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Needle-free injection of insulin powder: delivery efficiency and skin irritation assessment*

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Abstract: Insulin is widely used in treating diabetes, but still needs to be administered by needle injection. This study investigated a new needle-free approach for insulin delivery. A portable powder needleless injection (PNI) device with an automatic mechanical unit was designed. Its efficiency in delivering insulin was evaluated in alloxan-induced diabetic rabbits. The skin irritation caused by the device was investigated and the results were analyzed in relation to aerodynamic parameters. Inorganic salt-carried insulin powders had hypoglycemic effects, while raw insulin powders were not effective when delivered by PNI, indicating that salt carriers play an important role in the delivery of insulin via PNI. The relative delivery efficiency of phosphate-carried insulin powder using the PNI device was 72.25%. A safety assessment test showed that three key factors (gas pressure, cylinder volume, and nozzle distance) were related to the amount of skin irritation caused by the PNI device. Optimized injection conditions caused minimal skin lesions and are safe to use in practice. The results suggest that PNI has promising prospects as a novel technology for delivering insulin and other biological drugs.

Key words: Powder needleless injection, Insulin, Transdermal drug delivery, Skin irritation
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1 Introduction

Worldwide, the number of people suffering from diabetes was estimated to be 347 million in 2008, and more and more people use insulin to control their plasma glucose concentration (Danaei *et al*., 2011). However, insulin needs to be injected rather than orally administered because of its instability, like other biological drugs, in the presence of gastric acid and digestive enzymes. Thus, these drugs usually require needle injection delivery, which results in bleeding, pain, risk of cross-infection, and low compliance. The needle/syringe injection route is still the main administration method for the clinical application of insulin. To alleviate the pain and inconvenience of the patient, a variety of new non-injected routes for insulin delivery have been developed, such as insulin pumps, pulmonary inhalation, aerosol sprays of the nasal mucosa, oral mucosa attached agents, oral microsphere preparations, and oral

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liposomes or emulsions (Owens *et al*., 2003). Although these drug delivery technologies have made much progress and achieved some breakthroughs, developing new needle-free insulin delivery technology remains necessary.

A novel drug delivery technique named powder needleless injection (PNI) drew our interest. PNI uses high-speed gas flow to deliver vaccines or drugs in dry powder form into skin tissue without needles. It provides a promising approach for delivering drugs with both high bioavailability due to its special aerodynamic properties, and good compliance from patients due to its needle-free and pain-free advantages (Burkoth *et al.*, 1999; Chen *et al.*, 2001; Dean and Chen, 2004; Ziegler, 2008; Wang *et al.*, 2009). PNI is applicable to many types of drugs and especially to the administration of proteins, vaccines, and other biological agents (Mitchell *et al.*, 2003; Wang *et al.*, 2006; Zhou *et al.*, 2006). Preventive vaccines delivered by PNI have been reported (Wang *et al*., 2009); however, whether it is also effective for therapeutic biological drugs like insulin, and what level of delivery efficiency it can reach, are unknown.

The drug delivery efficiency of a previously reported PNI device, also called a transdermal powdered delivery (TPD) device (Sarphie *et al*., 1997; Burkoth *et al.*, 1999), is still uncertain. The bioavailability of our earlier prototype PNI device was also not desirable (Wang *et al*., 2007). In this study, we designed an automatic mechanical unit with a valve system, and used an enlarged de Laval nozzle to enhance the aerodynamic properties of the PNI device. Although they have the advantages of being needle-free and pain-free, PNI devices still have the potential to cause skin injury when used improperly. It is believed that the aerodynamic parameters of PNI devices are responsible for the skin irritation. Skin irritation caused by PNI has been reported (Sarphie *et al.*, 1997; Kendall *et al*., 2004; Arora *et al*., 2008), but assessments of the effects of gas pressure, gas flow speed, and the distance between the device and the skin have yet to be performed. The extent of skin lesions remains a concern when using this new device, and thus needs to be assessed.

PNI requires a drug to be in powdered form. Many drug carriers have been used to produce drugs in powdered form, such as solid liposomes, conjugates, nanoparticles, and microspheres (Agnihotri *et al*., 2004; Jaracz *et al*., 2005; Torchilin, 2005). However, drug powders for PNI, ideally, are expected to have high intensity, strength, hardness, and density, because delivery by PNI is totally dependent on physical impact. Polymer-based carriers of low density (nearly 1 g/cm^3 or lower) and a hollow-structure cannot meet the requirements of PNI delivery. So, we attempted to prepare an inorganic salt-based powder carrier with enough intensity, strength, and density, to achieve highly efficient delivery of insulin via PNI. We selected primarily three kinds of inorganic salts, aluminum hydroxide, calcium carbonate, and phosphate, to test as drug carriers for insulin. These inorganic salts are likely to be safe (Matheis *et al*., 2001; Li *et al*., 2007; Sivakumar *et al*., 2011). The use of an inorganic salt-based powder carrier in PNI delivery of insulin has not been studied before.

This study aimed to assess the delivery ability and skin irritation of PNI of insulin with a newly designed device and an inorganic salt-based drug powder.

2 Materials and methods

2.1 Ethics statement

This study was conducted in strict accordance with the recommendations of the Guidelines for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of China. The principles of laboratory animal care (National Institutes of Health (NIH) publication No. 85-23, revised in 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm) were also followed. The animal protocol was approved by the Committee on the Ethics of Animal Experiments of the 302 Military Hospital (Approval ID: 11-037). No surgery was performed and all efforts were made to minimize suffering.

2.2 Powder needleless injector

The newly designed powder needleless injector is primarily constructed of a gas cylinder, valve system, drug container, de Laval nozzle, and nozzle case (CN Patent 101829384 B). The structure scheme is shown in Fig. 1. Its working principle is similar to that of the previously reported PowderJect device (Sarphie *et al.*, 1997), but it has an automatic mechanical unit valve system and an enlarged de Laval nozzle.

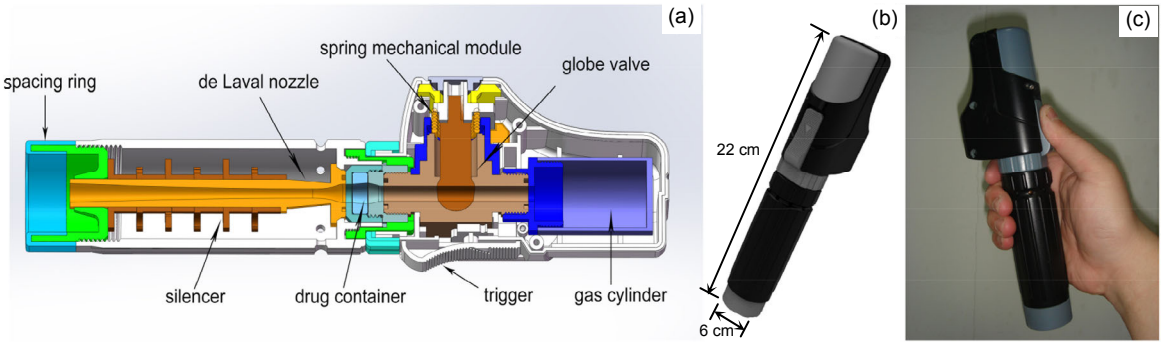

Fig. 1 Powder needleless injection (PNI) device (300.3 g)
(a) The structure scheme of the PNI device; (b) A 3D design sketch of the PNI device; (c) A prototype of the PNI device

The valve system consists of a globe valve controlled by a mechanical spring module. When the trigger is pushed, the mechanical spring module drives the valve automatically, and the gas stored in the cylinder is released. The designed pressure limit of the gas cylinder is 60 MPa. The drug container is pre-filled with drug powders and encapsulated with a plastic pellicle. The gas flow ruptures the plastic pellicle sealing the drug container, and then carries the drug powders through the de Laval nozzle. The two-phase flow of gas and powders accelerates in the de Laval nozzle into a supersonic flow. Upon exiting the supersonic flow, the gas and powders impact the skin surface. The gas flow then rebounds, and the drug powders immediately penetrate the skin. The nozzle case contains a spacing ring fixed on the end of the device and a silencer, which is used to decelerate the exhaust gas and reduce the noise.

2.3 Reagents and instruments

Porcine insulin was employed as the model drug. The raw insulin powder was supplied by the Wanbang Jinqiao Pharmaceutical Co., Ltd., Xuzhou, China. Alloxan (Sigma-Aldrich, USA) was used in the animal modeling. Sodium sulfide, vaseline, and nitrogen were prepared by the Fengtai Gas Supply Station, Beijing, China. Aluminum nitrate (analytical reagent (AR)) was prepared by the Shantou Xilong Chemical Factory, Guangdong, China. Ammonia water (0.25 g/ml), calcium carbonate, potassium dihydrogen phosphate, and dipotassium hydrogen phosphate were all of AR grade and supplied by the Beijing Chemical Company, China. Glucose assay kits were purchased from the Shanghai Rongsheng Biological Pharmaceutical Company, China. Distilled-deionized water was used throughout.

An AL-204 electronic analytical balance (Mettler-Toledo Co., Ltd., Shanghai, China), a DF-101S-type magnetic stirrer (Yuhua Instrument Co., Ltd., Henan, China), 2XZS-2-type vacuum pumps (Deying Co., Ltd., Shanghai, China), and an LGJ-18 type tetracyclic lyophilizer (Sihuan Scientific Instrument Factory, Beijing, China) were used in the experiments.

2.4 Preparation of inorganic salt-based insulin powder

2.4.1 Preparation of aluminum hydroxide-carried insulin powder

Aluminum hydroxide powder was prepared freshly before drug-loading. Aluminum nitrate (15 g) was dissolved in 200 ml deionized water at 60 °C. Ammonia water was added dropwise through a separatory funnel into the gently stirred aluminum nitrate solution. The addition of ammonia water ceased when a white flocculent precipitate was no longer generated. This solution was kept still for 30 min. The precipitate was filtered and washed with deionized water until odorless and then freeze-dried under vacuum at −40 °C to obtain a powder. The powder was then sieved between 150 mesh (0.1 mm) and 300 mesh (0.05 mm) screens.

Freshly made aluminum hydroxide powder (1.0 g) was put into a column (5 cm long and 1 cm in diameter) with a filter membrane at its end. The column was vibrated until the powder height no longer declined. Insulin (0.1 g) was dissolved in 1 ml of deionized water. Insulin solution was dripped into the aluminum hydroxide column vertically from top to bottom until the column became fully submerged. The valve of the column was then turned off and kept to reach adsorption equilibrium at 10 °C for 30 min.

Then, the valve was turned on and the residual solution removed. The aluminum hydroxide absorbed with insulin was freeze-dried under vacuum at −40 °C to obtain a powder. The powder diameter was in the range of 50–90 μm. The bulk density of the powder was 1.45 g/cm^3 and the particle density was 2.68 g/cm^3. The aluminum hydroxide-carried insulin powder obtained contained 76.6 mg of insulin (determined by the protein amount) per gram of powder.

2.4.2 Preparation of calcium carbonate-carried insulin powder

Pre-sieved calcium carbonate powder was put into a column as described above. The other preparation steps were the same as for the aluminum hydroxide-carried insulin powder. The powder was sieved between 150 mesh (0.1 mm) and 300 mesh (0.05 mm) screens. The powder diameter was in the range of 50–90 μm. The bulk density of the powder was 1.73 g/cm^3 and the particle density 2.95 g/cm^3. The calcium carbonate-carried insulin powder obtained contained 78.8 mg of insulin (determined by the protein amount) per gram of powder.

2.4.3 Preparation of phosphate-carried insulin powder

Potassium dihydrogen phosphate (8.34 g) and dipotassium hydrogen phosphate (0.87 g) were added to 1000 ml of deionized water to obtain a phosphate buffer (pH 5.8). Insulin (0.921 g) was added to 100 ml of phosphate buffer and mixed well. The mixed liquor was freeze-dried under vacuum at −40 °C to obtain a powder. The powder was sieved between 150 mesh (0.1 mm) and 300 mesh (0.05 mm) screens. The powder diameter was in the range of 50–90 μm. The bulk density of the powder was 1.17 g/cm^3 and the particle density 2.20 g/cm^3. The obtained phosphate-carried insulin powder contained 74.1 mg of insulin (determined by the protein amount) per gram of powder. The surface structure of the phosphate-carried insulin powder was observed by scanning electron microscopy (SEM).

2.5 Aerodynamic calculation and numerical simulation for PNI

In our test, the gas flowing through the de Laval nozzle could be considered approximately as an isentropic flow. Therefore, the exit pressure (P_e) and the linear velocity (v_e) of the exhaust gas out of the nozzle could be calculated using the equations of Clarke and Carswell (2007). The designed Mach number of the de Laval nozzle determines the maximal gas flow velocity. In our preliminary studies, the designed Mach number of 3 was acceptable to achieve adequate gas flow velocity and the nozzle length (about 9 cm) was appropriate.

$$P_e = P_0\left(1+\frac{\gamma-1}{2}\mathrm{Ma}_d^2\right)^{-\gamma/(\gamma-1)}, \quad (1)$$

$$v_e = \sqrt{\frac{TR}{M}\cdot\frac{2\gamma}{\gamma-1}\cdot[1-(P_e/P)^{(\gamma-1)/\gamma}]}. \quad (2)$$

The following terms and units were used in calculations: P_e, absolute pressure of the exhaust gas at the nozzle exit, Pa; P_0, inlet pressure, Pa; Ma_d, Mach number of the designed condition (equal to 3 in this study); γ, isentropic expansion factor (γ_{N2}=1.4); v_e, exhaust velocity at the nozzle exit, m/s; T, absolute temperature of the inlet gas, K; R, universal gas law constant, 8314.5 J/(kmol·K); M, gas molecular mass, kg/kmol; P, absolute pressure of the inlet gas, Pa.

Because the successive flow field in the de Laval nozzle cannot be calculated manually, a computer-aided numerical simulation (Fluent 6.0 software, Fluent Inc., USA) was used to calculate the aerodynamic flow and aerodynamic parameters. In addition, the motion of the powder at various initial radial positions in the de Laval nozzle was also investigated to explore the influence of the radial position on the drug powder's exit velocity.

To simulate the actual working conditions of PNI, a baffle was set up in front of the nozzle exit at a distance of 1 cm to simulate an injection site on the skin. It was hypothesized that the drug powder would be absorbed by the baffle that was impacted. All simulations used only half of the longitudinal section because the flow field was radially symmetrical.

2.6 Orthogonal test for skin irritation of PNI

Rabbits weighing (2±0.2) kg were shaved carefully with an electric hair clipper 24 h before the test to remove dorsal fur; residual fur was removed with sodium sulfide (0.075 g/ml) to expose a skin area of 6 cm×6 cm. After washing with water, the naked skin was oiled with Vaseline to protect the treated surface.

In an aseptic manipulation cabinet, the aluminum hydroxide-carried powder (3 mg) was poured into the drug container and encapsulated. The cylinder was charged with nitrogen. The volume of the

cylinder and the pressure of the gas charging could be modulated. The powder needleless injector was then assembled using these parts.

We designed a series of orthogonal experiments that differed in the gas pressure in the injection cylinder (P_0), the volume of the cylinder (V_0), and the distance between the nozzle exit and the skin surface (L_0), using three different levels for each factor. The degrees of external hemorrhage, subcutaneous hemorrhage, edema, and scab were identified using a lesion evaluation index, assessed using the Chemical Medicine Irritation Technical Guidelines Evaluation Criteria, and defined by a synthesis index of skin irritation (SISI), which is the sum of the abovementioned indices.

2.7 Modeling for experimental diabetes

Male and female adult New Zealand rabbits were purchased from the Laboratory Animal Centre (License No. SCXK2009-001), Academy of Military Medical Sciences, Beijing, China. The experimental diabetes model was induced by alloxan (120 mg/kg) injected through the ear vein of the rabbits. The blood glucose levels of the rabbits were determined 48 h after injection of alloxan. Modeling was considered successful when the serum level of postprandial glucose in the rabbits was above 15 mmol/L.

Before PNI injection, the dorsal fur of all experimental rabbits was removed using the abovementioned procedures.

2.8 Evaluation of the efficiency of PNI delivery of insulin

2.8.1 Comparison of hypoglycemic effects of different salt-carried insulin powders

The rabbits were divided randomly into six groups of six rabbits each. One group was assigned as the blank control group (N). The other five groups were modeled as diabetes and then assigned as the model group (M), the needle/syringe injection group (N/S), the aluminum hydroxide-carried insulin via PNI delivery group (PNI-Al), the calcium carbonate-carried insulin via PNI delivery group (PNI-Ca), and the phosphate-carried insulin via PNI delivery group (PNI-P), respectively. The group N/S was administered insulin (0.3 mg/kg) subcutaneously by needle/syringe and the groups N and M were administered the same volume of normal saline. The groups PNI-Al, PNI-Ca, and PNI-P were administered the corresponding salt-carried insulin (0.3 mg/kg) via PNI. Safe injection conditions of PNI were set according to the results of skin irritation tests: a gas pressure of 3.5 MPa, cylinder volume of 5 ml, and a nozzle distance of 3.5 cm.

2.8.2 Comparison of hypoglycemic effects of phosphate-carried insulin administered in different ways

Another 24 rabbits were modeled and then divided randomly into four groups of six rabbits each. One group was administered insulin (0.3 mg/kg) subcutaneously via needle/syringe and assigned as the group N/S. A second group was administered raw insulin powder (0.3 mg/kg) using the PNI device and assigned as the group PNI-Raw. A third group was administered phosphate-carried insulin powder (0.3 mg/kg) via the PNI device and assigned as the group PNI-P. A forth group was administered the re-dissolved phosphate-carried insulin solution (0.3 mg/kg) via needle/syringe and assigned as the group N/S-P. Safe injection conditions of PNI were set as described above.

2.8.3 Determination of plasma glucose concentrations

Blood samples were collected from the rabbits in each group for determination of plasma glucose levels using blood glucose assay kits. The area above the curve (AAC) of plasma glucose concentration versus time was calculated to evaluate the hypoglycemic effect of insulin delivered by PNI. AAC is defined as the area between the glucose concentration curve of the test pharmaceutics and the curve of the diabetic model group. The AAC was calculated by the trapezoidal method:

$$\mathrm{AAC}_{0-t}=\sum_{i=0}^{t}(C_i^{\mathrm{Model}}-C_i^{\mathrm{Test}})\times(t_i-t_{i-1}), \qquad (3)$$

where C_i^{Model} and C_i^{Test} are the plasma glucose concentrations of the model and tested rabbits, respectively; t is time.

2.9 Statistics

All the data were expressed as mean±standard deviation (SD). All data were analyzed using the Statistical Package for the Social Sciences for Windows, Version 13.0 (SPSS Inc., Chicago, IL, USA). The groups were compared using analysis of variance (ANOVA) and the significance probability was set at P=0.05.

3 Results

3.1 Skin irritation assessment

Using an orthogonal experimental design, three key factors related to skin irritation caused by PNI, i.e., the gas pressure in the injection cylinder (P_0), the cylinder volume (V_0), and the distance between the nozzle exit and the skin surface (L_0), were found to be correlated significantly with the extent of skin lesions (P<005). The gas injection pressure had a positive correlation, while the distance from the nozzle had a negative correlation with the extent of skin lesions. The effect of the volume of the cylinder was not linear: the lesions caused by the medium-size cylinder were the mildest, and those caused by both the bigger and the smaller cylinders were more serious. The results of the orthogonal test are summarized in Tables 1 and 2. Images of an impacted skin surface are shown in Fig. 2a. Notably, condition 8 (test No. 8 in Table 1) seemed to have no adverse effect (SISI=0). It has been demonstrated that the gas pressure is positively related to the powder velocity and delivery efficiency of PNI (Wang *et al*., 2007; 2009). Condition 8 used a relatively low gas pressure (2.5 MPa) and its delivery efficiency was not satisfactory. Although the distance from the nozzle had a statistically negative correlation with the extent of skin lesions, condition 8 with a short nozzle distance (1.5 cm) showed minimal skin lesion. This might be caused by an unknown interaction between nozzle distance and gas cylinder volume.

Table 1 L_9 (3^4) orthogonal test results of skin irritation

Test No.	P_0 (MPa)	V_0 (cm^3)	L_0 (cm)	Skin irritation score				
				EH	SH	Edema	Scab	SISI
1	4	10	1.5	2	2	3	3	10
2	4	5	2.5	1	1	2	1	5
3	4	3	3.5	1	1	1	1	4
4	3.5	10	2.5	2	1	1	1	5
5	3.5	5	3.5	1	1	0	0	2
6	3.5	3	1.5	2	3	3	3	11
7	2.5	10	3.5	1	1	1	1	4
8	2.5	5	1.5	0	0	0	0	0
9	2.5	3	2.5	1	1	1	1	4

Each test was performed twice. P_0: gas pressure in the injection cylinder; V_0: cylinder volume; L_0: distance between the nozzle exit and the skin surface; EH: external hemorrhage; SH: subcutaneous hemorrhage; SISI: synthesis index of skin irritation

Table 2 Statistical results of the orthogonal test

Source	DF	SS	F value	P value
P_0	2	49.33	6.56	0.0133
V_0	2	64.00	8.52	0.0058
L_0	2	41.33	5.50	0.0221
Model	6	154.67	6.86	0.0032
Error	11	44.33		
Corrected total	17	196.00		

P_0: gas pressure in the injection cylinder; V_0: cylinder volume; L_0: distance between the nozzle exit and the skin surface; DF: degree of freedom; SS: sum of squares

According to our experimental experience, a minimal hemorrhage (score=1, which presents some tiny hemorrhage dots either externally or subcutaneously, but not blood leakage) will heal in several hours and not leave any permanent lesions. Thus, minimal external and subcutaneous hemorrhage (SISI≤2) should be acceptable in the application of PNI devices. According to the results, safe injection conditions of this needleless powder drug delivery system are considered to be as follows: a gas pressure of 3.5 MPa, a cylinder volume of 5 ml, and a nozzle distance of 3.5 cm.

3.2 Aerodynamic parameters and their relationship with skin irritation

The relationship between the extent of skin lesions determined in the orthogonal tests and the theoretical aerodynamic parameters calculated using Eqs. (1) and (2) is shown in Figs. 2b, 2c, 2d, and 2e. The exit gas velocity (v_e) and the exit pressure (P_e) of the nozzle were positively correlated (Figs. 2b and 2c) with the extent of skin lesions, while the nozzle distance (L_0) showed a negative correlation (Fig. 2d). In addition, we identified a new variable, combining the product of exit velocity (v_e) and pressure (P_e) divided by the nozzle distance (L_0), which showed a higher positive correlation (Fig. 2e) with the extent of skin lesions than each single parameter.

The velocity (Mach number, m/s) and the temperature (Kelvin scale) of the gas flow through the de Laval nozzle of the PNI device were calculated using numerical simulation software. The velocity of the gas flow in the de Laval nozzle increased gradually and achieved a maximum velocity of Mach 2.69 at the exit of the nozzle (Fig. 3a), while the temperature

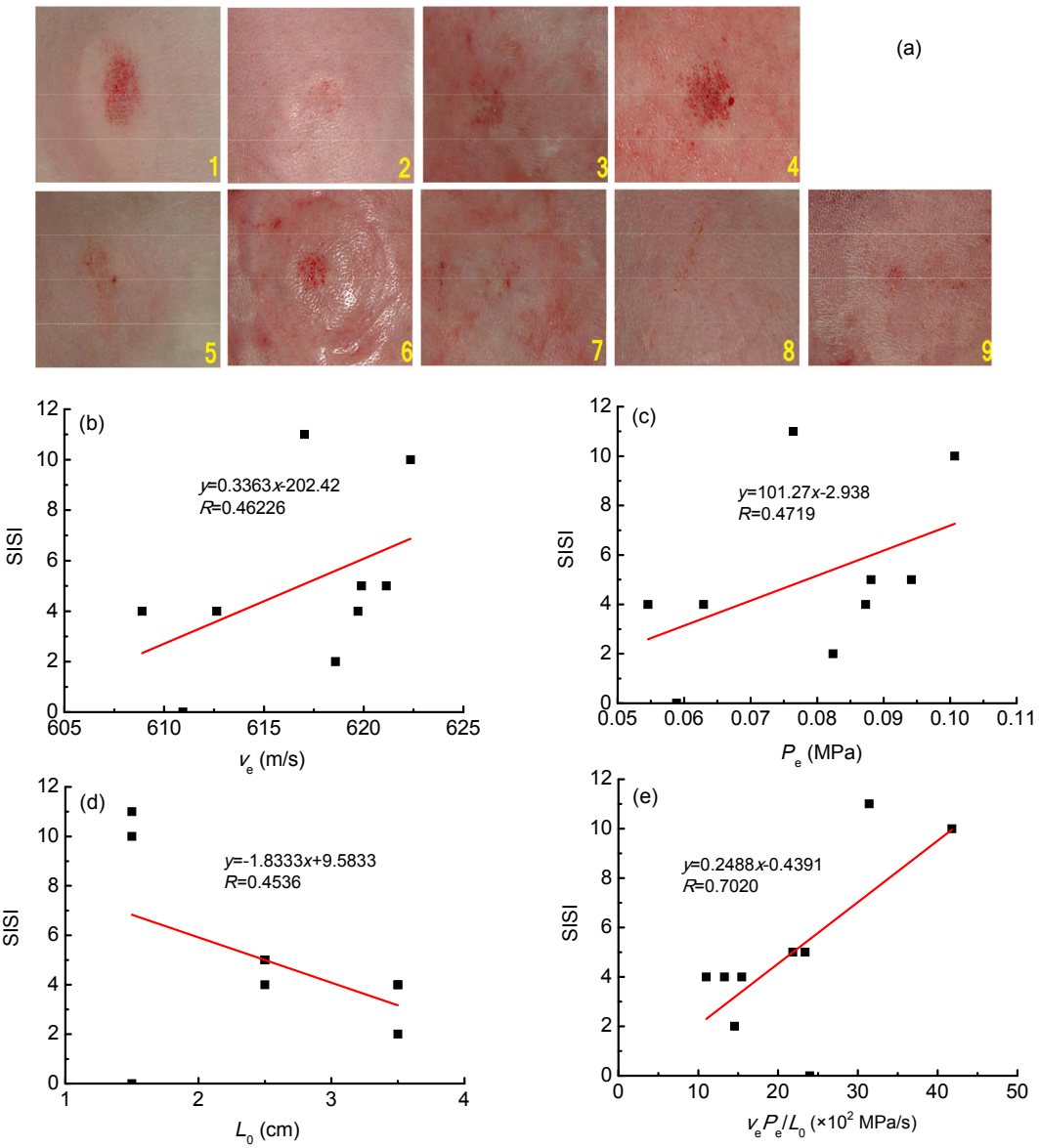

Fig. 2 Skin irritation of PNI and its relationship with aerodynamic parameters

(a) Skin trauma of PNI. The test numbers in the orthogonal design are shown in the lower right corner of each panel; (b, c, d, e) Correlations between the extent of skin lesion and aerodynamic parameters (exit gas velocity (v_e) (b), exit pressure (P_e) (c), nozzle distance (L_0) (d), and combining the product of v_e and P_e divided by L_0 (e)) of PNI

declined through the nozzle (Fig. 3b). Fig. 3c shows that the powder initially away from the nozzle axis moved towards the axis as it accelerated through the nozzle, while the powder initially on the axis remained there.

We expected a continuous decrease in gas temperature in the spacer ring due to gas expansion, but not a dramatic increase in gas temperature (Fig. 3b). Some of the kinetic energy of gas flow may have converted into heat and raised the gas temperature, since the gas flow impacted on the baffle (simulating the skin surface) and changed its direction of vertical movement in a narrow zone. Notably, the gas velocity also changed drastically from 2.69 Ma to 0.18 Ma when the gas flow impacted the baffle. Although the gas flow lost kinetic energy dramatically, the drug powder could retain enough kinetic energy when it passed through the space between the nozzle exit and the baffle. The ability of the powder to retain kinetic energy was related to the powder diameter (Fig. 3d).

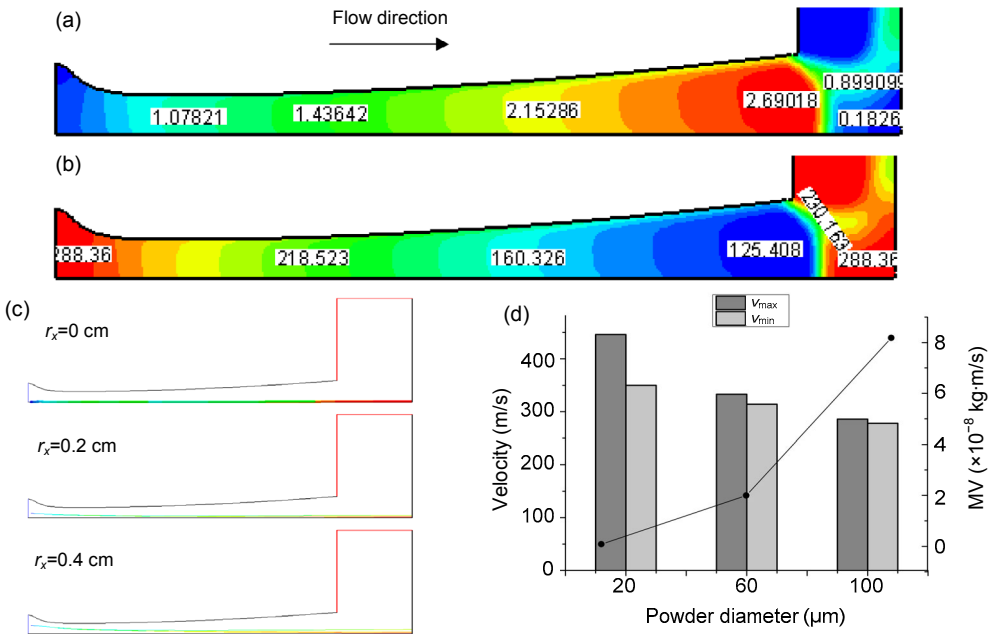

Fig. 3 Numerical simulation of the aerodynamic course of the PNI device

(a) The velocity (m/s) of the gas flow distributed in the de Laval nozzle. (b) The temperature (K) of the gas flow distributed in the de Laval nozzle. The numbers labeled in the diagram show the value at each point. (c) Simulated movements of the drug powder accelerated through de Laval nozzle from various initial positions. The colored lines indicate the tracks of powders moving through the nozzle. r_x (cm) indicates the radial position of the powder away from the nozzle axis. (d) The velocities and momentums of drug powders in different diameters accelerated through the de Laval nozzle. v_{max}: the maximal velocity of the powder achieved in the nozzle; v_{min}: the velocity of the powder when impacting the baffle; MV: momentum. The powder density was assigned as 1 g/cm^3 (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

3.3 Hypoglycemic effects of different salt-carried insulin powders delivered by PNI

The three different salt-carried insulin powders delivered through PNI all had hypoglycemic effects on alloxan-induced diabetic rabbits (Fig. 4a). Insulin delivered through the conventional needle/syringe method showed a high and rapid hypoglycemic effect in rabbits and caused hypoglycemia below normal plasma glucose concentration from 2 to 5 h after administration. After this very low glucose period, the plasma glucose concentration of the rabbits restored rapidly. On the other hand, all the PNI-delivered groups showed more gradual hypoglycemic effects. In addition, the phosphate-carried insulin powder showed a more potent hypoglycemic effect than the other salt-carried insulin powders administered via PNI. The ranking of the hypoglycemic effect of the three salt carriers based on their AAC values was: phosphate>aluminum hydroxide>calcium carbonate (Fig. 4b).

3.4 Relative efficiency of phosphate-carried insulin delivery by PNI

To calculate the relative delivery efficiency of phosphate-carried insulin administered by PNI, we re-dessolved the phosphate-carried insulin powder, and delivered the suspension by conventional needle injection. Phosphate-carried insulin powder delivered by PNI had a relative delivery efficiency of over 72% (Table 3). In contrast, the raw insulin powder delivered by PNI had a relative delivery efficiency of only 0.03%.

Table 3 Relative efficiency of phosphate-carried insulin delivery by PNI

Group	AAC (mmol·min/L)	RDE of PNI (%)	ADE of PNI (%)
N/S	152.34±33.87		
N/S-P	58.99±12.66		38.72
PNI-P	42.62±8.58	72.25	27.98
PNI-Raw	0.02±0.008	0.03	0.01

AAC are expressed as mean±SD (n=6). AAC: area above the blood glucose curve; RDE: relative delivery efficiency; ADE: absolute delivery efficiency

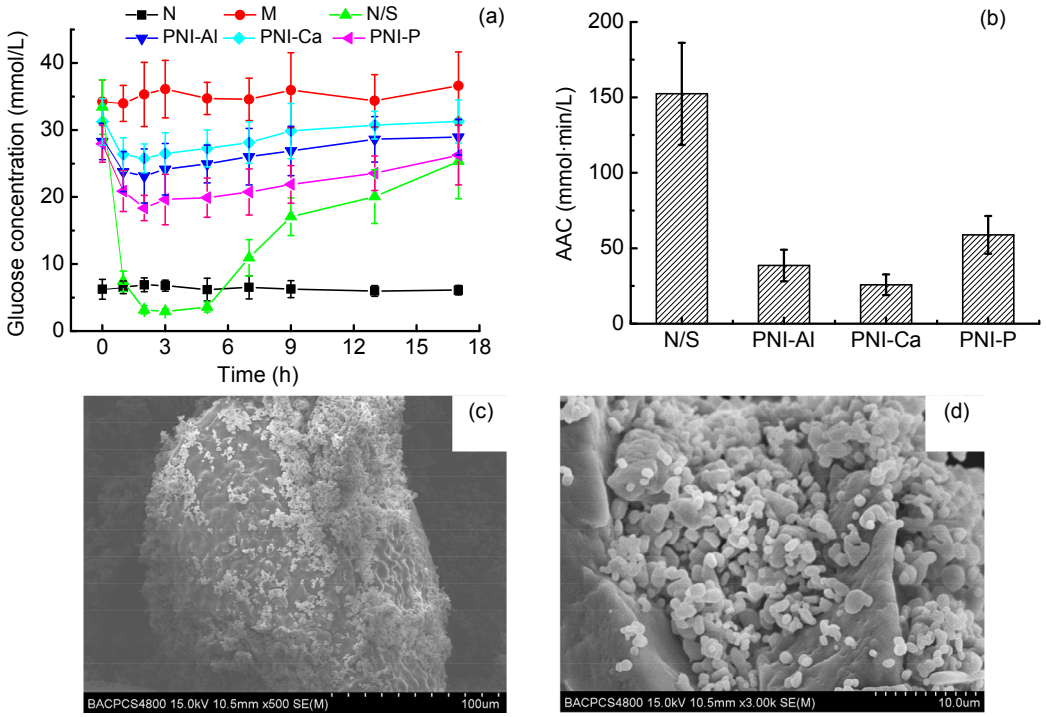

Fig. 4 Hypoglycemic effects of different salt-carried insulin powders delivered by PNI

(a) The change in glucose concentration caused by different salt-carried insulin powders delivered by PNI. (b) The area above the curve (AAC) of the glucose concentration of different salt-carried insulin powders delivered by PNI. Data are expressed as mean±SD. (c, d) Scanning electron microscope (SEM) images of the phosphate-carried insulin powder. The powder core is the phosphate crystal. The micro particles are insulin attached or adhered to the surface of the phosphate crystal

4 Discussion

In this study, we made a significant advance in PNI of insulin, achieving a relative delivery efficiency of 72.25% via a newly designed PNI device and insulin powder. Previously, the commercial application of PNI has been restricted by trying to balance high bioavailability with the limitations imposed by skin lesions. The de Laval nozzle is the most important part for producing supersonic gas flow. The new PNI device uses a de Laval nozzle with an enlarged "throat", where the cross-sectional area through the nozzle is minimal. The enlarged throat allows more gas flow and drug powder to pass through, and consequently is more efficient at accelerating drug powder to high velocity. Our previous PNI device with a small throat size could achieve only 34.9% of relative delivery efficiency (Wang *et al.*, 2007), markedly lower than that of the present device. The previously reported PowderJect injector was also designed with a small throat in the de Laval nozzle and its delivery efficiency was only 33% (Sarphie *et al.*, 1997). Therefore, we consider that the enlargement of the de Laval nozzle throat is important to ensure a high delivery efficiency of PNI. Apart from the de Laval nozzle, another improvement of the new PNI device is the valve system. In our preliminary studies, we found that the ventilation capability of the valve determines the delivery efficiency of the PNI device. A larger valve diameter permits more ventilation of the gas flow. A large valve diameter is necessary to match up with an enlarged de Laval nozzle. So we used a global valve with a normal diameter of 4 mm. To facilitate use of the device, we designed an automatic mechanical unit to drive the global valve from the closed to the open position. The mechanical unit is compact and easy to trigger. We also designed a safety ring to restrict the trigger when the PNI device is not in use. The current PNI device weighs about 300 g and can be operated with one hand.

However, the extent of skin lesions remains a concern when using this new prototype device, since the enlargement of the nozzle throat causes an increase in gas flow energy. Based on the results on rabbit skin, we found that the extent of skin lesions caused by PNI was related to the aerodynamic parameters of the gas flow. The gas flow velocity and exit pressure out of the de Laval nozzle showed positive

relationships with the extent of skin lesions (Fig. 2). As expected, the distance between the nozzle exit and the skin was negatively correlated with the extent of skin lesions. In this study, we found that lower pressure (3.5 MPa), wider spacing (3.5 cm), and a medium-size cylinder (5 ml) did not cause any obvious skin lesions on rabbit skin. Skin irritation was assessed using aluminum hydroxide powder. Since phosphate is soluble and of lower density compared with aluminum hydroxide, the skin irritation caused by phosphate powder was similar to (even weaker than) that caused by aluminum hydroxide powder. We also performed the test on ourselves under these injection conditions and felt no obvious injury.

The importance of the formulation of the powder has not been addressed previously in PNI delivery. We found that raw insulin powder was not effective when delivered by PNI, while the inorganic salt-carried insulin powders had hypoglycemic effects. The raw insulin powder was supplied by the manufacturer and was produced by freeze-drying a hollow powder structure. As stated above, a drug powder with a hollow structure will not meet requirements for PNI delivery. The results showed that the phosphate-carried insulin powder had the best efficacy among the three inorganic salt-carried powders investigated (Fig. 4a). The reason why phosphate showed a potent enhancement of the delivery of insulin via PNI, however, is unknown and deserves further study. Aluminum hydroxide is widely used as an adjuvant in vaccines. In our previous study (Wang *et al*., 2009), PNI delivery of aluminum hydroxide-carried tetanus toxoid could achieve a good immune response in mice. However, for therapeutic purposes, it is very likely that the use of aluminum hydroxide on a daily basis would exceed the safe limit of such a substance. Since aluminum hydroxide and calcium carbonate are insoluble and will degrade slowly in skin tissue, the phosphate formulation would be more feasible for drug delivery for therapeutic purposes. The SEM images of the phosphate-carried insulin powder (Figs. 4c and 4d) show that the insulin adhered to the surface of the phosphate crystal. The phosphate crystal provides a solid powder core of enough intensity, strength, and density. Thus, the insulin could be carried and delivered along with the phosphate powder into the skin by a PNI device. Besides the high hypoglycemic effect, the phosphate-carried powder is safe, since long-term administration of these powders will not accumulate insoluble particles.

However, the current procedure for producing phosphate-carried insulin powder is very crude. We noted that the re-dissolved phosphate-carried insulin powder administered by needle/syringe had a relatively low hypoglycemic effect compared with the raw insulin solution via needle/syringe delivery (Table 3). This result suggests that the amount of insulin recovered after powder manufacture is low. Since no protective reagent was added to the powder system when freeze-drying, the drying procedure might cause loss of biological activity of insulin. The other powder formulas using aluminum or carbonate share the same drying procedure and so may also suffer a loss of biological activity. Thus, the process of insulin powder-making needs to be optimized to enhance further the hypoglycemic effect of insulin delivered via PNI.

Another device, the gene gun, uses basically the same principle as PNI: it uses a high-velocity gas jet to deliver a gene plasmid-coated gold particle (1 μm in diameter) into skin tissue or cells. However, according to Jin *et al.* (2001) and our research, the drug delivery efficiency of the gene gun is lower than that of PNI. The principal difference between a PNI device and a gene gun lies in the gas accelerating nozzle: the PNI device uses a de Laval nozzle with a convergent-divergent internal shape, but the gun uses a divergent nozzle. The aerodynamic features of the two kinds of nozzle are totally different: a de Laval nozzle is suitable for producing supersonic gas flow, while a divergent nozzle is not. The ability of the gun to deliver drugs is unsatisfactory. However, the PNI technique might be suitable for use in gene delivery, as confirmed by some studies using the PowderJect device (Lesinski *et al.*, 2001).

In our experience, the particle diameter might affect the acceleration of drug powders in PNI devices. This was confirmed by the results of our numerical simulations. Small powders achieved higher velocity than large powders (Fig. 3d), but large powders achieved higher momentum than small powders. Thus, large-diameter drug powders would be delivered more easily into the skin by PNI devices than small-diameter powders (Sarphie *et al.*, 1997; Wang *et al.*, 2007). However, large particles cause more injury to the skin. In our experience, a drug powder diameter of 60 μm is appropriate for PNI delivery.

Besides PNI technology, there are many other potential approaches for alternative insulin administration, such as oral, pulmonary, buccal, nasal, and transdermal approaches (Trehan and Asgar, 1998). Oral administration of insulin offers relatively low

bioavailability, ranging from 0.5% to 10.0% (Cui *et al.*, 2007). Buccal delivery of insulin also achieved a limited bioavailability of 15%–29% (Xu *et al.*, 2002). Nasal administration of insulin with permeability enhancers achieved bioavailability of 8%–15% (D'Souza *et al.*, 2005; Shaha *et al.*, 2010). Pulmonary administration of insulin is a good alternative approach (Hussain *et al.*, 2004), achieving a relative bioavailability of 11%–57% (Agu *et al.*, 2001; Clark *et al.*, 2008). The irreversible lesion of lung alveolar cells, however, should be considered when insulin administration is long-term and repeated. Children and some patients with a limited ability for inhalation of insulin powders may not be suited to this approach.

It was reported that the powder delivered by the PowderJect device was deposited in the epidermal or dermal layer in the skin (Kendall *et al.*, 2004). Since the epidermal layer has fewer blood vessels than muscles, it could be expected that a drug delivered by PNI into the epidermal layer may be absorbed a little slower than a drug delivered by needle/syringe injection, which delivers drug into muscles. This might give PNI an additional advantage, as it may produce a more gradual hypoglycemic effect compared with the rapid hypoglycemic effect of conventional needle/syringe delivery (Fig. 4b). The gradual hypoglycemic effect of PNI-delivered insulin would decrease the risk of hypoglycemia caused by insulin in patients, and maintain a relatively steady plasma glucose concentration.

Apart from high delivery efficiency, another advantage of PNI is that the microscopic holes formed by drug powders are small enough to limit undesired effects, including pain, irritation, and infection (Arora *et al.*, 2008). Cost, however, is one of the important concerns for the use of PNI in practice. The PNI device used in this study offers a re-usable feature which can reduce the average cost for repeated drug delivery. Another concern is the safety of the device in respect of the use of pre-filled high pressure gas. The future development for PNI lies principally in device engineering to make the device smaller, more portable, and safer.

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Compliance with ethics guidelines

Chun-yu LI, Zhe-wei WANG, Can TU, Jia-bo WANG, Bing-qian JIANG, Qi LI, Ling-na ZENG, Zhi-jie MA, Ping ZHANG, Yan-ling ZHAO, Ya-ming ZHANG, Dan YAN, Rui TAN, and Xiao-he XIAO declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要：

本文题目：胰岛素粉末无针注射的给药效率和皮肤刺激性评价

Needle-free injection of insulin powder: delivery efficiency and skin irritation assessment

研究目的：胰岛素在临床上广泛用于糖尿病的治疗，但需要注射方式给药，给患者带来不便。本文报道了一种胰岛素粉末无针注射给药的新方法，并对其给药效率和皮肤刺激性进行评价。

创新要点：首次报道了胰岛素粉末无针注射给药方法，有望为糖尿病患者长期自我给药提供便捷。

研究方法：自主设计便携式自动无针粉末注射给药装置，采用计算机辅助方法计算给药装置的主要空气动力学参数；采用家兔评价无针粉末注射对皮肤的刺激性，并分析与空气动力学参数的相关性；采用四氧嘧啶诱导家兔糖尿病模型，评价不同无机盐载药的胰岛素粉末的无针注射给药降糖效果。

重要结论：采用自主设计便携式自动无针粉末注射给药装置，递送以无机盐为载体的胰岛素粉末具有显著的降血糖作用，而递送胰岛素原料药粉末无降血糖作用，说明无机盐载体对胰岛素粉末无针注射给药效果具有重要作用。皮肤刺激性实验表明，储气室气压、容积以及喷管与皮肤的间距三个参数显著影响无针粉末注射装置的皮肤损伤程度，优化后的注射条件对皮肤几乎没有损伤。优化给药条件下以磷酸盐载体的胰岛素粉末无针注射给药，相对给药效率为72.25%。研究结果表明无针粉末注射技术用于胰岛素或其它生物制品的无针给药具有良好应用前景。

关键词组：无针粉末注射；胰岛素；经皮给药；皮肤刺激性