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Shock waves for ballistic delivery of DNA droplets into living cells

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ABSTRACT

The biolistic approach of DNA/drug delivery was applied to deliver liquid DNA into living cells. The liquid DNA to be delivered was deposited as a drop on a thin aluminum foil and the posterior surface of the foil was ablated using an Nd:YAG laser. The ablation launched a shock wave through the foil. A part of this shock wave was transmitted to the drop, and a part was reflected back into the foil as an expansion wave. The wave motions caused the drug droplet to accelerate and acquire a sufficiently high velocity in the forward direction. A part of the propelled DNA droplet, on impacting the surface of a soft, living target, entered the target cells, accomplishing the drug delivery. The technique was tested on E-coli bacteria by delivering a plasmid DNA pUC119 into the bacterial cells. A few bacterial colonies could be transformed by this method of DNA delivery.

NOMENCLATURE

R	Reflection coefficient
z_1	Acoustic impedance of Aluminum
z_2	Acoustic impedance of water/DNA
T	Transmission coefficient

1. INTRODUCTION

The biolistic method has been widely used for the delivery of powdered vaccines into living cells. The method had its successful inception with the development of a particle gun to deliver nucleic acids, in particle form, into plant cells [1]. Due to the advantages associated with the delivery of powdered vaccines [2], the biolistic delivery was extended to clinical applications [3], and several devices were proposed to accelerate the drug-coated microparticles to sufficient momentum to penetrate soft targets [4, 5]. Administration of biolistic injections into skin has been proved to be one of the most efficient methods of protective immunizations [6, 7].

Gene therapy is used for the treatment of several inherent diseases such as cancer, cardiovascular and cerebrovascular diseases [8]. DNA vaccines are also used for protective immunizations against viral diseases [6]. These treatments are best realized when the vaccines/genes are delivered to the treatment sites locally, keeping a low systemic exposure to medication, thereby minimizing side effects, strokes and bleeding [9].

Biolistic delivery is a localized technique and can be employed to achieve the therapeutic purpose of introducing foreign genes into intact living cells. If the method of delivery is well controlled and non-intrusive, it can also be tried on internal treatment sites in human body [10]. Generally, in a biolistic approach, the drug is coated onto heavy metallic particles (such as gold), such that, on acceleration, the particles achieve sufficient momentum to penetrate the targets. There has been an apprehension that the remains of these heavy particles in the cells could cause some detrimental aftereffects and hence the biolistic approach, though quite efficient, remained limited to use on plant cells and external treatment sites in clinical procedures.

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The purpose of the present study is to apply a biolistic technique to delivery of liquid vaccines, thereby circumventing the inclusion of heavy particles in the process. The article presents the biolistic device, the method of drug delivery and some sample results.

2. MATERIALS AND METHODS

2.1. Experimental Procedure

A 1064 nm wavelength Nd:YAG laser beam that has a pulse duration and energy of 5.5 ns and 1.4 J/pulse, respectively, was used to ablate the posterior surface of a 100 μm thick aluminum foil, that contained 10 -15 μl of liquid DNA, in the form of a drop, on its anterior surface. A schematic of the setup is shown in Fig. 1. The surface of the foil that was exposed to the laser beam was covered by a BK7 glass overlay that was 2 mm in thickness. The BK7 glass is transparent to the light of 1064 nm wavelength and hence can provide an apt confinement to the ablation of the foil, thereby enhancing the ablation effects. The confined ablation was strong enough to launch a shock wave through the foil. The shock wave, at the foil-DNA interface, was partly transmitted into the DNA drop and was partly reflected back into the aluminum foil as an expansion wave due to the difference in the physical properties of aluminum and liquid DNA. The part reflection of the shock wave as an expansion wave into the aluminum foil, which moved in the backward direction, (in the direction opposite to that of the incident shock wave) unloaded the foil of the pressure induced by the incident shock wave. This unloading effect caused the foil to move suddenly in the forward direction (in the direction of the incident shock wave) at a substantial velocity. Moreover, the part of the shock transmitted to DNA drop propagated through the drop, and beyond the drop, at the drop-air interface, reflected back into the drop as an expansion wave. The two backward moving expansion waves, one in the foil and the second in the drop were collectively responsible for the forward movement of the drug drop. The drop was shattered into tiny droplets and acquired a high velocity towards the target surface. A schematic of the process of acceleration of the drop is shown in Fig. 2.

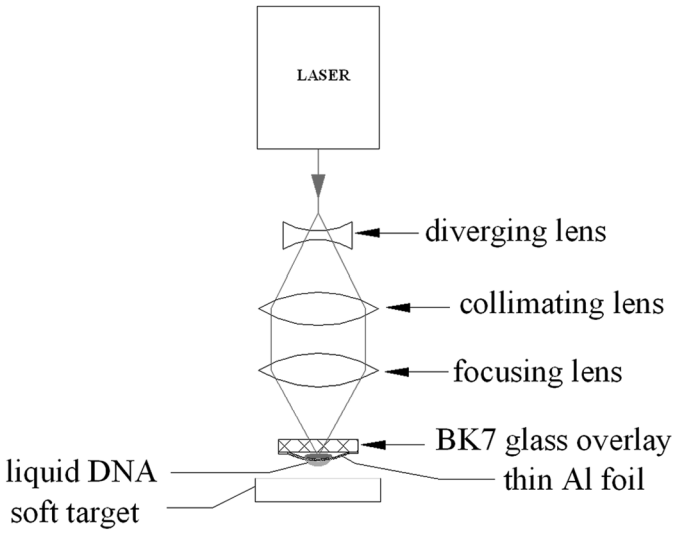

Figure 1. Arrangement of the experimental setup for drug delivery

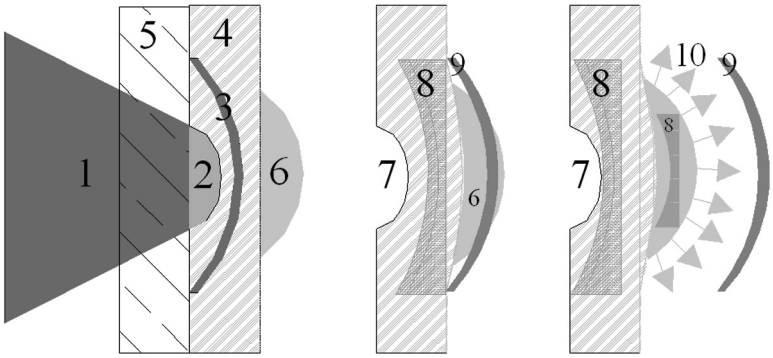

Figure 2. The physical process involved in the acceleration of the drug drop
[1 Laser beam. 2 Foil ablation. 3 Shock wave. 4 *Al* foil. 5 BK7 glass overlay.
6 Liquid-drug. 7 Micro-crater due to ablation. 8 Expansion wave.
9 Transmitted shock wave. 10 Acceleration of liquid drug drop.]

2.2 Materials

The drug used in this study was a plasmid DNA namely pUC119. About 10-15 µl (1-1.5 µg) of pUC119 was used in the form of a drop of about 3-4 mm diameter, when deposited on the foil surface. *E-coli* (Escherichia coli) bacteria (Strain: XL1Blue) were used as the target. *E-coli* were initially cultivated on an LB plate at 37 ^{0}C overnight. After delivering the liquid DNA into *E-coli*, these were spread on an LB containing Ampicillin, and were incubated at 37 ^{0}C for two days. The aluminum foil (99.2% purity; Nilaco) was used as the launch pad for the DNA drop. The acoustic speed of aluminum is quite high and this made the shock and expansion wave speeds very high in the foil.

3. RESULTS AND DISCUSSION

The liquid DNA drop in the present case is accelerated to high velocities using a laser ablation induced shock wave. Nd:YAG laser beam irradiates the surface of the foil, and in the process, a small portion of the foil surface, to the depth of its skin gets evaporated. The evaporated vapor is confined between the foil and the BK7 glass overlay, and hence gets pressurized and suddenly blows off launching a shock wave through the foil. The shock wave thus launched through the foil can be assumed to be a longitudinal compressive wave and this wave on reaching the foil-drop interface, gets partially transmitted into the drop. The amount of shock energy transmitted to the drop from the foil is dependent on the acoustic impedance match between the foil and the drop and can be calculated using the equations below:

$$R = \left[\frac{z_2 - z_1}{z_2 + z_1} \right]^2 \tag{1}$$

$$T = 1 - R \tag{2}$$

Since the sum of amount of reflected energy and the transmitted energy must equal the total amount of incident energy, the transmission coefficient T is calculated by subtracting the reflection coefficient from one (Eqn. 2). The density and longitudinal acoustic velocity for aluminum are 2700 kg/m^3 and 6198 m/s, respectively. Density and acoustic velocity for water (at standard conditions) are 1000 kg/m^3 and 1483 m/s, respectively. The physical properties of the DNA used are assumed to be very close to that of water. The acoustic impedance is the product of acoustic velocity and the density of the medium. Using the above values in Eqn. 1, the reflection coefficient for the foil-drop interface works out to be 0.7. Using Eqn. 2, the transmission coefficient for the interface works out to be 0.3. In other words, 30% of the incident shock energy gets transmitted to the drug drop, which again is in the form of a moving shock wave.

The 70% of the energy reflected back into the aluminum foil is in the form of an unloading wave, which rather gets converted into the kinetic energy of the foil. The energy transmitted to the drop

compresses the drop in the form of a moving shock wave and when this shock reaches the drop-air interface, again an unloading wave is sent back into the DNA drop. When the unloading wave propagates backwards through the drop, the drop gets expanded and shatters in the forward direction at a high velocity. In fact, the unloading waves induced in the foil and the drop are collectively responsible for the acceleration of the drop in the forward direction.

The acceleration of the drop was visualized using a high-speed, CCD video camera (Hyper-Vision HPV 1, Shimadzu Corporation, Japan) in a standard shadowgraph setup. The photography was carried out at a sampling rate of 500 kfps with a spatial resolution of 312×260 pixels. A continuous light source was used for the photography. The Nd:YAG laser was operated in a 2 Hz frequency mode. The first pulse was used to trigger the camera through the Q-switch, and the laser beam through this pulse was blocked from reaching the foil by a manual shield. The beam through the second pulse was used to ablate the foil and accelerate the drop. A delay generator was used to trigger the camera at the appropriate time and was connected between the trigger output from the Q-switch and the trigger input to the camera.

Figure 3 shows the photographs of the drug drop getting launched from the aluminum foil surface on laser ablation. The horizontal line in the first frame indicates the scale. The drop that is about 3 - 4 mm in diameter gets shattered into tiny droplets and expands in size as it moves away from the launch pad.

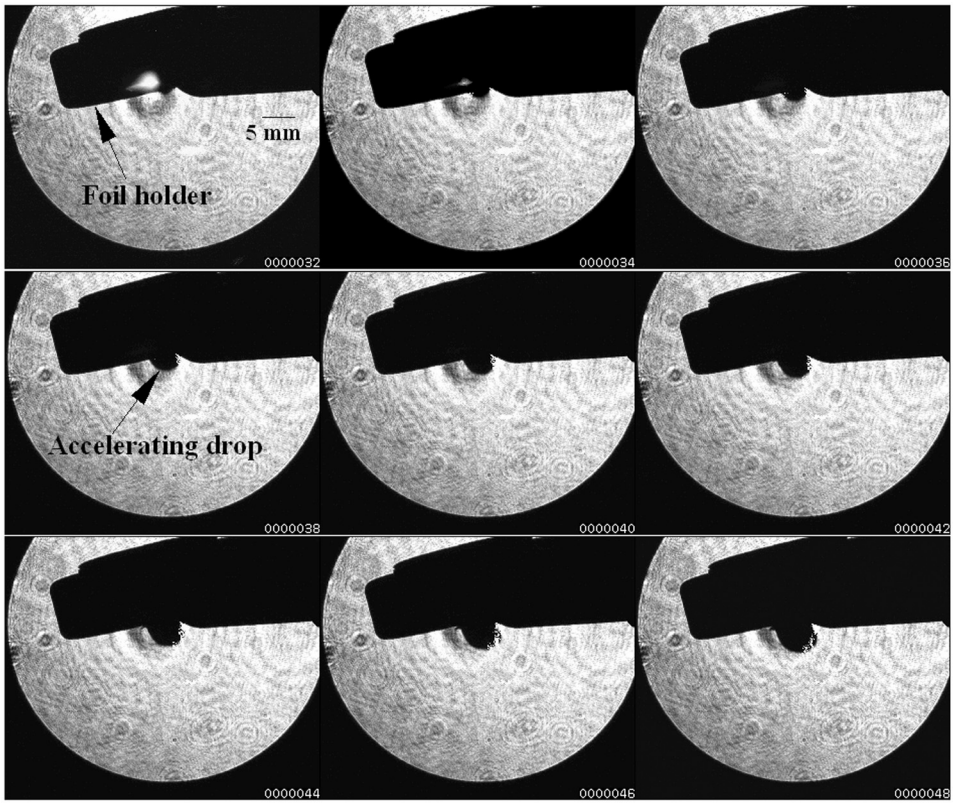

Figure 3. Visualized photographs of the accelerating drug drop on laser irradiation of the foil. The interframe interval is 2 μs. The frame-sequence is from left to right

The speed of the leading end (tip) of the drop with respect to distance from the launch pad was measured from the visualized pictures. Variation of the speed of the drop with the distance from the foil is plotted in Fig. 4. The y-axis (x=0) of the plot is the initial location of the tip of the drop, which is the reference line. The location of the foil is 0.95 mm upwards (x = -0.95 mm) from the leading end of the drop. The drop, on acceleration, attains an average speed of about 242 m/s, and is almost steady after traversing a distance of 2 mm from the reference line. Hence all the targets in the experiments were kept at a stand-off distance of 2mm from the reference line.

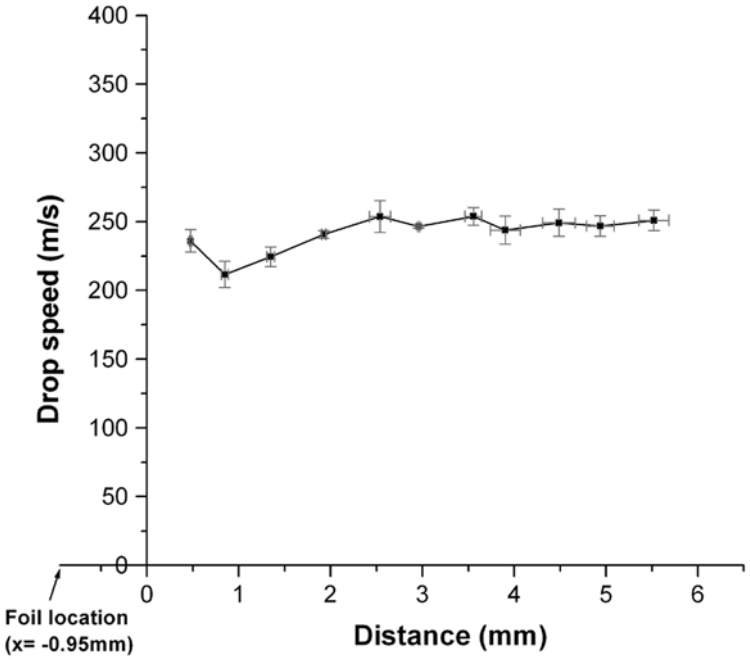

Figure 4. Velocity of the ejected drug drop measured from the visualized photographs

The transformed colonies of *E-coli* on delivering the DNA are shown in Fig. 5. The blue spots in the figure indicate the transformed colonies of the bacteria on LB plates containing Ampicillin. The plasmid DNA pUC119 develops immunity against antibiotics in *E-coli* and hence the bacteria that have received this DNA could survive and develop into colonies on LB plates containing Ampicillin, which is an antibiotic. The hydrodynamic pressure arising from the impact of the DNA droplets is believed to have forced the liquid DNA into the *E-coli* cells. A maximum of 10 *E-coli* colonies could be transformed by this method. The transfection efficiency of the process has been found less in comparison with the other liquid DNA delivery techniques into bacteria, such as electroporation and heat shock.

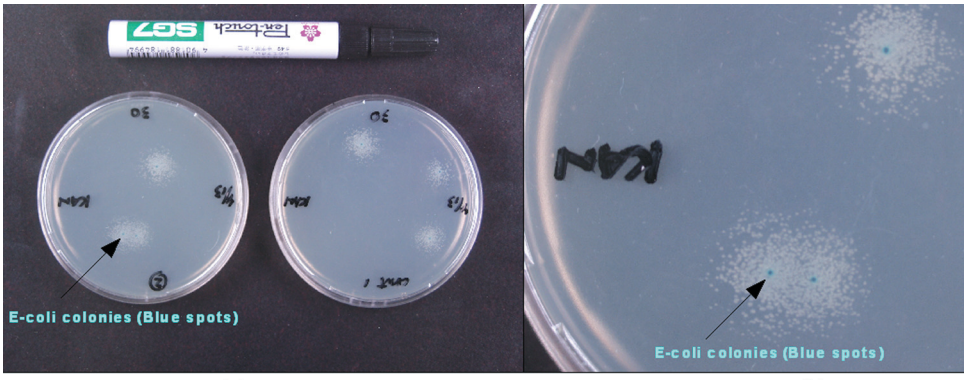

Figure 5. (a) The transformed *E-coli* colonies (blue spots) on LB plates containing Ampicillin. (b) Magnified view of the first plate in (a)

The present method can be used to deliver liquid drugs locally, even into interior treatment sites in human body. The delivery system can be miniaturized and a light guide or an optical fiber can be used to deliver laser pulses to the foil. Though, the transfection efficiency of the process is less at the moment, the technique can be used to accomplish certain intricate tasks such as intravascular drug delivery for cardiovascular, cerebrovascular and thrombolytic applications. The efforts to improve the transfection efficiency of the technique are underway.

4. CONCLUSION

We have demonstrated the feasibility of delivering liquid drugs into intact living cells through a biolistic approach, which is driven by laser ablation induced shock waves. Liquid plasmid DNA pUC119 has been delivered into *E-coli* cells successfully and the transfected *E-coli* could survive on an antibiotic medium and develop into colonies. The process of accelerating the liquid drug by laser irradiation of a metal foil has been studied through a high-speed photography and the speed of the propelled drug drop has been measured from the visualized pictures. This is a localized drug delivery technique with minimum invasion on the target, and may find potential applications in the treatment of vascular diseases, if the delivery device is miniaturized. Efforts to improve the transfection efficiency of this delivery method are underway.

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