氏　　　　名	小玉　哲也（こだま　てつや）
学 位 の 種 類	博士（医学）
学位授与年月日	平成 23 年 2 月 23 日
学位授与の条件	学位規則第 4 条第 2 項
最 終 学 歴	
学 位 論 文 題 目	Application of Bubbles and Waves to Medicine (気泡と波動の医学応用に関する研究)
論 文 審 査 委 員	主査　教授　福本　学 教授　小野　栄夫　　教授　荒井　陽一

論 文 内 容 要 旨

A bubble is a small particle that contains gas. In the biomedical field, bubbles are divided into two types of cavitation: stable and inertial cavitation (referred to as cavitation bubbles in the field of engineering). The former is the formation of pockets of gas in a liquid arising as a result of the creation of a new cavity, whereas the latter arises through the expansion of pre-existing gaseous nuclei. A stable gas bubble in a liquid will slowly dissolve because of the excess internal gas pressure required to balance the surface tension pressure.

When waves with larger than the threshold needed to induce cavitation bubbles propagate in the human body, impulsive pressures such as shock waves and liquid jets are generated

as a result of the bubbles' collapse, leading to tissue damage. Cavitation bubbles remain as gas bubbles for a certain time, and these gas bubbles interact with the subsequent waves, resulting in bubble oscillation, liquid jets, and shock waves. Therefore, the tissues surrounding bubbles are exposed to these superimposed impulsive pressures. On the other hand, the waves that interact with the bubbles are scattered and dissipated. Extracorporeal shock wave lithotripsy is a representative example that was developed based on controlling the impulsive pressures of waves and bubbles. On the other hand, contrast enhanced ultrasound imaging was based on the wave scattering.

In my doctoral dissertation, I describe the application of bubbles and waves to medicine in five chapters, and systematically elucidate biological responses to bubbles and waves.

Chapter 1 describes a rapid revascularization therapy using liquid jets generated by the interaction of gas bubbles and shock waves, which produce an impact on the thrombi. First, the interaction of a shock wave with a gas bubble attached to an artificial thrombus that is inserted into a tube model of a cerebral artery is investigated using a high-speed framing camera. Second, the fibrinolysis induced by the impact of the liquid jet on the urokinase is explored. It is shown that a rapid recanalization therapy for a cerebral embolism, using the impact of a liquid jet generated by the interaction of gas bubbles with shock waves, can potentially penetrate the thrombi in as little as a few μs with very efficient ablation.

Chapter 2 describes the mechanism of the tissue damage because of the cavitation bubbles generated behind shock waves during extracorporeal shock wave lithotripsy. First, I propose a new percutaneous transluminal shock wave source, which is an ideal shock wave source, to investigate the interaction of the tissue with a single shock wave. Next, I describe an investigation, using high-speed photography, of the behavior of cavitation bubbles attached to either a mimic or a tissue surface with a shock wave, and present a mechanism for bubble-mediated tissue damage.

Chapter 3 describes a molecular delivery method for cancer cells using shock waves. The pressure profile of a shock wave indicates its energy content, and the shock wave propagation is closely associated with tissue displacement, leading to cellular permeabilization and subsequent tissue damage. First, I investigate the relation between the shock wave profile and uptake of fluorophore into cells. I conclude that the impulse of the shock wave (i.e., the pressure integrated over time), rather than the peak pressure, is the dominant factor for the fluorophore uptake into living cells. Next, I show that shock waves can deliver molecules having a molecular weight of up to 2,000,000 into the cytoplasm of a cell without toxicity, and thus may have applications in gene therapy. Finally, I state that shock waves may have applications in promoting the cytoplasmic delivery of toxins into cancer cells after intratumoral injection.

Chapter 4 describes a molecular delivery method that uses microbubbles in the presence of ultrasound. First, I show that the cavitation bubbles created by the collapse of microbubbles are a key factor for transfection, and that their intensities are enhanced by the interaction of the superposed ultrasound with the decreasing height of the medium. Second, I evaluate the potential use of ultrasound and microbubbles for the delivery of oligodeoxynucleotides to human saphenous veins, with the long term objective of developing a way of preventing intimal hyperplasia in vein grafts.

Chapter 5 describes a molecular delivery method that uses nanobubbles in the presence of ultrasound. First, I develop acoustic liposomes (ALs) (nanobubbles) that encapsulate a phosphate buffer solution and perfluoropropane (C_3F_8) gas, and function as both ultrasound contrast agents and drug carriers. I elucidate the AL structure using transmission electron microscopy. Next, I investigate three types of nano/microbubbles—human albumin shell bubbles, lipid bubbles, and acoustic liposomes—in order to evaluate the efficiency of gene expression in skeletal muscle as a function of their physicochemical properties and the number of bubbles in solution. I report that acoustic liposomes show the highest transfection and gene expression efficiency among the three types of nano/microbubbles under ultrasound-optimized conditions.

Chapter 6 describes conclusions. The efficiency of fibrinolysis and molecular delivery into cells and tissue, as well as reduction in tissue damage, is regulated by impulsive pressures (shock waves, liquid jets, etc.) that are controlled by wave characteristics (pressure, wave profile, exposure time, repetition frequency, etc.) and bubble characteristics (size, surface materials, internal gas, etc.).

【所定様式⑬No.2】大学院の課程を経ない者（論博・医学）

審　査　結　果　の　要　旨

博士論文題名 Application of Bubbles and Waves to Medicine（気泡と波動の医学応用に関する研究）

受付番号 10B-1　　氏名 小玉　哲也

超音波や衝撃波などの波動が生体内を伝播する場合，ある条件下において，ガス気泡あるいはキャビテーション気泡が生体内に発生する．発生した気泡は衝撃波あるいは液体ジェットなどの衝撃圧を発生し，気泡周囲の生体組織に影響を与える．気泡と波動は相互に干渉し，組織損傷の発生や細胞への分子導入を可能にするものであるが，これまで，医学応用に関してこれらの作用機序が統一的に解明されてこなかった．本論文では，以下の医学研究課題に取組み，これらの作用機序を統一的に明らかにするものである．

第１章では，ガス気泡と衝撃波との作用で発生する液体ジェットを利用した脳塞栓血行再建術の開発を目的にした．血管内での液体ジェットの発生法を提唱し，この液体ジェットの利用により，既存の血栓溶解剤よりも血栓溶解率が有意に促進されることが実験的に明らかにされた．

第２章では，体外衝撃波結石破砕術で発生するガス気泡と衝撃波の相互干渉の解明を目的にした．高速度カメラ観察から，ガス気泡と衝撃波の干渉で発生する液体ジェットの組織損傷の機序を解明した．

第３章では，衝撃波の作用による細胞内への分子導入法の開発を目的にした．分子導入効率は衝撃波の撃力値に依存し，外来分子は分子量で最大 2,000,000 まで細胞内に導入されることが示された．また，リポゾーム不活化タンパク質をがん細胞に導入することで抗がん作用を有意に誘導されることが明らかにされた．

第４章では，超音波とマイクロバブルを用いた分子導入法の開発を目的に，外来分子の細胞内導入に求められる超音波の照射条件やマイクロバブルの濃度条件の最適化をおこなった．また，細胞膜の構造変化に寄与する，キャビテーション気泡の衝撃波の伝播距離を理論的に提示した．

第５章では，ナノバブルと超音波を用いた分子導入法の開発を目的にした．ガスと液体を同時に封入可能な音響性リポソーム（ナノバブル）を開発し，その構造を透過型電子顕微鏡で明らかにした．このナノバブルと超音波の組合せは，マイクロバブルよりも高い導入効率が得られることが明らかにされた．

第６章は結論である．

本論文は，気泡と波動の医学応用を目的に，脳塞栓血行再建術の開発，体外衝撃波結石破砕術，衝撃波による分子導入法，マイクロバブルと超音波を用いた分子導入法，ナノバブルと超音波を用いた分子導入法，における気泡と波動の生体効果を統一的に明らかにしたものである．本論文は，医学の進展に貢献すること多大であり，博士（医学）の学位論文として合格と認める。

学力確認結果の要旨

平成 22 年 12 月 1 日，審査委員出席のもとに，学力確認のための試問を行った結果，本人は医学に関する十分な学力と研究指導能力を有することを確認した。

なお，英学術論文に対する理解力から見て，外国語に対する学力も十分であることを認めた。