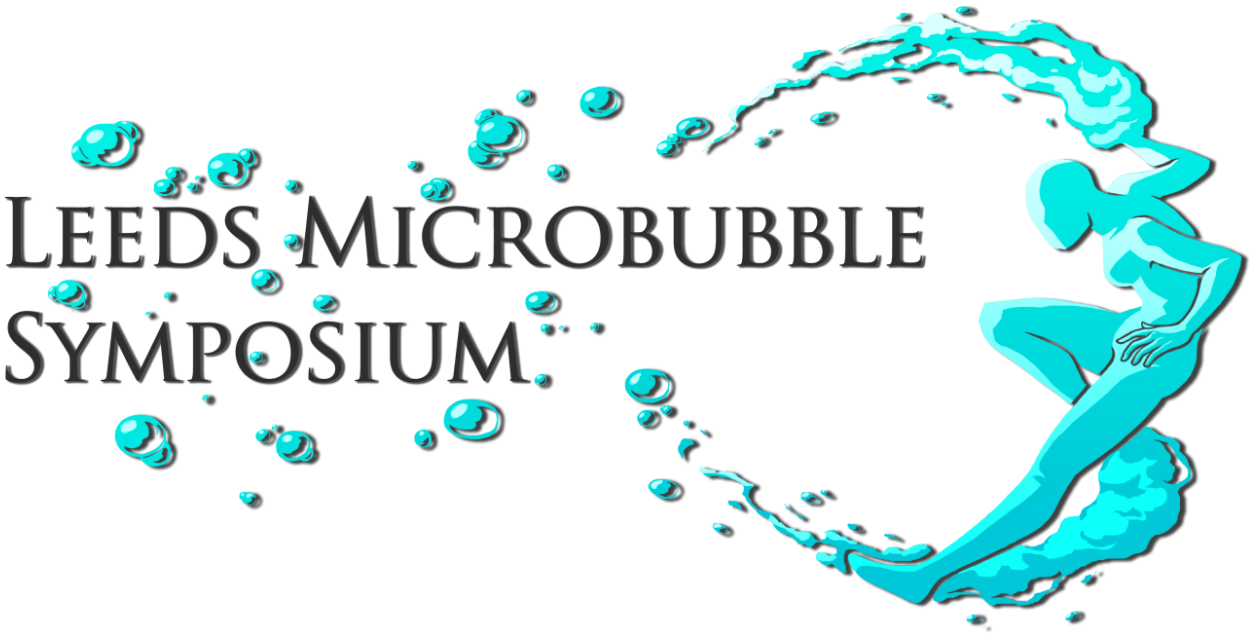
LEEDS MICROBUBBLE SYMPOSIUM



Microbubble and Nanobubbles:

From Fundamentals to Application

15th & 16th July 2024

Lawnswood Suite, Weetwood Hall, Leeds, LS16 5PS

Abstract Booklet

Abstract book can be downloaded from:
https://microbubbles.leeds.ac.uk/microbubble-symposium/

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Session 1	**Microbubble Fundamentals 1**
Name & Affiliation	**Michel Versluis,** Physics of Fluids dept, TechMed Centre, University of Twente
Title	**Contrast-enhanced blood flow quantification: from bench to bedside**

Abstract

Cardiovascular disease is the leading cause of death in Europe, with an increasing prevalence worldwide. Current monitoring and subsequent treatment options only consider very simple general geometric parameters, while patients could benefit substantially from integrating personalized features such as local blood flow patterns into therapy planning. Imaging endovascular flow patterns in the abdominal aorta and peripheral arteries bear a particular clinical relevance because of the relationship between local hemodynamics and the development of vascular diseases. Conventional Doppler ultrasound enables a one-dimensional blood flow velocity estimate in the direction of wave propagation. However, the vessels of interest typically lay parallel to the transducer surface, making it difficult to obtain reliable flow quantification. Ultrasound particle image velocimetry, dubbed echoPIV, can obtain two-dimensional blood flow velocity fields. Recent developments in the use of contrast-enhanced ultrasound have improved the clinical potential of quantifying blood flow with echoPIV. Here we discuss a series of studies that investigate the feasibility of echoPIV to quantify blood flow in the human abdominal aorta and peripheral arteries. We developed in vitro models of averaged and personalized aortic flow phantoms on the bench showing the potential of abdominal contrast-enhanced echoPIV at high frame rate. The system was further evaluated through the insertion of endovascular stents. The quantification of blood flow patterns within stented vessels, though challenging, is clinically important since it is suspected to be related to stent failure. Moreover, the current gold standard clinical modality, i.e. phase contrast MRI or 4D flow MRI, can be limited by metal-related artefacts. We found that high-frame rate echoPIV is a reliable method to quantify two-dimensional flow in both healthy and diseased subjects and can quantify flow velocities up to 1 m/s, that are typical for the human abdominal aorta and superficial femoral arteries. We will also discuss strategies to achieve time-resolved 3D blood flow quantification and higher precision through the development of novel contrast-based super-resolution imaging strategies.

Session 1	**Microbubble Fundamentals 1**
Name & Affiliation	**Nikos Pelekasis,** Dpt. Mechanical Engineering, University of Thessaly, Volos, Greece
Title	**Numerical simulations and stability of pulsating and translating coated microbubbles in the presence of viscoelastic, acoustic and adhesive forces**

Abstract

Nikos Pelekasis, Vlachomitrou, Lytra, Rosios Dpt. Mechanical Engineering, University of Thessaly, Volos, Greece

The impact that the onset of the compression-only behavior[1] of lipid shelled contrast agents bears on their dynamic interaction with a rigid wall under acoustic disturbances is investigated numerically in the context of axisymmetry. As the standoff distance from the wall is reduced it favors asymmetry by altering the compressed buckled shape around which the bubble oscillates. Above the amplitude threshold for parametric shape mode excitation, the onset of compression-only in the vicinity of a rigid wall typically interrupts the process of entrapment by reversing the direction of motion via the adverse pressure drug that is generated as a result of the emerging concave upwards buckled shapes[2]. Below this amplitude threshold symmetric shapes or asymmetric shapes that are concave downwards continue to translate towards the wall where they perform saturated trapped pulsations around nearly spherical flattened or concave downwards buckled shapes. The latter shapes perform compression only type pulsations and arise on the longer time scale required for the destabilization of the nearly spherical initially trapped shapes. Phase diagrams are constructed identifying regions of trapped pulsations, compression-only response and microbubble collapse, in the parameter space defined by sound amplitude and shell viscoelastic properties. Contour plots of Eulerian acoustic streaming patterns are also constructed.
A similar mechanism controls the translational motion of coated microbubbles in the presence of an imperfection in their initial shape, in the absence of wall interaction. Depending on the extent and location of the imperfection, buckling of the shell occurs at a small sound amplitude favoring the onset of asymmetric shapes that instigate translational motion in the direction of concavity. The speed of translation increases with increasing level of concavity as the amplitude increases, while exhibiting a maximum at the resonance frequency of the buckled shape for fixed sound amplitude. The above mechanism is conjectured to determine the experimentally observed translational motion of engineered microswimmer particles employed in various biomedical applications[3].
The impact of wall interaction on resonance frequency is also investigated in the context of coated microbubbles that adhere on a solid substrate, via a simplified approach that eliminates the need to discretize the contact region between the shell and the wall. Preliminary simulations are presented indicating the onset of dimpled shapes and the impact on resonance frequency is investigated via global stability analysis.

[1] Pelekasis, Vlachomitrou & Lytra "Acoustic interrogation of coated microbubbles: Viscoelastic properties and the onset of compression only behavior," Phys. Rev. Fluids 7, 003600, 2022.
[2] Vlachomitrou & Pelekasis, The compression-only behavior of coated microbubbles in a wall restricted flow, J. Acoust. Soc. Am. 155 (1), January 2024.

[3] Chabouh, Mokbel, van Elburg, Versluis, Segers, Aland, Quilliet & Coupier, Coated microbubbles swim via shell buckling, Communications Engineering, (2023) 2:63.

Session 1	**Microbubble Fundamentals 1**
Name & Affiliation	**Sam Sloan,** University of Southampton
Title	**Using High-Speed Imaging and Digital Image Correlation to Compare Microbubble-Induced Deformation in Stiff and Soft Cells**

Abstract

Sonoporation is a critical mechanism underpinning the therapeutic effects of microbubbles. It refers to the permeabilisation of a cell likely caused by the strain imparted by ultrasound-stimulated microbubbles. While sonoporation is a well-documented behaviour, it remains poorly understood how microbubbles induce cell strain and how this correlates with membrane permeabilisation and drug delivery. Here, we use high-speed imaging of microbubble-cell interactions alongside digital image correlation (DIC) to better elucidate the mechanical interactions between ultrasound-stimulated microbubbles and cells. Furthermore, we use this technique to determine the effect of the disruption of cytoskeletal structure in cells on stress cytoplasmic propagation.

MG63 osteosarcoma cells were cultured on a glass slide integrated with a PDMS manifold. Microbubbles, made through sonication of DSPC:PEG40s (9:1 molar ratio) lipid films, were then exposed to ultrasound generated by a 1MHz transducer. Imaging was performed using a Hypervision HPV-X high-speed camera attached to an Olympus XI-71 inverted microscope and captured at 5Mfps. DIC was performed using MatchID 2D software to resolve the 2D displacement of the cells' surface between each frame. The displacement magnitude against time was then fitted to a sine curve, and parameters, such as displacement amplitude, phase change and R^2 of fit can be mapped across the cell surface.

R^2 was used to determine the "goodness of fit" to the sine curve for each point, and points with an $R^2 < 0.8$ were excluded from further analysis. Cell deformation decayed rapidly with increasing distance from the microbubble. Three possible strain decay models with distance from the microbubble were fit across all data sets, and R^2 values were used to determine the model that most accurately captures the experimental behaviour. Exponential decay was found to fit the best, $R^2 = 0.71 \pm 0.21$, when compared to linear, $R^2 = 0.55 \pm 0.19$ ($p < 0.0001$), and inverse square $R^2 = 0.65 \pm 0.25$ ($p < 0.001$). "Half decay distance" ($D_{1/2}$) was used as a measure of the rate of decay between untreated and cytochalasin-treated cells. A non-significant decrease in $D_{1/2}$ was observed in the cytochalasin-treated cells, $D_{1/2} = 3.7 \pm 1.8$ µm (n=35), when compared to the untreated control cells, $D_{1/2} = 3.1 \pm 1.7$ µm (n=15).

Microbubble-cell interactions can be imaged at 5Mfps with a high enough resolution and contrast to successfully resolve the 2D displacement of the cell surface using DIC. Further, displacement amplitude decays exponentially with distance from the microbubble, indicating that damping is the driving factor in the decay. Cell deformation in the cytochalasin-treated cells appears to decay quicker than in the untreated control group. These results could have a large impact in cell and tissue, targeting and stiffness modification to optimise microbubble-enhanced ultrasound-mediated drug delivery.

Session 2	**Microbubble Fundamentals 2**
Name & Affiliation	**Mark Borden,** University of Colorado Boulder
Title	**Nanobubble Echogenicity and Microbubble Pharmacokinetics**

Abstract

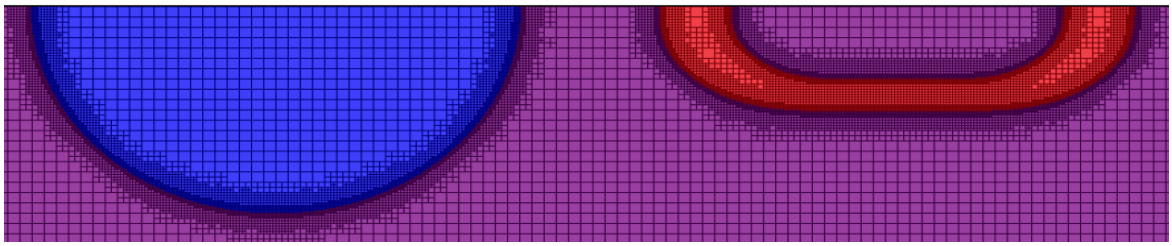
The first part of this talk will focus on our work regarding nanobubble echogenicity. Microbubbles (1–10 μm diameter) have been used as conventional ultrasound contrast agents (UCAs) for applications in contrast-enhanced ultrasound (CEUS) imaging. Nanobubbles (<1 μm diameter) have recently been proposed as potential extravascular UCAs that can extravasate from the leaky vasculature of tumors or sites of inflammation. However, the echogenicity of nanobubbles for CEUS remains controversial owing to acoustics modeling that has shown very low ultrasound backscatter. We hypothesize that microbubble contamination in nanobubble formulations may explain the discrepancy. To test our hypothesis, we examined the size distributions of lipid-coated nanobubble and microbubble suspensions using multiple sizing techniques, examined their echogenicity in an agar phantom with fundamental-mode CEUS at 7 MHz and 330 kPa peak negative pressure. We found that nanobubble formulations contained a small contamination of microbubbles. Once the contribution from these microbubbles is removed from the acoustic backscatter, the acoustic contrast of the nanobubbles was shown to be near noise levels. This result indicates that nanobubbles have limited utility as UCAs for CEUS.

The second part of this presentation will focus on results from our recently developed technique to directly measure the microbubble size and concentration in blood samples following intravenous injection. Using this technique, we have investigated the effects of lipid-coated microbubble size, dose and PEG content on their pharmacokinetic (PK) profile. We found that representing the data as microbubble volume dose (MVD) in units of μL/kg versus time was most beneficial for fitting the PK models. Our results showed that at low doses and/or small sizes, the curves are mono-exponential and follow a single compartment PK model. At larger doses and sizes, however, the curves are bi-exponential and follow a two-compartment PK model. Additionally, we studied the effect of PEG content on the lipid shell. Surprisingly, our results showed that increasing PEGylated lipids on the microbubble shell can increase complement C3 protein activation in vitro and lead to faster elimination kinetics in vivo.

Bio. Mark Andrew Borden is a Professor of Biomedical Engineering and Mechanical Engineering at the University of Colorado, Boulder. He received the B.S. in Chemical Engineering from the University of Arizona in 1999 and the Ph.D. in Chemical Engineering from the University of California Davis in 2003. He then worked as a postdoctoral researcher in Biomedical Engineering at UC Davis and visiting scientist in Radiology at the Arizona Cancer Center. He was appointed Assistant Professor of Chemical Engineering at Columbia University in 2007 before moving to CU Boulder in 2010. He served as the Inaugural Director of Biomedical Engineering at CU Boulder, leading the program from conception through accreditation. He currently serves as a technical program committee member of the IEEE International Ultrasonics Symposium and the European Ultrasound Contrast Symposium. His honors include an NSF CAREER Award, James D. Watson Investigator Award and multiple department awards.

Session 2	**Microbubble Fundamentals 2**
Name & Affiliation	**Andreas Papoutsakis,** University of Hertfordshire.
Title	**Investigation of inertia bubble collapse for the solid liquid gas interactions observed in bacteria incapacitation configurations.**

Abstract



Water sanitization [1] and sterilization [2] constitute a global scale human activity with major implications on public health. Non-chemical approaches to water treatment reduce the evolutionary pressure responsible for Drug-Resistant infections. Furthermore, Ultra-Sound (US) treatment of water presents the scalability potential towards widespread application. US water treatment takes advantage of the induced cavitation of microbubbles dispersed in the water. The energy focusing properties of microbubbles during US excitement result in impinging micro-jets which are intrinsically directed towards the closest solid boundary and mechanically incapacitate bacteria and protozoa [3].

Bacterial incapacitation, along with drug delivery by inertia bubble collapse, involves the interfacial interaction between the gaseous and the liquid phases in a bubbly environment in the proximity of soft matter. In these multi-material configurations, the different materials and phases interact across a dynamic network of interfaces, i.e.: Fluid-Gas and Fluid-Vapour interfaces, Gas-Gas shocks, Cracks and Boundary Layers, which introduce fine temporal and spatial scales [4].

Traditionally, multi-material configurations are dealt by the weak coupling of Lagrangian domains and are solved independently. The coupling is achieved by immersed boundary approaches or Arbitrary Eulerian Lagrangian methods. Recently, Fully Eulerian approaches, like the Diffused Interface Method (DIM) have been introduced towards a unified model [5].

In the simulation framework suggested here, we utilize the Diffused Interface Method (DIM) in a multiscale adaptive framework that allows for the modelling of all participating phases that models the full elastic stress tensor for the fluid phase. In the DIM approach, each material is modelled with an individual Equation of State [6].

Here, the scope of our analysis is to investigate the efficacy of representative cell and bubble topologies, in respect to the US wave orientation.

References:

[1] WHO "Drinking Water", Fact Sheets, Mar 2022.

[2] Wang, G. et.al. "The promise of low-intensity ultrasound" Ultr. Sonoch. Nov 2021.

[3] Zupanc, M. et.al. "Effects of cavitation on different microorganisms". Ultr. Sonoch. 2019 Oct; 57:147-165

[4] A. Papoutsakis, et. al An efficient Adaptive Mesh Refinement (AMR) algorithm for the Discontinuous Galerkin method: applications for the computation of compressible two-phase flows. JCP 363 399-427, 2018.

[5] S. Richard, C. Pantano. Diffuse-Interface Capturing Methods for Compressible Two-Phase Flows. Annu. Rev.Fluid Mech. 105-130, 50(1) 2018.

[6] E. Koukas, A. Papoutsakis, M. Gavaises. Numerical investigation of shock-induced bubble collapse dynamics and fluid–solid interactions during shock-wave lithotripsy. Ultrasonics Sonochemistry. Volume: 95, 2023.

Session 2	**Microbubble Fundamentals 2**
Name & Affiliation	**Theresa Kosmides,** Case Western Reserve University
Title	**Nanobubble Formulation and Size Isolation: An Investigation into Critical Process Steps**

Abstract

Nanobubbles (NBs) are submicron, lipid shelled nanoparticles which encapsulate a gas core. These nanoparticles can be used as ultrasound (US) contrast agents in a variety of medical applications ranging from disease diagnosis to treatment. Currently, there are multiple methods used to produce NBs, such as mechanical agitation, extrusion, and microfluidics. In this work, we investigate how the dispersion of lipids in the precursor emulsion affects the formation of NBs created by mechanical agitation as well as the effect of pressurized size isolation on NBs.

First, lipid emulsion samples were subjected to three sonication conditions: no sonication, bath sonication, and probe sonication. The size of particles in the lipid emulsions was determined by dynamic light scattering (DLS). The samples were then used to make NBs. For the size isolation investigation, the polydisperse activated suspension was transferred to a non-pressurized tube prior to differential centrifugation. The resulting NB size and concentrations were determined by DLS and resonant mass measurement (RMM) respectively, and ultrasound signal intensity and stability were tested in an agarose phantom under the following conditions: 18MHz fc, 4% power, 1fps on a VisualSonics Vevo2100 system.

As the sonication intensity increased (no, bath, and probe sonication), the lipid emulsion polydispersity index (PDI) decreased (Fig. 1A). Despite the difference in PDI, there was no significant difference in diameter or concentration of the NBs between sonication conditions. The NBs from all sonication conditions were stable under US. Bath and no sonication had comparable US signal intensities while the probe sonicated condition had a lower US signal intensity (Fig. 1B). There was no significant difference in diameter or concentration between NBs that were size isolated in a non-pressurized system compared to a pressurized isolation (Fig. 1C). The NBs from the non-pressurized isolation had a lower nonlinear contrast (NLC) US signal intensity and higher signal variability compared to NBs from pressurized isolation (Fig. 1D).

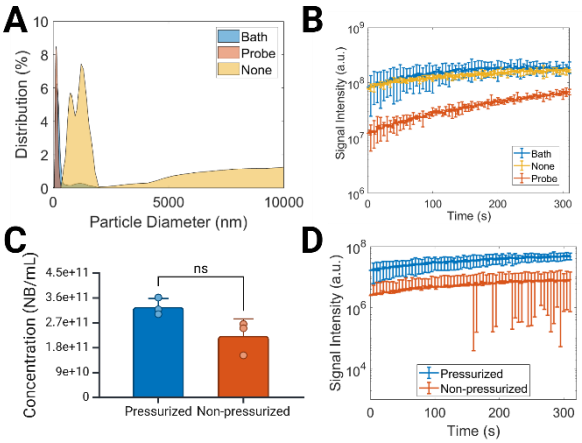


Figure 1. (A) Intensity weighted size distribution of precursor lipid emulsion. (B) Acoustic characterization of NB from sonicated precursor emulsions under US in agarose phantom. (C) Concentration of buoyant particles from pressurized, non-pressurized size isolation. (D) Acoustic characterization of NB from pressurized, non-pressurized size isolation under US in agarose phantom.

We demonstrated that a monodisperse precursor emulsion is not required to produce monodisperse mechanically agitated NBs. Additionally, the data indicate that pressurized size isolation is not essential to separate NBs from a polydisperse suspension. However, NBs from non-pressurized size isolation have a lower signal intensity, and higher signal variability. This work suggests that there may be other critical factors in the formation and size isolation of NBs that affect size and stability under US.

Theresa Kosmides1, Agata Exner1,2
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Session 2	**Microbubble Fundamentals 2**
Name & Affiliation	**Martin van den Broek,** BIOS/Lab on a Chip group, Max Planck Center Twente for Complex Fluid Dynamics, University of Twente, The Netherlands
Title	**Interfacial rheometer for microbubble dilatational shell parameter characterization**

Abstract

The acoustic response of phospholipid-coated microbubbles is strongly affected by the viscoelastic properties of their stabilizing shell. Both the shell elasticity and shell viscosity are dependent on surface dilatation through the lipid packing density. However, there is currently no fast, reliable, and user-friendly method available to probe the surface dilation-dependent shell parameters akin to a Langmuir trough that is typically used for macroscopic flat lipid monolayers. In this work, we introduce a method to measure the shell parameters of lipid coated monodisperse microbubbles as a function of surface dilatation. The method combines ambient pressure-dependent acoustic attenuation spectroscopy, which probes the microbubble resonance behavior, with a light obscuration technique based on Mie scattering for fast and accurate microbubble sizing. This integrated approach enables swift characterization of surface dilatation-dependent shell parameters within a single 1-s experiment. We demonstrate the high potential of the method by reporting dilatation-dependent shell parameters, specifically shell viscosity, shell elasticity, and the corresponding surface tension curve as a function of microbubble surface area. Our results include data for a microbubble suspension with palmitic acid added to the shell and for varying medium salinities.

Session 3	**Microbubble Ultrasound Techniques 1**
Name & Affiliation	**Eleanor Stride**, University of Oxford
Title	**Challenges in Classifying Cavitation**

Abstract

Both the biological effects and acoustic emissions generated by cavitation are produced by a range of phenomena associated with bubble activity. Monitoring of acoustic emissions is therefore desirable to improve treatment safety and efficacy. However, the relationship between the emission spectra and bubble dynamics is complex. The aim of this study was to characterise this relationship for single microbubbles using simultaneous ultra-high-speed optical imaging and passive acoustic mapping of cavitation emissions. As expected, both the number of discrete harmonics and broadband content in the emissions increased with increasing amplitude of bubble oscillation. However, the spectral content was also dependent upon other variables, including the frequency of bubble collapse and transducer characteristics. Phenomena such as fragmentation and microjetting could not be distinguished from spherical oscillations from the integrated emission spectra. There was also no correlation between the detection of broadband noise and widely used thresholds for distinguishing bubble dynamics. It is therefore concluded that binary categorisations such as stable and inertial cavitation should be avoided and different types of bubble behaviour should not be inferred on the basis of frequency content alone. Treatment monitoring criteria should instead be defined according to the relevant bioeffect(s) for a particular application.

Session 3	**Microbubble Ultrasound Techniques 1**
Name & Affiliation	**Tim Segers**, BIOS/Lab on a Chip group, *Max Planck Center Twente for Complex Fluid Dynamics,* University of Twente, The Netherlands
Title	**Controlling the stability of monodisperse lipid-coated microbubbles by tuning their buckling pressure**

Abstract

In recent years, advancements in the microfluidic production of phospholipid-coated monodisperse microbubbles have resulted in a long shelf life, raising hopes for their in vivo applications such as enhanced contrast imaging, controlled therapy, and noninvasive pressure measurement. However, the stability of these microbubbles is highly sensitive to ambient pressure changes, posing a challenge for applications that require a stable acoustic bubble response, such as non-invasive blood pressure sensing. Upon intravenous injection, physiological pressures can cause bubble dissolution, compromising their uniformity. In this study, we demonstrate a direct relationship between shell buckling and bubble dissolution by acoustically measuring the buckling pressure and monitoring the response of bubbles to controlled pressure changes. We found that the fraction of PEGylated lipid, essential for stable coalescence-free microfluidic bubble production, can be adjusted to tune the buckling pressure, thereby enhancing bubble stability. This adjustment can be made either during bubble production or more conveniently through a post-production heating process. The heating step exploits the increased mobility of phospholipids within the shell upon their phase change, enabling the selective expulsion of PEGylated lipids. Reducing the molar fraction of PEGylated lipid from 10 mol% to 1.5 mol% increases the buckling pressure from 0 kPa to 27 kPa, exceeding physiological pressure levels and significantly improving bubble stability for clinical applications.

Session 3	**Microbubble Ultrasound Techniques 1**
Name & Affiliation	**Hilde Metzger,** University of Glasgow
Title	**Characterising the steady-state cavitation of a range of UCA formulations with high-speed imaging and parallel acoustic detection**

Abstract

Hilde Metzger, Faraz Amini Boroujeni, Paul Prentice, University of Glasgow

The deployment of ultrasound contrast agents (UCAs) for therapeutic and diagnostic applications is rapidly increasing. This is resulting in new generations of UCA formulations and architectures that are more targeted towards their specific application. The exploration of the effects of the gas core composition, shell structure and compounds bound to the shell is a pertinent subject. Although these properties are important for the chemical and physical attributes of UCAs in the specific context of their applications, their effects on their cavitation behaviour have not been studied in detail.

In this study, the steady state cavitation behaviour is characterised for a range of UCAs (both commercial and novel ones designed by different research groups) by means of high-speed imaging with parallel acoustic detection. Each UCA suspension was injected into a 500 µm diameter polycarbonate capillary at a concentration low enough to allow for single bubble cavitation in the acoustic field-of-view of the hydrophone. The capillary was placed in the focus of a HIFU transducer (H-149, Sonic Concepts) which was driven with 200 cycles at a frequency of 692 kHz over a range of peak-negative pressures (220-540 kPa), via a signal generator (DG4102, Rigol, Agilent) and power amplifier (1040L, E&I). The high-speed camera (HPV-X2, Shimadzu) with a 5x objective lens observed the cavitation at a frame rate of 10 million frames per second to allow for temporal resolution of the bubble oscillations.

As way of analysing the cavitation dynamics for the different UCAs, the high-speed imaging frames, acoustic spectra and time domain shockwave emissions of each are compared for the range of pressures. The pressure threshold at which f0/2 subharmonics are detected in the spectrum, with their corresponding period-doubled oscillations in the high-speed imaging and period-doubled shockwave emission in the time domain signal, is used for quantitative comparison. The results from this suggest that after the onset of cavitation, the bubble oscillations and resulting acoustic emissions are dependent on the driving pressure amplitude, regardless of the UCAs' chemical and physical properties.

Session 3	**Microbubble Ultrasound Techniques 1**
Name & Affiliation	**Guillaume Lajoinie**, University of Twente
Title	**Cascaded waves for flow imaging**

Abstract

Quantitative imaging of blood flow in paramount to improving the diagnosis of cardiovascular diseases such as stenoses or aneuryms. Accurate flow imaging can allow for investigating the pathogenesis of the diseases, better assess the risks, predict the development, and design personalized treatment therapies. Vector flow imaging has improved flow quantification by improving both the spatial and the temporal resolution, while overcoming the directivity limitations of Doppler. However, vector flow imaging suffers from low SNR and requires extensive spatiotemporal filtering, even when using ultrasound contrast microbubbles. This low SNR has a direct impact on flow quantification accuracy.

Here, we develop the concept of cascaded waves introduced in [1-2]. We introduce a new time-delay encoding strategy and the associated frequency-domain decoding. The technique is compatible with nonlinear contrast and allows for using single pulse trains, thereby solving the issue of motion decorrelation for fast arterial flows. Furthermore, we show both experimentally and for synthetic data that the use of a single pulse train can provide a 6 dB SNR improvement, which translates into an up to tenfold decrease in flow quantification uncertainty.

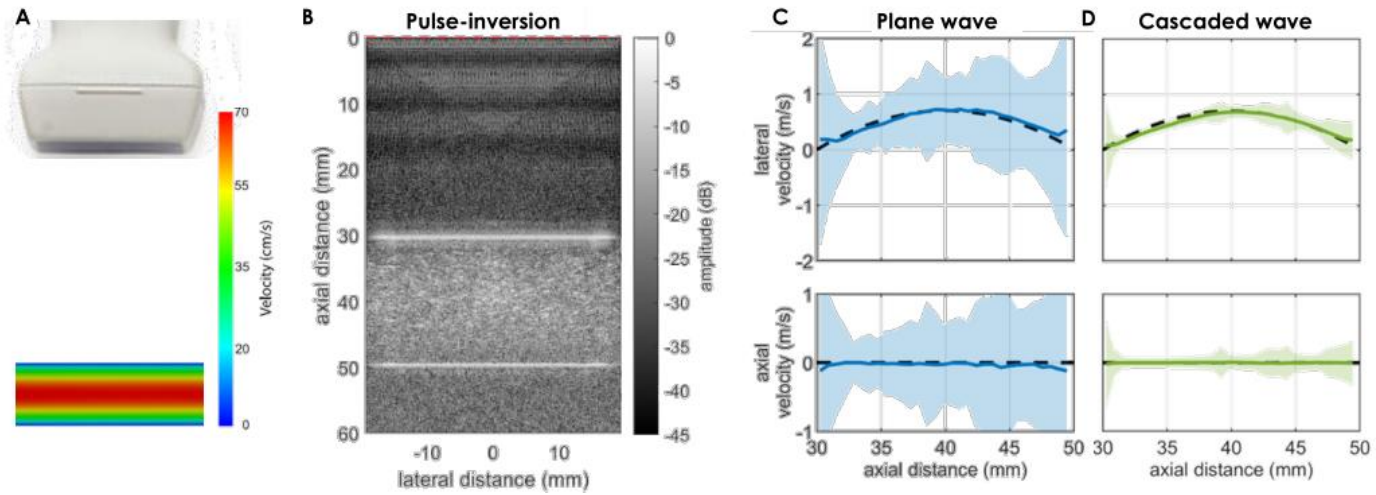


Figure 1: A. simulated geometry and flow. 50 thousand nonlinear bubbles flow in a 2 cm-diameter pipe with a peak velocity of 0.7 m/s. The bubbles are irradiated by a digital L12-3v transducer at 500 kPa. B. Simulated pulse-inversion image showing the nonlinear bubbles in the tube. C. Velocity profiles extracted through PIV processing. The black dashed line is the ground truth. The solid line is the mean estimated velocity, showing the systematic error. The shaded area is the standard deviation, representing the random error. D. Same information as in C., when using cascaded waves.

[1] Zhang Y, Guo Y, Lee WN. Ultrafast Ultrasound Imaging With Cascaded Dual-Polarity Waves. IEEE Trans Med Imaging. 2018 Apr;37(4):906-917. doi: 10.1109/TMI.2017.2781261. PMID: 29610070.

[2] N. Bottenus, "Improving signal-to-noise ratio through generalized multipulse transmit encoding," 2021 IEEE International Ultrasonics Symposium (IUS), Xi'an, China, 2021, pp. 1-4, doi: 10.1109/IUS52206.2021.9593443.

Session 4	**Microbubble Ultrasound Techniques 2**
Name & Affiliation	**Emma Harris,** Institute of Cancer Research, London, UK
Title	**Preclinical investigations of microbubbles for improving radiotherapy**

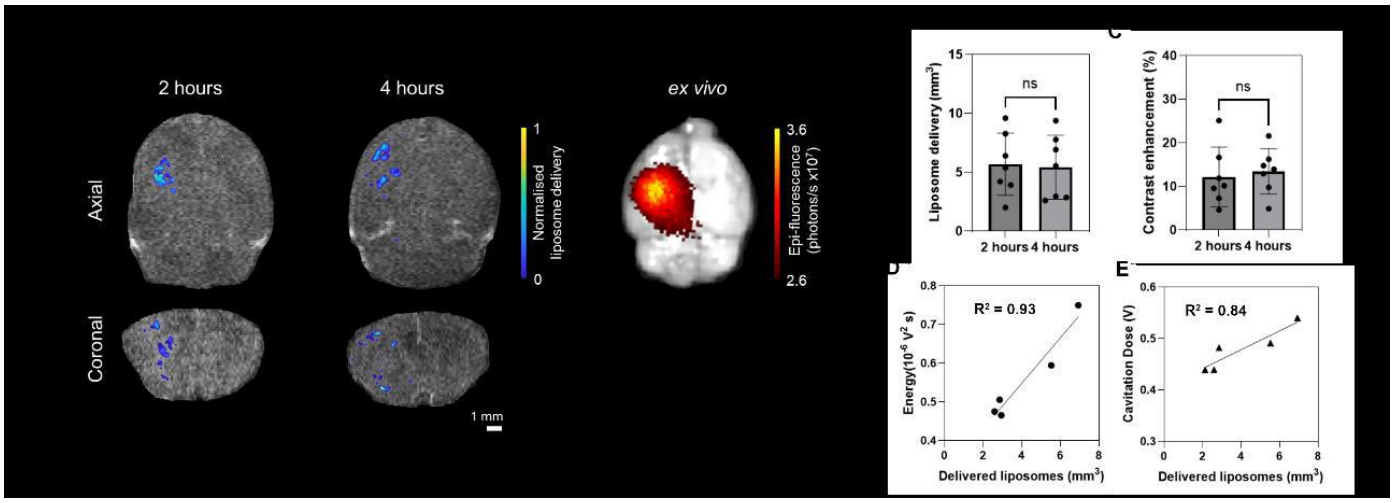
Abstract

The efficacy of radiotherapy is likely to influenced by the tumour vasculature. Poor blood supply can limit oxygen transport leading to hypoxia, which reduces the effectiveness of radiation therapy to damage DNA. Furthermore, tumour infiltrating lymphocytes require functioning vasculature to elicit an anti-tumour immunogenic response to radiation. This talk will focus on two ways in which microbubbles injected into the vasculature may be used to improve how we treat cancer with radiotherapy. The first part investigates the potential for dynamic contrast enhanced ultrasound (DCE-US) metrics as predictive imaging biomarkers of tumour vascular response to radiation to help clinicians manage treatment. We explored differences in DCE-US metrics both before and immediately after radiation in two models of cancer (head and neck cancer and cervical cancer). We investigated sources of variability in the measurement of DCE metrics, and compared metrics derived from 2D and 3D DCE-US. The second part of this talk explores how microbubbles stimulated with low-intensity ultrasound may be used to radiosensitise tumours. We explored the effect of ultrasound-stimulated microbubbles (USMB) on the tumour microenvironment including the vasculature, in two syngeneic murine models of head and neck cancer with different immune microenvironment phenotypes. We trialled DCE-US and photoacoustics for characterisation of the effect of USMB in vivo as well as exploring histopathological read-outs of response.

Session 4	**Microbubble Ultrasound Techniques 2**
Name & Affiliation	**Chris Payne**, Kings' College London
Title	**Thermosensitive liposome delivery to the brain after FUS-induced blood-brain barrier opening for brain tumour treatment**

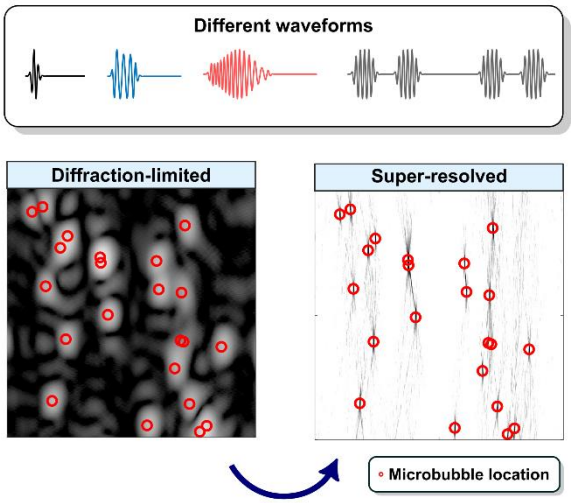
Abstract

Objectives
To enhance and quantify the delivery of thermosensitive liposomes (iTSL's) to the brain of wild-type mice using MRI and fluorescence (NIRF) imaging after FUS-induced BBB opening.
Methods
FUS-induced BBB opening was performed in 7 mice (frequency = 0.5 MHz, PNP = 320 ± 30 kPa, cycles = 500, PRF = 5 Hz, pulses = 600, location = -2 mm lateral, +2 mm ventral from lambdoid suture). SonoVue microbubbles and iTSL's were administered intravenously during sonication. Passive cavitation detection (PCD) was performed during sonications for real-time monitoring. T_1-weighted MRI was performed 2 and 4 hours post sonication. NIRF imaging was performed on ex vivo brains.
Results
MRI and NIRF imaging confirmed localized delivery of iTSLs where BBB opening was performed compared to the contralateral control side (Fig 1A). Delivered liposome volume and liposome-induced contrast enhancement (Fig 1B-C) was 5.7 ± 2.6 mm_3 and 12.2% at 2 hours, 5.4 ± 2.7 mm_3 and 13.4% at 4 hours. No significant difference was observed between time points (P = 0.86 and P = 0.70 respectively, two-tailed t test). Total acoustic energy and cavitation dose (Fig 1D-E) correlated linearly with delivered liposome volume observed from MRI (R_2 = 0.93 and 0.84 respectively, n = 5).
Conclusion
These results suggest that MRI/NIRF-tagged drug loaded liposomes are suitable drug carriers for the treatment of brain tumors using FUS-induced BBB opening and both PCD and MRI provide useful prognostic information on treatment success. Future work will focus on delivering drug-loaded iTSL's in orthotopic brain tumor mouse models.
Acknowledgements
This project was funded by The Little Princess Trust and Innovate UK.



Session 4	**Microbubble Ultrasound Techniques 2**
Name & Affiliation	**Rienk Zorgdrager,** University of Twente
Title	**Waveform-Specific Performance of Deep Learning-Based Ultrasound Super-Resolution Models**

Abstract



Resolving arterial flows is essential for understanding cardiovascular pathologies, improving diagnosis, and monitoring patient condition. Ultrasound contrast imaging uses microbubbles to enhance the scattering of the blood pool, allowing for real-time visualization of blood flow [1]. Recent developments in vector flow imaging further expand the imaging capabilities of ultrasound by temporally resolving fast arterial flow [2]. The next obstacle to overcome is the lack of spatial resolution. Super-resolved ultrasound images can be obtained by deconvolving radiofrequency (RF) signals before beamforming, breaking the link between resolution and pulse duration. Convolutional neural networks (CNNs) can be trained to locally estimate the deconvolution kernel and consequently super-localize the microbubbles directly within the RF signal [3]. However, microbubble contrast is highly nonlinear, and the potential of CNNs in microbubble localization has not yet been fully exploited. Assessing deep learning-based deconvolution performance for non-trivial imaging pulses is therefore essential for successful translation to a practical setting, where the signal-to-noise ratio is limited, and transmission schemes should comply with safety guidelines. In this study, we train CNNs to deconvolve RF signals and localize the microbubbles driven by harmonic pulses, chirps, or delay-encoded pulse trains. We find that, whereas the CNNs can accurately localize microbubbles for all pulses, a short imaging pulse offers the best performance in noise-free conditions. However, chirps offer a comparable performance without noise, but are more robust to noise and outperform all other pulses in low-signal-to-noise ratio conditions.

Sources:

[1] E. Quaia, "Assessment of tissue perfusion by contrast-enhanced ultrasound," *Eur. Radiol., vol. 21, pp. 604–615, Oct. 2010.*

[2] K. L. Hansen, M. B. Nielsen, and J. A. Jensen, "Vector velocity estimation of blood flow – a new application in medical ultrasound," *Ultrasound*, vol. 25, no. 4, pp. 189–199, Nov. 2017.

[3] N. Blanken, J. M. Wolterink, H. Delingette, C. Brune, M. Versluis, and G. Lajoinie, "Super-resolved microbubble localization in single-channel ultrasound RF signals using deep learning," *IEEE Trans. Med. Imag.*, vol. 41, no. 9, pp. 2532–2542, Sep. 2022.

Session 5	**Microbubbles: Towards Translation 1**
Name & Affiliation	**Klazina Kooiman,** Erasmus MC, Biomedical Engineering, Dept. of Cardiology
Title	**Elucidating mechanisms of microbubble-mediated vascular permeabilization for local drug delivery**

Abstract

Ultrasound-activated microbubbles can locally increase vascular permeabilization to enhance drug delivery. Vascular permeabilization includes the poration of the cell membrane (i.e., sonoporation), which promotes drug uptake into endothelial cells, and endothelial gap formation by either tunnels through cells or openings between cells, which increases vascular permeability [1,2]. However, the microbubble–cell–drug interaction is not fully understood, which is hindering controlled and optimal therapeutic outcomes. With the use of vessels-on-a-chip and a custom-built Nikon A1R+ microscope coupled to an ultra-high-speed camera [3], we have been simultaneously studying the cellular response to ultrasound-activated microbubbles in micrometer and microsecond resolution. Endothelial cells in a 100% confluent monolayer, either grown in 2D or 3D on a chip, were used to assess sonoporation, tunnel formation, cell-cell contact opening and F-actin remodeling upon ultrasound-activated microbubble treatment (2 MHz, 90-750 kPa PNP, 10 or 1000 cycles). When endothelial cells were transfected with fluorescent cytoskeleton F-actin and grown in 2D, disruption of the F-actin by oscillating microbubbles (350 kPa, 10 cycles) was only observed when cells were also sonoporated. When the F-actin was disrupted only, i.e., there was no recovery of the F-actin during the 4-min microscopy recording, tunnel formation was mainly induced (75%). On the other hand, when F-actin stress fibers were severed and as a consequence recoiled, cell-cell contact opening within 15 s upon treatment (54%) and tunnel formation (15%) were mainly induced. The severing of F-actin stress fibers occurred when they were within reach of the microbubble's maximum radius during oscillation, requiring normal forces of ≥230 nN. As it is challenging to investigate drug delivery beyond the endothelial layer in the current 2D models, we developed a microvessel-on-chip model with a perfused lumen and extravascular space. Although sonoporation could be induced in the microvessel-on-chip with both 10 cycles and 1000 cycles of ultrasound, albeit it at different PNP namely at 750 kPa for 10 cycles and at 220-750 kPa for 1000 cycles, there were more distinct differences in the induction of vascular permeability between these two cycle lengths. At 10 cycles, the vascular permeability did not significantly increase until 750 kPa PNP, while this was 550-750 kPa PNP for 1000 cycles. Both the onset time and rate of increased vascular permeability were slower for 10 cycles in comparison to 1000 cycles. For both cycle lengths, the microvessels showed varying numbers and uneven distributions of initial spots of increased permeability, likely due to differences in the concentration of microbubbles. Together, these findings give new insights into the microbubble-cell-drug interactions needed to induce vascular permeabilization for local drug delivery.

References: [1] Kooiman et al., Ultrasound Med Biol 2020, 46:p.1296; [2] Beekers et al., J Control Rel 2022, 347:460; [3] Beekers et al., Ultrasound Med Biol 2019, 45:p.2575.

Session 5	**Microbubbles: Towards Translation 1**
Name & Affiliation	**Damien Batchelor,** University of Leeds
Title	**Characterisation of microbubble-mediated *S. aureus* biofilm removal in central venous catheter models**

Abstract

Staphylococcus aureus is a bloodstream infection that has mortality rates of 17-46% and is a daily occurrence, with over 12,700 cases in England per year. A leading cause of bloodstream infections is due to the growth of *S. Aureus* biofilms within central venous catheters (CVC). Traditionally, CVC infections are treated through the means of an "anti-biotic lock". Here, we use ultrasound (US) and microbubbles (MB) to reduce biofilm biomass, to increase effectiveness and reduce treatment time required for anti-biotic lock therapy.

S. Aureus biofilms were grown in the inner lumen of polyurethane catheter mimics, and treated with MBs and US (1.1 MHz, 240 – 2500 kPa,1 kHz PRF, 10 µs pulse length). US treatment was performed by rastering (1 mm/s) a focused ultrasound transducer across the entire length of the catheter, filled with MBs (4x10^8/ mL). MB behaviour within the catheter during insonation was investigated using optical imaging and passive cavitation detection (PCD), whilst biofilm biomass was assessed using a crystal violet assay.

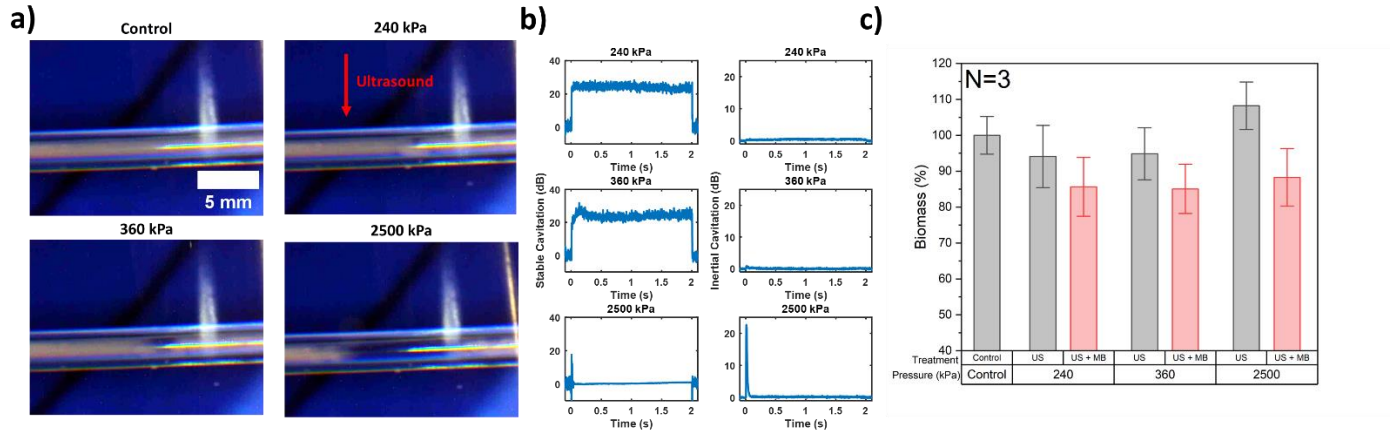


Figure 2 a) Optical images of microbubble-filled (4x10^8 /mL) catheter sections before and after ultrasound treatment, at varying peak negative pressures. b) Passive cavitation data assessing the occurrence of stable and inertial microbubble cavitation during ultrasound treatment. c) Biofilm biomass after ultrasound (HIFU) and microbubble (HIFU + MB) treatment.

At lower pressures (240, 360 kPa) microbubbles were observed to translate towards the distal side of the catheter, due to the acoustic radiation force whilst PCD data showed the occurrence of stable cavitation throughout the US exposure, with minimal inertial cavitation. At 2500 kPa, there was near instant MB destruction correlating with PCD data: minimal stable cavitation was recorded, likely due to an initial spike in broadband noise, and hence MB destruction.

For all US and MB treatment conditions, biofilm biomass dropped by ~ 15 % compared to no treatment and US only controls. Future work will consist of combining US + MB treatment with antimicrobials, assessment of biofilm metabolic activity, as well as the use of affimer-coated MBs, targeted specifically to S. Aureus.

Session 5	**Microbubbles: Towards Translation 1**
Name & Affiliation	**Paul Cressey**, King's College London (KCL)
Title	**Microbubble blood brain barrier opening for the delivery of imageable thermosensitive liposomes for glioblastoma treatment.**

Abstract

Glioblastoma is an extremely aggressive brain tumor and currently has limited effective treatment options, with median survival of only 14 months.[1] This low survival is partially attributed to the blood-brain barrier (BBB), especially in early-stage glioblastoma.[2] In this study, we formulated a PET imageable drug-loaded thermosensitive liposomes (iTSL, fig 1A) and tested their efficacy against U87-MG *in vivo* (subcutaneous). We performed focused ultrasound (FUS)-induced BBB opening (BBBO) to deliver the iTSLs into wild-type mice. Liposome uptake in the brain following BBBO was determined and quantified using PET imaging and gamma counter measurements.

To incorporate the ^{89}Zr radioisotope into the formulation, a DSPE-PEG$_{2000}$.-DFO lipidic chelating agent was synthesized. The radiolabeling of the iTSL was carried out at 60 °C for 1 h with a radiolabeling efficiency of 97 ± 2%. iTSLs were loaded with SN-38 (1.11 ± 0.08 mg/ml) and carboplatin (0.84 ± 0.14 mg/ml). BBBO was performed in mice using a 1.5 MHz transducer and 500 kPa peak-negative pressure. SonoVue microbubbles (2 ml/kg) were administered I.V during FUS treatment, the iTSLs (8 ml/kg) were administered I.V after FUS. PET images were taken at 4, 24, 48 and 72 h after BBBO, to allow time for iTSLs to accumulate in the brain for optimal contrast. Blood samples were also taken at various timepoints and measured to determine the blood PK. Finally, the ability of drug loaded liposomes to suppress tumor growth was investigated with single treatments against U87-MG subcutaneous flank xenograft tumours (10 mg/kg SN38, 42 °C, 10 min).

Thermosensitive iTSL were successfully formulated with the DSPE-PEG$_{2000}$.-DFO with a size of 96 ± 3 nm, PDI 0.25 ± 0.01. Drug-loaded iTSL (10 mg/kg SN-38) treatment caused an increase in survival of mice with xenograft tumours when administered alone and a significant increase in survival and tumor uptake when combined with FUS (42 °C, 10 min) (Fig. 1. B). PET imaging confirmed localized increased uptake of the iTSL following FUS-induced BBBO, when compared to non-sonicated controls (Fig 1. C). These results suggest that drug loaded liposomes are a suitable treatment option for glioblastoma when combined with FUS-induced BBB opening. Future work includes iTSL delivery in orthotopic brain tumours and evaluation of survival benefit in tumor-bearing mice.

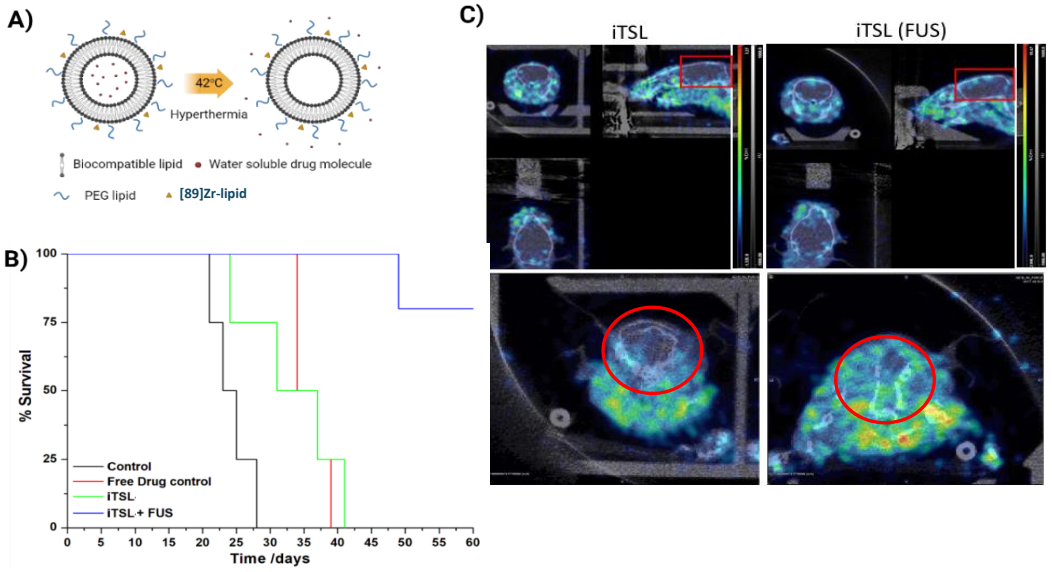


Fig.1 A) Graphical representation of iTSL design. B) Keplan-Meier curve representing treatment of: PBS, carboplatin + irinotecan (10 mg/kg), iTSL (10 mg/kg SN-38) and iTSL +FUS (10 mg/kg SN-38, 42 °C, 10 min) against U87-MG subcut xenograft tumors. C) Example brain PET images of a non-tumour bearing mouse 4h after iTSL administration with and without BBBO (FUS) (Red circles are used to highlight the brain area).

References: 1. S. Mohammed, et al., Rep. Pract. Oncol. Radiother., 2022, 27, 1026–1036, 2. J. N. Sarkaria, et al., Neuro Oncol, 2018, 20, 184–19, 3. Ingram et al. Theranostics, 2020, 10973-10992. **Acknowledgements**: Catapult for helping with the PET imaging and radiolabeling. All animal procedures were conducted under the U.K. Home Office regulations and the Guidance for the Operation of Animals (Scientific Procedures) Act (1986).

Session 5	**Microbubbles: Towards Translation 1**
Name & Affiliation	**Thomas McKaig,** Ulster University
Title	**Ultrasound targeted microbubble destruction using docetaxel and Rose Bengal loaded Microbubbles for targeted Chemo-Sonodynamic therapy treatment of prostate cancer**

Abstract

Docetaxel (DTX) chemotherapy is commonly used in the treatment of patients with advanced prostate cancer demonstrating modest improvements in survival [1]. As these patients are often elderly and the chemotherapy treatment is not targeted, it is often poorly tolerated. More targeted approaches that increase therapeutic efficacy yet reduce the amount of toxic chemotherapy administered are needed [2], [3]. In this manuscript, we investigate the potential of ultrasound targeted microbubble destruction (UTMD) to deliver a combination of docetaxel chemotherapy and Rose Bengal mediated sonodynamic therapy (SDT) in pre-clinical prostate cancer models. A Rose Bengal modified phospholipid was synthesized and used as a component lipid to prepare a microbubble (MB) formulation that was also loaded with DTX. The DTX-MB-RB formulation was used in the UTMD mediated treatment of androgen sensitive and androgen resistant 3D spheroid and murine models of prostate cancer. Results from the 3D spheroid experiments showed UTMD mediated DTX-MB-RB chemo-sonodynamic therapy to be significantly more effective at reducing cell viability than UTMD mediated DTX or SDT treatment alone. In an androgen sensitive murine model of prostate cancer, UTMD mediated DTX-MB-RB chemo-sonodynamic therapy was as effective as androgen deprivation therapy (ADT) at controlling tumour growth. However, when both treatments were combined, a significant improvement in tumour growth delay was observed. In an androgen resistant murine model, UTMD mediated DTX-MB-RB chemo-sonodynamic therapy was significantly more effective than standard DTX monotherapy. Indeed, the DTX dose administered using the DTX-MB-RB formulation was 91% less than standard DTX monotherapy. As a result, UTMD mediated DTX-MB-RB treatment was well tolerated while animals treated with DTX monotherapy displayed significant weight loss which was attributed to acute toxic effects. These results highlight the potential of UTMD mediated DTX-MB-RB chemo-sonodynamic therapy as a targeted, well tolerated alternative treatment for advanced prostate cancer.

1. P. Rawla. Epidemiology of prostate cancer. World J. Oncology., 10 (2) (2019), pp. 63-89
2. D. Dearnaley, I. Syndikus, H. Mossop, V. Khoo, A. Birtle, D. Bloomfield, et al. Conventional versus hypofractionated high-dose intensity-modulated radiotherapy for prostate cancer: 5-year outcomes of the randomised, non-inferiority, phase 3 chhip trial. Lancet Oncol., 17 (8) (2016), pp. 1047-1060
3. V. Beckendorf, S. Guerif, E. Le Prisé, J.-M. Cosset, A. Bougnoux, B. Chauvet, et al. 70 gy versus 80 gy in localized prostate cancer: 5-year results of GETUG 06 randomized trial. Inter. J. Radiation Oncology*biology*physics., 80 (4) (2011), pp. 1056-1063

Session 6	**Microbubbles: Towards Translation 2**
Name & Affiliation	**Jacques Lux, Ph.D., CChem, MRSC** Associate Professor Assistant Director, Translational Research in Ultrasound Theranostics (TRUST) Program Faculty Member, Organic Chemistry & Biomedical Engineering Graduate Programs
Title	**MUSIC has Healing Power: Ultrasound-Guided STING Activation for Cancer Immunotherapy**

Abstract

The recent success of immunotherapy for treatment-refractory metastatic melanoma, lung cancer and renal cell carcinoma has provided a new hope that immunotherapy can be generalized to a broader range of cancers. However, many cancers do not respond to immune checkpoint inhibitors, which has limited their use to a subset of patients.

Recent studies have shown that the activation of the innate immune sensor cyclic GMP–AMP synthase–stimulator of interferon genes (cGAS-STING) promotes antigen presentation and T-cell activation, thus transforming cold tumors to immunologically responsive (or "hot") tumors [1]. However, because STING is a cytosolic protein and a key mediator of inflammation, activation of STING needs to be specific to antigen-presenting cells (APCs) in tumor tissues. To address this technical challenge and meet the clinical need, we developed a technology termed MUSIC (Microbubble-assisted Ultrasound-guided Immunotherapy of Cancer) that utilizes gas-filled microbubbles (MBs) conjugated with APC-targeting antibodies, and loaded with the STING activator cGAMP.

Our results show that, upon exposure to US, MUSIC produces robust STING activation and type I interferon responses in APCs and more efficiently primes antigen-specific CD4+ and CD8+ T cells in vitro. These immune stimulatory effects of MUSIC directly translated into antitumor responses in vivo, where we showed that the MUSIC was able to generate antitumor effects against syngeneic orthotopic primary (EO771) and metastatic (4T1) murine breast cancer models [2]. Both models showed dramatic antitumor responses following local treatment of the primary tumor. Specifically, 6 out of 10 EO771 tumor-bearing animals were tumor-free after 50 days while 4T1 tumor-bearing mice exhibited a significant decrease of the systemic disease burden including lung metastases. We also confirmed the establishment of systemic immune memory following MUSIC treatments as mice rejected tumor cells upon re-challenge.
We recently evaluated the efficacy of MUSIC in two syngeneic murine melanoma models (B16-F10 and D4M) with 100% of D4M-tumor bearing animals and 50% of B16-tumor-bearing animals achieving complete remission when treated with a combination of MUSIC and immune checkpoints inhibitors [3].

1. Khorsandi S, Huntoon K, Lux J. Putting the sting back in STING therapy: novel delivery vehicles for improved STING activation. Frontiers in Chemical Biology. 2024;3. doi: 10.3389/fchbi.2024.1386220.
2. Li X, Khorsandi S, Wang Y, Santelli J, Huntoon K, Nguyen N, Yang M, Lee D, Lu Y, Gao R, Kim BYS, de Gracia Lux C, Mattrey RF, Jiang W, Lux J. Cancer immunotherapy based on image-guided STING activation by nucleotide nanocomplex-decorated ultrasound microbubbles. Nat Nanotechnol. 2022;17(8):891-9. Epub 2022/06/01. doi: 10.1038/s41565-022-01134-z. PubMed PMID: 35637356; PMCID: PMC9378430.

3. Khorsandi S, Huntoon K, Wang Y, Woodward A, Antony A, Endsley C, Hafeez N, Kim BYS, Jiang W, Lux J. Targeted STING Activation using Modified Ultrasound-Responsive Microbubbles Enhances Immune Checkpoint Blockade against Melanoma. Under Revisions.

Session 6	**Microbubbles: Towards Translation 2**
Name & Affiliation	**Alessandra Vitaliti**, University of Rome Tor Vergata
Title	**Targeting glioblastoma vasculature using PVA-microbubbles: *in vivo* evidence**

Abstract

The development of echogenic microbubbles (MBs) with biocompatible polymeric shells has redefined ultrasound contrast agents as a versatile platform with theranostic application. Our group has developed MBs with an air core and a crosslinked polyvinyl alcohol (PVA) shell, which exhibit high robustness and are easily derivatizable with ligands and/or drugs [1]. Taking advantage of PVA-MBs micrometric dimensions (around 3 µm), we have built a safe and non-invasive multifunctional injectable system to improve diagnosis and therapy of Glioblastoma (GBM), a highly aggressive and incurable brain cancer for which the disease recurrence is very common and challenging to treat.

In this scenario, we have improved PVA-MBs to actively target GBM microvasculature, by binding on their surface a cyclic pentapeptide containing the Arginine-Glycine-Aspartate ("RGD") sequence. Such peptide interacts with a_vb_3 integrins which are overexpressed in tumor-associated endothelium, been crucial in the tumor angiogenesis. Previously, our group demonstrated *in vitro* that RGD-modified PVA-MBs adhere efficiently to a microchannel coated with a layer of human umbilical vein endothelial cells (HUVEC) to reproduce the physiological blood flow and the size of postcapillary vessels [2]. To translate *in vivo* these results, mice were orthotopically implanted with GL261 brain tumors and intracardiac injected with PVA-MBs, labeled with the Fluorescein isothiocyanate (FITC) to detect their distribution. Our data demonstrated that fluorescent RGD-modified PVA-MBs efficiently localize into the mouse brain tumor capillaries as identified by Pecam-1 (endothelial marker) or Gfap (astrocyte marker) immunostainings. Additionally, we functionalized PVA-MBs as microcarriers of the radioactive Y90 by engineering the shell with the bifunctional chelator S-2-(4-Isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane tetraacetic acid (p- SCN-Bn-DOTA) able to complex Y isotopies. Finally, we demonstrated that PVA-MBs improved with RGD and DOTA can be successfully injected via microcatheter within a microchannel coated with HUVEC cells, highlighting a promising approach for *in situ* accumulation onto the GBM vascular tissues [3].

Acknowledgments: the research was funded by INAIL under the grant agreement BRiC2022 ID53.

References

1. Oddo, L. *et al.* Next generation ultrasound platforms for theranostics. *J. Colloid Interface Sci.* 491, 151–160 (2017).
2. Paradossi, G. *et al.* Toward a theranostic device for gliomas. *Biochem. Biophys. Res. Commun.* 671, 124–131 (2023).
3. Da Ros, V. *et al.* PVA-Microbubbles as a Radioembolization Platform: Formulation and the In Vitro Proof of Concept. *Pharmaceutics* 15, (2023).

Session 6	**Microbubbles: Towards Translation 2**
Name & Affiliation	**Sally A. Peyman,** Heriot-Watt University
Title	**Organ-on-chip; towards animal-free models of disease to investigate therapeutic microbubble potential**

Abstract

Microbubbles (MBs)are well known contrast agents in diagnostic ultrasound (US) and have become of interest as drug delivery vehicles due to their biocompatibility, imaging modality and their ability to release a drug payload at a region of interest using a short US 'destruction' pulse. During this drug release process, MBs also produce localized shockwaves that can puncture holes in nearby cell membranes, potentially increasing drug uptake.

Drug delivery in cancer, and in particular in solid tumours, is one area of research that MBs have gained much interest. The ability to image the MBs in the tumour vasculature, and use a destruction pulse to release a drug payload in the region of interest, thus reducing off-site toxicity, makes MBs a promising therapeutic agent. However, like many new therapies, translation to the clinic is difficult, with most drugs failing to produce outcomes observed in early pre-clinical studies. It is widely accepted this is due to inter-species differences between humans and animals, with an estimated 90% of animal testing in medicine not leading to any human benefit and also current in vitro models that do not represent in vivo conditions.

Organ-on-chip is a developing technology that shows promise in improving in vitro models of disease by recapitulating the biochemical and biophysical microenvironment of the body. Human cell lines and even patient tissues can be grown in devices in which cell-cell interactions, fluid pressures and chemical gradients can be precisely controlled. Not only does this improve current in vitro models, but it allows scientists to control experimental parameters and observe drug-tissue interactions, not currently possible in pre-clinical studies.

Here, we show two organ-on-chip devices that model two regions of interest in cancer therapy; the tumour microvasculature and fibrotic stroma of solid tumours. We use these models to study the potential of MBs to improve drug delivery. As a first example, we demonstrate a tumour-associated vasculature to investigate the specific targeting of MBs to diseased vasculature and delivery of a liposome pay-load. In a second example, we use an organ-on-chip model of pancreatic cancer to show how MB destruction can potentially disrupt the rigid, collagenous tumour matrix to improve interstitial flow and drug penetration.

These two models demonstrate the potential of organ-on-chip technology to be a powerful tool in improving in vitro modelling and in the replacement of animal models, towards better predicting patient outcomes and translation of new drugs to the clinic.

References.

1. Bourn, Peyman et al. *Lab on a Chip* (**2023**) 23 (6) 1674
2. Kpeglo, Peyman et al. *Lab on a Chip* (**2024**) 24 (4) 854
3. Kpeglo, Peyman et al. *In preparation*.

Session 6	**Microbubbles: Towards Translation 2**
Name & Affiliation	**Jack Wright,** Ulster University and SonoTarg Ltd.
Title	**Process Development of a Drug-Loaded Microbubble Formulation for use in a Phase 1 Clinical Trial**

Abstract

Authors: Jack Wright[1], Keiran Logan[1], Thomas McKaig[1], Sukanta Kamila[1], Chloe McClenaghan[1], Mark Taylor[2], Mark Love[3], Eleanor Stride,[4] Nigel R Trim[5], Jamie X Chorlton[5], Anthony P. McHale[1], John F. Callan[1*]

Affiliations: 1. Biomedical Sciences Research Institute, University of Ulster; 2. Department of HPB Surgery, Mater Hospital; 3. Imaging Centre, The Royal Victoria Hospital; 4. Institute of Biomedical Engineering, University of Oxford; 5. SonoTarg Ltd.

Currently, in the UK, pancreatic cancer has a 5-year survival of less than 7%, giving it the lowest survival statistics of all common cancers (Pancreatic Cancer UK, 2024). As such, a targeted delivery system capable of maximizing chemotherapeutic drug concentrations at the tumour is of great need. Whilst the microbubble is capable of this, drug loading is often limited by the capacity of the hydrophobic tails of the lipid layer (Delaney et al., 2022). However, if a hydrophobic chemotherapeutic is first dissolved in a triglyceride, drug loading can be substantially increased through incorporation of the triglyceride within the microbubble shell. This has facilitated a five-fold increase in the loading capacity of paclitaxel within the microbubble formulation, with additional positive effects on microbubble size and concentration. Paclitaxel encapsulation was quantified by HPLC analysis where it was found that 82.0% was contained within the microbubbles.

Attempts to translate this formulation into a clinically feasible model have necessitated the investigation of several approaches, such as pre-microbubble emulsions and bi-phasic storage approaches. This led to the design of a two-vial approach, with vial 1 containing the paclitaxel-triglyceride solution and vial 2 being filled with a lipid blend comprising a sonosensitiser and a perfluoropropane headspace. Bedside preparation of the activated microbubble suspension is facilitated through the addition of vial 1 to vial 2 prior to subsequent mixing.

Preparation for Phase 1 clinical trials has required scale-up process development for the sterile manufacture of vial 1 and vial 2, which has included studies into appropriate sterilisation techniques. This has also necessitated the development of an alternative method to the conventional thin-film approach. Here, propylene glycol is used to dissolve the lipids prior to liposomal formation through the addition of a PBS-glycerol solution. Thus far, batch sizes of 100 mL have been produced with plans to produce a three-litre batch in the future.

References

Pancreatic Cancer UK. (2024). *Pancreatic cancer statistics*. [online] Available at: https://www.pancreaticcancer.org.uk/what-we-do/media-centre/pancreatic-cancer-statistics/ [Accessed 21 Jun. 2024].

Delaney, L.J., Selin Isguven, Eisenbrey, J.R., Hickok, N.J. and Forsberg, F. (2022). Making waves: how ultrasound-targeted drug delivery is changing pharmaceutical approaches. *Materials advances*, [online] 3(7), pp.3023–3040. Available at: https://pubs.rsc.org/en/content/articlehtml/2022/ma/d1ma01197a [Accessed 21 Jun. 2024].

Poster Presentations

Number	Name	Title
1.	Kathryn Burr	Targeted delivery of antibiotics using proteins that bind to bacterial biofilms
2.	Qi Chen	Ultrasound-triggered NO therapeutic microbubbles for tumour drug penetration and multidrug resistance reversal in cancer therapy
3.	Amelia Claxton	Investigating the acoustic properties of ultrasound activatable nanodroplets for BBB opening.
4.	Joseph Fox	Microbubble Enhanced Delivery of Vitamin C for Treatment of Colorectal Cancer
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Poster Number 1	**Kathryn Burr**, University of Leeds
Title	**Targeted delivery of antibiotics using proteins that bind to bacterial biofilms**

Abstract

Staphylococcus aureus is a versatile human pathogen and a significant cause of infected indwelling medical devices such as catheters and prosthetic valves. The ability of *S. aureus* to infect these sites is mediated by biofilm formation, a multi-layered, aggregated community of bacteria embedded within a protective matrix. Higher rates of treatment failure are associated with biofilm-related infections, highlighting the need for improved therapeutic strategies. Microbubbles are micron-sized gas bubbles encased by a lipid, polymer or protein shell that are traditionally used as contrast agents for ultrasound imaging, however there is interest in their use for treating bacterial infections. The aim of the BETATRON project is to use microbubbles encapsulated with an antimicrobial peptide, which specifically target *S. aureus* biofilms and subsequently allow treatment of the infection using ultrasound-mediated drug release.

My project addresses the first objective of the BETATRON project, to evaluate the binding interactions of our Affimer library which was raised against *S. aureus* biofilms, and to determine which of these Affimers would be the optimal targeting agents. Affimers are small, heat-stable proteins with antibody-like hypervariable loops conferring binding specificity. Preliminary work investigating the use of Affimers as biofilm targeting agents demonstrated significantly enhanced binding of microbubbles to *S. aureus* biofilms (Caudwell et al., 2022). This showed that the Affimer A-ClfA1 bound specifically to ClfA (clumping factor A) on the surface of *S. aureus*, a cell wall-anchored adhesin involved in the initial stages of biofilm formation. However, the remaining Affimers in our library were uncharacterised. With the use of proteomics, my project has identified ligands for the remaining Affimers using a biotin-Neutravidin pull-down assay. Our Affimers bind to one of three cell wall proteins: ClfA, ClfB or SdrD. However, protein expression is highly variable between *S. aureus* strains, growth conditions, age of the biofilm or in planktonic cells. Therefore, profiling the expression of cell wall proteins under different conditions could indicate which Affimers might be the best to use on the microbubbles.

Poster Number 2	**Qi Chen**, University of Leeds
Title	**Ultrasound-triggered NO therapeutic microbubbles for tumour drug penetration and multidrug resistance reversal in cancer therapy**

Abstract

Pancreatic cancer is one of the deadliest malignant tumours in humans and chemotherapy is an important approach to treat it. However, traditional chemotherapy suffers from various drawbacks such as limited penetration and multi drug resistance (MDR) that still greatly undermine its effectiveness although many measures have been taken to improve it. Nitric oxide (NO), a gaseous molecule, has multiple physiological functions, including its ability to overcome the dense extracellular matrix (ECM) barrier and alleviate or reverse MDR through multiple pathways. But its low stability and short half-life greatly limit its widespread clinical application. Microbubbles (MBs) with a gas core are a good carrier to solve these problems. Therefore, the aim of this work is to develop an ultrasound-triggered NO therapeutic MBs to improve tumour drug penetration and alleviate tumour MDR. In this work, the chemotherapeutic drug, irinotecan will be loaded into targeted liposomes conjugated to NO MB (NO thMBs). Targeting molecules will be investigated for optimal uptake in pancreatic cancer. The NO thMBs will be delivered to the pancreatic tumour, and upon ultrasound (US) exposure, the oscillating and rapid collapse of the NO thMBs will increase the permeability of tumour vascular endothelium and promote the release of the irinotecan-loaded liposomes to the target site. The NO released from the MBs should freely diffuse into the tumour region, break down the ECM barrier, counteract MDR, and enhance tumour cell sensitivity to the effects of irinotecan.

Poster Number 3	**Amelia Claxton,** King's College London
Title	**Investigating the acoustic properties of ultrasound activatable nanodroplets for BBB opening.**

Abstract

Amelia Claxton, Paul Cressey, Antonios Poulipoulos and Maya Thanou. King's College London

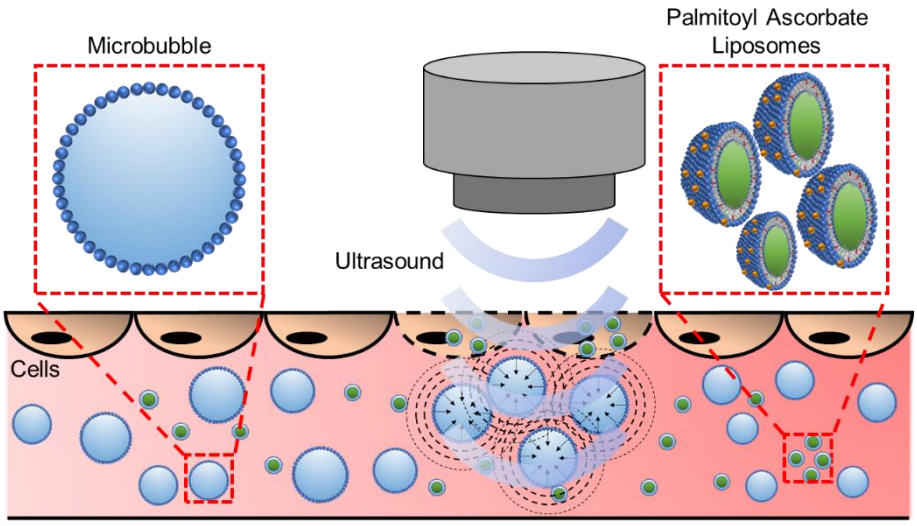
Microbubbles, minute gas-filled spheres, find widespread application in ultrasound imaging and targeted drug delivery. Several methodologies, such as sonication, amalgamation, and saline shaking have been employed for microbubble synthesis. These methods yield highly concentrated microbubble suspensions; however, offer limited control over size and polydispersity. Addressing these challenges, capillary-embedded T-junction microfluidic (CETM) devices have emerged as a promising technique for generating monodisperse microbubbles, offering control over their polydispersity. Nevertheless, the limitations of CETM devices, such as low production rates (~200 bubbles/s) and large microbubble sizes (~300 µm), restrict their use in biomedical applications. Therefore, in this study, we introduce an innovative approach to overcome these challenges by integrating CETM devices with ultrasound, with the objective to enhance the production of narrow-sized microbubble suspension. Our methodology involved connecting two CETM devices in parallel, coupled by an ultrasonic horn, to facilitate the production of lipid-coated SF6 core microbubbles within the size range of 1–5 µm. Interestingly, the incorporation of ultrasound (100% amplitude) significantly enhanced the rate of microbubble production from 180 microbubbles/s to (6.5 ± 1.2) × 10^6 microbubbles/s. B-mode imaging was performed using tissue-mimicking flow phantoms to evaluate the proficiency of freshly prepared microbubble suspensions in enhancing the contrast of ultrasound images. Our observations revealed that the contrast-to-noise ratio (CNR) increased from 8.1 ± 1.4 to 29.5 ± 1.2 with the increase in microbubble concentration, from 10^5 to 10^7 microbubbles/ml. These findings indicate that microbubbles prepared using CETM devices and ultrasound combined possess the capability to enhance the contrast of ultrasound images.

Poster Number 4	**Joseph Fox**, University of Leeds
Title	**Microbubble Enhanced Delivery of Vitamin C for Treatment of Colorectal Cancer**

Abstract

Colorectal cancer is the 3rd most common malignancy and the 2nd leading cause of cancer death globally[1]. In the case of advanced or metastatic disease, the chemotherapy regimens typically followed use mixtures of drugs that can be systemically damaging, leading to a range of adverse side effects[2]. High doses of vitamin C (ascorbic acid) have shown selective anti-cancer effects[3], improvements to the efficacy of a range of chemotherapy drugs[4] and enhanced cytotoxicity towards KRAS-mutated colorectal cancers[5].

This work explores microbubble-enhanced delivery of the ascorbic acid derivative, palmitoyl ascorbate, to KRAS-mutated colorectal cancer cells *in vitro*. The interaction between microbubbles and ultrasound can generate pores in nearby tumor cells[6], permitting enhanced drug uptake in the region the ultrasound is applied. It was shown that ultrasound-triggered microbubbles enhanced the efficacy of liposomal palmitoyl ascorbate treatments 1.7- and 2.2-fold in LS174T and HCT116 colorectal cancer cell lines, respectively. This enhancement was achieved without increasing the drug dosage or exposure time. Furthermore, additional exposures to microbubbles and ultrasound further amplified the therapeutic effect of the palmitoyl ascorbate, which was shown to be localized to the area that received the ultrasound pulse, aiding in the reduction of off-site toxicity.



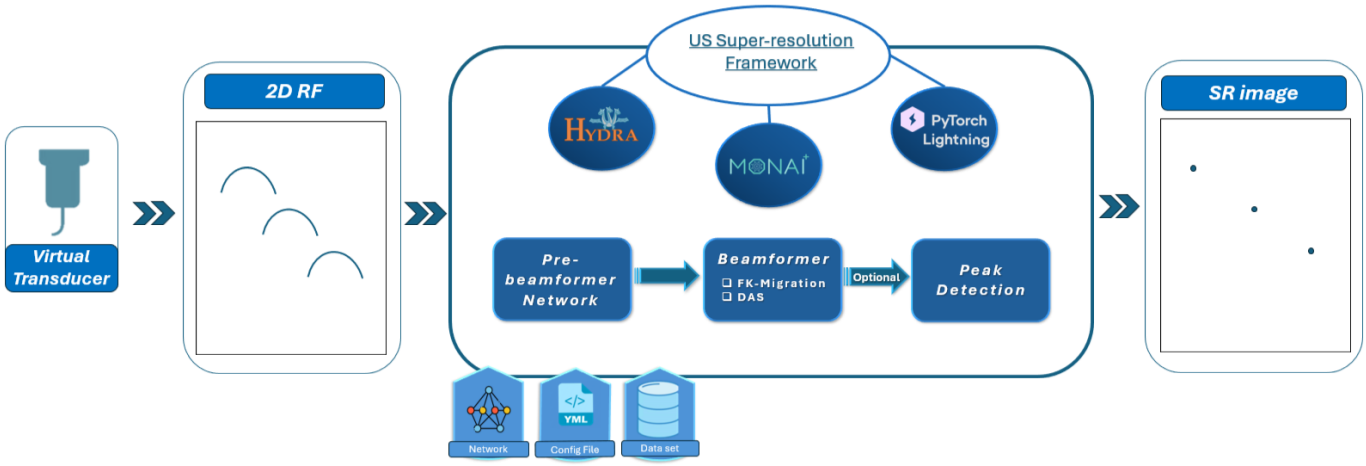
References

1. Keum, N. et al. Nature Reviews Gastroenterology & Hepatology 2019, 16 (12), 713-732.
2. CRUK Folinic acid, fluorouracil and oxaliplatin (FOLFOX). (Accessed 05/07/2024).
3. Chen, Q. et al. PNAS 2005, 102 (38), 13604.
4. Kurbacher, C. M. et al. Cancer Letters 1996, 103 (2), 183-189.
5. Yun, J. et al. Science 2015, 350 (6266), 1391.
6. Kooiman, K. et al. Journal of Controlled Release 2011, 154 (1), 35-41.

Poster Number 5	**Anass Hameddine**, Physics of Fluids (POF), Faculty of Science and Technology (TNW), University of Twente. **Bram de Wilde**, Physics of Fluids (POF), Faculty of Science and Technology (TNW), University of Twente.
Title	**Multichannel end-to-end AI-based super-resolution**

Abstract

Ultrasound localization microscopy (ULM) has been a major advancement in the field of pre-clinical ultrasound imaging. However, ULM requires sparse microbubble distributions, which in turn, imposes prolonged acquisition times to adequately image the full microvasculature. Recently, we have introduced a novel super-resolution methodology that leverages a deep learning, physics-based approach to overcome traditional ULM limitations. Specifically, the neural network learns to deconvolve single RF lines to localize microbubble signatures.
Here, we take a step forward in both, the optimization of the technique, and its practical implementation by creating a modular pipeline for deep learning-based super-resolution using the full 2D RF data. The pipeline, designed for open-source release, integrates several deconvolution algorithms, example synthetic datasets, and a Stolt's FK migration beamforming algorithm. We focus on low-frequency ultrasound for deep imaging of arterial flow, i.e., on dense bubble cloud with speeds in excess of 1 m/s. The synthetic data comprises a broad range of acoustic pressures (5-250 kPa) that elicit nonlinear responses of resonant, lipid-coated microbubbles. We expect this approach to reduce detection uncertainty and to allow for super-resolution imaging of denser bubble clouds, leading to better RF-based super resolution in more realistic scenarios.



Poster Number 6	**Kristian Hollingsworth,** University of Leeds
Title	**Multi-targeting system for microbubbles for the eradication of *Staphylococcus aureus* biofilms**

Abstract

Kathryn Burr, Damien Batchelor, Anjali Lad, Bruce Turnbull, Jonathon Sandoe, Stephen Evans, Steven Freear, Zhan Ong

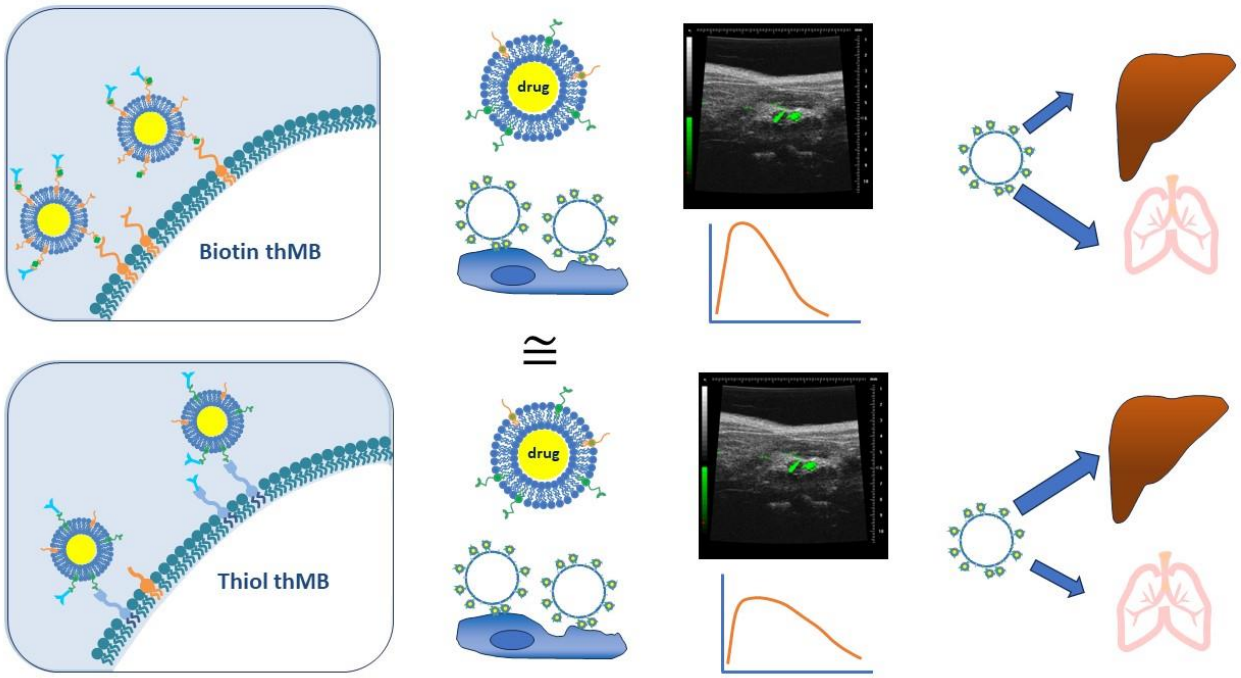
Staphylococcus aureus biofilms present a significant challenge in clinical settings due to their resistance to conventional antibiotics. Under ultrasound stimulation microbubbles can physically disrupt the structure of biofilms and enhance the penetration of antibiotics. This study aims to increase the effectiveness of these strategies by specifically targeting microbubbles to S. aureus biofilms. Given the diverse composition of S. aureus biofilms across different strains, relying on a single biomolecule for targeting may prove insufficient. Thus, the strategy involves targeting multiple constituents within the biofilm. Various targeting agents were selected to address the three primary components of S. aureus biofilms: proteins, polysaccharides, and DNA. Employing a high-throughput method, the diverse library of targeting agents was screened to assess their binding affinity and specificity towards S. aureus biofilms. Promising complementary agents have been identified, and ongoing efforts are directed towards evaluating their collective efficacy in tandem.

Poster Number 7	Nicola Ingram, University of Leeds
Title	Maleimide–Thiol Linkages Alter the Biodistribution of SN38 Therapeutic Microbubbles Compared to Biotin–Avidin While Preserving Parity in Tumoral Drug Delivery

Abstract

Therapeutic microbubbles (thMBs) contain drug-filled liposomes linked to microbubbles and targeted to vascular proteins. Upon the application of a destructive ultrasound trigger, drug uptake to tumour is improved. However, the structure of thMBs currently uses powerful non-covalent bonding of biotin with avidin-based proteins to link both the liposome to the microbubble (MB) and to bind the targeting antibody to the liposome–MB complex. This linkage is not currently FDA-approved, and therefore, an alternative, maleimide–thiol linkage, that is currently used in antibody–drug conjugates was examined. In a systematic manner, vascular endothelial growth factor receptor 2 (VEGFR2)-targeted MBs and thMBs using both types of linkages were examined for their ability to specifically bind to VEGFR2 in vitro and for their ultrasound imaging properties in vivo. Both showed equivalence in the production of the thMB structure, and the in vitro specificity of binding and safety profiles. In vivo imaging showed subtle differences for thMBs where biotin thMBs had a faster wash-in rate than thiol thMBs, but thiol thMBs were longer-lived. The drug delivery to tumours was also equivalent, but interestingly, thiol thMBs altered the biodistribution of delivery away from the lungs and to-wards the liver compared to biotin thMBs, which is an improvement in biosafety.

Ingram 2024, 16, 434. https://doi.org/10.3390/ pharmaceutics16030434

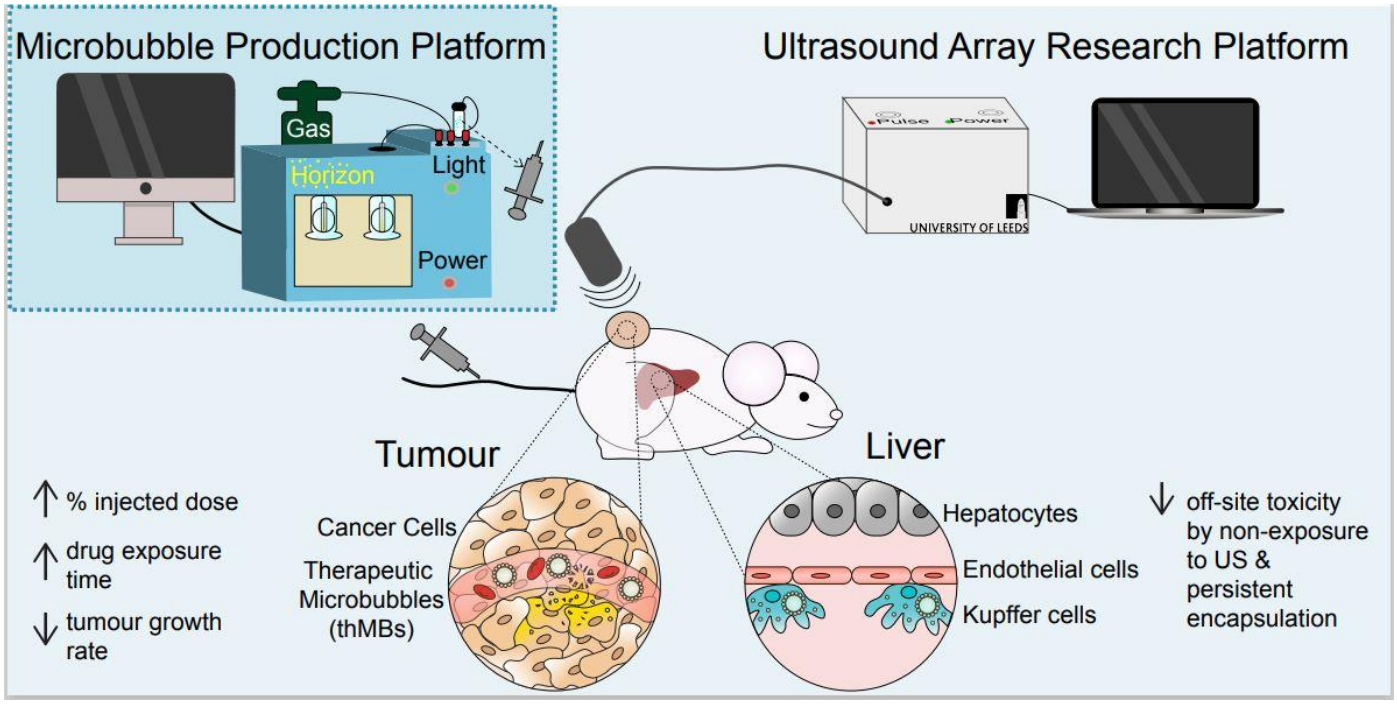


Poster Number 8	Nicola Ingram, University of Leeds
Title	Ultrasound-triggered therapeutic microbubbles enhance the efficacy of cytotoxic drugs by increasing circulation and tumor drug accumulation and limiting bioavailability and toxicity in normal tissues

Abstract

The majority of cancer patients receive chemotherapy at some treatment stage which makes improving the efficacy of cytotoxic drugs an important goal. Despite large numbers of potent anti-cancer agents entering clinical development, a major obstacle to clinical translation remains the inability to deliver therapeutic doses to a tumour without toxicity in normal tissues causing intolerable side effects. Therefore, there has been intense interest in nanoformulations such as liposomes and nanoparticles to overcome these problems. So far however, few nanoformulated drugs have delivered consistent improvements in therapeutic index. This reflects inadequate tumour drug uptake and uncontrolled biodistribution, which prevent the use of more potent but toxic molecules. Herein, we describe the use of therapeutic microbubbles (thMBs), which combine VEGFR2 targeting and a localized ultrasound (US)-trigger for tumour-specific, enhanced cytotoxic drug delivery.We show that thMBs improve tumour responses to low dose irinotecan or its active metabolite, SN38 in mouse tumour models. Sensitive LC-MS/MS quantification of drugs and their metabolites showed that thMBs extended drug exposure in tumours but limited exposure in normal tissues by persistent encapsulation of the drug in areas not exposed to US, prior to elimination. ^{89}Zr PET radiotracing showed that the percentage injected dose (%ID) in tumours achieved with thMBs was twice that from VEGFR2-targeted SN38 liposomes alone. This demonstrates how US-triggered thMBs significantly increase drug delivery to tumours and provides a generic platform for effective targeted delivery of otherwise toxic therapeutics.

Ingram 2020; 10(24): 10973-10992. doi: 10.7150/thno.49670



Poster Number 9	Theresa Kosmides, Case Western Reserve University
Title	Novel EDB-FN Targeted Nanobubble Ultrasound Contrast Agents for Pancreatic Cancer Diagnosis

Abstract

Currently, the only treatment for non-resectable pancreatic ductal adenocarcinoma (PDAC) is resection, however few patients qualify for this treatment due to advanced disease state. Thus, there is a need for tools which detect PDAC lesions in early stages for increased treatment options. Nanobubbles (NB) are a submicron ultrasound (US) contrast agent which can extravasate through permeable vasculature, increasing accumulation in tumors. The addition of a targeting moiety which specifically and selectively binds to PDAC biomarkers increases accumulation and retention of contrast agents in tumors. Of many biomarkers, extra domain B fibronectin (EDB-FN) is overexpressed in PDAC. We propose the addition of ZD2, an EDB-FN targeting peptide, to increase signal intensity in PDAC lesions, thus improving visibility and ease of diagnosis via US.

Nanobubbles with ZD2 (ZD2-NB) were prepared by adding ZD2 solution to precursor emulsion prior to activation and size isolation. The stability of ZD2-NB was confirmed with US in an agarose phantom under the following conditions: 18MHz fc, 4% power, 1fps on a VisualSonics Vevo2100 system. Diameter of NBs was measured using dynamic light scattering (DLS). The targeted NBs' uptake in cells was determined by co-incubating rhodamine tagged ZD2-NBs with human PDAC cell lines (Capan-1 and BxPC3) and measuring fluorescence intensity. The NBs' targetability to PDAC tumors was examined using a flank tumor model in immunocompromised mice inoculated with Capan-1 cells. Both targeted and untargeted NBs were administered via tail vein injection and signal in the tumor and kidney was monitored in both B and NLC mode via US (18MHz fc, 4% power, 1fps) for 20 minutes post injection.

ZD2 peptide was incorporated into the NB shell resulting in an average diameter of 240 nm compared to untargeted NB diameter of 238 nm (Fig. 1A). ZD2-NBs had approximately 2x higher fluorescence signal compared to untargeted NBs in BxPC3 cells. The ZD2-NBs had comparable US contrast signal to the untargeted NBs and the addition of the peptide did not negatively affect signal intensity or stability for 300 s (Fig. 1B). The ZD2-NBs had 2.86x higher tumor NLC signal at 16 minutes after injection compared to untargeted NBs (Fig. 1C).

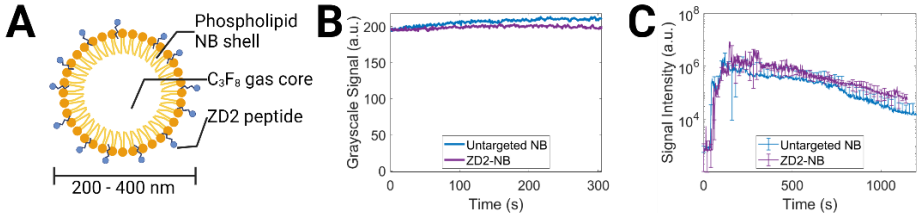


Figure 3. (A) Schematic of ZD2-NBs. (B) Acoustic characterization of ZD2-NB and NB under US in agarose phantom. (C) Nonlinear contrast signal in flank tumor model of ZD2-NB and NB.

We successfully incorporated an EDB-FN targeting peptide, ZD2, into our ultrasound contrast agents. The addition of ZD2 did not significantly alter the size, charge, or stability under US of the NBs. Results demonstrate an increase in cellular uptake of ZD2-NBs by cells which express the target, EDB-FN. NBs targeted to EDB-FN had higher signal intensity compared to untargeted NBs in mice.

Theresa Kosmides1, Pinunta Nittayacharn1, Songqi Gao1, Zheng-Rong Lu1, Agata Exner1,2

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Poster Number 10	**Delanyo Kpeglo**, University of Leeds
Title	**A microfluidic pancreatic ductal adenocarcinoma (PDAC) culture model for investigating microbubble-mediated drug delivery of gemcitabine against PDAC cells**

Abstract

We present a microfluidic pancreatic ductal adenocarcinoma (PDAC) culture model, with PDAC's biophysical barriers to therapeutics, to test microbubbles (MBs)-mediated drug delivery approach.

There are many promising new drugs and delivery methods in development against PDAC, the most prevalent pancreatic cancer with poor prognosis. However, there remains a critical gap in knowledge when translating drug studies to the clinic: a rigid fibrotic stroma which hinders drug penetration into the tumour, leading to treatment failure. High densities of cells and extracellular matrix (ECM) proteins form a rigid mass with high interstitial pressure, leading to the collapse of vasculatures and the development of a fibrotic, hypoxic, mechanically stiff stroma with reduced interstitial flow. Despite this, most pre-clinical drug tests are performed on cellular models without these biophysical characteristics, which are central to ineffective drug delivery. Microfluidics provides a means to develop adequate culture models, mimicking tumour biophysical features with appropriate flow conditions, and allows accurate test beds to assess new drugs and emerging delivery approaches such as MBs-mediated drug delivery. The cavitation and collapse of the micron-sized phospholipid-shelled gas bubbles with ultrasound (US) exert forces in the local ECM and cell membranes to increase drug uptake.

Our microfluidic PDAC model features co-culture of PANC-1 with PSCs, the fibroblasts responsible for overproducing collagen for the rigid fibrotic stroma. Off-chip investigation found a 21-day culture was required to develop the stiff PDAC stroma (~1kPa). Translated on-chip, immunostaining of our model found a collagenous matrix by day 21 of culture, leading to increased mechanical rigidity, reduced interstitial flow, and a hypoxic environment. When the chemotherapeutic gemcitabine was delivered with MBs and repeated US, gemcitabine effectiveness increased by ~15%.

This work shows the significance of modelling disease biophysical characteristics for drug testing and the use of MBs for successful drug delivery to cells.

Poster Number 11	**Anjali Lad,** University of Leeds
Title	**Polymeric Microbubbles for Tackling Antimicrobial Resistance (AMR)**

Abstract

The dependence and overuse of antibiotics when treating infections have led to the proliferation of antimicrobial resistance (AMR) in society. *Staphylococcus aureus (S.aureus)* is a bloodstream infection that has already demonstrated resistance in the 50s and 60s to the antibiotic methicillin and penicillin. *S.aureus* forms biofilms, a community of microbial cells that are part of an extracellular matrix, which can grow on medical devices and internal organ surfaces. Biofilms protect the bacteria making it harder for drugs to penetrate and for treatment. Alongside this, the increase in AMR has led to the need to find alternative methods and drugs to treat this type of infection.

Microbubbles (MBs) are commonly used as contrast agents and drug delivery vessels to treat tumours. Research has moved to using MBs alongside ultrasound, to penetrate biofilms allowing for more targeted drug delivery and destruction of biofilms. Layer-by-layer (LbL) assembly has become an increasingly popular technique that has evolved from flat surfaces to structures such as MBs. The technique involves the build-up of sequential materials on a surface through interactions including electrostatic interaction. The use of LbL assembly for drug delivery has allowed increased drug loading and easier manipulation of properties.

Using a LbL assembly technique, a novel Antimicrobial Peptide (AMP), which has been shown to have adverse effects on *S.aureus* biofilms, will be loaded around the lipid-covered microbubble in order to be delivered to the biofilm and burst using ultrasound. However, the AMPs has been seen to be broken down by enzymes in the bloodstream before reaching the site of the infection. Therefore, it is proposed the AMP will be protected by a polyelectrolyte. This will allow multilayers to be created around the MB increasing the drug loading and then be burst with ultrasound, delivering the drug to the biofilm.

Poster Number 12	**Georgina Lee**, Physics and Astronomy, University of Leeds
Title	**Modelling the Biophysical Barriers to Drug Delivery in Cancer**

Abstract

Intravenous cancer therapies encounter multiple biological barriers to drug delivery, particularly tumour vasculature and crossing the endothelial barrier into tissues [1]. In addition, results seen in animal studies often are not reflected in clinical trials, and many new molecular entities are biologic therapies targeting human molecular sequences [2, 3]. This project aims to develop a human vasculature-on-chip model to assess new drug delivery mechanisms.

Microfluidic devices were fabricated using PDMS soft lithography techniques. To measure flow rates within the device, fluorescein solution or fluorescent green magnetic particles were perfused through devices and recorded using an epifluorescent microscope. On-chip, GFP-HUVECs and NHLFs were grown in a self-assembly system in a fibrin gel while media was perfused through side channels. GFP-HUVECs were imaged on-chip with confocal microscopy. A perfusion assay with 70 kDa Texas Red dextran was performed to assess vessel leakage [4].

Hydrostatic flow rate in the microfluidic device can adjusted by changing the media height difference in reservoirs, and interstitial flow across the cell culture chamber is induced by media channel pressure gradient. Hydrostatic flow rate in media channels was consistent across multiple devices, though this and the interstitial flow rate differed from previous measurements [4]. While GFP-HUVECs show some network formation after 8 days on-chip, culture and flow conditions require further optimisation to induce lumen formation and vessel perfusion.

Future work shall focus on further optimising conditions on-chip to support vasculature maturation, modelling tumour vasculature, and characterising cells grown on-chip with molecular biology techniques. Once functional, the model shall be used to investigate microbubble-assisted drug delivery, and whether microbubble sonoporation is able to rupture vessels in healthy and tumour vasculature models.

1. Azzi, S., J. Hebda, and J. Gavard, *Vascular Permeability and Drug Delivery in Cancers.* Frontiers in Oncology, 2013. **3**.
2. Low, L.A., et al., *Organs-on-chips: into the next decade.* Nature Reviews Drug Discovery, 2021. **20**(5): p. 345-361.
3. Ingber, D.E., *Human organs-on-chips for disease modelling, drug development and personalized medicine.* Nature Reviews Genetics, 2022. **23**(8): p. 467-491.
4. Bourn, M.D., et al., *Tumour associated vasculature-on-a-chip for the evaluation of microbubble-mediated delivery of targeted liposomes.* Lab Chip, 2023. **23**(6): p. 1674-1693.

Poster Number 13	**Giulia Perilli,** University of Rome Tor Vergata
Title	**PVA-shelled perfluorocarbon droplets as a versatile radio-sensitive platform for potential occupational and biomedical use**

Abstract

In the vast imaging and theranostic field, phase-change perfluorocarbon microdroplets (MDs) play a pivotal role thanks to their capability to efficiently transform into echogenic and optically detectable microbubbles upon interaction with ultrasound (US), laser and ionizing radiation (IR) beams [1]. In the most recent scenario, the exploitation of MDs has emerged as an exciting and invaluable new radiosensitive tool for conceiving novel external and internal IR dosimetry [2]. In this context, it is important to underline that professional use of IR has highly increased in the past two decades [3]. People working with IR are exposed to a high tumor risk [3] during the production, testing and use of IR source machines. Hence, it is fundamental to evaluate the exposure by using imaging dosimetry systems that can process the signal rapidly and in real-time. Significant improvement in this respect can be achieved by the optimization of MDs stability, sensitivity, chemical versatility and synthesis efficiency.

Here we show the development of a new dosimetry system based on meso-droplets (DPs) with a polymer shell of polyvinyl alcohol (PVA) and a perfluorocarbon core (DFP, decafluoropentane). The latter can undergo liquid-vapor phase transition once exposed to US, protons or C ions and potentially X rays, leading to the formation of echogenic microbubbles. Vaporization events can be detected by echography and optical imaging rapidly, giving the chance to intervene immediately in case of accidental high-dose exposure. Furthermore, these techniques can be coupled to artificial intelligence (AI) to analyze the images faster. We developed and optimized a synthesis method using two homogenization techniques. In order to characterize the platform, the final product has been studied by optical and confocal laser scanning microscopy (CLSM), exploiting the chance to introduce different fluorescent dyes inside the core (Nile-Red) or linked to the shell surface (FITC).

Following this protocol, we obtained $\sim \mathbf{10^5 - 10^6\ DPs/ml}$ with a spherical morphology and a mean diameter of approximately 160 µm. Further, we were able to introduce these DPs inside ergonomic gels based on different compositions before exposing them to US and hadronic irradiation at various intensities. Our results show that the platform morphology is modified after exposure both in water and in gel matrices and that the signal is clearly detectable in rapid time scales.

The system biocompatibility was verified by different biological assays (MTT and Trypan Blue) on HaCaT cells and we were able to define proper working conditions for a safe topical application of the platform as a professional dosimeter. The system was also tested for an alternative approach: exploiting the cells affinity for the biocompatible shell of PVA, the DPs were studied as scaffolds for tridimensional cell growth, giving preliminary promising results.

Acknowledgments: the research was funded by INAIL under the grant agreement BRiC2022 ID53.

[1] TOUMIA, Yosra, et al. Potential of BPA functionalized poly (vinylalcohol)-shelled perfluorobutane nanodroplets towards enhanced boron neutron capture therapy and in-situ dosimetry. *Applied Materials Today*, 2024

[2] TOUMIA, Yosra, et al. Ultrasound-assisted carbon ion dosimetry and range measurement using injectable polymer-shelled phase-change nanodroplets: in vitro study. *Scientific Reports*, 2022

[3] MORTAZAVI, S. M. J., et al. An artificial neural network-based model for predicting annual dose in healthcare workers occupationally exposed to different levels of ionizing radiation. Radiation Protection Dosimetry, 2020

Poster Number 14	**Suraj Prashad Sharma,** Brunel University London
Title	**Air Nanobubble Generation in Gasoline and Fundamental Spray Characteristics**

Abstract

Suraj Prashad Sharma, Abinash Biswal, Xinyan Wang

This study explores the use of nanobubbles to improve engine performance by enhancing spray characteristics and employing hydrodynamic cavitation for the generation of air nanobubbles in gasoline. Due to their high stability and large specific surface area, nanobubbles significantly improve fuel atomization, resulting in efficient combustion and reduced emissions. Key parameters for hydrodynamic cavitation crucial to nanobubble formation are examined with regards to the personally designed recirculation nanobubble generation system. The Malvern Zetasizer is used to precisely measure nanobubble size and concentration. Experimental activities have demonstrated notable changes in spray characteristics with nanobubble-assisted fuel spray, including increased penetration length, variations in radial spray development (cone angle), and spray area, which all contribute to fuel atomization efficiency. These findings highlight the potential of nanobubbles to improve atomization and fuel efficiency of internal combustion engines, paving the way for future research aimed at achieving low-carbon engines.

Key Words: Nanobubbles, Spray characteristics, fuel atomization, Hydrodynamic Cavitation.

References

Etchepare, R., Oliveira, H., Nicknig, M., Azevedo, A., & Rubio, J. (2017). Nanobubbles: Generation using a multiphase pump, properties and features in flotation. Minerals Engineering, 112, 19–26. https://doi.org/10.1016/j.mineng.2017.06.020

Nakatake, Y., Kisu, S., Shigyo, K., Eguchi, T., & Watanabe, T. (2013). Effect of nano air-bubbles mixed into gas oil on common-rail diesel engine. Energy, 59, 233–239. https://doi.org/10.1016/j.energy.2013.06.065

Oh, S. H., Han, J. G., & Kim, J. M. (2015). Long-term stability of hydrogen nanobubble fuel. Fuel, 158, 399–404. https://doi.org/10.1016/j.fuel.2015.05.072

Xuan, R., Guo, L., Cai, N., Sun, W., Yan, Y., Zhang, H., Chen, Y., Wang, H., Zhang, J., & Ge, Y. (2024). Study on spray characteristics of micro-nano bubble premixed fuel. Fuel, 363. https://doi.org/10.1016/j.fuel.2024.131035

Poster Number 15	**Yuhong Shi**, King's College London
Title	**mRNA lipid nanoparticle for focused ultrasound assisted diffuse midline glioma treatment**

Abstract

Diffuse midline glioma (DMG) is an incurable malignant brain tumor predominantly found in pediatric patients. Originating from pons, thalamus and spinal cord, surgical removal of DMG is impossible while the blood-brain barrier (BBB), which restricts the delivery of a majority of therapeutics into brain area, is another predominant obstacle for the treatment of DMG, leading to a dismal prognosis of DMG treatment. Thus, exploiting a novel and effective treatment modality for DMG becomes a necessity.

Microbubble assisted focused ultrasound (MB+FUS) is a novel technique which can open the BBB in a targeted and non-invasive manner. Once sonicated by focused ultrasound, the microbubble will oscillate and generate a series of mechanical forces, disrupting the tight junctions in BBB, therefore facilitating the delivery of therapeutics into brain areas. Clinical studies have proven the safety and feasibility of this promising tool in BBB opening and have successfully delivered trastuzumab, a large molecular drug into brain tumors.

Exploring the application of nucleic acid-based therapeutics for brain cancer treatment has drawn much attention in recent years. Among a variety of therapeutic candidates, messenger RNA (mRNA) has displayed an outstanding potency in treatment due to its simple functional mechanism and superior safety profile. Once delivered into cytoplasm, mRNA can be translated into therapeutic proteins or tumor antigens, paving the way for the further development of cancer vaccine or protein replacement therapy.

In this study, a lipid-based nanoparticle (LNP) in novel formulation for mRNA loading was developed, showing a superior ability in mRNA protection and DMG transfection when compared with the commercial LNP for covid vaccine. FUS+MB was further applied to an in vitro BBB model to investigate its impact on the permeability of mRNA-LNP across the BBB model, laying a foundation for the followed in vivo study.

Poster Number 16	**Fleur Vialle,** University of Twente
Title	**Dynamics of ultrasound-driven coated microbubbles confined in viscoelastic capillaries**

Abstract

The dynamics of phospholipid-coated microbubbles used as ultrasound contrast agents are well-known in free space. However, when these microbubbles are contained in the microcirculation, they are confined within viscoelastic capillaries, that may change their behavior. Several studies have shown such a change in microbubble dynamics. However, these numerical models are complex while in experiment the role of the underlying physics remains unclear [1,2,3]. Thus, more precise models are needed to increase the sensitivity of diagnostic ultrasound in small vessels. This study aims to investigate the effects of microbubble size, vessel diameter, vessel mechanics, and bubble shell properties on microbubble dynamics in confinement. To this end, we have developed a 3D finite element model of an ultrasound-driven microbubble held within a (visco)elastic capillary (see Figure 1). Two-way coupling between the fluid and the (visco)elastic walls is implemented using a partitioned fluid-structure interaction model, as described in [4]. To account for mesh deformations at the bubble-fluid and fluid-solid interfaces, the underlying physics are solved within an arbitrary Lagrangian-Eulerian (ALE) framework. The shell is modeled as a pressure boundary condition applied at the microbubble surface, where the surface tension follows the behavior as described in the Marmottant model [5]. To validate the numerical model, we conduct experiments in artificial microcapillaries with a diameter ranging from 25 to 100 µm. We combine ultrasound imaging and optical high-speed imaging using both hollow glass beads (as linear reference) and monodisperse microbubbles. We utilize Pulse Inversion (PI) contrast imaging to differentiate the fundamental and harmonic components of the ultrasound backscatter responses. Preliminary results show that we can measure the acoustic response from microbubbles and beads flowing through the capillaries and that in the present setup, even at low pressure, nonlinear propagation is a significant confounding factor for the measured acoustic response.

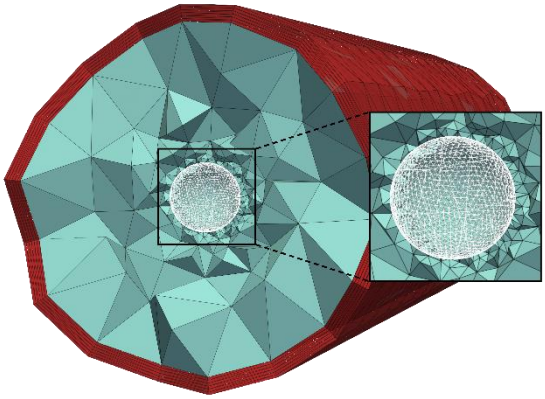


Figure 4: Mesh geometry of microbubble in capillary.

[1] X. Zhao, A. Wright, and D. E. Goertz, "An optical and acoustic investigation of microbubble cavitation in small channels under therapeutic ultrasound conditions," *Ultrason. Sonochem.*, vol. 93, p. 106291, Feb. 2023, doi: 10.1016/j.ultsonch.2023.106291.
[2] C. Chen, Y. Gu, J. Tu, X. Guo, and D. Zhang, "Microbubble oscillating in a microvessel filled with viscous fluid: A finite element modeling study," *Ultrasonics*, vol. 66, pp. 54–64, Mar. 2016, doi: 10.1016/j.ultras.2015.11.010.
[3] N. Hosseinkhah and K. Hynynen, "A three-dimensional model of an ultrasound contrast agent gas bubble and its mechanical effects on microvessels," *Phys. Med. Biol.*, vol. 57, no. 3, pp. 785–808, Feb. 2012, doi: 10.1088/0031-9155/57/3/785.
[4] A. Goetz, P. Jeken-Rico, Y. Chau, J. Sédat, A. Larcher, and E. Hachem, "Analysis of Intracranial Aneurysm Haemodynamics Altered by Wall Movement," *Bioengineering*, vol. 11, no. 3, p. 269, Mar. 2024, doi: 10.3390/bioengineering11030269.
[5] M. Versluis, E. Stride, G. Lajoinie, B. Dollet, and T. Segers, "Ultrasound Contrast Agent Modeling: A Review," *Ultrasound Med. Biol.*, vol. 46, no. 9, pp. 2117–2144, Sep. 2020, doi: 10.1016/j.ultrasmedbio.2020.04.014.

Poster Number 17	**Macy Vreman,** BIOS/Lab on a chip group, University of Twente, the Netherlands
Title	**Control of lipid phase separation in the shell of monodisperse microbubbles**

Abstract

Phospholipid-coated microbubbles with a uniform acoustic response are a promising avenue for functional ultrasound sensing, e.g. of blood pressure and cell phenotype. A uniform acoustic response requires both a monodisperse size distribution and uniform viscoelastic shell properties. Recently, it has been shown by Spiekhout et al. (Appl. Phys. Lett 124, 231601. 2024) that monodisperse microbubbles formed by microfluidic flow focusing can have non-uniform viscoelastic shell properties leading to a non-uniform acoustic response. In the same work, it was also shown that monodisperse microbubbles can have non-uniform phase-separated shell microdomains, which ultimately may explain their non-uniform viscoelastic shell properties. Here, we aim to understand and thereby control phase separation in the shell of monodisperse microbubbles to increase their acoustic uniformity. We hypothesize that phase separation occurs during the diffusive stabilization of the microbubbles, which initially have a loosely packed shell until their size has reduced by a factor of 2 to 3. The stabilization timescale relative to the timescale of domain nucleation and growth is then assumed to be key in the final shell microstructures on the stable bubble. To test our hypothesis, we control the stabilization time (compression rate) of the microbubbles over 4 orders of magnitude in time by filling the bubbles with three different gases, with different solubility in water: CO_2 , N_2 and C_3F_8 . Our results demonstrate that microbubbles with a stabilization time of the order of 1 second do not show distinctive shell microdomains. In contrast, microbubbles that stabilized in the order of 10^2 seconds have small shell microdomains with a nearly uniform size. For a stabilization time of the order of 10^4 seconds, the shell microstructures were larger and their sizes became non-uniform. Our work shows that the compression rate of the lipid shell is key in the formation of shell microstructures and future work should further elucidate the relation between shell microstructure and acoustic response.

Poster Number 18	**Jacob Wilson,** Kings College London
Title	**Formulation and characterisation of MRI-contrast phase-change nanodroplets with a 'theranostic' view of glioblastoma treatment**

Abstract

Background: Glioblastoma is an extremely aggressive brain tumor and currently has limited effective treatment options, with median survival of only 14 months. This limited survival is primarily attributed to the blood-brain barrier (BBB), which blocks the uptake of nearly all medications. A potential application to transiently 'open' the BBB to allow the entry of potential treatments is microbubbles (MBs) combined with focused ultrasound to cause cavitation mediated BBB opening. However, while MBs work in the clinic they have drawbacks such as poor pharmacokinetics and limited distribution throughout the tumour due to their large size. Therefore, we propose using nanodroplets (NDs), which have been shown to have higher stability than MBs in in vivo conditions. In addition, by incorporating MRI contrast lipid into can be tracked *in vivo and* potentially 'tagging' the tumour, as a secondary confirmation of BBBO.

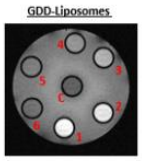
Methods: The MRI-contrast lipid, Gadolinium-DOTA-DSA (GDD), was synthesised using a similar method to *Kamal et al.* The MRI-NDs were prepared using modified methods outlined in *Zhang et al*, characterisation and stability studies were carried out using DLS and ^{19}F-NMR, DSC, and TXRF. NMR relaxivity was determined using a 9.4T NMR spectrometer and MRI phantom images were obtained using a 9.4T MRI. Cavitation analysis of the nanodroplets was completed using PCD, comparing the nanodroplets to controls (blank nanodroplets and clinically approved SonoVue®

Results: The GDD MRI lipid was successfully synthesised as confirmed by mass spectrometry. The MRI-NDs were successfully formulated, and their characterization data summarized in figure 1. Stability studies over a 21-period showed that the nanodroplets were stability, keeping a consistent size on DLS and their entire perfluorohexane content (>90%) as shown by 19F-NMR spectrometry.

NMR relaxometry showed similar T_1 relaxivity values (3.89 mMs^{-1}) for the nanodroplets to that of the commercially available small molecule agent GadoVist® (4.04 mMs^{-1}). MRI phantoms showed that MRI-NDs contrast can be seen 0.38 mg/mL lipid concentration (circle 4 in the figure). Cavitation analysis showed a significant difference between control and the nanodroplets at a pressure as low as 200-300 kPa and showed similar cavitation behaviour to the commercially available SonoVue® microbubbles.

Conclusions: GDD was successfully synthesized and incorporated into MRI-NDs. The MRI-NDs displayed suitable size, [Gd], perfluorohexane concentration and stability over a 21-day period. The nanodroplets showed comparable MRI contrast to clinically available contrast agents, as well as a good cavitation profile.

Nanoparticle	Z-average diameter (nm)	PDI	%v/v Perfluorohexane	[Gadolinium] (mg/mL)
GDD-NDs	111.2 (± 7.9)	0.306 (± 0.037)	0.404 (± 0.005) (Theoretical= 40%)	0.287 (± 0.014) (Theoretical= 86%)
GDD-Liposomes	120.6 (± 12.9)	0.217 (± 0.040)	N/A	0.783 (± 0.013) (Theoretical= 79%)
Blank NDs	93.9 (± 8.3)	0.255 (± 0.035)	0.470 (± 0.020) (Theoretical= 47%)	N/A
Blank Liposomes	108.4 (± 8.6)	0.188 (± 0.020)	N/A	N/A



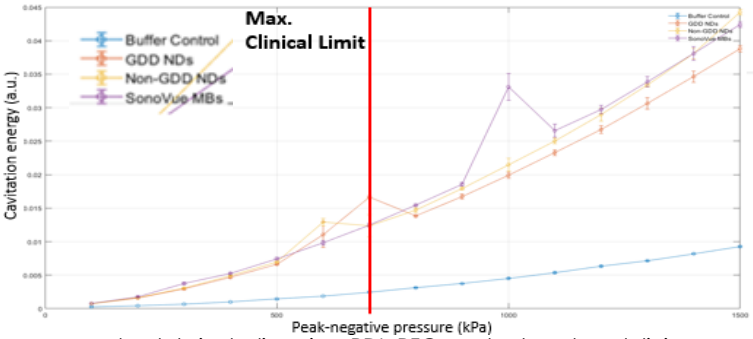


Figure- A) Characterisation data of the MRI-contrast phase-change nanodroplets including size, PDI, PFC content and gadolinium concentration. B) T1 MRI phantoms of the MRI-NDs and liposomes 1: 1.5, 2: 0.75, 3: 0.38, 4: 0.19, 5: 0.9, 6: 0.45 mg/mL lipid, c= buffer control. C) Cavitation energy for the MRI-NDs vs commercial MBs and buffer is shown at various acoustic pressures.

References- **1**. Argersinger DP et al., Cancers. 2021 . **2**.E. V .Rosca, et al., *Mol. Pharm.*, 2015, **12**,335–1346. **3.** D. V. Voronin *et al., Int. J. Mol. Sci.* 2021. **4.** Y. Meng *et al., Nature Reviews Neurology.,* 2020, **17**, 7-22. **5**. F. Maghsoudinia *et al., Europ. J. Pharm, Sci.* 2022, **174**. **6.** Kamaly, N *et al., Or. Biomol. Chem.* 2010, **8**, *201-211.* **7**. Zhang, W *et al. Ultrasonics Sonochemistry.* 2023, **97**, 106445.

Delegates

Meshary	Alotibi	University of Sheffield
Faraz	Amini	University of Glasgow
Damien	Batchelor	University of Leeds
Aya	Ben Issa	The University of Southampton
Abinash	Biswal	Brunel University, London
Mark	Borden	University of Colorado, USA
Kathryn	Burr	University of Leeds
John	Callan	Ulster University
Paul	Campbell	University of Dundee
Qi	Chen	University of Leeds
Amelia	Claxton	King's College London
Jolyon	Cleaves	Specialised Imaging
Louise	Coletta	University of Leeds
Paul	Cressey	King's College London
Bram	de Wilde	University of Twente
Melanie	Disher	Analytik
Antoine	Drolet	Bracco Suisse SA
Stephen	Evans	University of Leeds
Joseph	Fox	University of Leeds
Steve	Freear	University of Leeds
Anass	Hameddine	University of Twente.
Emma	Harris	Institute of Cancer Research, UK
Kristian	Hollingsworth	University of Leeds
Nicola	Ingram	University of Leeds
Ben	Johnson	University of Leeds
Klazina	Kooiman	Erasmus MC
Danial	Kordbacheh	University of Dundee
Theresa	Kosmides	Case Western Reserve University
Delanyo	Kpeglo	University of Leeds
Alex	Kramer	Croda
Anjali	Lad	University of Leeds
Guillaume	Lajoinie	University of Twente
Georgina	Lee	University of Leeds
Kieran	Logan	Causeway Chemicals
Mi-Ye	Lowes	University of Leeds
Jacques	Lux	UT Southwestern, USA
Alex	Markham	University of Leeds
Thomas	McKaig	Ulster University
James	McLaughlin	University of Leeds
Helen	McNamee	F2 Chemicals
Hilde	Metzger	University of Glasgow

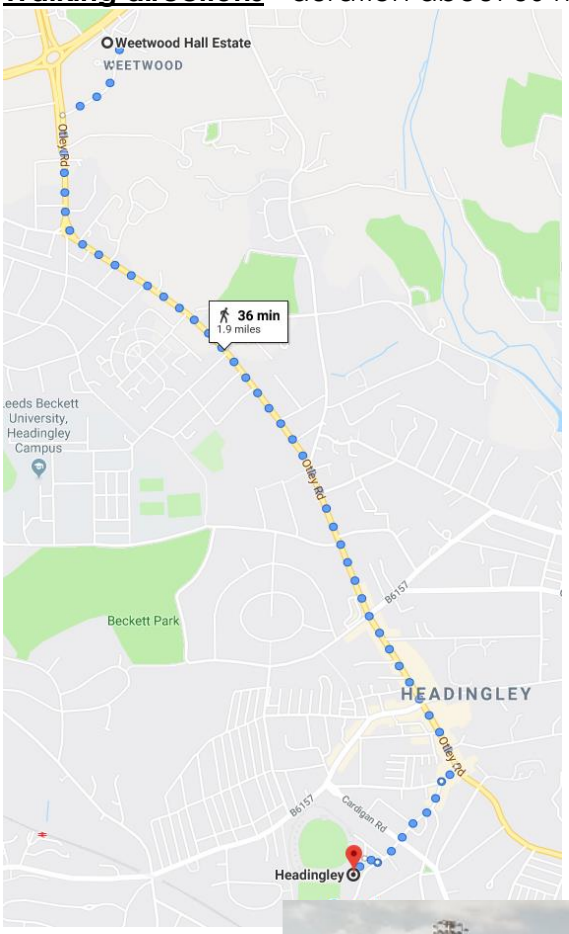
Andreas	Papoutsakis	University of Hertfordshire
Chris	Payne	Kings' College London
Nikos	Pelekasis	University of Thessaly
Giulia	Perilli	University of Rome Tor Vergata
Sally	Peyman	Heriot-Watt University
Suraj	Prashad Sharma	Brunel University London
Isabelle	Rogers	University of Leeds
Jonathan	Sandoe	University of Leeds
Tim	Segers	University of Twente
Yuhong	Shi	King's College London
Sam	Sloan	University of Southampton
Brian	Speyer	Shimadzu
Eleanor	Stride	University of Oxford
Bruce	Turnbull	University of Leeds
Martin	van den Broek	University of Twente
Michel	Versluis	University of Twente
Fleur	Vialle	University of Twente
Alessandra	Vitaliti	University of Rome Tor Vergata
Macy	Vreman	University of Twente
Jacob	Wilson	King's College London
Jack	Wright	Ulster University
Yuang	You	King's College London
Rienk	Zorgdrager	University of Twente

Conference Dinner – Directions:

Main Venue: Weetwood Hall Estate, Otley Rd, Leeds LS16 5PS
Dinner Venue: Headingley Cricket Ground (c/o Headingley Experience – Directors Suite, St Michael's Lane, Headingley, LS6 3BU).

Arrival from 19:00; Meal to be served at 19:30 (with wine / soft drinks)
****Please note**: The bar is cashless, so will only accept card payments****

Walking directions - duration about 30 minutes



- Turn left onto Otley Rd/A660: 1.4 mi
- Turn right onto St Michael's Rd: 200 ft
- Turn left onto St Michael's Ln: 0.2 mi
- Turn right through the entry gates (ENTRANCE B), then turn left towards the Headingley Experience Building, where you will be met by one of the Microbubble team.

We have a coach going from the front of Weetwood Hall departing at 18:55 and returning after the meal at approximately 21:45.