast imaging and therapy studies in various animal models, altering the lifetime stability and kinetics of the bubble population and moderately to severely depressing cardiovascular func¬tion. These changes give rise to dose and time dependent alterations in physiological parameters potentially affecting gas saturation (particularly oxygen), blood pressure and perfusion. Acoustic cluster therapy (ACT) employs both gas-filled microbubbles and oil microdroplets, electrostatically bound together in clusters. Under insonation using diagnostic ultrasound pulses, activation occurs, i.e., the microbubbles are destroyed and the droplets undergo a phase change to result in large (20-30 micron) bubbles in the tumour vasculature which are then modulated using low frequency (~ 500 kHz) ultrasound to enhance therapeutic delivery into the tumour. In preclinical ACT studies to date we have employed injectable anaesthesia to simplify animal handling, minimise changes to blood gas concentration and limit breathing motion. There are potential advantages, however, in switching to gaseous formulations. Isoflurane, an inhalation anaesthetic, offers moderate cardio de¬pressive effects compared to those of injectable agents, and is frequently employed for surgical interventions and short-term experimenta¬tion with more rapid recovery times. Material and Methods Here, using dynamic contrast enhanced ultrasound (DCEUS), we compare the effects of inhalation anaesthesia (Isoflurane plus medical air) against a standard injectable anaesthetic (Hypnorm: Hypnovel), and have studied the additional effect of giving an oxygen challenge during isoflurane and air anaesthesia. Echo time-intensity curve (TIC) properties were measured to characterise the dynamics of ACT bubble evolution in normal murine kidney, following tail vein injection of ACT clusters in mice, 3 injections per animal, 6 animals, randomised with different anaesthesias on different days. Imaging and activation of ACT were achieved with a Toshiba Aplio XG standard clinical US scanner and 1204BT linear array providing simultaneous interleaved, non-linear contrast mode and fundamental B-mode imaging at 8 MHz, MI = 0.31. Images were decompressed (linearised) and background (pre-contrast) subtraction applied. Regions of interest were drawn over the whole kidney and time-intensity curves (TIC) characterised to return curve peak and area under curve (AUC). The kidney was employed as a surrogate organ for imaging as it is relatively shallow, well perfused and clearly delineated on US imaging. Results and Discussion B-mode TIC peak and AUC were significantly greater (~ 70%, Brown-Forsythe and Welsh ANOVA, $p = 0.011$ and $p = 0.034$ respectively) in Isoflurane compared to injectable anaesthetised mice. In Contrast mode the TIC peak was however not significantly different, and AUC was higher (~ 70 %, $p = 0.095$) in injectable anaesthetised mice compared to Isoflurane. Imaging with the ACT formulation includes backscatter from the ACT bubbles and free Sonazoid component in the formulation. Due to the larger ACT size compared to Sonazoid (~ 10 times in diameter, 1000 times in volume), nonlinear contrast mode is dominated by backscatter from Sonazoid, whereas the converse is true in B-mode contrast (8 MHz) (ACT bubbles behaving as linear scatterers well above their resonance frequency of ~0.3 MHz). ACT bubbles are also more robust to destruction by US compared to microbubbles. Oxygen challenge following ACT activation under Isoflurane and air produces a significant reduction in B-Mode contrast which confirms previous isoflurane studies which suggest blood oxygen saturation strongly affects bubble lifetime. Greater breathing motion was observed with Isoflurane and air compared to injectable anaesthesia and a 0.7 Hz modulation in B-mode contrast was observed potentially resulting from changes of ACT bubble volume with pressure changes due to breathing. Conclusions A significant increase (70%) in ACT backscatter in B-mode is observed for isoflurane and air compared to injectable Hypnorm:Hypnovel anaesthesia, indicating more effective ACT activation. There is no strong reason to prefer Isoflurane and air or injectable anaesthesia for pre-clinical ACT studies in terms of imaging properties. Switching to Isoflurane and Oxygen can be used to rapidly clear ACT bubbles in vivo. In comparative pre-clinical ACT or contrast studies it is important to standardise anaesthetic dose and formulations.

Nigel Bush, Andrew Healey, Per Christian Sontum, Svein Kvåle, Jeffrey Bamber

Poster Number: 8	Gonzalo Collado Lara, **Biomedical Engineering, Erasmus**
Title	**The Effect of Radiotherapeutic Radiation on the Properties of Clinically Approved Microbubbles**

Abstract

Dosimetry is a key step in radiotherapy to compare the treatment plan with the actual delivered irradiation. However, no gold standard has emerged in the clinic. In this study we wanted to assess the dosimetric properties of clinically approved Ultrasound Contrast Agents since a decrease in the acoustic attenuation was shown for the experimental agent Targestar-P after irradiation for doses of 4-30 Gy (Verboven et al., IEEE IUS Proceedings 2014). SonoVue, Definity and Optison samples were irradiated by a 6 MV radiotherapeutic beam for a total dose of 15 Gy. During the irradiation process, acoustic pulses with 2.25 MHz centre frequency and peak negative pressures of 25 and 50 kPa were used to measure the evolution of the acoustic attenuation and scattering of the samples. After this process, the concentration and size distribution were measured using a Coulter Counter. Fresh samples from the same vials were used for control measurements, without triggering the radiotherapeutic beam. No transient effects were observed in the acoustic response either at the start or end of the irradiation. Furthermore, no significant difference was found in the final concentration or size distribution between the irradiated and control samples. However, a permanent decrease in the attenuation was observed during the measurements. The total decrease was in the order of 0.1 dB/cm larger for the irradiated samples. This change is below relevance for clinical use. These results suggest that SonoVue, Definity and Optison are not sensitive enough to be directly used for dosimetry.

Gonzalo Collado, Sophie V. Heymans, Koen Van Den Abeele, Klazina Kooiman, Hendrik Vos, Jan D'hooge, Nico de Jong

Poster Number: 9	**Joke Deprez,** Ghent University
Title	**Evaluation of liposome loaded microbubbles as theranostic tool for rheumatoid arthritis.**

Abstract

Rheumatoid arthritis is an autoimmune disease that is characterized by severe inflammation of the synovium, joint swelling, cartilage destruction and bone deformation. The inflammatory cytokines present in the inflamed regions promote the leakiness of the vasculature and the occurrence of new vasculature, so-called neovascularization. For monitoring the inflammation, angiogenesis and pannus growth, the use of contrast enhanced ultrasound (CEUS) has already shown potential in humans. Therefore, we wanted to evaluate whether drug-loaded-microbubbles have potential in rheumatoid arthritis monitoring and treatment. In this study, the use of CEUS is investigated to quantify neovascularization in arthritic mice. Subsequently, we verified if we were able to enhance liposomal delivery to the inflamed knee-joints by making use of liposome loaded microbubbles and ultrasound. For this purpose, we loaded lipid microbubbles with fluorescently labeled liposomes and injected them i.v. in arthritic mice. Subsequently we exposed the knee-joints with ultrasound (1 MHz, 30s, 2W/cm2 to 4W/cm2, 2% DC). The biodistribution of the liposomes was monitored up to 24 hours after ultrasound exposure and compared with the biodistribution of liposomes only to define the impact of microbubble loading and the ultrasound on the biodistribution of the liposomes. We could observe an influx of microbubbles in the inflamed joints but we did not observe an improved microbubble influx due to neovascularization. The presence of microbubbles in the synovial vasculature did not result in an additional accumulation of liposomes in the inflamed joints. In contrast, microbubble loading of the liposomes lead to a much faster clearance of the liposomes and prevented further accumulation of liposomes in the joints. We hypothesize that use of microbubbles to visualize neovascularization in small research animals might be hampered due to size restraints and that more optimized transducers and acoustic settings are required to stimulate microbubble cavitation and liposomal shedding in the joints.

J. Deprez, S. Roovers, R. Verbeke, K. Debusschere, B. Descamps, C. Vanhove, G. Lajonie, M. Versluis, H. Dewitte, P. Jacques, S.C. De Smedt, I. Lentacker

Poster Number: 10	**Fabio Domenici,** Dipartimento di Scienze e Tecnologie Chimiche, Università degli Studi di Roma
Title	**DODAB-Shelled Microdroplets For Antitumour Drug Delivery**

Abstract

The introduction of phase change perfluorocarbon microdroplets (MDs) in the vast theranostic scenario is evaluated through the key opportunity they offer to conjugate drugs cargo capability with echogenicity promoted by acoustic core vaporization [1]. Significant improvement in this respect mainly passes through the optimization ofthe MDs shell with respect to stability, chemical versatility, and synthesis efficiency. We present decafluoropentane MDs stabilized by a shell of dimethyldioctadecylammonium bromide (DODAB) cationic surfactant. The long saturated hydrocarbon chains, deeply penetrating the hydrophobic core, stabilize the droplet and the drug cargo within, and the high positive ζ-potential favours the colloidal stability of MDs. The biological attractiveness of DODAB shell lies in the ability to bind anionic antigenic proteins, nucleotides or DNA, and nanoparticles, for remarkable immunoadjuvant, gene therapy, and antitumour action [2,3]. 3•1010 droplets/ml are readily provided in a few seconds by pulsed high-power insonation, resulting in low polydispersed 1μm sized droplets, exhibiting a ζ-potential of ~ 100mV. We show that this system acts as efficient reservoir for doxorubicin in vitro. Specifically, the droplets shell, interacting with the cell membrane of fibroblasts and melanoma cell models, favours the drug internalization within cells to kill them up to 45% in only 24 hours. Equivalent concentrations of either free doxorubicin or unloaded MDs do not produce any effect on the cell viability. More important, the transition from doxorubicin loaded MDs to microbubbles further modulates the level of the drug penetration providing remarkably enhanced cytotoxicity. The selectivity of cell-MDs interaction will be also discussed. Our results may be relevant in greatly amplifying the benefit-to-risk ratio of chemotherapeutics a well as in facilitating real-time monitoring of the treatments.

[1]. Capece S, Domenici et al, Phys.Chem.Chem.Phys. 18:8378-8388, 2016
[2]. F. Domenici, C. Castellano et al, Colloids Surf.B 88:432-439, 2011
[3]. P.S. Dubey, Srinivasan et al, Sci.Rep. 8:1-16, 2018

Damiano Palmieri, Elisabetta Tortorella, Letizia Oddo, Francesco Brasili, Yosra Toumia, Angelico Bedini, Fabio Domenici, Gaio Paradossi

Poster Number: 11	**Georg Feichtinger,** Division of Oral Biology, School of Dentistry, University of Leeds
Title	**Intra-articular in vivo gene delivery for regenerative medicine applications**

Abstract

Joint-destructive conditions such as osteoarthritis inevitably lead to tissue erosion, loss of joint function, pain and disability. Currently, there are no treatments available offering a permanent cure. Non-viral gene delivery of anabolic growth factor genes or inflammation modulating cytokines has recently shown promising effects for treatment, leading to partial regeneration and reversal of disease symptoms. It would be possible to develop novel economical and safe therapeutics for arthritic diseases if effective, minimally invasive and translation-geared approaches to non-viral gene delivery could be developed. Therefore, we investigated the potential of sonoporation for the delivery of a novel infrared-emitting luciferase model gene for deep within tissue imaging of transgene expression after intra-articular gene delivery. To this end, healthy rat joints as well as joints harbouring an acute osteochondral defect were sonoporated using a probe emitting columnar-beam 1MHz ultrasound in conjunction with pDNA and Optison® clinical-grade microbubbles. An optimised protocol based on previous work and available literature was developed. Positive controls were carried out by additionally sonoporating the tibialis anterior muscle in each limb. In order to compare established luciferases (CBRLuc) with the performance of the novel reporter gene, CBRLuc was sonoporated in contralateral limbs as control. 24hrs, 48hrs and 14 days post gene delivery AkaLuc luciferase was imaged on the IVIS Spectrum. Strong expression of AkaLuc was observed in sonoporated animals at all timepoints, originating from the knee joint and positive control sites. CBRLuc was not as effective nor reliable in reporting transgene activity due to the inferior tissue penetration properties. In conclusion, an effective gene delivery modality was developed and characterised in vivo, demonstrating that sonoporation is a viable approach for the delivery of translation-geared gene therapeutics to treat arthritic conditions in the future. Detailed biodistribution of the reporter transgene is currently under investigation via immunohistochemistry.

G. A. Feichtinger, X. B. Yang

Poster Number: 12	**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. We also show how these microbubbles perform as well as biotin-avidin microbubbles in vivo in terms of time-intensity curve parameters and longevity.

Nicola Ingram, Radwa Abou-Saleh, Steve Evans and P. Louise Coletta

Poster Number: 13	**Joop Kouijzer,** Biomedical Engineering, Erasmus MC
Title	**Vancomycin-targeted microbubbles as a novel treatment of Staphylococcus aureus biofilms**

Abstract

Due to an aging population increasingly more cardiac devices are implanted, such as pacemakers. Life-threatening bacterial infections associated with these devices are a major problem because removal of the infected devices is either not possible or a major undertaking, with a risk of death or significant complications. In most cases the infection is caused by the bacteria Staphylococcus aureus (S. aureus). A new, targeted, and less invasive treatment is highly desirable but still unavailable. This study aimed to produce targeted microbubbles to treat S. aureus biofilms in combination with ultrasound. Vancomycin, a glycol-peptide antibiotic, was chemically conjugated via its primary amine group to the lipid DSPE-PEG(3400)-NHS and incorporated in the coating of the targeted microbubbles. The microbubble coating mainly consisted of DSPC and had a perfluorobutane gas core. To mimic the surface of the cardiac devices, IbiTreat µ-Slides (ibidi GmbH, Germany) were used to grow S. aureus biofilms in vitro using a clinical isolate from a patient. During ultrasound insonification (2MHz, 250kPa, 10.000 cycles), microbubble behavior was recorded with the Brandaris 128 ultra-high speed camera coupled to a custom-built Nikon A1R+ confocal microscope. Vancomycin-targeted microbubbles bound to the S. aureus biofilms, whereas non-targeted microbubbles did not. Furthermore, a vancomycin competition assay showed that increasing concentrations of vancomycin added prior to the addition of the targeted microbubbles significantly reduced the amount of targeted microbubbles bound to the biofilm. During insonification, the targeted microbubbles formed clusters by migrating towards each other resulting in deformations and detachment of bacteria from the biofilms. Also, a greater amount of movement of bacteria was observed along the path where the targeted microbubbles had been. Mechanically disrupting the bacterial biofilm by using the vancomycin-targeted microbubbles in combination with ultrasound can assist the treatment of cardiac-device related infections in a more efficient and less invasive manner.

Joop J.P. Kouijzer, Kirby R. Lattwein, Inés Beekers, Nico de Jong, Antonius F.W. van der Steen, Alexander L. Klibanov, Willem J.B. van Wamel, Klazina Kooiman

Poster Number: 14	**Delanyo Kpeglo,** School of Physics & Astronomy, University of Leeds
Title	**On-chip Modelling of Pancreatic Cancer and its Stromal Environment**

Abstract

The prognosis of pancreatic cancer is dismal, with few patients selected for surgery due to late stage diagnosis. A hallmark of the disease is the pervasive growth of dense fibrous tissue around the tumour, and cells of the tumour environment such as the fibroblast cells of the pancreas, pancreatic stellate cells (PSCs), are reported to centrally play in the fibrous network impeding the delivery of chemotherapy [1]. However, current in vitro models, both 2D and 3D do not often account for cellular complexity or the essential fibrotic microenvironment found in vivo. Therefore, new methods are needed to study the disease for the development and assessment of new therapeutic approaches. In understanding more about diseases, microfluidic devices provide a versatile way of modelling and studying diseases in 3D. Due to the micron-scale network of defined channels that can be engineered, physical parameters such as in vivo flow rate and shear stress on tissues can be accurately mimicked. Microfluidics allows the recapitulation of disease tissues much more representative of those in vivo in comparison to static well formats. Diseases including cancers of e.g. the lungs, gut and breast has been modelled with microfluidics in learning more of the diseases [2, 3, 4]. On-chip modelling of pancreatic cancer aims to mimic the disease tissue with focus on the tumour microenvironment for therapeutic assessment with new approaches such as micro- and nanobubbles and nanoparticles. So far, microfluidic devices have been designed to mimic the pancreas for the on-chip 3D culture of pancreatic cancer cells and PSCs. Off-chip, 3D spheroids were cultured and cytotoxicity assay performed to determine the optimum drug concentration for on-chip therapeutic assessments. Furthermore, due to the fibrotic nature of pancreatic cancer it has been shown that the disease tissue is contributing to chemoresistance [5]. Thus, the mechanical properties of cultures are being measured to determine if the presence of PSCs contributes to the matrix stiffness of cultures.

[1]. Apte, M. V., Pirola, R. C., & Wilson, J. S. (2012). Pancreatic stellate cells: A starring role in normal and diseased pancreas. Frontiers in Physiology. https://doi.org/10.3389/fphys.2012.00344

[2]. Huh, D. (2015). A human breathing lung-on-a-chip. In Annals of the American Thoracic Society (Vol. 12, pp. S42–S44). American Thoracic Society. https://doi.org/10.1513/AnnalsATS.201410-442MG

[3]. Sontheimer-Phelps, A., Hassell, B. A., & Ingber, D. E. (2019, February 1). Modelling cancer in microfluidic human organs-on-chips. Nature Reviews Cancer. Nature Publishing Group. https://doi.org/10.1038/s41568-018-0104-6

[4]. Jeon, J. S., Bersini, S., Gilardi, M., Dubini, G., Charest, J. L., Moretti, M., & Kamm, R. D. (2015). Human 3D vascularized organotypic microfluidic assays to study breast cancer cell extravasation. Proceedings of the National Academy of Sciences, 112(1), 214–219. https://doi.org/10.1073/pnas.1417115112

[5]. Rice, A. J., Cortes, E., Lachowski, D., Cheung, B. C. H., Karim, S. A., Morton, J. P., & Del Río Hernández, A. (2017). Matrix stiffness induces epithelial-mesenchymal transition and promotes chemoresistance in pancreatic cancer cells. Oncogenesis, 6(7), page 352 https://doi.org/10.1038/oncsis.2017.54

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

Poster Number: 15	**Maia Munteanu,** Medicines Discovery Catapult
Title	**Dose response effect of liposome encapsulated SN38 therapeutic microbubbles in a colorectal cancer xenograft mouse model**

Abstract

A big challenge in drug development is achieving delivery of drugs at appropriate concentrations to target tissues with little or no side effects. In cancer, the limiting drawbacks of chemotherapy are its poor bioavailability, narrow therapeutic indices and adverse side effects. Targeted delivery platforms could address these issues by carrying drugs to the desired sites of therapeutic action and allowing controlled drug release reducing systemic side effects previously seen. Medicines Discovery Catapult (MDC) have partnered with the University of Leeds to help to advance their therapeutic microbubble (ThMb) platform to a clinically relevant drug delivery system. The ThMb platform consists of lipid shelled gas filled microbubbles that are coupled to drug encapsulating liposomes and functionalised with anti VEGFR2 antibodies, preferentially targeting the tumour site and releasing their payload after exposure to focused ultrasound. The average size and concentration of drug loaded liposomes for in-vivo injection was determined as 139.8 ± 4.2 nm and 1.4E+13 ± 1.7E+12 respectively using nanoparticle tracking analysis, while that of the whole microbubble complex was acquired using optical microscopy (average size of 2.1±1.3µm and concentration of 1.7E8). The drug loading efficiency in the liposomes and in the whole ThMb complex was determined by liquid chromatography mass spectrometry. A dose response efficacy ThMb study was performed in CD1 mice bearing the SW480 colorectal xenograft model. Mice were injected i.v. with ThMbs (vehicle, 0.15, 0.3 and 0.6mg/kg) every 72 hours (in total 5 doses). A dose-dependent efficacious response to ThMBs was observed, with a 44% reduction in tumour growth in the 0.6mg/kg SN38 ThMb compared to saline control ($p = 0.02$ and 0.01 vs vehicle at the 3rd, 4th and 5th imaging time point respectively). These results suggest that ThMBs represent a promising delivery system and may increase therapeutic efficacy and specificity of drug compounds while reducing side effects.

Maia Munteanu, Duygu Yilmaz, Malcolm Haddrick, Will Townley, Juliana Maynard, Sally Price, Peter Simpson, Nicola Ingram, Radwa Abou-Saleh, Louise Coletta, Benjamin Johnston, Stephen Evans

Poster Number: 16	**Luzhen Nie,** Institute of Robotics, Autonomous Systems and Sensing, School of Electronic and Electrical Engineering University of Leeds
Title	**Motion Compensation for High-Frame-Rate Contrast-Enhanced Echocardiography Using Diverging Waves**

Abstract

It was shown that the efficacy of diverging wave (DW) imaging of the heart is reliant on carefully designed motion compensation algorithms capable of correcting for incoherence between steered DWs. Despite the availability of different solutions for motion compensation in high-frame-rate contrast-enhanced echocardiography (HFR CEE) using DWs, little is known about their relative performance both in vitro and in vivo. The aim of this study was to compare any improvements in CR, CNR or CTR when using an image registration-based method and a correlation-based method for motion compensation in HFR CEE.

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

Poster Number: 17	Neil Thomson, **School of Physics & Astronomy, University of Leeds**
Title	**Molecular effects of glycerol on lipid monolayers: Impact on microbubble physical and mechanical properties**

Abstract

The production and stability of microbubbles (MBs) is enhanced by increasing the viscosity of both the formation and storage solution, respectively. Glycerol is a good candidate for biomedical applications of MBs, due to its biocompatibility, but the exact molecular mechanisms of its action is not fully understood. We investigated the influence glycerol has on lipid-shelled MB properties, using a range of techniques. Population lifetime and single bubble stability were studied using optical microscopy. Bubble stiffness measured by AFM compression was compared with lipid monolayer behaviour in a Langmuir-Blodgett trough. We found that glycerol has multiple effects on the lipid monolayer at the molecular level but its viscosity is the dominant effect on MB solution stability. We deduced that increasing glycerol concentrations enhances stability of MB populations through a three-fold mechanism. Firstly, binding of glycerol to lipid headgroups in the interfacial monolayer up to 10% glycerol, increases MB stiffness but has limited impact on shell resistance to gas permeation and corresponding MB lifetime. Secondly, increased solution viscosity above 10% glycerol slows down the kinetics of gas transfer, markedly increasing MB stability. Thirdly, above 10%, glycerol induces water structuring around the lipid monolayer, forming a glassy layer which also increases MB stiffness and resistance to gas loss. At 30% glycerol, the glassy layer is ablated, lowering the MB stiffness but MB stability is further augmented. Although the molecular interactions of glycerol with the lipid monolayer modulate the MB lipid shell properties, MB lifetime continually increases from 0 to 30% glycerol due to its high viscosity. This work gave new insight into the action of glycerol on lipid monolayers at the gas-liquid interface. The three-fold action and biocompatibility makes glycerol ideal for therapeutic MB formation and storage.

Radwa H Abou-Saleh, James R McLaughlan, Richard J. Bushby, Benjamin R.G. Johnson, Steven Freear, Stephen D. Evans and Neil H. Thomson

Poster Number: 18	**Jordan Tinkler,** School of Physics & Astronomy, University of Leeds
Title	**Targeted microbubbles for S.aureus biofilm treatment**

Abstract

Biofilms are structurally organised pathogenic communities embedded within a self-made protective extracellular matrix (ECM). This protective matrix consists of a mixture of lysed cells, proteins, extracellular DNA, and polysaccharides. These bacterial colonies have shown an enhanced resistance to traditional antimicrobial treatment methods and the host's immune system. Biofilm infections have shown an affinity for implanted medical devices, such as replacement heart valves, catheters, and arterial stents. Infections of this type can lead to inflammatory diseases, such as endocarditis in relation to infected heart valves, and in some cases, total device failure. These infections may also act as a reservoir for chronic repeated infections. The primary treatment method currently available is the total removal and replacement of the infected implant device. This process can be expensive, surgically invasive, and potentially traumatic for the patient. Microbubbles (MBs) are 1-10 μm diameter lipid-stabilised parcels of gas. MBs have seen extensive use and development since their discovery as ultrasound contrast agents (UCAs). More recent modifications to bubble compositions and the potential for the addition of targeting proteins and liposome-loaded drug payloads to the outer shell has expanded the field of drug delivery and targeted therapeutics. This work investigates the use of targeted MBs for the microfluidic-based treatment of in vitro S.aureus biofilms through the use of a novel targeting affimer protein. Through the application of ultrasound, MB destruction may be applied to rupture bacterial cells and break apart the biofilm structure, thereby allowing for enhanced drug delivery and uptake. This method aims to provide an alternative therapeutic route to those currently available, reducing healthcare costs and improving patient recovery.

Jordan M Tinkler, Dr J A T Sandoe, Prof S Freear, Prof S D Evans

Delegate List

Name		Affiliation
Radwa	Abou-Saleh	University of Leeds
Tahani	Albogami	University of Leeds
Amjad	Aljaloud	University of Leeds
Benedetta	Arno	Medicines Discovery Catapult
Alexander	Arnstein	University of Leeds
Damien	Batchelor	University of Leeds
Faith	Bonner	University of Leeds
Matthew	Bourn	University of Leeds
Veerle	Brans	University of Oxford
Christa	Brown	University of Leeds
Nigel	Bush	Institute of Cancer Research
Richard	Bushby	University of Leeds
Louise	Coletta	University of Leeds
Gonzalo	Collado Lara	Erasmus MC
Christian	Coviello	OxSonics Ltd
Miguel	de Vargas Serrano	Tide Microfulidics
Joke	Deprez	Ghent University
Fabio	Domenici	Università degli Studi di Roma
Stephen	Evans	University of Leeds
Agata	Exner	Case Western Reserve University
Georg	Feichtinger	University of Leeds
Joe	Fox	University of Leeds
Steve	Freear	University of Leeds
Odd Helge	Gilja	National Centre for Ultrasound in Gastroenterology
Martin	Greenhall	F2 Chemicals

Judith	Hadley	Malvern Instruments
Nicola	Ingram	University of Leeds
Ananda	Jadhav	University of Birmingham
Ben	Johnson	University of Leeds
Jithin	Jose	FUJIFILM Visualsonics
Alexander	Klibanov	University of Virginia
Klazina	Kooiman	Erasmus MC
Joop	Kouijzer	Erasmus MC
Delanyo	Kpeglo	University of Leeds
Marie Pierre	Krafft	University of Strasbourg (CNRS)
Ine	Lentacker	UGent
Alex	Markham	University of Leeds
Juliana	Maynard	Medicines Discovery Catapult
Anthony	McHale	Ulster University
Maia	Munteanu	Medicines Discovery Catapult
Luzhen	Nie	University of Leeds
Marcus	Orton	Healthcare Innovation
Damiano	Palmieri	Università degli Studi di Roma
Georgios Andreas	Papagiannidis	University of Birmingham
Andy	Penman	F2 Chemicals
Sally	Peyman	University of Leeds
Paul	Prentice	University of Glasgow
Marcus	Salmon	FUJIFILM Visualsonics
Jonathan	Sandoe	University of Leeds
Tim	Segers	University of Twente
Elliott	Smith	University of Leeds
Brian	Speyer	Speyer Photonics Ltd

Eleanor	Stride	University of Oxford
Neil	Thomson	University of Leeds
Jordan	Tinkler	University of Leeds
Bruce	Turnbull	University of Leeds
Liz	Valleley	University of Leeds
Alexandra	Vasilyeva	University of Oxford
Michel	Versluis	University of Twente
Anthony	Woodhead	Healthcare Innovation
Duygu	Yilmaz	Medicines Discovery Catapult