INOJEX™ Study

Assessment of the biological performance of the needle-free injector INOJEX using the isolated porcine forelimb.

*Study was performed using INJEX™ system. INJEX™ system has changed it's Trademark name to INOJEX™

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Cutaneous Biology

Assessment of the biological performance of the needle-free injector INOJEX using the isolated porcine forelimb

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Summary

Background The development and utilization of novel needle-free injection devices in order to minimize needle stick injuries make increasing demands for suitable assay systems, which reflect the physiological situation in humans as close as possible.

Objectives It was therefore the goal of the present study to test the biological performance of a needle-free injector (inojex) by the use of porcine skin as a model with a high predictive value for the feasibility in humans because of its close similarity to human skin.

Methods In order to use porcine skin in the context of the underlying tissues, the isolated porcine forelimb was chosen as an assay model for use with the INOJEX injector. Ink or the fluorescent dye fluorescein-isothiocyanate was injected and the penetration depth was determined metrically and dye distribution histologically. To assess the resorption of heparin, needle injection was compared with needle-free injection in a perfused limb model.

Results Increasing amounts of ink increasingly penetrated into subcutaneous tissue layers in a cone-shaped manner mainly following lead structures. Penetration was hampered by skin thickness and by the deep muscle fascia, which served as a penetration barrier. Resorption of heparin was similar irrespective of injection by the use of a needle or the INOJEX device.

Conclusions The isolated porcine forelimb serves as a versatile tool for the assessment of the biological performance of needle-free injection devices such as INOJEX. Further studies are necessary to correlate the model for drug delivery in humans.

Key words: INOJEX, needle-free injection, porcine skin, transdermal drug delivery

The World Health Organization estimates that 12 billion hypodermic injections are given in healthcare settings around the world each year.[1,2] Accidental needle stick injuries (NSIs) continue to pose a significant risk of occupational exposure to blood-borne pathogens, including human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and others to millions of workers in the healthcare industry (estimation of the OSHA, U.S. Department of Labor's Occupational Safety and Health Administration).[3] As a result of the NIOSH Alert in November 1999, reporting 600 000–800 000 NSIs per year associated with severe infections of at least 60 000 hospital employees during the last decade in the U.S.A., strong efforts are seen world wide to develop safer injection technology.[4]

As well as these drawbacks, needles and syringes often become the object of fear and avoidance among patients who have to inject themselves frequently, thus adversely affecting compliance with recommended therapies. Therefore, in order to search for new, easy and safer ways to administer drugs, needle-free injection systems have been explored as an alternative to conventional intra- or subcutaneous medication delivery devices. After technical improvements beginning in the 1950s, needle-free injection (jet injection) was introduced for mass immunizations,[5,6] the application of insulin,[7,8] local anaesthetics[9,10] and hormones.[11]

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However, there is only limited availability of adequate models to study the biological performance of needle-free injection systems during the developmental process of such systems. Porcine skin has been found to have similar morphological and functional characteristics to human skin.[12,13] Therefore, in the present study the perfused porcine forelimb as a close-to-human *in vitro* assay model developed by Vitro-Tec Entwicklungs-GmbH (Berlin, Germany) was used because of the similarity between porcine and human skin to investigate the feasibility of the needle-free INOJEX system (Ro¨sch AG Medizintechnik, Berlin, Ger-many) for subcutaneous injection of liquids. The aim was to determine the depth of penetration and tissue distribution of injected dyes representative (also with respect to viscosity) of the delivery of liquid pharma-ceuticals. In addition, in a perfused forelimb model, transdermal delivery by using the needle-free INOJEX system and the accumulation of heparin in the perfusion medium was monitored and compared with delivery by conventional needle application. It could be demonstrated that the porcine forelimb served as a versatile tool to study the biological performance of needle-free injection devices in an *in vitro* model during preclinical development.

Materials and methods

Organ and blood collection as well as preparation and transportation were described in detail previously.[14] The skin area between the olecranon and the ossa carpi, termed the injection area, was carefully sheared. This area was chosen for drug application, as hair density and the thickness of the skin are comparable to human skin.[15] The limbs for ink application were treated additionally for 2 min with a peeling cream (Aok Seesand Peeling, Kräuter aktiv, 4110 Z 92, Schwarzkopf & Henkel, Art. 4015000 260 145, Düsseldorf, Germany) in order to remove the upper horny layers.

INOJEX

The INOJEX system consists of a spring-loaded variable-dose injector to which a disposable plastic ampoule containing the medication is attached. When activated the trigger releases the spring propelling a liquid drug with high velocity through a microorifice (diameter 0·17 mm) in the tip of the ampoule. The jet stream of medication traverses the skin (140 ms) and the drug disperses subcutaneously. Spring pressure and orifice diameter are designed for a depth of penetration of about 3–9 mm at the usual subcutaneous injection sites.

Injection of fluorescein-isothiocyanate and ink for the determination of penetration depth

After transport to the laboratory each limb was rinsed with 900 mL oxygenated electrolyte solution (37 °C). The amount of ink (black, Herlitz no. 1; Herlitz AG, Berlin, Germany) or fluorescein-isothiocyanate (FITC) indicated was poured into 0·3-mL ampoules (Rösch AG Medizintechnik), which were then mounted to a Standard-INOJEX (Standard-INOJEX serial no. 3 10180 A; Ro¨sch AG Medizintechnik).

The injection areas on the inner side of the porcine forelimb were marked using a waterproof marker (Fig. 1). Skin thickness was determined at each injection site after peeling using a mobile ultrasound device (Caris, Esaote Biomedica, Neufahrn-München, Germany; ultrasound gel: Sonogel® contact gel, Sonoring Deutschland GmbH, Berlin, Germany).

Immediately after performing the injection, the injection area was cut vertically, preferably through the injection channel, using a scalpel. The depth of ink penetration and the distance between skin and muscle fascia were determined using a caliper. To estimate ink distribution two tissue samples for each volume were collected immediately after the injection was performed and processed for histological evaluation. Samples were fixed in 4% formalin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), embedded in paraffin, stained with haematoxylin and eosin according to standard procedures, and examined by bright-field microscopy.

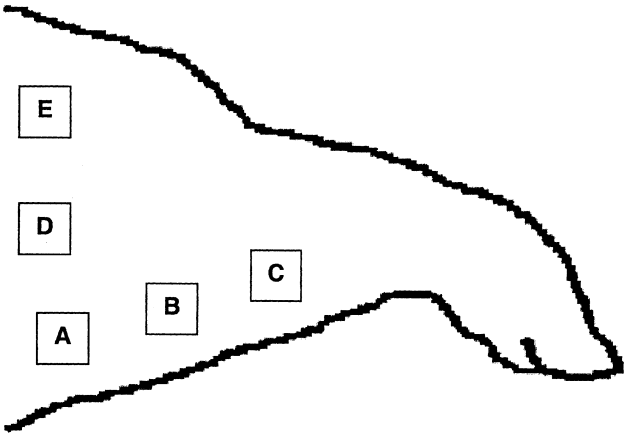

Figure 1. Schematic drawing of the medial view of the porcine forelimb. Five different injection sites (A–E) were chosen and skin thickness and the distance from the skin surface to the deep muscle fascia determined as described under Materials and methods.

Immediately after the injection of FITC (fluorescein 5-isothiocyanate, Sigma-Aldrich Chemie GmbH, Cat. no. F 7250, 10%, dissolved in PBS), skin samples were taken, snap-frozen in 2-methylbutane (Sigma-Aldrich Chemie GmbH, Cat. no. 27,034-2) under liquid-nitrogen cooling. The samples were stored on dry ice at − 80 °C until shipment.

Injection of heparin into perfused limbs for the study of percutaneous absorption

Perfusion set-up. The perfusion system, as developed by Vitro-Tec Entwicklungs-GmbH, comprises two circuits, a perfusate and a dialysate circuit as described in detail elsewhere.[14] The perfusate, running freely off the venous and capillary vessels of the organ, was collected in a reservoir. From there it was transported by a first roller pump into the dialysis module (Fresenius Polysulphone UF 6·4; Fresenius Medical Care, Bad Homburg, Germany), where it was heated to approximately 39 °C (normothermic for pigs) and equilibrated with nutrients and appropriate gas conditions derived from the dialysate circuit. A second roller pump recirculated the perfusate into the limb again via the arteria brachialis. Flow, pressure, temperature and pH were monitored to assess the proper performance of the limb perfusion system.

Perfusion conditions (limbs for heparin kinetics). After 3–3·5 h of ischaemia which was needed for the preparation procedure and transportation, the limbs were connected to the perfusion system. The limbs were perfused with a flow of 250 mL min^{-1} and the perfusate was oxygenated with an oxygen/room air mixture resulting in an oxygen saturation of 98–100%. No data are available for the blood flow in porcine forelimbs *in vivo*, but 230–250 mL min^{-1} are comparable with the flow in porcine hind limbs measured in the arteria iliaca.[16]

The limbs were perfused with dialysate supplemented with 4% bovine serum albumin. The dialysate was prepared from the acid concentrate SK-F203 (Fresenius Medical Care) and mixed with the basic concentrate BC-F 8·4% (Fresenius Medical Care). At the beginning of the experiment 3 g of glucose were added to the dialysate reservoir (3 L) and subsequently 1 g of glucose was added each hour.

Subcutaneous injection of heparin. After an adaptation period of 1 h, 0·3 mL (1500 IU) Liquemin N 25000 (PZN-3441331; Hoffman-La Roche, Grenzach-Wyhlen, Germany) was injected medially at the injection area with the standard INOJEX ($n = 6$). Another six limbs were injected with 0·3 mL (1500 IU) Liquemin N 25000 subcutaneously by the use of a conventional needle system (Terumo Europe NV, Leuven, Belgium, 0·4 × 20).

For the determination of heparin concentrations, aliquots were taken from the venous perfusate every hour during a total perfusion period of 6 h. Aliquots were centrifuged at 1500 ***g*** for 10 min and the resulting supernatant frozen at − 18 °C until further analysis by a colorimetric endpoint assay (Accucolor™ Heparin; Sigma-Aldrich Chemie GmbH).

Statistical analysis

Data are presented as mean ± SD from the number of experiments given in the figure legends. Significant differences were estimated by the use of Student's *t*-test for unpaired values.

Results and discussion

Determination of penetration depth

To corroborate the setting as well as the medico–technical claims for the INOJEX system in an *in vitro* assay model, the penetration depths of different ink volumes injected into the isolated pig forelimb were determined. In this experimental series injection sites were chosen randomly without focus on the specific anatomical topology of a single injection site.

Different volumes ranging from 0·05 mL to 0·20 mL were injected as described under Materials and methods. Ink penetration increased linearly with increasing volumes. While 0·05 mL penetrated to a depth of 3·88 ± 0·58 mm, 0·20 mL penetrated to a depth of 6·96 ± 0·40 mm into subcutaneous layers. Intermediary volumes reached intermediary penetration depths (Table 1). The dye deposition was primarily in the subcutaneous fat tissue without penetration through the deep muscle fascia. Thus, the physical performance

Table 1. Increase of penetration depth with increasing injection volume

Volume (mL)	0·05	0·10	0·15	0·20
Penetration depth (mm)	3·88 ± 0·58	5·12 ± 0·40	6·12 ± 0·73	6·96 ± 0·40

Injections were performed as described under Materials and methods'. Different injection volumes were applied as indicated. Values are mean ± SD from five injections each.

Table 2. Correlation of penetration depth with skin thickness and distance from the skin surface to the muscle fascia

Injection site	Skin thickness (mm)	Distance skin/fascia (mm)	Penetration depth (mm)
A	2·91 ± 0·27	9·15 ± 2·85	7·44 ± 2·12
B	3·35 ± 0·45	7·68 ± 2·69	6·14 ± 1·43
C	3·10 ± 0·39	7·06 ± 1·20	6·08 ± 1·02
D	2·66 ± 0·35	13·00 ± 4·45	15·58 ± 3·35
E	2·70 ± 0·45	16·23 ± 2·42	14·38 ± 2·17

Ink (3 mL) was injected at the different injection sites A–E. Penetration depth as well as distance from skin surface to the muscle fascia was determined by the use of a caliper, whereas skin thickness was determined by ultrasound. Values are mean ± SD from eight different limbs with injection sites A–E on each limb.

of the INOJEX system allowed for the reliable and reproducible delivery of the injected material to the desired target tissue in a volume-dependent manner.

Correlation of anatomical features of the injection site to the depth of penetration

Principally, the anatomical topology of an injection site could affect the performance of the injection. For example, the thickness of the skin could restrict the injection or the deep muscle fascia could limit penetration of an injected material into the muscle. Five distinct areas (A–E) were chosen on the inner side of the limb, as shown schematically in Figure 1. Skin thickness and distance from the surface of the skin to the deep muscle fascia were determined for each injection site as described and correlated to the penetration of injected ink.

It was shown that the injected ink penetrated to deeper layers if larger volumes were used (see above). Therefore, in order to reach even deeper layers, 0·3 mL of ink was used. Mean skin thickness at the sites A–E ranged from 2·66 mm to 3·35 mm (Table 2).

A nonlinear correlation between skin thickness and penetration depth at the various injection sites (Fig. 2) was observed. Skin thickness < 3·0 mm resulted in a larger penetration depth, whereas between 3·0 and 3·4 mm the penetration depth changed only slightly.

Depending on the site of injection the mean distance between skin surface and deep muscle fascia ranged from 7·06 mm at site C to 16·23 mm at site E. Correlation of this distance to the penetration depth revealed a nearly linear relationship, showing increasing penetration with increasing distance between skin surface and muscle fascia (Fig. 3). Beyond 13 mm, the distance seemed to have no impact on the penetration depth. If the ink reached the fascia, it was not penetrated.

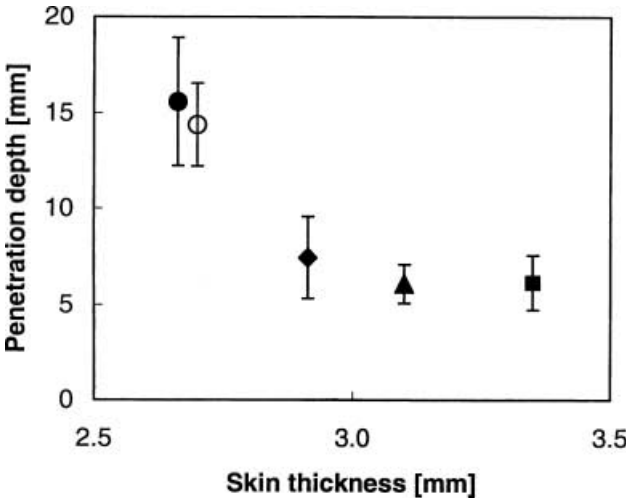

Figure 2. Relationship of penetration depth and skin thickness at the injection sites A–E (in Figure 1) as mean ± SD ($n = 8$, using eight porcine forelimbs from four different animals). Ink (0·3 mL) was injected using the standard INOJEX system. Penetration depth of the injected ink was measured by the use of a caliper at the five different injection sites (A, ◆ B, ■; C , ▲; D , ● E, ○) as described under Materials and methods.

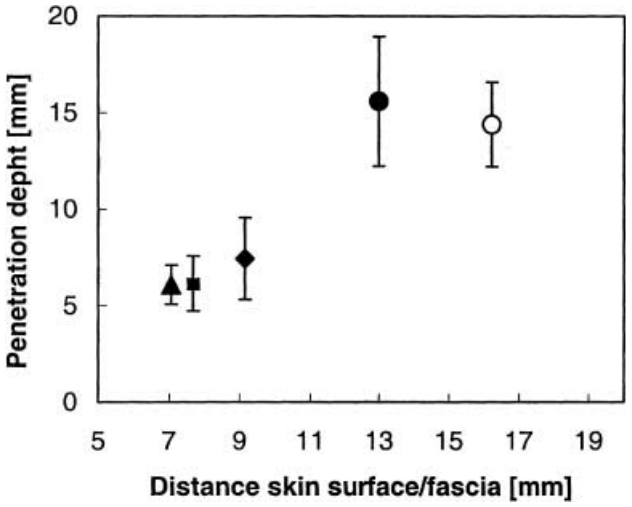

Figure 3. Correlation of penetration depth and distance from the skin surface to the muscle fascia at the injection sites A–E (in Figure 1) as mean ± SD ($n = 8$, using eight porcine forelimbs from four different animals). Ink (0·3 mL) was injected using the standard INOJEX system. Penetration depth of the injected ink was measured by the use of a caliper at the five different injection sites (A, ◆ B, ■; C , ▲; D, ● E, ○) as described under Materials and methods.

Thus, it can be concluded that both parameters, skin thickness as well as distance from the skin surface to fascia, influenced the penetration depth. The skin as

well as the muscle fascia served as natural barriers. The data show that the injection force using the standard INOJEX system under the applied conditions is necessary and sufficient for deposition of a medication into subcutaneous tissue layers.

Histological evaluation of dye distribution

Sporadically published studies in animals with different devices have demonstrated that fluids administered with jet injection technology followed the path of least resistance and did not pass into the substance of bone, into the walls of blood vessels, or into the nerve fibres.[17] In order to demonstrate the distribution of ink administered with the INOJEX system in detail, 0·1 mL of ink was injected into the porcine forelimb and vertical sections through the injection site were prepared. Medial–lateral sectioning through the injection site revealed a cone-like distribution of the ink with narrow dispersal underneath the epidermis and broadening through the subcutaneous fat tissue. Occasionally the ink reached the muscle fascia, which, however, was not penetrated. The epidermis was entirely intact and the subcutaneous fat tissue did not show any obvious lesions (Fig. 4A).

Injection of FITC showed that the dye penetrated mainly along lead structures such as connective tissue fibres or hair shafts. In the subcutaneous fat tissue the dye did not stain the fat deposits in the fat cells, indicating that these cells were omitted. No obvious tissue lesions were observed (Fig. 4B). This is consistent with findings observed from injections using needle and syringe (not shown). The data are also consistent with findings from a human trial, which showed no difference of tissue distribution between injection of contrast medium into the upper arm either by the use of the INOJEX or needle and syringe (unpublished results).

Taking the results together it can be assumed that the spatial three-dimensional reconstruction of the injected ink would reveal a bulb-shaped distribution with the broad side facing the muscle fascia and the narrow side underneath the epidermis. This, however, is only true for the situation where the distance between the surface of the skin and the muscle fascia is large with ample interspersed subcutaneous fat tissue and the volume of the injected material is small enough to allow for unrestricted distribution. In the case of only narrow distance between skin and muscle fascia the fascia serves as barrier (see above), which forces the injected material to spread along the fascia (data not shown). This clearly demonstrated that the site of injection has great impact on the delivery of a medication at a given volume.

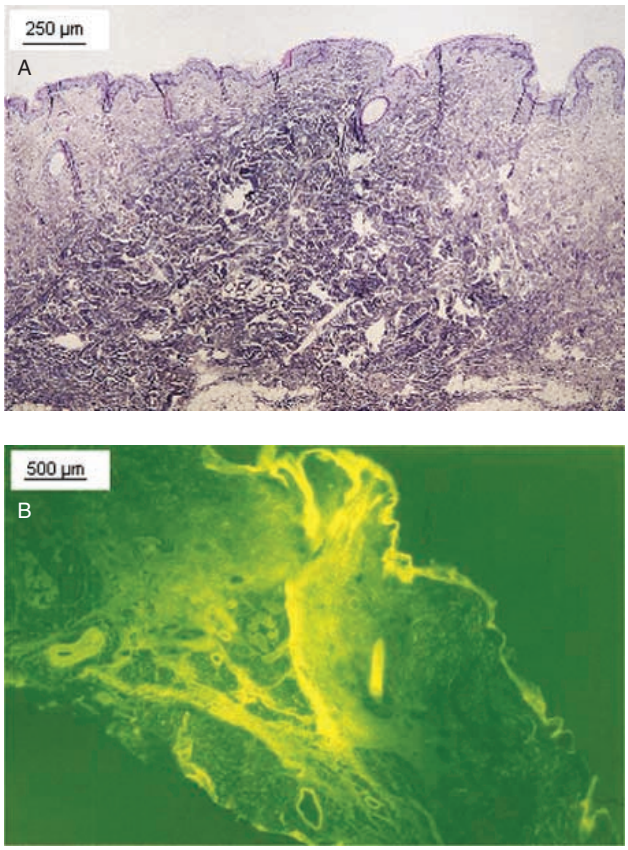

Figure 4. Histological distribution of ink or fluorescein-isothiocyanate (FITC) injected into the porcine forelimb; 0·1 mL of either dye was injected by using the INOJEX system. (A) Injection of ink and (B) injection of FITC. The injection site was cut vertically next to the centre of the injection site for histological processing. Dye distribution is mainly along lead structures underneath the epidermis and in the subcutaneous fat tissue. Bar: 250 µm (A) or 500 µm (B); (A) haematoxylin and eosin staining or (B) fluorescent microscopic image.

Comparison of heparin resorption after injection with the needle-free INOJEX *vs. conventional needle injection*

The perfused porcine forelimb has been used successfully for the study of transdermal delivery of medications from therapeutic patches.[18–21] Therefore, in order to demonstrate whether resorption of the test substance heparin after application with the needle-free INOJEX system was comparable to the resorption after subcutaneous injection of heparin using a conventional needle and syringe, a pharmacokinetic profile of heparin resorption was recorded.

Using needle and syringe, heparin concentration increased 1 h after injection and remained elevated for

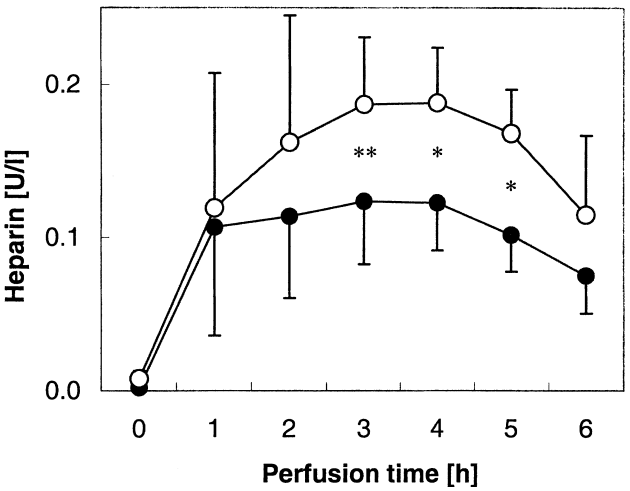

Figure 5. Resorption kinetics of heparin after administration of the drug with the needle-free injection system INOJEX (●) or with con-ventional needle and syringe (○). High-molecular-weight heparin (0·3 mL) was injected subcutaneously and heparin accumulation in the perfusion medium determined at the time points indicated. Values are mean ± SD from six separate experiments for either application mode. $^{*}P \leq 0{\cdot}01$; $^{**}P \leq 0{\cdot}05$.

the whole experimental time course up to 6 h after injection. Using the INOJEX, heparin increased 1 h after injection and remained elevated for the following 5 h of perfusion. However, levels were always significantly lower compared with the injection by needle and syringe (Fig. 5).

This effect might be due to the fact that the limbs were not peeled (using a peeling cream) before performing the injections. As mentioned in the Rösch AG technical report (06/2001) (unpublished data), the penetration depth of ink injected using the INOJEX was higher after peeling of the limbs compared with no peeling.

The histological findings demonstrated that the subcutaneous deposition of ink after application with the INOJEX or with the needle was very similar. It was shown in human trials that injection of heparin with gas-driven needle-free injectors resulted in the maximal accumula-tion of the drug in the blood 3–4 h after injection,[22,23] which is comparable to the results from the present study using the porcine forelimb. Thus, it can be assumed that subcutaneous administration of a medication with the needle-free INOJEX system results in similar release from the deposition and resorption into the circulation compared with conventional needle administration. This is consistent with results from previous studies in human trials using heparin as a test substance for injection with different needle-free devices.[22,24,25]

Conclusion and perspectives

Skin thickness in humans might vary between body regions, races and gender, therefore the development of a model covering all injection areas might be difficult. The results presented show that the porcine forelimb might be a versatile tool for the early technical assessment of needle-free injection systems. Three major claims could be corroborated, such as the proper deposition of a given medication at the desired target tissue, the adequate resorption of the medication from the deposition, and finally the lack of any tissue damage due to the use of the INOJEX in comparison with the use of needle and syringe. It is mandatory that the mechanical and physical performance of a needle-free injection system shall be verified by clinical data, with special emphasis on the desired clinical endpoint. As such, the assessment of the biological performance of a needle-free injection system like the INOJEX by the use of the porcine forelimb might give valuable information on the feasibility for use in humans of devices already in early phases of product development.

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