日本超音波医学会平成 20 年度第 1 回超音波分子診断治療研究会抄録

代　表：立花克郎（福岡大学医学部解剖学教室）
日　時：平成 20 年 5 月 27 日（火）
場　所：福岡大学医学部情報センター 4 階　映写室 2（福岡市）
【招待講演】座長　立花克郎（福岡大学医学部解剖学教室）

1）The Physics of Sonoporation

Dr Paul A. Campbell（Reader in Physics, Div. Electronic Engineering & Physics, Ewing Building, University of Dundee, Dundee DD1 4HN, Scotland. UK）

Microbubble contrast agents were originally developed to enhance echogenicity in diagnostic sonography. However, their somewhat unique acoustic response and facility to transduct energy into spatially focussed regimes, together with the clinical demand for non-invasive adjuncts and options to conventional therapy, have seen interest in exploring the therapeutic potential of microbubbbles grow steadily within this past decade or more. For the purposes of the present paper, the author has sought to select several key aspects of acoustically driven microbubble interactions,（both with o0117 ther bubbles, and with cells also）and to assess what we have learned, and perhaps more importantly, what we still require to understand better. This is presented in the context of both in vitro sonoporation experimentation, and also with more clinically related areas such as transdermal drug delivery

2）High speed observation of microbubbles

Nobuki Kudo（Hokkaido University, Japan）

Membrane perforation of endothelial cells with attached microbubbles caused by exposure to single-shot short pulsed ultrasound is described, and the mechanisms of membrane damage and repair are discussed. Real-time optical observations of cell-bubble interaction during sonoporation and successive scanning electron microscope observations of the membrane damage with knowledge of bubble locations revealed production of micron-sized membrane perforations at the bubble locations. High-speed observations of the microbubbles visualized production of liquid microjets during non-uniform contraction of bubbles, indicating that the jets are responsible for cell membrane damage.

日本超音波医学会平成 20 年度第 2 回超音波分子診断治療研究会抄録

代　表：立花克郎（福岡大学医学部解剖学教室）
日　時：平成 20 年 8 月 8 日（金）
場　所：北海道大学情報科学研究科（札幌市）
共　催：第 2 回基礎技術研究会
第 2 回基礎技術研究会共催の為，「超音波医学」36 巻 3 号に掲載されていますので，ご参照下さい．

日本超音波医学会平成 20 年度第 3 回超音波分子診断治療研究会抄録

日　時：平成 20 年 11 月 29 日（土）
場　所：日本学士会館（東京都千代田区）
共　催：第 7 回日本超音波治療研究会　Japanese Society of Therapeutic Ultrasound（JSTU）

1）マイクロバブルを用いた医用超音波の新展開

松本洋一郎（東京大学工学系研究科）

悪性腫瘍の治療法として，低侵襲であるというメリットを持つ HIFU（HighIntensity Focused Ultrasound）が注目されており , 数多くの臨床例が報告されている．しかし，超音波が伝播する過程で様々な組織で反射，屈折，減衰を起こすため，体内深部に存在する悪性腫瘍などには十分なエネルギーが届かず，治療が困難となる問題もある．その解決には超音波造影剤などマイクロバブルを用いて HIFU による加熱効果を増強する試みも行われている．ここでは，マイクロバブルを用いた HIFU 治療において，意図した部位のみを効率よく治療する手法を紹介する．特に，マイクロバブルの発熱作用と高温領域形成，生体組織におけるマイクロバブルの加熱凝固作用について考察とともに，超音波診断による加熱凝固領域の同時観察の可能性などについても紹介し，今後の技術の動向などを展望する．

2）パルス超音波とターゲティング気泡を用いた標的治療の可能性について

工藤信樹[1]，八木智史[1]，萩沢康介[2]，鈴木　亮[3]，丸山一雄[3]，
山本克之[3]（[1]北海道大学大学院情報科学研究科，[2]防衛医科大学，[3]帝京大学薬学部）

超音波照射により一時的に細胞膜の透過性を向上させることにより，細胞内に遺伝子や薬剤を導入するソノポレーションが注目を集めている．この手法では，一般に連続超音波が用いられているが，我々は，微小気泡存在下でパルス超音波照射により生じるソノポレーションについて検討を行っている．この手法では，あらかじめ気泡が接触した状態にある細胞にのみ損傷を生じるため，特定の細胞に付着する標的気泡の実現が大きな意味を持つ．そこで本発表では，特定の細胞の表面に発現するインテグリンに特異的に結合するリガンドを持つターゲット気泡の細胞選択性について検討を行なった結果について述べ，パルス超音波を用いたソノポレーション法の有用性を示す．

3）AG73 ペプチド修飾バブルリポソームの調製とその応用

根岸洋一[1]，角田由佳[1]，濱野信人[1]，遠藤葉子[1]，鈴木　亮[2]，野水基義[1]，丸山一雄[2]，新槇幸彦[1]（[1]東京薬科大学薬学部，[2]帝京大学薬学部）

これまで我々は，生体適合性，血中安定性に優れた PEG 修飾リポソームに注目し，これに超音波造影ガスを封入したバブルリポソームに超音波を併用する遺伝子導入法の開発を行ってきた．これは超音波照射によりバブルリポソームが瞬時に崩壊することでキャビテーションが誘導され，それに伴うマイクロジェット流により細胞膜に一過性の小孔が生じ，遺伝子などを細胞内に送達させる方法である．しかしながら，バブルリポソームは超音波造影ガスが封入されているために浮上しやすく，in vitro での遺伝子導入の効率化には，細胞膜付近でのキャビテーション誘導が望ましいと考えられる．そこで，本研究では，癌細胞や新生血管に高発現するシンデカンに特異的なリガンドであるラミニン由来ペプチド（AG73）で修飾したバブルリポソームを新たに調製し，その遺伝子導入効果，ターゲッティング型超音波造影剤としての可能性について検討を行ったので報告する．

社団法人日本超音波医学会平成 20 年度第 4 回超音波分子診断治療研究会抄録

代　表：立花克郎（福岡大学医学部解剖学教室）
日　時：平成 21 年 1 月 9 日（金）
場　所：名古屋駅前イノベーションハブ（名古屋市）
共　催：日本ソノケミストリー学会

1）微小気泡のふるまいの音響的・光学的観察　Evaluation of microbubble behavior using acoustical and optical methods

工藤信樹，山本克之（北海道大学大学院情報科学）

In this study, two methods for evaluating behavior of microbubbles under exposure to ultrasound were examined. One is an optical method that directly visualizes bubble behavior using a high-speed camera, and the other is an acoustical method that estimates bubble behavior by measurement of transmission characteristics of a bubble suspension at different pressure amplitudes of probing pulses. These methods have complementary roles because the acoustical method can evaluate characteristics of a bubble suspension that contains bubbles of various sizes, whereas the optical method can evaluate only one or two bubbles at a time. According to the experimental results, acoustic properties of microbubbles of ultrasound contrast agents estimated using the two methods showed good agreement, suggesting the usefulness of these complementary methods.

2）超音波キャビテーションのその治療応用　Acoustic cavitation and its therapeutic use in High Intensity Focused Ultrasound（HIFU）

吉澤　晋，梅村晋一郎，松本洋一郎（東北大学工学部）

In the medical ultrasound applications, acoustic cavitation and microbubbles are closely tied to the diagnostic and therapeutic uses. The therapeutic use of microbubbles has recently been the subject of much interest. In a HIFU treatment, the heat generated by the bubble oscillation contributes to an enhanced localized heating effect. In the lithotripsy of renal calculi, the acoustic cloud cavitation contributes to the comminution of the renal stones. In all these applications, it is essential to understand the microbubbles and bubble cloud dynamics. The bubble motion and bubble cloud behavior are strongly influenced by the internal phenomena of the bubbles, such as thermal diffusion, mist formation, mass diffusion and heat and mass transfer through the bubble wall. Temperature monitoring with thermocouples and a thermal liquid crystal sheet are conducted in in vitro experiments to investigate the behavior of the microbubbles and its effect on the therapy. In in vivo experiments, monitoring with an ultrasound diagnostic system, microbubble enhanced HIFU are applied. It is shown that microbubbles are utilized to enhance the heating efficiency in the HIFU process.

3）リポソーム型微小気泡（バブルリポソーム）の開発とドラッグデリバリーへの応用　Development of liposomal bubbles（Bubble liposomes）as novel tools in drug delivery system

鈴木　亮，小田雄介，宇都口直樹，丸山一雄（帝京大学薬学部）

In cancer immunotherapy with dendritic cells（DCs）, which are the most potent antigen-presenting cells, it is important that DCs present peptides derived from tumor-associated antigens on MHC class I molecules and activate tumor-specific cytotoxic T lymphocytes（CTLs）. However, MHC class I molecules generally present endogenous antigens expressing in cytosol. Development of an excellent antigen delivery system capable of inducing efficient presentation of exogenous antigens via MHC class I in DCs is required for the establishment of effective cancer immunotherapy with DCs. Recently, we developed the novel liposomal bubbles（Bubble liposomes（BLs））entrapping perfluoropropane gas which is utilized for contrast enhancement in ultrasonography. In addition, we reported that BLs are promising as effective gene delivery tools by the combination with ultrasound（US）exposure in vitro and in vivo1-2）. In the gene delivery mechanism, the mechanical effect based on the disruption of BLs by US exposure, which results in generation of some pores on plasma membrane, might be associated with direct delivery of extracellular plasmids into cytosol. Therefore, we thought that the combination of BLs and US exposure would directly deliver exogenous antigens into cytosol of DCs（Fig. 1）. In fact, this approach could efficiently deliver exogenous antigens into MHC class I antigen presentation pathway（Fig. 2）, and induce potent anti-tumor effect by the immunization of antigen-delivered DCs（Fig. 3）. In this presentation, we would like to discuss cancer immunotherapy based on BLs-mediated antigen delivery to DCs.

4）Novel Microbubbles: Evaluation and Bioeffects

Mariame A. Hassan, Takashi Kondo（Department of Radiological sciences, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama）

As a consequence to the ample applications of microbubbles in ul-

trasonography and therapeutic ultrasound and; meanwhile, the lack of coherent mathematical models that govern the bubble stability and behavior outside or within the acoustic field, the area of research has been wide open for novel microbubbles to be introduced, unfortunately, on random bases. Many studies have emerged with new procedures and setups for composing microbubbles using different shell materials and gases. In fact, it is no longer the issue of tailoring microbubbles that concerns alone, but the method for evaluating these products does. The strict evaluation offers the way of using these efforts exerted and forcing them on the track for clinical applicability.

The evaluation of novel microbubbles can be generally classified into two stages, the first one deals with the physical stability of the bubble suspension before irradiation. In this stage, the stability should be guaranteed not only to ensure that the microbubbles are uniformly provided each time they are manufactured but also to ensure that they will survive in-vivo until they reach the target organ where they will be irradiated. The second stage involves the determination of the safety of the microbubbles on the surrounding tissues after irradiation. Microbubbles have been shown to elicit numerous biological responses, ranging from cytolysis to subtle membrane changes, as controlled by the nature of their constituents and the their behavior in the acoustic field. These effects, however, could also offer some advantages as they open the way for therapeutic uses in cancer therapy or gene delivery. Thus the assessment of the bioeffects of microbubbles doesn't result in an all-or-none decision but redirects the new products to their proper use.

Here, we will demonstrate an example of the evaluation of two different groups of novel microbubbles. The first group consisted of air-filled bovine serum albumin（BSA）microbubbles of different sizes for which stability studies were conducted using a simple, yet sensitive method based on light scattering. The validity of the method was compared to another conventional methods in use. The second groups of microbubbles consisted of three microbubbles, namely, AS-0100, BG6356A and BG6356B, which possessed different shells and gas cores. These bubbles had already their stability assessed and they were tested for their biological and chemical effects including the enhancement of ultrasound-induced apoptosis and cell lysis and free radical production.

5）マイクロバブルを用いた微細血流の可視化技術　Ultrasound Imaging of the parenchymal blood flow using microbubbles

神山直久（東芝メディカルシステムズ㈱ 超音波臨床応用研究開発グループ長）

Ultrasound contrast imaging associated with microbubble-based contrast agent is explained in this manuscript. When microbubbles in liquid are stimulated by the ultrasound pulse, the echo signals from the microbubbles include higher-order frequency components which does not include in the transmission pulse. Principal of the microbubble imaging is based on the extraction of such signals, which is conventionally called "harmonic imaging". As well as imaging, bubble-distraction method is also one of the unique techniques in the ultrasound microbubble imaging. By the transmission at higher acoustic power, microbubbles in the scan fields rapidly disappear and the reperfusion of the vascular and organ perfusion will be visualized in real-time.

6）Ultrasonic Synthesis and Characterisation of Air- and Oil-filled Lysozyme Microspheres

Muthupandian Ashokkumar,[1] Meifang Zhou[1] and Francesca Cavalieri[2]（[1]School of Chemistry, University of Melbourne, VIC 3010, Australia; [2]2Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma）

The emulsification and chemical effects generated during acoustic cavitation have been used for synthesising air- and oil- filled protein microspheres［1, 2］. When lysozyme is used as the protein, stable microspheres are formed. The stability of the air-filled microbubbles is studied using pulsed sonoluminescence technique. Sunflower oil-, tetradecane- and perfluorohexane- filled lysozyme microspheres have also been synthesised successfully. Among the three different oils used, perflurohexane-filled microspheres are found to be relatively stable with a narrow size distribution. In order to explore the possibility of encapsulating biofunctional molecules（e.g., drugs）, a fluorescent dye dissolved in oil is encapsulated and its ultrasound-induced release has been investigated.

7）マイクロバブルと超音波治療　Microbubbles and Therapeutic Ultrasound

立花克郎（福岡大学医学部解剖学教室）

Therapeutic ultrasound catheters and extracorporeal ultrasound probes are under development specifically for this purpose, some already in clinical trials. Such examples as enhancement of thrombolytic agents by ultrasound have proven to be beneficial for acute stroke patents and peripheral arterial occlusions. Non-invasive focused ultrasound in conjunction with anti-cancer drugs may help to reduce tumor size, lessen recurrence as well as reduce severe drug side effects. Chemical activation of drugs by ultrasound energy for treatment of atherosclerosis and tumors is another new field recently termed as "Sonodynamic Therapy". Lastly, advance in molecular imaging has also initiated great expectations in applying ultrasound for both diagnosis and therapy at the same time. Microbubbles or nanobubbles targeted at the molecular level will permit medical doctors to make a final diagnosis of a disease by ultrasound and immediately proceed to therapeutic ultrasound.