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RESEARCH PAPER

Measuring Tissue Back-Pressure - *In Vivo* Injection Forces During Subcutaneous Injection

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ABSTRACT

Purpose Limited information is available on injection forces of parenterals representing the *in vivo* situation. Scope of the present study was to investigate the contribution of the subcutaneous (sc) tissue layer to injection forces during *in vivo* injection.

Methods Göttingen minipigs received injections of isotonic dextran solutions (1–100 mPas) into the *plica inguinalis* using different injection rates and volumes (0.025–0.2 mL/s and 2.5 vs. 4.5 mL).

Results The contribution of the sc back-pressure to injection forces was found to increase linearly with viscosity and injection rate ranging from 0.6 ± 0.5 N to 1.0 ± 0.4 N (1 mPas), 0.7 ± 0.2 N to 2.4 ± 1.9 N (10 mPas), and 1.8 ± 0.6 N to 4.7 ± 3.3 N (20 mPas) for injection rates of 0.025 to 0.2 mL/s, respectively. Variability increased with viscosity and injection rate. Values are average values from 10 randomized injections. A maximum of 12.9 N was reached for 20 mPas at 0.2 mL/s; 6.9 ± 0.3 N was determined for 100 mPas at 0.025 mL/s. No difference was found between injection volumes of 2.5 and 4.5 mL. The contribution of the tissue was differentiated from the contribution of the injection device and a local temperature effect. This effect was leading to warming of the (equilibrated) sample in the needle, therefore smaller injection forces than expected compensating tissue resistance to some parts.

Conclusions When estimating injection forces representative for the *in vivo* situation, the contribution of the tissue has to be considered as well as local warming of the sample in the needle during injection.

KEY WORDS injection device · injection forces · minipig · subcutaneous drug administration · viscosity

INTRODUCTION

Subcutaneous (sc) drug administration of parenterals is a convenient way for easier drug application compared to the intravenous (iv) route of injection (1–7). This offers the possibility of home-treatment by the patient him-/herself or treating healthcare professional, especially if combination products like pre-filled syringes, autoinjectors or injection pumps are used (8–10). The development of these combination products requires a comprehensive assessment and understanding of parameters contributing to injection forces. These include (1) device components, (2) drug solution properties, and (3) human factors, such as the capability of the end-user to apply the product. In particular, device components

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contribute to the hydrodynamic component of injection forces, which can be described by the Hagen-Poiseuille's law. These are the syringe diameter, the needle inner diameter, and the needle length. Dynamic viscosity of the solution (2) is itself dependent on temperature (described by Arrhenius equation), formulation, and concentration of the active substance (9,11–14). Besides hydrodynamic forces, frictional forces between stopper and plunger have to be considered as well. These are usually influenced by siliconization of the syringe barrel and plunger dimensions among others (11,15).

An anthropometric strength study showed that the force that a user can exert onto a syringe plunger is determined by multiple (human) factors including the strength of the individual, the upper limb, and hand posture required for injection (health status) (16). It should also be considered that the personal preference and training of the individual may influence their actual behavior (16). In reality, users are able to moderate the injection force (when using pre-filled syringes) by adjusting their injection speed and may choose a slower injection resulting in a lower injection force to fit their capability or preference. Human factor studies usually do not target actual sc administration but usage of injection pads. Thus, deriving injection force data from these studies lack a potential impact of sc back-pressure. The same applies for *in vitro* testing of injection forces as well as syringe functionality testing (break loose and glide force) which is usually performed into air, thus, ignoring the *in vivo* situation and potential impact of sc back-pressure.

Predictive *in silico* models were described in literature to estimate injection forces of parenterals (9,11,13). However, only limited data is available dealing with the contribution of the tissue during injection. Cilurzo *et al.* have recently performed an experiment where they injected a 19 mPas and a 101 mPas solution into the abdominal skin (sc) from an Eurasian female who underwent cosmetic surgery. They found an increase in injection force by a factor of 1.1 compared to injection into air due to tissue resistance. This experiment was performed *ex vivo* at a constant injection speed of 1 mm/s, which was equivalent to approximately 0.03 mL/s (17). Vosseler and co-workers have measured the in-line pressure during intradermal injection into pig ears using microneedles (*ex vivo*). They found significant back-pressure of the intradermal tissue when tested for a viscosity of 1 and 55 mPas at (slow) injection rates of 0.1 and 0.5 mL/h. The authors reported that the back-pressure is a non-linear function of flow rate (18). In different studies, the intestinal fluid pressure in the forelimb of Yorkshire pigs (3.9 ± 1.4 mmHg) (19), in human foot skin (5.9 ± 2.9 mmHg) (20), and in human sc tissue of the lower limb (2.5 ± 3.0 mmHg) (21) was measured giving a hint towards a contribution of the tissue pressure to injection forces. However, up to date there is no *in vivo* study available which characterizes the contribution of the sc back-pressure to injection forces.

The aim of the present study is to investigate the contribution of the back-pressure of the sc tissue layer to injection forces during injection into the *plica inguinalis* of Göttingen minipigs. Göttingen minipigs are considered as a relevant animal model to study sc tissue as the structure of their hypodermis resembles that in humans more than any other investigated animal (e.g. rodent, monkey) (2,22–24). Different concentrations of isotonic dextran solutions corresponding to viscosities of 1, 10, 20, and 100 mPas were administered at injection rates of 0.025, 0.1, and 0.2 mL/s and two injection volumes (2.5 and 4.5 mL) were tested. The contribution of the sc back-pressure to injection forces was defined and determined by comparing subsequent *in vitro* and *in vivo* measurements of injection force profiles corrected by a temperature factor by use of an instrumental set-up built for this purpose. After termination, the injection sites were dissected and inspected macroscopically as well as histologically.

MATERIALS AND METHODS

Materials

Dextran Solutions

Isotonic dextran solutions were prepared in concentrations of 0%, 14.9%, 20.8%, and 34.8% by dissolution of dextran 40 (BioChemica, Darmstadt, DE) in water for injection and by addition of sodium chloride (Merck, Darmstadt, DE). The pH was determined as 5.5 ± 0.4. The dextran concentrations corresponded to viscosities of 1, 10, 20, and 100 mPas at room temperature characterized by plate/cone rheometry as described previously (11). For the tested injection rates, the dextran solutions showed Newtonian flow behavior.

Disposable Injection Equipment

Disposable 5 mL plastic syringes (BD, Franklin Lakes, NJ) from a single lot with luer-lock tip were connected by male or female luer fittings (1/16″, Nylon) to a one-way stopcock with luer connection obtained from Cole-Parmer (Vernon Hills, IL) and non-expanding PTFE tubing with an inner diameter of 1.6 mm and a length of 100 cm (Scat Europe, Mörfelden, DE). The tubing was connected to a Microlance™ 3 26 G 3/8″ needle (BD, Drogeda, IRL) as shown in Fig. 1a.

Methods

Injection Force Measurements and Data Analysis

The instrumental set-up for the injection force measurements is shown in Fig. 1a. A PHD 2000 Infusion syringe pump from Harvard Apparatus (Holliston, MA) was used in combination

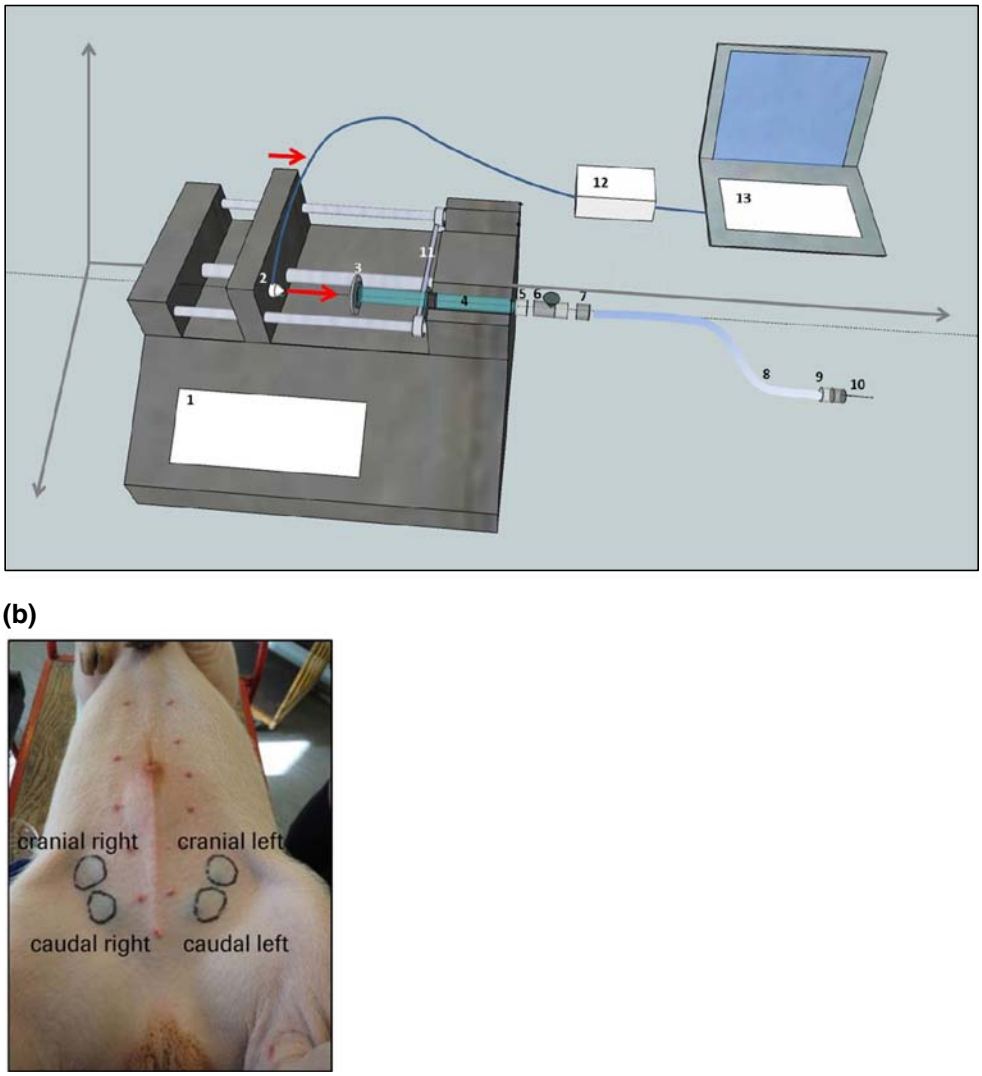
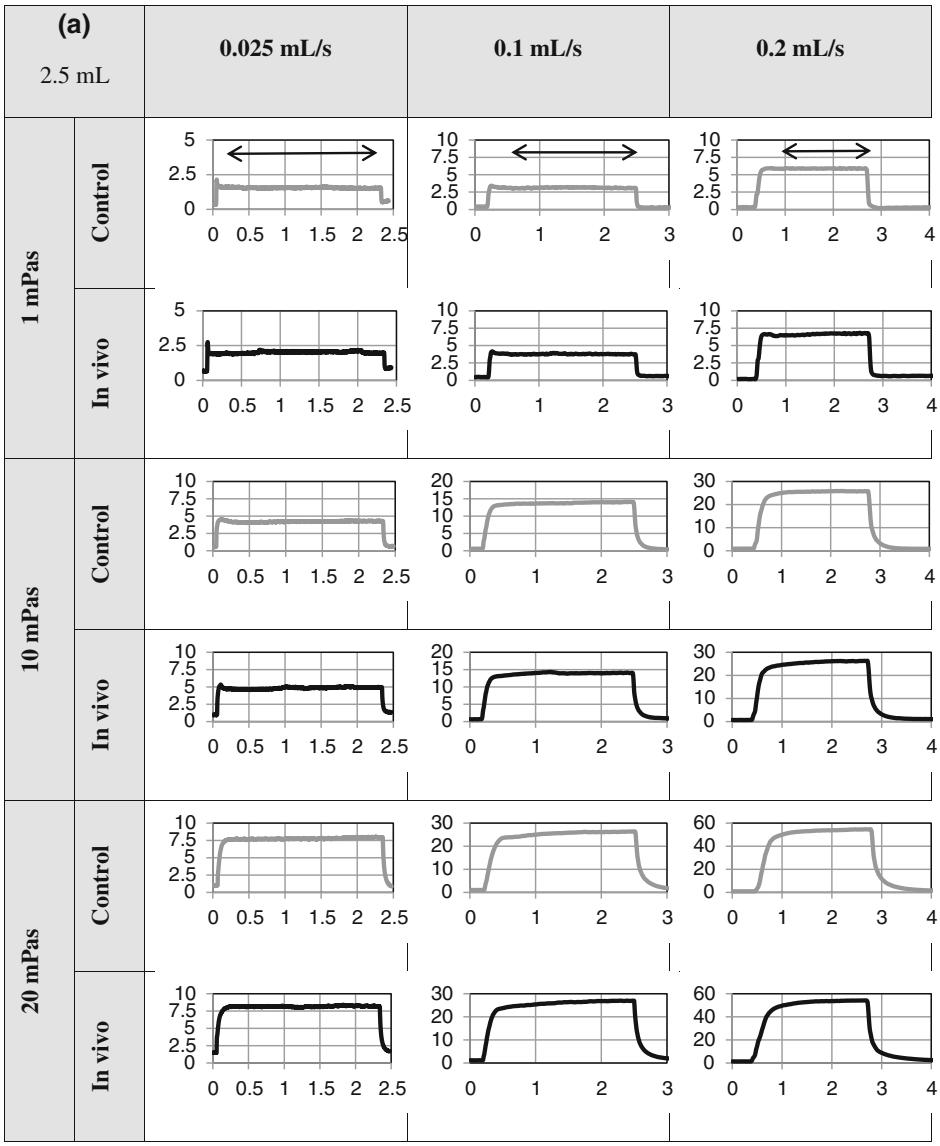


Fig. 1 Instrumental set-up for the injection force experiments (**a**), and injection sites for sc injection into the *plica inguinalis* of the minipig (**b**). (*1*) Syringe pump, (*2*) load cell (force sensor), (*3*) sticking metal platelet, (*4*)/(*5*) syringe with luer-lock tip, (*6*) one-way cock, (*7*)/(*9*) luer lock connections, (*8*) PTFE tubing, (*10*) needle, (*11*) fixation, (*12*)/(*13*) computer and interface.

with a RSB5 Subminiature load cell (force sensor) with an accuracy of ±0.5% of the full calibrated scale (0–100 N). The load cell was connected to a DI-100U interface obtained from Loadstar Sensors (Fremont, CA). Injection force measurements were performed at constant injection rates of 0.025, 0.1, and 0.2 mL/s applied by the syringe pump. An injection rate of 0.1 mL/s was chosen which is considered as appropriate for the end-user and commonly used and accepted for functionality testing (11,16). 0.2 mL/s was chosen as a maximum test rate, whereas 0.025 mL/s represents a slow injection rate as e.g. used by injection devices for high-volume sc dosing (8). The force exerted to the plunger of the syringe was recorded by the load cell each 0.1 s using the software 'Single-channel LV-1000 LoadVUE Pro' (Loadstar Sensors, Fremont, CA). The injection equipment and the test solutions were equilibrated to controlled room temperature overnight. Before each experiment, the injection equipment was rinsed with the sample under consideration. Directly before the *in vivo* measurements, control measurements into air (*in vitro*) were performed to exclude differences in injection forces caused by differences in temperature as well as differences in injection equipment. The temperature was monitored and found as 20°C ±1.5 (MIN 17.6°C and MAX 23.6°C).

Data analysis of the injection force profiles was performed by calculating the average value of the injection force plateau which was reached for all profiles between 0.25 and 2.5 mL for an injection rate of 0.025 mL/s, between 0.5 and 1 mL for 0.1 mL/s, and between 1 and 2 mL for 0.2 mL/s. Representative profiles are shown in Fig. 2 for *in vitro* as well as for *in vivo* measurements for different injection rates, viscosities, and injection volumes tested. Arrows indicate the range used for data analysis to determine the average value of the plateau of the force profile.


(a)
2.5 mL
0.025 mL/s
0.1 mL/s
0.2 mL/s
1 mPas
10 mPas
20 mPas
Control
In vivo
(b)
2.5 mL
Control
In vivo
Control (39°C)
100 mPas
0.025 mL/s
(c)
4.5 mL
Control
In vivo
10 mPas
0.1 mL/s

◀ **Fig. 2** Representative examples of injection force profiles for *in vitro* (control) and *in vivo* measurements. The injection force is presented as a function of injection volume for all profiles shown. (**a**) Different viscosities (1–20 mPas) and injection rates (0.025–0.2 mL/s) for an injection volume of 2.5 mL. (**b**) Viscosity of 100 mPas at 0.025 mL/s (2.5 mL) including injection force profile from temperature control measurement (39°C). (**c**) Injection volume of 4.5 mL for a viscosity of 10 mPas at 0.1 mL/s. The arrows indicate the range used for data analysis to determine the average value of the plateau of the profile which was 0.25–2.5 mL for an injection rate of 0.025 mL/s, 0.5–1 mL for 0.1 mL/s, and between 1 and 2 mL for 0.2 mL/s.

Control for Local Temperature Effect at the Injection Site

To account for the effect of body temperature on solution temperature in the needle and thus viscosity, control measurements were performed (*in vitro*, $N=3$) by injection of dextran solutions into water which was tempered to 39°C - equal to the body temperature of minipigs (25). Only the needle was inserted into the water to simulate *in vivo* injection. The instrumental set-up is shown in the 'Supplementary Material' Figure S-1. A representative example of the injection force profile is shown in Fig. 2b. The measurements were performed dependent on viscosity and injection rate, and were compared to subsequent injections into air at room temperature. Based on the difference, the decrease in injection force per degree Celsius caused by warming of the sample in the needle was calculated using the Arrhenius equation (26). This correction factor per degree Celsius was then applied to each individual *in vivo* measurement. (No difference was measured between injections into air and injections into water at the same temperature.)

For final data analysis, the correction factor was substracted from the difference between injection forces determined by subsequent *in vitro* and *in vivo* measurement. To give an order of magnitude, correction factors are presented in Table S-1 of the 'Supplementary Material' dependent on viscosity and injection rate for a temperature increase from 20 to 39°C (minipig) and additionally from 20 to 37°C (humans).

In Vivo Testing Set-Up

Ten Göttingen minipigs (male) with a body weight between 21.5 and 27.3 kg received four sc injections per dosing occasion into the *plica inguinalis* as shown in Fig. 1b. There were three dosing occasions per pig during the study with a recovery period of at least 48 h before the next four injections. Prior to dosing, the minipigs were anaesthetized using Zoletil®dosed as 0.1 ml/kg. Before each injection, the injection sites were inspected for absence of infections. Upon puncture, it was verified visually that the needle was in the sc tissue layer indicated by loose movement of the needle in the tissue. The injection angle was in parallel with the body surface to avoid penetration of the sc tissue layer. During and/or after each injection, the injection site was inspected for successful injection into the sc tissue layer by (temporarily observed) blister formation.

The dextran concentrations were administered with different injection rates and volumes and performed ten times for each condition. The injections were randomized for minipig, injection site, viscosity, and injection rate. The study was approved and conducted in accordance with local legislation for animal welfare (Pipeline Biotech A/S license number 2011/561-2006, schedule C2 and extension).

Macroscopic Evaluation After Dissection of the Injection Sites and Histology

At termination after the third dosing, macroscopic evaluation of the dissected injection sites was conducted to ensure successful injection into the sc tissue layer. To facilitate the recognition of the injection sites, 1 drop of ink (blue stamp ink, Carfa, Richterswil, CH) was added to 20 mL of sample (which was determined to not affect viscosity). The length, width, and height of the injection sites (as shown in Fig. 5b and c) was measured and recorded by the same person.

For histological characterization of the injection sites, the dissected tissue was transferred and fixed in 10% neutral buffered formalin, and embedded in paraffin. Slices of 4 µm were prepared, stained with standard hematoxylin-eosin, and investigated under the light microscope.

RESULTS

In Vivo Characterization of sc Back-Pressure

The contribution of the back-pressure of the sc tissue layer to injection forces was investigated in Göttingen minipigs dependent on solution viscosity (1–20 mPas) and injection rate (0.025–0.2 mL/s). Figure 3a shows the force related to back-pressure as a function of viscosity for the different injection rates for an injection volume of 2.5 mL. It is presented as mean values from 10 randomized measurements with standard deviation as well as minimum and maximum values. Overall, the force related to sc tissue back-pressure was measured between 0.1 and 12.9 N. In detail, it was found in the range of 0.6–1.0 N for 1 mPas, 0.7–2.4 N for 10 mPas, and 1.8–4.7 N for 20 mPas for an injection rate of 0.025 to 0.2 mL/s (mean values). Variability of mean values increased with higher viscosity and injection rate as indicated by error bars in Fig. 3. Maximum contribution of tissue back-pressure to injection forces of 1.7 N, 6.4 N, and 12.9 N were found for

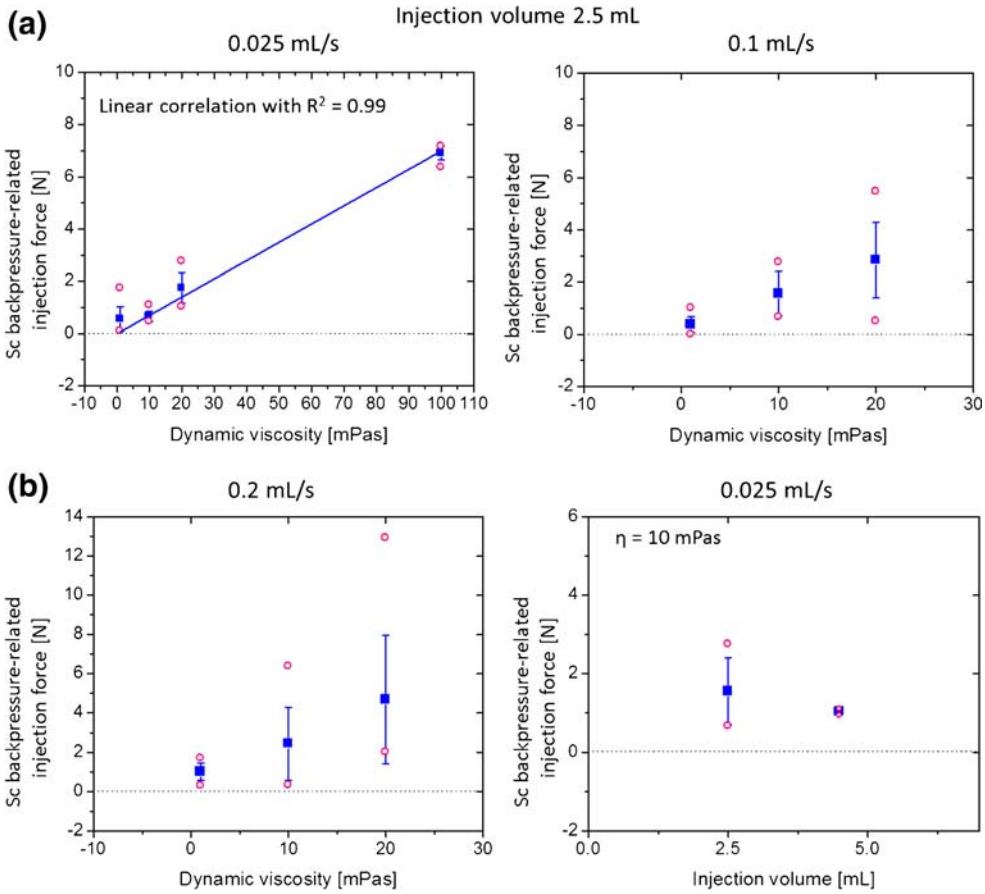


Fig. 3 Contribution of sc back-pressure to injection forces dependent on injection rate (0.025, 0.1, and 0.2 mL/s), viscosity (1–100 mPas) of dextran solutions, and injection volume (2.5 mL, A, or 4.5 mL, B). The contribution of back-pressure to injection forces was analyzed as value of the injection force plateau. Measurements were performed as $N = 10$ randomized by minipig, injection site, injection rate, and viscosity and were reported as mean value with standard deviation (*squares*), minimum and maximum value (*circle*).

the samples with 1, 10, and 20 mPas for the highest injection rate, respectively. The highest dextran concentration with a viscosity of 100 mPas was found to show an additional force related to back-pressure of 6.7 N (mean value) with a maximum value of 7.2 N measured at an injection rate of 0.025 mL/s.

Figure 3a shows that a linear increase of the contribution of sc back-pressure to injection forces was found with increasing viscosity for all three injection rates. This is emphasized in Fig. 3a for the lowest injection speed of 0.025 mL/s indicating a linear increase ($R^2 = 0.99$) of sc back-pressure related contribution to injection forces between 1 mPas up to the highest viscosity tested, which was 100 mPas. A linear increase was also found dependent on injection rate for the tested solution viscosities (correlation not shown separately).

In a follow-up experiment, the injection volume was increased to 4.5 mL and the sc back-pressure was tested for a viscosity of 10 mPas at an injection rate of 0.1 mL/s, in order to study potential impact of injection volume on sc tissue back-pressure. Figure 3b shows the corresponding mean values from 10 injections as well as minimum and maximum values with comparison of the 2.5 and 4.5 mL. No difference in the contribution of injection forces of sc back-pressure was found between the two injection volumes, however variability as indicated by error bars of the mean value decreased for the larger injection volume indicating a stabilization of the measured injection force signal.

For all tested conditions, the injection force profile reached a plateau as shown in Fig. 2 for the different viscosities and injection rates, including the higher injection volume of 4.5 mL (Fig. 2c). This indicates that - up to the maximal tested injection volume - the visually observed blister formation for some injection sites of the tissue due to the injected volume itself has a negligible influence on injection force or is in the magnitude of the measurement error.

It has to be highlighted that the contribution of the sc back-pressure was quantified in this study and differentiated from the contribution of the injection device as well as separated from a local temperature effect. This temperature effect was quantified as a temperature correction factor as outlined in the Method's section. To our knowledge, this is the first study to report a local temperature effect at the injection site leading to warming of the (equilibrated) sample during sc injection in the needle and smaller injection forces than expected. This will be discussed in detail later in this article.

Injections were performed ten times for each condition and were randomized for minipig, injection site, viscosity, and injection rate. Figure 4 displays the box plots of the contribution of sc back-pressure to injection forces (average values) dependent on minipig (named as 1 to 10, Fig. 4a), injection

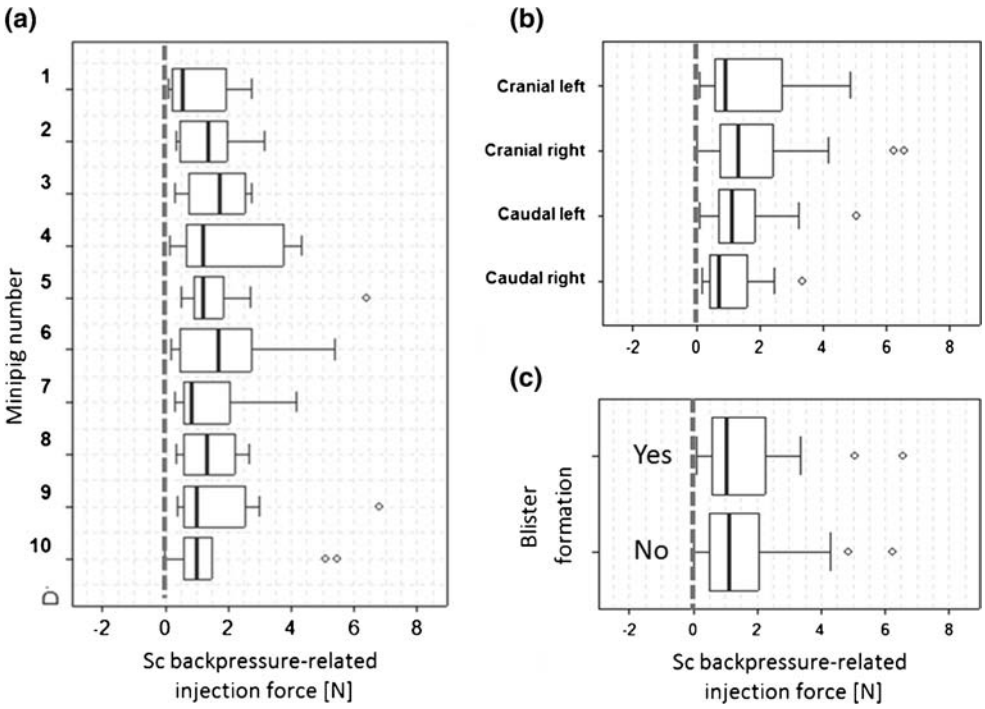


Fig. 4 Box plots of the contribution of sc back-pressure to injection forces (mean value) dependent on (**a**) minipig (1 to 10), (**b**) injection site, and (**c**) blister formation after injection. The box plot represents mean, upper and lower quartile (*box*), minimum and maximum values (*whiskers*) as well as outlier defined by > 1.5 times of the interquartile range (circle).

site (Fig. 4b), and observed blister formation after injection (Fig. 4c) showing no differences in sc back-pressure related injection force between these parameters. Most interestingly, Fig. 4c does not show a difference in the contribution of back-pressure to injection forces between injections where blister formation after injection was observed and those where it was not observed. This confirms the previous findings, that the sc back-pressure does not increase with increasing injection volume (2.5 vs. 4.5 mL) and that the visually observed blister formation due to the injected volume itself during injection has a negligible contribution or is within the variation of injection force measurements for the conditions tested. Thereby, the number of injection sites showing blister formation after injection was found to decrease with increasing bodyweight of the minipig as shown in the 'Supplementary Material' in Figure S-2.

Verification of Injection into the sc Tissue Layer

As the *plica inguinalis* has only a thickness of a few mm, a short needle (3/8″) was chosen to facilitate injection into the sc tissue layer. It was verified for all injections that they were indeed occurring into the sc space by: (1) visual inspection before *in vivo* injection indicated by loose movement of the needle inserted into the tissue. (2) During injection, where differentiation between sc and intradermal (id) injection is possible as id injection manifests in characteristic blister formation of the skin. Besides the visual inspection of the injection sites, sc injection was verified on the last study day for the full set of injections, including all different conditions for injection, by dissection of the injection sites followed by (3) macroscopic evaluation and (4) histology.

Macroscopic Evaluation After Dissection of the Injection Site

On the last study day, a dye was added to the dextran solutions prior to injection and each injection site was dissected and inspected. Each injection was recovered as defined, well-localized injection site in the sc tissue layer for all conditions tested. Representative pictures of the injection sites are shown in Fig. 5a, b. The width, height, and length of the sites were measured by the same person (indicated by arrows in Fig. 5a).

Figure 6 shows the dimensions sorted according to viscosity and injection speed as well as injection volume. For an injection volume of 2.5 mL, dimensions of 1.7–4.2 cm, 2.7–5.6 cm, and 0.3–1.9 cm were found for width, length, and height, respectively. With higher injection volume (4.5 mL), the dimensions increased to 2.0–3.3 cm, 2.7–9.0 cm, and 0.4–1.3 cm (width, length, and height). The calculated distribution volume in the sc tissue based on these dimensions assuming oval spreading was found as 3.7 and 7.2 mL for an injection volume of 2.5 and 4.5 mL. This corresponds to an increase in distribution volume in the sc space by 50% compared to the initial injected volume. Overall, the macroscopic evaluation of the injection sites at termination showed that all injections were located in the subcutaneous tissue layer.

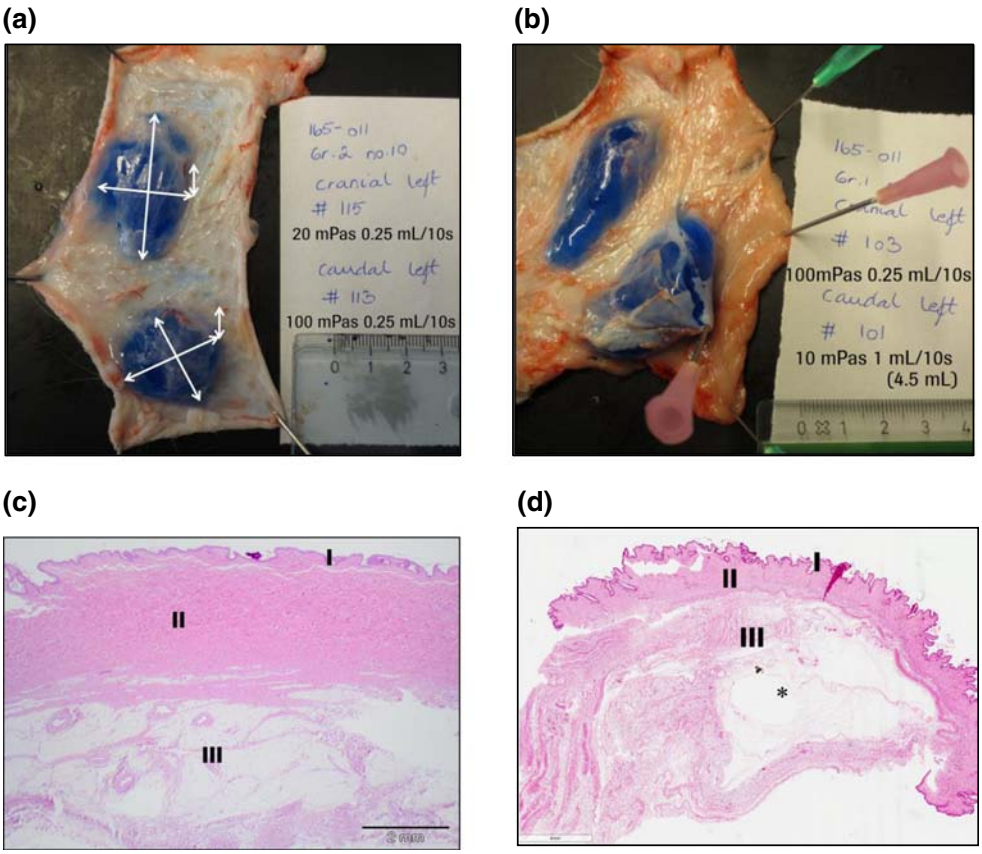


Fig. 5 (**a**, **b**) Representative pictures of dissected injection sites (*plica inguinalis*) after injection of a colored dextran solution (20 and 100 mPas/0.025 mL/s/2.5 mL and 10 mPas/ 0.1 mL/s/4.5 mL). The white arrows (**a**) indicate how the measurement of the dimensions of the injection sites was performed. (**c**, **d**) Representative histological sections before (**c**) and after (**d**) injection of an isotonic dextran solution with a viscosity of 10 mPas at an injection rate of 1 mL/10s. I: epidermis, II: dermis with adnexe e.g. hairs and sebeceous glands, III: subcutis, (*asterisk*) subcutaneous edema due to injection.

Histology

Figure 5 shows histological slices of the skin of the injection site (*plica inguinalis*) before (C) and after (D) injection of a dextran solution with a viscosity of 10 mPas injected at an injection rate of 0.1 mL/s. The histology shows the epidermis, dermis with adnexa like hairs and sebaceous glands, and subcutis. Figure 5d clearly shows a subcutaneous edema due to the

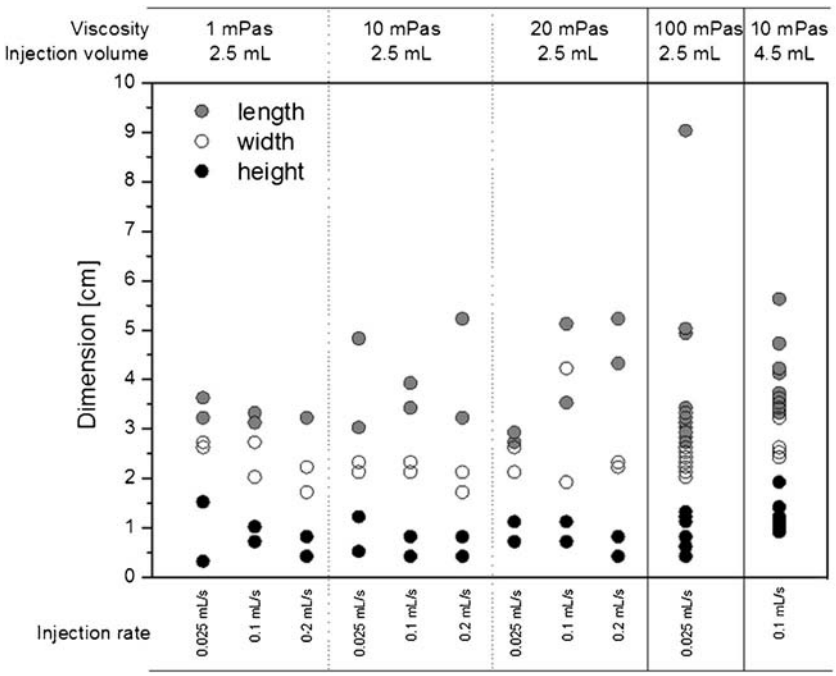


Fig. 6 Dimensions of dissected injection sites after termination with length, width, and height dependent on viscosity, injection rate, and injection volume. Data are presented as single values and were measured as duplicates or $N = 10$, the latter for the high viscous (100 mPas) and high volume (4.5 mL) experiments.

dextran injection when compared to Fig. 5a. In general, there were no differences found in histology between the four injection sites.

DISCUSSION

Göttingen Minipigs as *In Vivo* Model for sc Tissue

The minipig is currently considered as the most appropriate translational animal model to study the pharmacokinetics of biotherapeutics after sc injection e.g. for studies of monoclonal antibody therapeutics (2,27,28). Most importantly, the structure of the hypodermis of the pigs was reported to resemble that in humans more than any other animal (e.g. rodent, monkey) (2,22–24). The skin of the pig is, like in humans, connected to the deep fascia via a fibrous network which defines the spreading behavior of a sample. A relevant difference between humans and e.g. rodents, monkeys, and pigs is the presence of the *panniculus carnosus* for the latter three animal species (3,22,24,29). However, the *panniculus carnosus* is missing in some parts of the pig, like in the groin, and therefore ressembling human tissue structure (22). Thus, the *plica inguinalis* was chosen as injection site in minipigs for this study. Only the height of the subcutaneous tissue layer differs between the human skin (approximately 11 mm) (30) and the *plica inguinalis* of the minipig (few mm). Therefore, a short needle with a length of 3/8″ was chosen to control and ensure injection of the solutions in the sc tissue space in our study. Adsorption of the dextran was not investigated and not in scope of our study as only the injection process was considered. The injections were randomized for each minipig animal, injection rate, viscosity, and injection site. In summary, mean values of the contribution of tissue back-pressure to injection forces were determined in the range of 0.6 to 4.7 N for injection rates between 0.025 and 0.2 mL/s and viscosities between 1 and 20 mPas. A minimum value of 0.1 N and a maximum value of 12.9 N were measured. The injection force related to sc tissue back-pressure was found to be linear dependent on injection rate as well as on viscosity. Injection force profiles reached a plateau for all conditions as outlined tested. No increase in the contribution of sc back-pressure was observed for an increase in injection volume from 2.5 to 4.5 mL. Moreover, the occurrence of blisters in the sc tissue during/after injection due to the injected volume per se was found to be independent on the force related to sc back-pressure. These findings together indicate that the contribution of blister formation during/after injection to injection forces is smaller or in the magnitude of the variation of the force measurement for the conditions tested. Based on these data, we may assume that the dextran solution might rapidly diffuse to the connective tissue. However, the macroscopic evaluation of the injection sites on the last study day has shown well-localized compartments for all conditions tested.

Influence of Body Temperature on Injection Forces

As outlined previously, the data analysis of the contribution of sc back-pressure to injection forces was performed by subtraction of the *in vivo* from the *in vitro* measurement which were determined subsequently, corrected by an individual temperature factor. The *in vitro* and the *in vivo* experiment were performed subsequently under same temperature conditions using the same injection equipment. The injection equipment was rinsed before start of the experiment with the sample to be injected which was equilibrated to room temperature over several hours in the facility.

An interesting observation was made when injecting the 100 mPas dextran solution. The data suggested that the measured injection forces of the control measurements were significantly higher than the injection forces of the *in vivo* experiment. However, as the *in vitro* experiment was performed prior the *in vivo* experiment under same temperature conditions using the same injection equipment, the data suggested parameter to additionally influence to injection forces under *in vivo* conditions. As the sample was equilibrated to room temperature for several hours and most importantly the tubing/injection equipment was not in contact with the body surface of the minipig at any time (except of the needle tip), the hypothesis was that a local, fast decrease in viscosity in the needle tip may consequently lead to the reduction in injection forces. This local decrease might be a result of the fast warming of the sample in the needle during the injection process due to the body temperature of the minipig (39°C). To proof this, a simple control measurement was performed. The dextran samples were injected into tempered water of 39°C equal to the body temperature of minipigs (25), with only the needle inserted into the water bath. Prior to this experiment as a control, the same injections were performed into air for comparison under the same conditions (sample, temperature, equipment). The experiments confirmed that injection forces were significantly decreased if the needle tip - although only few millimeters in length - faces warmer environmental conditions than room temperature during injection than compared to injections into air (at room temperature). This additional negative contribution to injection forces was found and quantified to be dependent on viscosity and injection rate indicating a hydrodynamic process. In detail, the contribution of the body temperature of the minipig to injection forces was separated for each individual experiment from the contribution of the sc back-pressure and the hydrodymanic component as follows: the decrease in injection force per degree Celcius was obtained from the experimental data of the control measurements (waterbath vs. air) using the Arrhenius equation dependent on injection rate and viscosity.

Based on these factors, the decrease in injection force was calculated (Arrhenius equation) for each individual experiment based on the temperature difference between control measurement (39°C) and the actual monitored temperature during the *in vitro*/*in vivo* experiment. Examples for a temperature difference of 20–39°C and 20–37°C are given in Table S-1 in the Supplementary Material. In summary, we concluded, that the observed temperature effect manifests as a localized temperature effect at the injection site. This leads to warming of the (equilibrated) sample in the needle during injection, thus smaller viscosity and smaller injection forces than expected. As the temperature factor was found to be dependent on viscosity and injection rate, this indicates a hydrodynamic process and strengthens our conclusion that the temperature at the animal body/at the injection site leads to adjustment of the fluid temperature in the needle during sc injection.

Implications of sc Back-Pressure and Local Warming in Context of User Capability

As outlined in the introduction, injection forces of highly concentrated protein products are influenced by device dimensions like needle inner diameter, product properties like viscosity - itself dependent on temperature and concentration of active substance, and by the injection rate which is defined by the capability of the end-user. Also frictional forces between plunger and syringe barrel contribute to injection forces. These parameters are well understood and were investigated in detail in the past. Current literature models use these factors to predict injection forces, however limited to the *in vitro* situation neglecting actual *in vivo* conditions (11). Dependent on the indication, patients might be limited in their strength like e.g. for rheumatoid arthritis patients, stating a challenge to expel a syringe. Therefore, the limits for maximal required injection force have to be carefully evaluated before design of an injection device. A highly concentrated protein therapeutic with a viscosity of 100 mPas has been previously studied (16). This protein therapeutic was tested for injectability by Sheikhzadeh and co-workers in an anthropometric study. The authors reported that rheumatoid arthritis patients were capable to exert a force of approximately 17 N (95% percentile) and 48 N (mean) in average onto the syringe plunger (16). Setting this into relation to our findings assuming a constant, slow injection speed of 0.025 mL/s, the injection force of this product would be higher by approximately 7 N due to the contribution of the sc back-pressure compared to *in vitro* injection forces which are in general tested against air. The local temperature effect for a body temperature of 37°C leading to warming of the sample in the needle would decrease injection forces by approximately 4 N assuming similar viscosity-temperature dependence of the therapeutic as dextran. This would result in an overall higher injection force by 3 N compared to the estimated or measured injection force in the *in vitro* situation. Testing of injection force *ex vivo* still remains questionable of being representative of *in vivo* injection forces. Moreover, it may lead to unexpected modifications/distortions and may therefore not be representative for the *in vivo* situation.

The data indicate that consideration of the actual *in vivo* sc back-pressure is essential when assessing injection forces. Moreover, compensation of the increase in injection forces by the tissue back-pressure due to the local temperature effect, as observed for the minipig, should be considered. Based on the above findings, the current *in silico* model to predict injection forces (11) can be extended to:

$$F_{total} = F(Q;\eta)_{hydrodynamic} + F(Q)_{friction} + F(Q;\eta)_{scbackpressure} - F(Q;\eta)_{bodytemperature}$$

(F = force, Q = volumetric flow, η = dynamic viscosity)

Sc Injection Volume

The present study showed that injection volumes up to 4.5 mL into the sc tissue space were forming localized, well-defined injection sites, without any observed leakage. This is of high importance as the sc tissue layer of the *plica inguinalis* of the minipig is even thinner than the one of humans. The localized injection sites were found for all conditions tested independent on viscosity and injection rate. Compared to literature, the current sc injectable volume is considered to be limited to 1 to 1.5 mL for human use (31). Frost *et al.* described the volume for sc injection to be generally limited to less than 2 mL due to compliance of the tissue space to injected fluids (32). This is driven by the fibrous bands in the *panniculus adiposus* that reach into the deep fascia for humans and other furless animals (22,33,34) which might lead to pain and tissue distortion upon sc injection. Current marketed products for sc administration are to date limited to 1 to 1.5 mL volume of administration (1).

Recent approaches have shown that higher injection volumes are feasible for sc injection by co-injection with recombinant human hyaluronidase, an enzyme that temporarily and reversibly degrades hyaluronan which is a major component of the extracellular matrix of the skin besides collagen. This facilitates the penetration and diffusion of the co-administered drug (34–37). Shpilberg and colleagues showed that administration of up to 15 mL of a co-formulation of hyaluronidase and a monoclonal antibody into healthy volunteers (phase Ib study) was feasible (7,38).

The present study showed that injection of up to 4.5 mL was feasible from the point of view that the sc tissue was capable to keep the solution at a defined injection site as outlined above. Moreover, another study suggested local

tolerance in beagles with good tolerability up to an injection volume of 5 mL into the sc tissue space (39).

CONCLUSION

The development of injection devices for combination products (e.g. autoinjectors or pre-filled syringes) requires a detailed understanding of injection forces dependent on device dimensions like needle diameter, and formulation properties like viscosity. Current literature provides experimentally verified *in silico* models for prediction of injection forces into air. To our knowledge, this is the first study which provides quantitative data on the back-pressure of the sc tissue contributing to injection forces measured during *in vivo* injection. In summary, mean values were determined in the range of 0.6 to 4.7 N for injection rates between 0.025 and 0.2 mL/s and viscosities between 1 and 20 mPas. A minimum value of 0.1 N and a maximum value of 12.9 N were measured. These data demonstrate that the sc tissue back-pressure during injection can be significant. All force profiles reached a plateau during injection, even for the higher injection volume of 4.5 mL forming well-localized compartments in the sc tissue layer. Moreover, we reported that a local temperature effect led to warming of the (equilibrated) drug solution in the needle due to the body temperature of the minipig. This led to smaller measured injection forces than expected relieving the increase in injection forces due to sc back-pressure - to some parts. As the structure of the sc tissue layer was shown to be comparable between the *plica inguinalis* of minipigs and humans, the current data set presents - to our knowledge - the first appropriate quantitative data on injection forces during sc administration considering *in vivo* conditions representative for humans.

The present study separates the contribution of the tissue back-pressure from the contribution of the injection device as well as from the local temperature effect which was quantified dependent on viscosity and injection rate. Based on these findings, the current *in silico* model to predict injection forces (11) can be extended to:

$$F_{total} = F(Q;\eta)_{hydrodynamic} + F(Q)_{friction} + F(Q;\eta)_{scbackpressure} - F(Q;\eta)_{bodytemperature}$$

(F = force, Q = volumetric flow, η = dynamic viscosity)

To conclude, this knowledge is of key importance to further develop and define limits and setup for testing of device robustness during the evaluation, planning, and design phase of the development of injection devices.

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