

Relationships between the Particle Velocity and Introduction of Drug-Loaded Microparticles into the Skin in a Microparticulate Bombardment System

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Recently, it has been suggested that a microparticulate bombardment system would be a very useful tool for the delivery of a variety of powdered drugs as an alternative to parenteral injection *via* a needle. However the relationship between the particle dynamics and introduction into the skin has not been researched using this system. In the present study, we analyzed the velocity of microparticles bombarded by the HeliosTM gun system under various conditions using particle image velocimetry (PIV). The particle kinetic energy, which depended on the particle velocity and particle mass, was increased with increasing helium pressure and particle size, decreasing bombardment dose, resulting in the increased percentage introduction and relative bioavailability (F_{0-24h}). The particle velocity had a greater influence than the particle mass. Therefore, in order to be the most effective system for introduction into the skin, it is necessary to use a high helium pressure and microparticles of high density. However, it is also necessary to consider the skin damage after bombardment.

Key words microparticulate bombardment system; HeliosTM gun system; particle velocity; particle mass; particle image velocimetry

We have already reported that the bombardment with microparticles containing drug and antigen using a microparticulate bombardment system (HeliosTM gun system) is a very useful tool for the introduction of drug and antigen in microparticles into the skin.^{1–3)} The underlying principle is to accelerate microparticles to an appropriate velocity in a transonic gas jet, directed towards the skin, such that they can pass through the outer skin and lodge in deeper layers of tissue.⁴⁾ Therefore, this system is a very attractive alternative to traditional injection by needle and syringe. We have also demonstrated that introduction of drug- and antigen-loaded microparticles into the skin obtained by this system depends on the helium pressure, particle size and bombardment dose.^{1–3)} However, the relationship between the particle dynamics and introduction into the skin has not been researched using the HeliosTM gun system. This study is necessary to design the most suitable device and prepare the most suitable microparticles in this system.

Currently, a wide variety of flow analyzing techniques, such as laser doppler velocimetry (LDV), particle tracking velocimetry (PTV) and particle image velocimetry (PIV),⁵⁾ has been introduced to study the flow fields, for example, fluid mechanics for air and water flow, turbulent boundary layers, vortex shedding, impinging jets, two-phase flow and cavitation microstreaming flow induced by sonophoresis.^{5–9)} In particular, one advantage of the PIV technique over others like LDV is the short time, of the order of milliseconds, required to obtain the velocity maps, which permits the study of very rapid transients or flow oscillations in a plane.

In the present study, we applied PIV technology to the analysis of velocity of microparticles bombarded by the HeliosTM gun system under the various conditions. Indomethacin (IDM)-loaded poly-L-lactic acid microspheres (PLA MS) with different particle sizes were used as microparticles for introduction into the skin as well as tracer

particles. We also investigated the effects of helium pressure, particle size and bombardment dose on the particle velocity and the relationships between the particle velocity, mass and % introduction, bioavailability of IDM.

Experimental

Materials Indomethacin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Poly-L-lactic acid (molecular weight *ca.* 10000) was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). A polyvinyl chloride film (thickness 10 μm), used as the rupture membrane, was purchased from Hitachi Chemical Filtec Inc. (Tokyo, Japan). All other chemicals were of reagent grade.

Animals Male WBN/ILA-Ht hairless rats (body weight 240–290 g) were supplied by the Life Science Research Center of Josai University (Saitama, Japan). All experimental animal protocols met the Guidelines for Animal Experimentation approved by the Japanese Association of Laboratory Animal Science and the Japanese Pharmacological Society. The animals were maintained at 24 ± 1 °C under a 12-h light–dark cycle and had free access to a standard rodent diet and clean water.

Preparation of IDM-Loaded PLA MS IDM-loaded PLA MS were prepared by an oil/water (O/W) solvent evaporation method as described previously.^{1,2)} Briefly, 100 mg IDM and 300 mg PLA were dissolved in 3 ml dichloromethane (CH_2Cl_2). This solution was then added to 150 ml cold 0.5% polyvinyl alcohol aqueous solution while stirring at 2500 rpm. The mixture was then stirred at 2000 rpm for 25 min at room temperature. Thereafter, the mixture was continuously stirred at 1000 rpm for 5 h at 37 °C to evaporate CH_2Cl_2 . The hardened IDM-loaded PLA MS were collected by centrifugation (3500 rpm) and washed 6 times with distilled water. After freeze drying, the harvested PLA MS were divided into the required particle size ranges using stainless-steel mesh sieves (20–38, 44–53, 75–100 μm in diameter). The size distribution in all three kinds of sieved PLA MS was measured. The mean particle size in PLA MS of 20–38, 44–53 and 75–100 μm in diameter were 30.3 ± 7.33 , 49.8 ± 6.41 and 92.2 ± 12.6 μm , respectively (volume-weighted mean \pm S.D.). The density of the IDM-loaded PLA MS with different size ranges was measured by liquid displacement.¹⁰⁾ The density of microspheres was about 1.2 g/cm³ for all size ranges.

Determination of IDM Content in PLA MS Five milligrams IDM-loaded PLA MS was weighed precisely and dissolved in 5 ml acetone and the solution was diluted with acetonitrile to a total volume of 50 ml. Then, 200 μl of this mixture was added to 200 μl acetonitrile containing *n*-hexyl *p*-hydroxybenzoate (Tokyo Chemical Industry Co., Tokyo, Japan) as an internal standard. The amount of IDM in the solution was analyzed by HPLC to

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determine the IDM content of the PLA MS. The HPLC system used involved a pump (LC-10S), a UV detector (SPD 10A), an integrator (CRSA), a column oven (CTO-10A), a system controller (SCL-10A), an auto injector (Sil-10A) (Shimadzu Corp., Kyoto, Japan), and a reverse-phase column (Inertsil ODS-2, 5 μm , 4.6 \times 250 mm, GL Sciences Inc., Tokyo, Japan). The mobile phase was 0.1% phosphoric acid-acetonitrile (45:55, v/v) and the flow rate was 1.2 ml/min. The UV detector was operated at 262 nm and the column temperature was maintained at 40 °C. The IDM content of PLA MS with a diameter of 20–38, 44–53 and 75–100 μm was 10.4, 18.8 and 22.2%, respectively.

Particle Bombardment with IDM-Loaded PLA MS of the Skin Using the Helios™ Gun System Particle bombardment with IDM-loaded PLA MS was performed as described previously.^{1,2)} Briefly, after anesthetizing (25% (w/v) urethane, 1.0 g/kg, intraperitoneally (i.p.)) hairless rats, 0.5, 1.0 and 3.0 mg of IDM-loaded PLA MS (20–38, 44–53 and 75–100 μm in diameter) were transferred to the cartridge container fitted with a polyvinyl chloride film (thickness 10 μm) as a rupture membrane. The cartridge containers were placed in a cartridge holder with twelve holes. IDM-loaded PLA MS in the cartridge container was accelerated by the high velocity helium gas in the Helios™ gun system to bombard the shaved abdominal skin. In the analysis of particle flow, IDM-loaded PLA MS was bombarded in the absence of the rat skin. The helium pressures used were 100, 200 and 300 psi, respectively (1 psi=6890 Pa).

Image Analysis of Particle Flow In order to analyze the particle flow, image analysis was performed by the following system. The metal halide light from light source devices (HVC-SL, Photron Ltd., Japan) was used to irradiate the bombarded microparticles from the nozzle of the Helios™ gun system. The distance between the Helios™ gun system and the light devices was about 0.6 m. The intensity of illumination was about 800000 lx per single device and four devices were used to obtain a suitable intensity of illumination. The scattered light of microparticles bombarded from the Helios™ gun system under the various conditions was recorded using an ultra high-speed camera with a monitor (FASTCAM-ultima-I², Photron Ltd., Japan). The recordable frames in this system were 40500 frames/s. The frame pictures were used to calculate the particle velocity immediately after bombardment. The particle velocity was estimated by the two pictures taken at different times: since the position of the top of the mass of microparticles bombarded by the Helios™ gun system was different between two pictures taken, it was calculated by using the distance that microparticles were bombarded and the times. The particle velocity was measured at the spot supposed that the particles collided with the skin. This experiment was performed at least three times under the same particle bombardment conditions.

Measurement of Percentage Introduction of IDM into the Skin After bombardment of IDM-loaded PLA MS as described above, the skin (diameter 2.5 cm, area ca. 5 cm²) was excised immediately, and the surface was washed and cleaned using physiological saline (pH 7.2). The excised skin was homogenized with an adequate amount of pH 7.4 phosphate buffered saline (PBS) (2.5 or 5.0 ml) in a homogenizer (POLYTRON® PT 3000, KINEMATICA AG, Switzerland). The resulting skin homogenate was mixed with an adequate amount of ice cold acetone-acetonitrile (1:4) to give a volume of 7.5 ml and 15.0 ml and then shaken for 5 min. The supernatants were collected following centrifugation at 18000 $\times g$ at 4 °C for 5 min and then stored in a refrigerator until use.

Supernatants containing IDM (200 μl) were mixed with an aliquot (200 μl) of acetonitrile containing *n*-hexyl *p*-hydroxybenzoate as an internal standard. The amount of IDM in the mixture (50 μl) was determined by HPLC as described above. The percentage introduction of IDM into the skin was calculated as describe previously.¹⁾ The recovery of IDM from skin lysate was almost complete (99.8 \pm 0.66%, $n=6$).

Determination of Relative Bioavailability with Respect to Intracutaneous (i.c.) Injection Time courses of IDM plasma concentrations after i.c. injection of IDM solution (25 μl PBS, 100 $\mu\text{g}/\text{kg}$ in dose) and particle bombardment of IDM-loaded particles were measured as described previously.²⁾ Briefly, blood samples were collected from the jugular vein using a heparinized syringe at predetermined times and centrifuged at 13600 rpm (18000 $\times g$) for 5 min at 4 °C to obtain plasma. Plasma samples were mixed with acetonitrile containing *n*-hexyl *p*-hydroxybenzoate as an internal standard (1:2) and then centrifuged at 13600 rpm (18000 $\times g$) for 5 min at 4 °C. The obtained supernatants were stored in a refrigerator until analysis. IDM concentrations were determined by HPLC as described above.

Plasma data were analyzed by a non-linear least squares regression program (Algorithm: Damping Gauss-Newton method).¹¹⁾ The areas under the blood concentration-time curves (*AUC*) were calculated by the trapezoidal rule. The $F_{0-24\text{h}}$ (relative bioavailability with respect to i.c. injection) was

calculated as described previously.²⁾

Statistics Statistical analysis was performed using the two-tailed Student's *t*-test. Correlations were derived from linear regression analysis. A *p*-value of less than 0.05 was considered statistically significant.

Results and Discussion

Effect of Helium Pressure on the Particle Velocity

Figures 1a and b show the effect of helium pressure on the particle velocity, and the relationship between the particle velocity and % introduction, $F_{0-24\text{h}}$, respectively after bombardment with 1.0 mg IDM-loaded PLA MS, 75–100 μm in diameter, at different helium pressures. The particle velocity was measured at the spot supposed that the particles collided with the skin because the velocity of larger particles was more decreased during a long traveling distance generally. There was no change in the particle velocity within this field (data not shown). Therefore, the particle velocity was not influenced by air resistance in this study. The particle velocity increased on increasing the helium pressure (Fig. 1a). In our previous study, the percentage introduction and $F_{0-24\text{h}}$ increased on increasing the helium pressure.^{1,2)} Therefore, the higher particle velocity produced the higher percentage introduction and $F_{0-24\text{h}}$. These results suggest that the increased particle velocity by helium pressure affects the kinetic energy of microparticles, resulting in an increased % introduction into the skin and $F_{0-24\text{h}}$.

Effect of Particle Size on the Particle Velocity Figures 2a and b show the effect of particle size on the particle velocity, and the relationship between the particle velocity and % introduction, $F_{0-24\text{h}}$, respectively after bombardment with 1.0 mg IDM-loaded PLA MS, different particle size ranges, at 300 psi of helium pressure. The particle velocities were about 400 m/s, almost the same for each particle size range ($p>0.05$). Nevertheless, in our previous study, the percentage introduction and $F_{0-24\text{h}}$ tended to increase on increasing the particle size, although there was no significant difference between them at each particle size ($p>0.05$).^{1,2)} This result obviously differed with the above result which the percentage introduction and $F_{0-24\text{h}}$ increased on increasing the particle velocity. These results suggest that there are some factors that is related to the introduction of microparticles into the skin apart from the particle velocity. It seems that one of

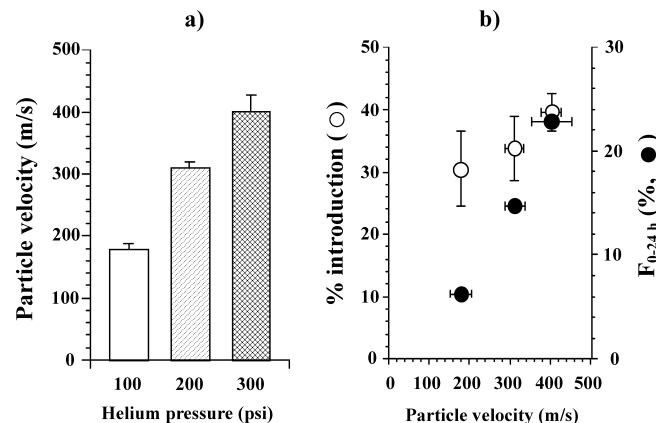


Fig. 1. Effect of Helium Pressure on the Particle Velocity (a), and the Relationship between the Particle Velocity and the % Introduction, $F_{0-24\text{h}}$ of IDM (b)

Particle size range; 75–100 μm , Bombardment dose; 1.0 mg. Each data represents the mean and standard error ($n=3-4$).

them is the particle mass related to the particle size and particle density.

Effect of Bombardment Dose on the Particle Velocity

Figure 3 shows the effect of the bombardment dose on the particle velocity after bombardment with different doses of IDM-loaded PLA MS, 75–100 μm in diameter, at 300 psi of helium pressure. Table 1 summarizes the relationship between the bombardment dose and % introduction, F_{0-24h} . The particle velocity tended to reduce on increasing the bombardment dose (Fig. 3). In our previous study, the percentage introduction tended to reduce and the F_{0-24h} reduced on increasing the bombardment dose.^{1,2)} Therefore, the reduced percentage introduction and F_{0-24h} were produced by decreasing the particle velocity (Table 1). These results suggest that the reduced percentage introduction and F_{0-24h} by in-

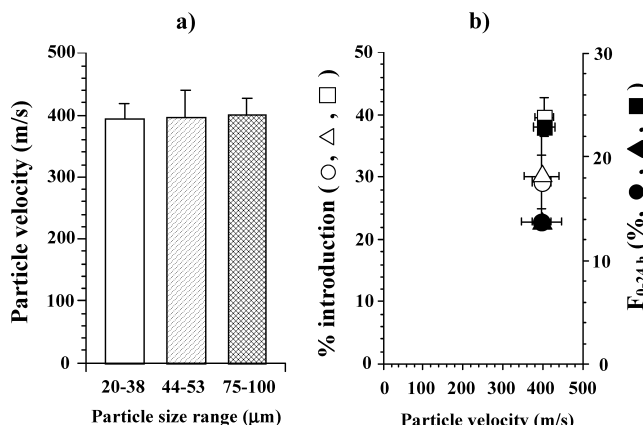


Fig. 2. Effect of Particle Size on the Particle Velocity (a), and the Relationship between the Particle Velocity and the % Introduction, F_{0-24h} of IDM (b)

Helium pressure; 300 psi, Bombardment dose; 1.0 mg. ○, ●: 20–38 μm, △, ▲: 44–53 μm, □, ■: 75–100 μm in diameter. Each data represents the mean and standard error ($n=3-5$).

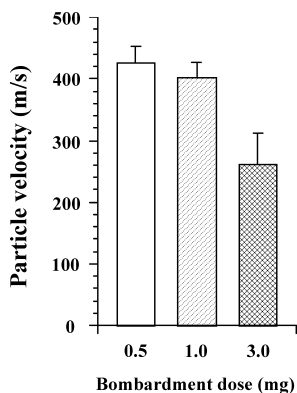


Fig. 3. Effect of Bombardment Dose on the Particle Velocity

Particle size range; 75–100 μm, Helium pressure; 300 psi. Each data column represents the mean and standard error ($n=3-6$).

Table 1. Relationship between Bombardment Dose and % Introduction, F_{0-24h} of IDM

Particle size (μm)	Bombardment dose (mg)	Helium pressure (psi)	Particle velocity (m/s)	Introduction (%)	F_{0-24h} (%)
75–100	0.5	300	426.3±27.1	35.6±4.2	25.0
75–100	1.0	300	402.3±24.3	39.6±3.0	22.9
75–100	3.0	300	261.2±50.4	20.1±1.7	14.7

Each data represents the mean and standard error ($n=3-6$).

creasing the bombardment dose depend on the particle velocity. However, the particle velocity in 0.5 mg of bombardment dose was slightly faster than that in 1.0 mg and the F_{0-24h} was also increased on increasing the particle velocity, but the % introduction tended to reduce against the above result. This result may be influenced by introduction depth of IDM-loaded PLA MS into the skin as shown in the absorption rate constant (k_a) in our previous report.²⁾

Relationships between the Particle Velocity and the % Introduction, F_{0-24h} of IDM

The above results indicated that the percentage introduction into the skin and F_{0-24h} were affected by the particle velocity and the particle mass. We investigated the effects of the particle velocity and the particle mass on the % introduction into the skin and F_{0-24h} . To evaluate the effect of particle mass, the masses of IDM-loaded PLA MS recorded at a particle velocity of about 400 m/s were chosen and estimated as the relative particle mass, that is, it assumed that the mass of the smallest IDM-loaded PLA MS (20–38 μm in diameter) calculated from the mean particle size was equal to 1. Figure 4 shows the effects of the particle velocity and the relative particle mass on the % introduction into the skin and F_{0-24h} in the microparticulate bombardment system. The percentage introduction into the skin and F_{0-24h} tended to increase on increasing the particle velocity and the relative particle mass.

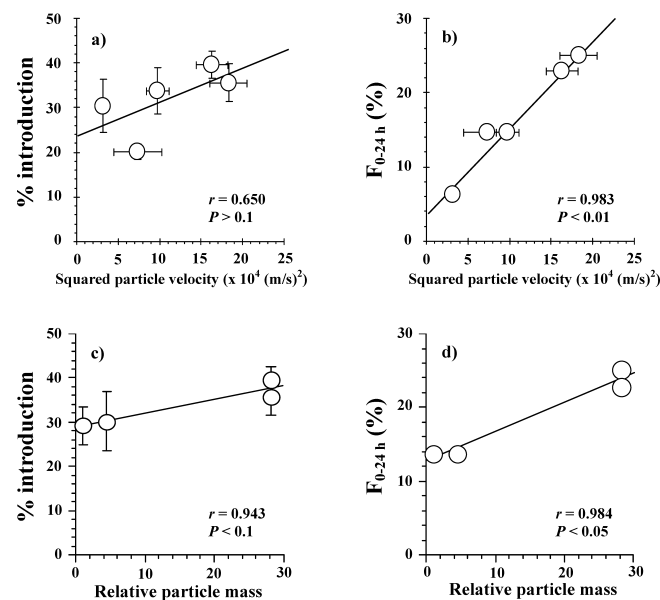


Fig. 4. Relationships between the Squared Particle Velocity, and the Relative Particle Mass and % Introduction, and F_{0-24h} of IDM

a) Effect of particle velocity on the % introduction, particle size range; 75–100 μm. b) Effect of particle velocity on the F_{0-24h} , particle size range; 75–100 μm. c) Effect of relative particle mass on the % introduction, particle velocity; about 400 m/s. d) Effect of relative particle mass on the F_{0-24h} , particle velocity; about 400 m/s. Each data point represents the mean and standard error ($n=3-6$).

In particular, there was a good relationship between F_{0-24h} and the squared particle velocity ($r=0.983$), the relative particle mass ($r=0.984$). This may be due to buried microparticles on the skin surface observed after bombardment (data not shown). Namely, the buried microparticles may be not reflected in the transport to the systemic circulation. The slopes between the squared particle velocity and % introduction into the skin, F_{0-24h} were clearly greater than those between the particle mass and % introduction into the skin, F_{0-24h} . These results suggest that the particle velocity has a greater effect on the % introduction into the skin and F_{0-24h} compared with the relative particle mass.

However, the particle mass had a little effect on the % introduction into the skin and F_{0-24h} . Although an increase in particle size is needed to increase the particle mass, the increase in particle size may cause a reduction in the % introduction into the skin because of an increased the particle frontal area. Indeed, the kinetic energy/particle frontal area ratio had a better correlation with the % introduction into the skin and F_{0-24h} ($r=0.657$ ($p>0.1$) and 0.960 ($p<0.001$), respectively) compared with the kinetic energy ($r=0.597$ ($p>0.1$) and 0.864 ($p<0.05$), respectively) (data not shown). Therefore, there may be an optimum particle size for the introduction into the skin. In order to enhance the % introduction into the skin, the particle density may be an important factor rather than the particle mass and the particle size.

Conclusion

In conclusion, the helium pressure and bombardment dose affected the particle velocity, and the particle size affected the particle mass, resulting in the influenced % introduction

into the skin and F_{0-24h} in the microparticulate bombardment system. The particle velocity more influenced the % introduction and F_{0-24h} compared with the particle mass. Therefore, in order to be the most effective system for introduction into the skin, it is necessary to use a high helium pressure and microparticles of high density. However, it is also necessary to consider the skin damage after bombardment.

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