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研究課題名（英文）Micro-plasma induced DNA/drug delivery

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研究成果の概要（和文）：DNA /薬剤/ワクチンをデリバーする新しい衝撃波アシスト非侵襲方法を開発することを目的とした。モデル構築、実験解析と最適化、生体組織のモデルとデリバーのイン・ビトロでの評価、および非侵襲的なデリバリーの皮膚モデル化がなされた。本研究に最適なパルスパワーシステムが用いられ、それらの効率、安全性、および費用対効果を分析した。パルスパワー生成衝撃波を用いて、新しい高効率マイクロ/ナノ粒子デリバリーシステム、衝撃加速及び針を用いないDNA /薬剤/ワクチンデリバリーのための自由表面の急速な動きを調べた。研究成果は、PLoS one、Theranosticsなどの主要な科学雑誌に掲載された。

研究成果の概要（英文）：The research aimed to investigate novel techniques using pulsed power and micro-plasma for developing a shock wave assisted non-invasive method for DNA/drug/vaccine delivery.According to the research plan, design and construction of the model system, experimental analysis and optimization of the techniques, tissue model and in-vitro evaluation of the delivery, and skin model non-invasive needle free delivery, have been performed.
Interesting results from the research have been obtained. The results include a new high efficiency micro-plasma micro/nano-particle delivery system, novel impulsive acceleration and rapid motion of free surface for DNA/drug/vaccine delivery, and new needle-free drug/vaccine delivery using pulsed power shock wave. The research outcome have been published in leading scientific journals, including PLoS one, Applied Physics Letter, Theranostics, and Biotechnology & Bioengineering Journals.

研究分野：　工学

キーワード：　パルスパワー　バイオエレクトリクス　衝撃波　DNA /薬剤/ワクチンデリバリー

1. 研究開始当初の背景

Physical methods for DNA/drug delivery have been an attractive research subject in recent years. Several advanced systems for vaccine/drug delivery into skin have been investigated and tried. The strategies included delivery of heavy particulate drug (biolistic) [1], operation of needleless jet injectors, patches with arrays of drug coated micro-projections, ultrasound permeabilizers to enhance vaccine diffusion into skin, laser and thermal ablation, and abrasive devices. The needle-free injectors have the advantage of controlled release to the depth of the dermis with a minimum and recoverable breach of the target. The minimum breach of the target cells will improve the uptake of drug, enhancing the effectiveness of drug delivery.

Pulsed power, including shock waves, pulsed ultrasound, and pulsed laser, suggest novel possibilities for new advanced DNA/Drug delivery systems. Shock wave is a phenomenon that has sufficient strength to compress any matter in nature. Shock waves in condensed media have been intensively studied in connection with their applications to several industrial and medical procedures/devices. Shock waves in liquids were used for kidney and gall stone disintegration (in Lithotripters), rejuvenation of human cells, cure of heart and bone diseases, and as a mode of drug transfer by increasing permeability of cell wall. Interaction of underwater shock waves and pulsed ultrasound with micro-bubbles have been investigated to generate micro-fluid flow for DNA transfection to biological cells. Shock compression of solids has been investigated for drug delivery into soft living targets.

2. 研究の目的

Purpose of the research has been to investigate the use of a novel technique of pulsed power and laser micro-plasma for developing a shock wave assisted micro/nano-particle DNA/drug delivery method suited for non-invasive or less-invasive therapies.

The existing sophisticated drug delivery devices coupled with their skilled operators and conducive ambience increase the cost of operation, and may deter their extensive use in global health care. Extending non-invasive efficient drug delivery to a widespread primary use level, has been a thrust area of research, and a substantial effort is being devoted to achieve this objective worldwide. In this backdrop, we developed pulsed power micro-plasma, and shock wave driven needleless system for delivery of DNA and liquid/colloidal drugs into human skin, and soft body targets.

3. 研究の方法

For flow visualization, a high-speed framing camera (ULTRA-Neo, 5 ns exposure time, and 200MHz framing rate, 1000 x 860 pixels per image, nac Company, Japan) in a shadowgraph optical setup was used to observe the micro-plasma generation, high-speed particles and jets, and the underwater shock wave dynamics. An inter-frame time of 25–50 ns was adopted for the visualization that generated 12 frames in each operation.

For micro particle DNA/drug coated delivery, experimental set-up consisted of an Nd:YAG Laser at 1064 nm wavelength and 5.5 ns pulse duration, equipped with lenses and mirrors for fine focusing. The DNA coated gold particles of 1 um size were deposited on the posterior surface of a foil as a thin layer. In this new method, the ablation caused a micro-plasma, the sudden blow-off of which launched a shock wave through the foil and also ruptured the foil through an opening of a size of a millimeter, due to heat and pressure. Due to shock wave loading, the micro-particles deposited on the posterior surface of the foil accelerated, and when the foil ruptured, the ionized vapor from the anterior side of the foil rushed out as a tiny jet, further carrying the already accelerated particles onto the targets placed in the vicinity of the foil.

In order to study mechanism of shock wave loading and transmission in a drug delivery device, shock waves were generated by electric discharge through a magnetic pulse compression (MPC) circuit with 5 J/pulse energy. Tungsten electrodes of 800 um diameter and 15 mm length were used to generate a micro-plasma and lunch the shock wave. Degassed water with a salinity of 10% and a temperature of 295 K was used for an effective operation. The current and voltage during the operation were measured using a current monitor and a high-voltage probe, respectively. The typical peak voltage across the tungsten electrodes was approximately 16 kV with 0.42 kA of current value, over a full width at half maximum (FWHM) pulse duration of 250 ns. The shock acceleration of free surface directed us to design and construct a new needle-less drug delivery system.

4. 研究の成果

The operation of the device was analyzed through photography of the particle acceleration process using the high-speed camera aligned with the Shadowgraph optical setup. For the analysis of micro-particle acceleration, tungsten particles of 1 um size were used, as the tungsten particles have the same density as that of gold particles, and the particle dynamics did not change, while the cost of the test was reduced substantially due to the use of tungsten particles instead of gold. About 500 ug of tungsten particles (Bio-Rad) were deposited on the posterior surface of a 100 um thick aluminum (99.2% purity) foil and the anterior surface of the foil was ablated using the laser beam as explained in the methods. High-speed images of the launch of tungsten micro-particles from the foil surface on laser ablation were taken. The average velocity of the tip of the particle cloud were measured from the visualized photographs. The particle cloud accelerated on leaving the launch pad over a distance of about 5 mm, then attained a quasi-steady, average velocity of 1100 m/s for a distance of 10 mm, before declining. The acceleration and maintenance of a high velocity by the micro-particles for a substantial period is the main advantage of the present mode of operation of the device in comparison with our previous studies, where the particles decelerated quickly on leaving the launch pad, limiting the use to soft targets.

The particle delivery was initially tested on a gelatin slab of 3% concentration to analyze the impact on the target. Tungsten particles of 1 um size were used on this in vitro target, which was placed at a standoff of 3 mm from the launch pad. The 3% gelatin (strength: 20–25 bloom; cooled at 10^{o}C for 1 hour) represents human thrombus. The results showed the spread of the particles on the target surface on bombardment. The effective area of bombardment in which the particles penetrated the target was observed to be about 6 mm^{2} in gelatin. The effective area was expected to change with the target standoff from the launch pad, as the particle cloud diverged under the influence of plasma jet. For in-vivo DNA delivery, plasmid DNA coated gold micro-particles of 1 um size were used. The GUS activity in the cells confirmed the effective delivery. More details can be found in "PLoS one" article listed on publications.

Displacement of the free-surface of water on reflection of the rarefaction wave was observed in the high-speed magnified visualizations. The movement of the interface was highly impulsive with an average displacement of about 115 um over an average duration of 250 ns. The micro-plasma generated between electrodes after the discharge was well observed. The micro-plasma drove the shock wave in water. The shock movement observed was corresponding to a shock velocity of 1920 m/s, which was an average shock velocity just before its impingement on the water-air interface. At the point of interaction with the free-surface, the incident underwater shock wave could be discretized as a planar shock wave. The pressure and velocity of mass motion behind the shock wave just before reflection from the interface could be calculated. The pressure and particle velocity for a shock when interacted with interface with velocity of 1920 m/s are 0.444 GPa and 231 m/s, respectively. The initial average velocity of the free-surface of water on the reflection of the rarefaction wave from the water-air interface was measured to be 450 m/s, based on the visualized, time-resolved images of the free-surface. The time variation of the displacement of the free-surface of water for three experiments were ploted. The rise-time of the interface correlates well with the duration of shock positive impulse, which follows the time duration of energy release during the discharge. For shock pressure of order 0.5 GPa, the values of experimental and calculated free interface velocities are almost twice the particle velocities behind the shock waves, which confirm that free-surface velocity of two times of particle velocity gives a good approximation for the weak to moderate shock strengths. In a liquid micro-jet, the particle acceleration offers necessary impulse for the instantaneous emanation of liquid mass from the nozzles. Velocity of a jet determines its penetration depth in a soft target. In addition, predetermination of jet velocity based on known incident shock strength enables appropriate design and regulation of the power required to accomplish the task of needle-free, liquid drug delivery. Modulation of the shock strength based on the requisite jet velocity can minimize damage and invasion on the target cells, making the drug dissemination efficient. In diffusion based drug delivery methods, where the delivery is effected by enhancing the permeability of cell wall by shock impingement, the acceleration and displacement of the shocked liquid surface controls the volume of

drug delivery. This study, which was published in Applied Physics Letter, directed us to an advanced needle-free drug/vaccine delivery system.

The real-time images of development of the liquid micro-jet designed for an efficient needle-free drug delivery were optained. The velocity of the jet was measured from the visualized images, which is the average velocity of the tip of the jet. The diameter of the emanating jet was observed to be in the range of 140–160 um over the distance between the nozzle and the target. Variation of the jet velocity with respect to distance from the nozzle was measured. The test conditions could be varied by the variation of fill-gas pressures in the syringe. The velocity of the jet was of the order of 60–80 m/s and was observed to be nearly constant over a distance of 6 mm from the tip of the nozzle. The stand-off distance of the targets was within 6 mm from the nozzle tip. A 6.67% gelatin soft-tissue-mimicking model was used for jet delivery. The jet penetrated through about 10 mm in the gelatin. The 6.67% gelatin that has a bloom number of 250 g, models a soft tissue in human body. The device could deliver 18 uL of liquid in each pulse, out of which about 4 uL of liquid was found penetrated into the target. The idea behind this development was the delivery of a clinically acceptable small volume of liquid at a high-speed. The injection volumes are typically of the order of 1-10 uL in such delivery techniques. This ensures a controlled release with minimal pain.

The ex-vivo experiments of jet delivery were performed on the human skin. The images of the skin placed above a 6.67% gelatin soft-tissue mimicking block were obtained. The liquid of the jet was colored by methylene blue to identify the spot of its piercing through the skin onto the gelatin. The spots of jet penetration in the front and rear surfaces of the skin after the delivery indicated the extent of breach of the skin during jet penetration. The jet penetrated through a depth of 200 um in the skin samples (n=7), which is the depth of dermis, which contains skin's microcirculation system. The gelatin block placed beneath the skin during the jet delivery showed that the jet solution passed through the skin, confirming the penetration. The stand-off distance of the skin from the nozzle was adjusted at 1.5 mm in order that the jet impinged onto the target without spreading, rather maintaining all of its liquid mass in the potential core. The results has been published in Biotechnology and Bioengineering journal.

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6. 研究組織

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