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Penetration and delivery characteristics of repetitive microjet injection into the skin

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Abstract

Drugs can be delivered transdermally using jet injectors, which can be an advantageous route compared to oral administration. However, these devices inject large volumes deep into the skin or tissues underneath the skin often causing bruising and pain. This may be prevented by injecting smaller volumes at lower depth in a repetitive way using a microjet injection device. Such a device could be used to apply drugs in a controllable and sustainable manner. However, the efficacy of microjet injection has been rarely examined. In this study, the penetration and delivery capacity was examined of a repetitive microjet injection device. Various experiments were performed on epidermal and full-thickness human as well as porcine skin samples. Results revealed that microjets with a velocity exceeding 90 m/s penetrated an epidermal skin sample with a delivery efficiency of approximately 96%. However, in full-thickness human skin, the delivery efficiency drastically decreased to a value of approximately 12%. Experiments on full-thickness skin revealed that the microjets penetrated to a depth corresponding to the transition between the papillary and reticular dermis. This depth did not further increase with increasing number of microjets. In vivo studies on rats indicated that intact insulin was absorbed into the systemic circulation. Hence, the microjet injection device was able to deliver medication into the skin, although the drug delivery efficiency should be increased.

Keywords

Microjet injection; transdermal drug delivery; jet penetration; skin damage; delivery efficiency

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1. Introduction

Jet injectors have been used to deliver various drugs, such as vaccines, insulin and growth hormones, into the skin or into tissues underneath the skin (Mitragotri 2006). This route of administration has several advantages over oral administration of drugs that are digested before entering the circulation. Moreover, it obviates the need for hypodermic needles to achieve intramuscular or subcutaneous injection, with its association with needle phobia. However, current available jet injectors may cause pain and bruising of the skin, which are assumed to be caused by its relative deep penetration and large injection volumes (Mitragotri 2006). Particularly in the case of chronic diseases, such as diabetes and Alzheimer's, where medication has to be frequently applied, patient acceptability and compliance needs to be improved. Skin damage and irritation should be minimized and the delivery precisely controlled. A microjet injector that is able to repetitively inject smaller volumes of medication superficially is thought to overcome the disadvantages of the current jet injector (Arora et al. 2007). These microjet injectors are potential wearable transdermal drug delivery devices that allow for a precisely, continuously controlled and sustainable delivery of drugs for patients with chronic diseases.

In both jet and microjet injectors, a drug solution is ejected through a nozzle with a diameter ranging from 30 to 560 µm at a velocity of approximately 100 m/s (Mitragotri 2006). Various techniques have been used to generate this high velocity jet, such as spring-driven devices (Schramm-Baxter & Mitragotri 2004; Michinaka & Mitragotri 2011; Resik et al. 2015), piezo actuation (Arora et al. 2007; Stachowiak et al. 2009), compressed gas (Shergold et al. 2006; Resik et al. 2015), Lorentz force actuation (Taberner et al. 2012) and laser-induced jet generation (Tagawa et al. 2013; Jang et al. 2014). Most jet injectors eject volumes of 10 to 500 µl. However, the injection depth has been shown to be correlated to the injection volume (Baxter & Mitragotri 2005). Therefore, for superficial injection the injected volume should be reduced, resulting in a microjet, which is designed to inject volumes in the nanolitre range (Arora et al. 2007; Stachowiak et al. 2007; Jang et al. 2014).

For the jet injection devices the penetration characteristics have been examined. In addition to the injected volume, it has been reported that jet velocity, nozzle diameter, and stiffness of the penetrated material influence the penetration depth (Schramm-Baxter & Mitragotri 2004; Schramm-Baxter et al. 2004; Baxter & Mitragotri 2005). Contrary, there is a paucity of data on the penetration process and efficiency of microjet injectors. Nevertheless, it has been demonstrated that a microjet injector is feasible to penetrate the epidermis and deliver fluid into the skin (Arora et al. 2007; Stachowiak et al. 2009).

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There are still unanswered questions about microjet penetration. Specifically, what proportion of the ejected dose is delivered into the skin? And further, what makes the microjet penetrate to a specific depth? What kind of damage is induced in the skin? Is the delivered drug still intact? These questions provide motivation for the current study to assess the feasibility of a microjet drug delivery device for controlled and sustained drug delivery. Various aspects of the penetration process of microjets into the skin were examined using ex vivo porcine and human skin. A threshold value for the microjet velocity and the amount of microjets to penetrate the epidermis were determined. Moreover, the penetration depth and damage were assessed with histology and confocal microscopy. The delivery efficiency was examined using a radioactive tracer. In addition, the ability of the microjet injection device to deliver medication that is taken up in vivo was assessed by measuring the effect on blood glucose levels in a rat model after administration of insulin.

2. Materials and Methods

2.1 Microjet drug delivery system

A microjet drug delivery device was developed at Philips Research (Eindhoven, the Netherlands), which was able to repetitively eject small volumes of a solution at high velocity, with the aim of delivering a drug into the superficial layers of the skin.

The system consists of a piezoelectric actuator that is attached to a plunger (Fig. 1). When a voltage is applied to the piezoelectric actuator with a pulse generator, the plunger will be displaced and quickly compresses a solution in the chamber of the replaceable cartridge. This almost instantaneous expansion allows a high pressure build up in the chamber. The high pressure results in the ejection of a high velocity microjet through a nozzle with a diameter of 50 µm. Without any actuation, capillary forces prevent the liquid from leaking out of the chamber. After extension, the piezoelectric actuator and plunger are placed back to their original position by a mechanical spring and the chamber in the cartridge is refilled with a micropump via the refill channel. Excess fluid from the refilling process was removed using a vacuum pump (Fig. 1b). A spacer of 1 mm thickness prevented the nozzle to come in direct contact with the skin. This microjet injector produces repetitive microjets with a volume of 7-30 nl as determined with a radioactive tracer (section 2.5). In this study, all repetitions within one series of experiments were performed with the same cartridge.

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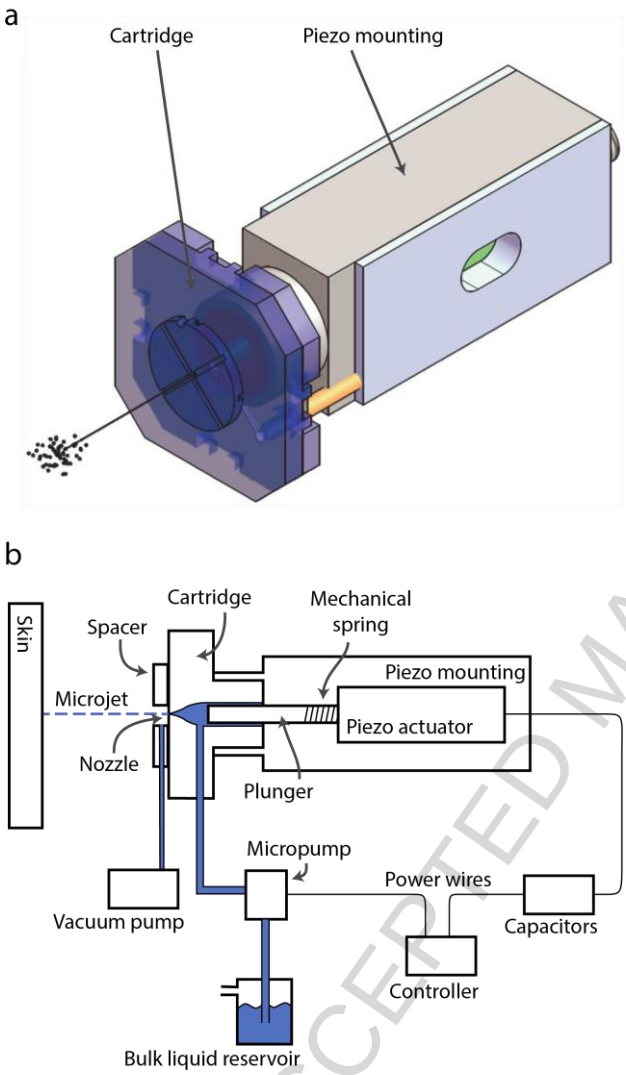

Figure 1. Schematic pictures of the microjet drug delivery system. A piezo actuator displaced a plunger, compressing a solution in the chamber of the replaceable cartridge. Subsequently, a microjet was ejected through the nozzle into the skin. The cartridge was refilled using a micropump. Excess fluid at the nozzle was removed using a vacuum pump.

2.2 Preparation of skin samples

Fresh porcine ears were obtained from a local abattoir. These ears were removed from the cadaver before it was exposed to the high temperature cleaning procedure to maintain their integrity. After transfer to the laboratory, the porcine ears were washed and hairs were trimmed. Subsequently, skin slices with a thickness of approximately 800 µm were obtained using a dermatome (Humeca, Enschede, the Netherlands). The epidermis was separated from the dermis using enzymatic separation with dispase II (Stenn et al. 1989; Geerligs 2010). The samples were then wrapped in plastic and aluminium foil and stored at -80°C until further processing.

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Human skin of patients who had undergone abdominoplasty was obtained from the Catharina Hospital in Eindhoven, the Netherlands, with approval of the Medical Ethics Committee of the same hospital. Human skin samples with a thickness of approximately 0.2 and 1.2 mm were prepared in a similar manner as porcine samples using a dermatome. In addition, samples containing only the epidermis were prepared using enzymatic separation. All samples were stored at -80°C wrapped in plastic and aluminium foil.

2.3 Penetration of porcine and human skin

To assess the ability of the microjet injector to penetrate the skin, single microjets were injected into porcine epidermal samples at jet velocities ranging from 45 to 130 m/s. Before injection, these samples were thawed and subsequently placed on top of a 0.4% agarose gel to support the tissue and to prevent it from dehydration. Deionized water was used as injection liquid. The penetration depth of a single microjet penetrating the epidermal layer into the agarose gel was measured to determine the minimal velocity of the microjet necessary to penetrate the epidermal layer. This velocity was determined by recording the microjet with a high speed camera mounted perpendicularly to the direction of the microjet. Using the distance travelled between successive frames and the known frame rate, the microjet velocity was calculated. The velocity could be controlled by varying the voltage applied to the piezoelectric actuator.

Based on the results on porcine skin, the ability of the microjet to fully penetrate human skin samples was assessed using a microjet velocity of 105-125 m/s. Since the single microjet did not always fully penetrate the human skin, multiple microjets were injected at the same location at a frequency of 0.25-1 Hz. Injections were performed on both human epidermal samples and skin sample with a thickness of approximately 200 µm, which included a part of the human dermis. All human samples were also placed on top of a 0.4% agarose gel.

2.4 Delivery depth and skin damage

In order to examine the precise depths of penetration into the skin, fluorescent particles with a diameter of 100 nm (FluoSpheres, F8888, Invitrogen) were diluted in deionized water to a concentration of 0.2 mg particles per ml. This solution was injected in a full-thickness human skin sample at various sites located in a 5 x 5 array separated by a distance of 0.5 mm. At each site, 20 consecutive jets were ejected. Subsequently, images of the sample were recorded at multiple depths in the plane parallel to the surface of the skin using a confocal microscope.

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To determine the amount of skin damage caused by repetitive injections, 1000 to 4000 microjets of a saline solution were injected at a single location in full-thickness human skin samples of 1.2 mm thick (n = 1-2 per number of microjets). These samples were subsequently sliced and stained with a haematoxylin-eosin staining (H&E) and the tissue damage assessed using brightfield microscopy.

2.5 Delivery efficiency

The delivery efficiency of microjets into the human epidermis and into full-thickness human skin was determined using a radioactive tracer. Epidermal samples were placed on top of a 0.4% agarose gel, while full-thickness samples with a thickness of approximately 1.2 mm were placed on top of a hydrophilic polymer matrix hydrated with a saline solution. To determine the delivery efficiency, 5 MBq of Technetium-99m (Tc-99m), a γ-ray emitter, was dissolved in 10 ml saline solution (0.9% NaCl). The microjet injection system was loaded with this solution and placed 1 mm above each of the skin samples. Subsequently, 100 to 1000 microjets were ejected at a single location. After injection, the surface of the skin was wiped off using cotton swaps. Subsequently, the amount of activity in the epidermis, agarose gel, and cotton swaps was measured for the epidermal samples using a gamma counter (1480 Wizard, PerkinElmer Inc., Waltham, MA, USA). For the full-thickness skin, in addition, the most superficial layers of the stratum corneum were removed using tape stripping and the amount of radiation of the skin sample, cotton swaps, and tape was measured. To determine the delivery efficiency, the delivered amount was compared with total volume ejected by the microjet device. This volume was estimated by performing series of microjet ejections before and after the experiments. For this calibration, the same radioactive tracer was ejected in series of 100 to 1000 microjets on a piece of cotton wool in a vial. The volume of a single ejection in each series was averaged and used to estimate the total ejected volume.

2.6 In vivo administration of insulin in rat

To evaluate the use of the microjet injector to deliver a drug into and through the skin and to assess if the functionality of the drug was affected by high speed injection, insulin was delivered to two Sprague Dawley rats in vivo. Prior to the experiment, the rats were anesthetized using gas (isoflurane in filtered compressed air) and a blood sample was collected from the tail vein. The abdomen of the rat was shaved and the microjet injector was placed against the skin. Subsequently, an insulin solution (100 U/ml, Novorapid) was administered with repetitive microjet injection for 20 minutes at a frequency of 1 Hz. Blood samples were collected every 10 to 60 minutes from the start of the experiment up to 2.5 hours. Blood glucose levels of each sample were directly assessed with an i-STAT system (Abbott Point of Care Inc., IL, USA) with an expected error of less than 1%. Skin irritation was assessed before and after the

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injection period and after collection of the last blood sample by imaging the injection site. Subsequently, a biopsy of the injection site was collected and processed for histological analysis. The experimental protocol was approved by the Animal Experiments Committee of Maastricht University (DEC-UM).

3. Results

3.1 Penetration of porcine and human skin

The capability of a single microjet to fully penetrate the porcine epidermis depended on the jet velocity (Fig. 2). Above a velocity of 90 m/s almost all microjets penetrated the epidermis and were injected into the underlying agarose gel. At microjet velocities below 70 m/s most jets did not penetrate the epidermis, but were rebounded from its surface. In the velocity range between 70 and 90 m/s some microjets penetrated the porcine epidermis while others did not.

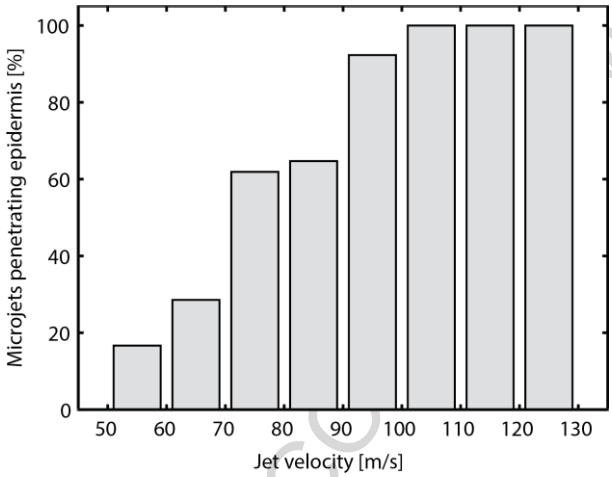

Figure 2. The percentage of a single microjet that fully penetrated the porcine epidermis into a 0.4% agarose gel at various microjet velocities. Above 90 m/s more than 90% of the microjets penetrated the epidermis. A total of 82 measurements were performed on four skin samples.

In contrast to the porcine epidermis, the human epidermis was not always fully penetrated with a single shot at a velocity of 105 - 125 m/s. At these speeds, up to 9 jets were needed before the epidermis was penetrated (n = 4 on a single sample). The number of jets to fully penetrate human skin samples of approximately 200 µm varied from a single or few microjets to no penetration at all after many microjets (n = 30 on three samples).

3.2 Delivery depth and skin damage

Confocal microscopy images of multiple locations in a single skin sample showed that fluorescent particles were injected into the full-thickness human skin (Fig. 3). At a depth of 50-60 µm (n = 3) below

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the skin surface, deposits of fluorescent particles with disc-like shapes were detected. These areas were spaced 0.5 mm apart, as were the injection sites. The discs had a diameter of approximately 160 µm (n = 8) and were oriented parallel to the surface of the skin, indicating sideways dispersion of the injected particles. In addition, some fluorescence was identified at the surface of the samples, indicating that part of the fluorescent solution was not delivered into the skin but was deposited on its surface.

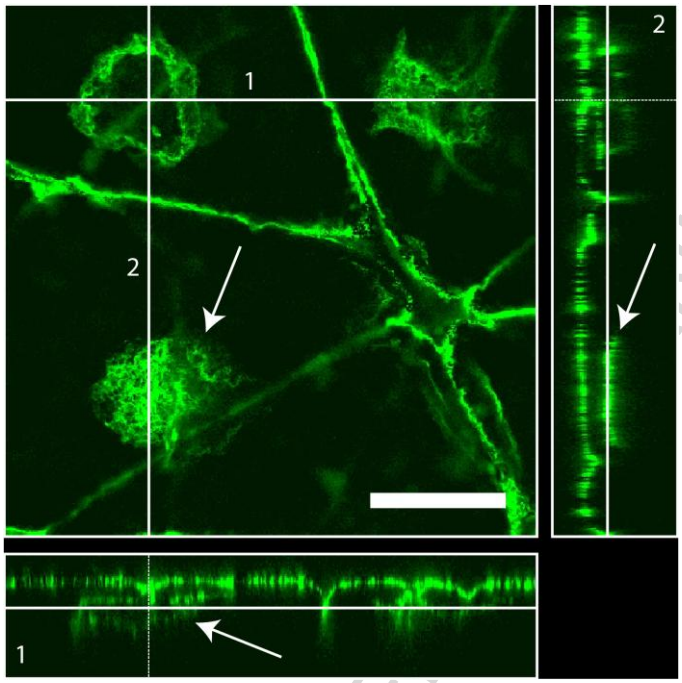

Figure 3. Confocal fluorescence microscopy image of sites injected with fluorescent particles (green) by 20 microjets at a depth of 50-60 µm below the surface of the human skin. The insertions at the bottom and right of the image show the cross sections at line 1 and 2, respectively. The injected particles were deposited in disc like shapes 50-60 µm below the surface, as indicated with arrows, and had a parallel orientation to this surface. This suggests that repetitive injections were dispersed sideways and did not penetrate deeper into the skin. Part of the fluorescent solution was not delivered into the human skin and is visible as a layer on the surface of the skin. Bar indicates 200 µm.

Although a different number of microjets was applied to a single location, the histological images of injection sites indicated similar disc shaped damage in the dermal layer of the skin compared to those of the fluorescent images. The histological images revealed the formation of a cavity at the boundary between the papillary and reticular dermis in the human skin (Fig. 4). In addition, these images showed that increasing the number of jets from 1000 up to 4000, did not increase the penetration depth, but tended to increase the width of the cavity.

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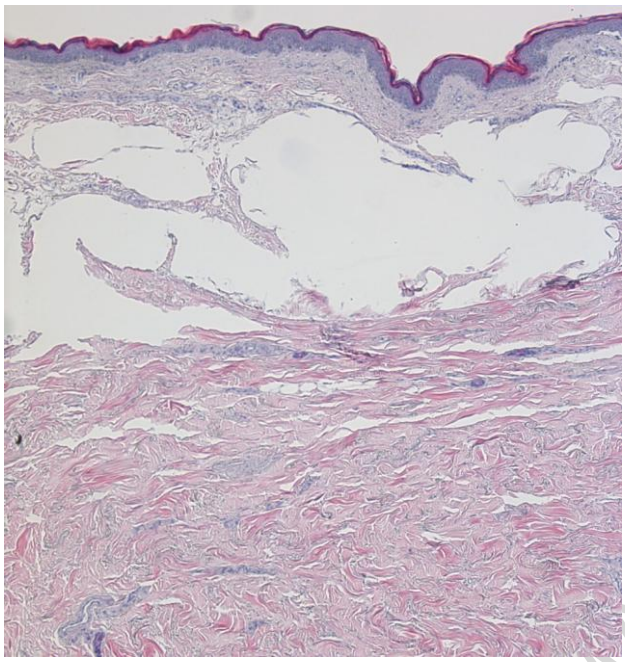

Figure 4. Histological image of human skin damage formed by 2000 microjet injections. A cavity was formed at the boundary between the papillary and reticular dermis. The size of the cavity might be overestimated due to sectioning artefacts, however, the depth of the damage is clearly visible.

3.3 Delivery efficiency

The delivery efficiency of the radioactive tracer fully penetrating the epidermis into the agarose gel was approximately 96%, as can be seen in Figure 5a. The efficiency was independent of the amount of microjets delivered. In contrast, the delivery efficiency drastically decreased in full-thickness human skin samples with values ranging from 8 to 18% and a mean of 12% (Fig. 5b). The part not delivered into the skin was deposited on its surface or splashed back onto the cartridge. These findings were again independent of the number of microjets delivered. The volume of a single ejection was 29 nl and 11 nl for the two experiments, respectively.

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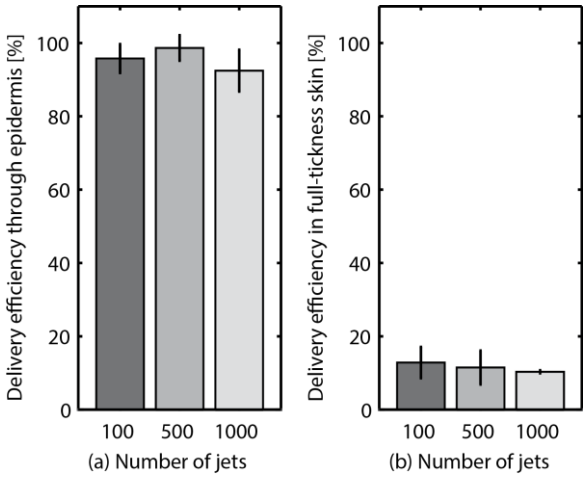

Figure 5. Delivery efficiency of microjets into the human skin. (a) Delivery of a radioactive tracer fully penetrating a human epidermal layer on top of an agarose gel. The percentage detected in the agarose gel is presented. (b) Delivery into full-thickness human skin. The percentage detected inside the skin sample is presented. n = 3 per bar.

3.4 In vivo administration of insulin

Prior to repetitive microjet injection, the rat skin did not show any sign of irritation (Fig. 6a). However, after 20 minutes of insulin injection a white swollen oedematous area was observed at the injection site (Fig. 6b). Histological images revealed multiple cavities throughout the depth of the skin (data not shown). These observations suggest that one or multiple fluid deposits were formed in the skin of the rat.

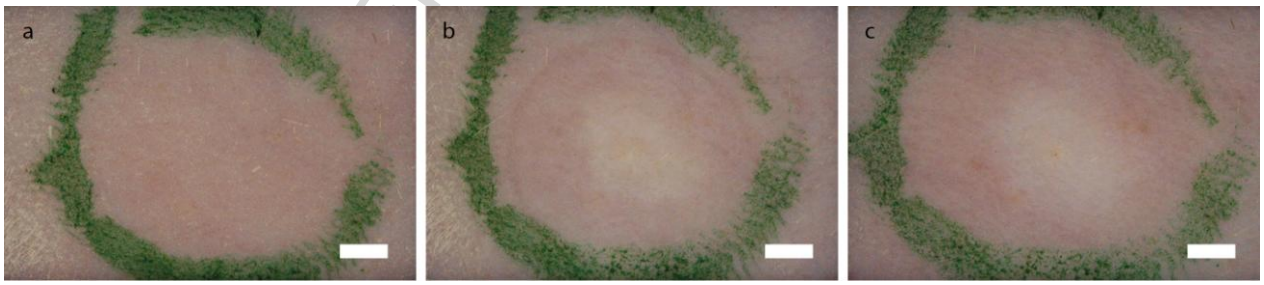

Figure 6. Images of a single location on the rat skin. (a) Before the start of the injections. (b) Directly after 20 minutes of repetitive microjet injections. (c) At the end of the experiment. The location of the injection site is circled with green. A white and swollen area is visible after microjet injection. Bars indicate 2 mm.

The blood glucose level of each rat was reduced during insulin delivery (Fig. 7), indicating the uptake of intact insulin molecules into the circulation. Recovery of blood glucose levels began to occur after 30 to 60 minutes following injection. These are typical changes in blood glucose levels as would be expected after insulin delivery (Arora et al. 2007; Qinna & Badwan 2015), therefore, these experiments indicated that the microjet injector was feasible to deliver a drug into the systemic circulation.

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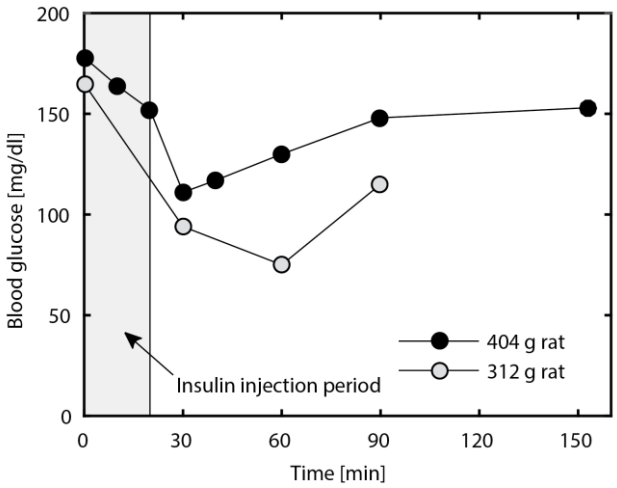

Figure 7. Blood glucose levels during and after repetitive microjet injection of insulin into two rats. Insulin injection started at t = 0 and lasted for 20 minutes.

4. Discussion

A microjet injection device was designed and built that enabled injection of multiple microjets with a volume of a few nanolitres into the skin in a repetitive manner. Results revealed an effective delivery by fully penetrating the epidermal layer. However, the delivery efficiency in full-thickness human skin was less impressive with a mere value of 12%. Moreover, results suggest that the reticular dermis provides an important barrier, preventing the microjets from penetration further than the transition between the papillary and reticular dermis. This barrier was also observed to be the location corresponding to the greatest skin damage, and may explain the low delivery efficiency. Nevertheless, in vivo studies in rats indicated that the solution injected into the skin was taken up into the circulation.

At velocities exceeding 90 m/s, a single microjets fully penetrated the epidermal layer of the porcine skin, although, in some cases, multiple jets were necessary when using human skin. This corresponds to previous reports (Arora et al. 2007). Repetitive microjets penetrated further into the dermis, up to a depth of approximately 50-60 µm, which corresponded to the transition of the papillary and the reticular dermis. At this level subsequent microjets were obstructed for deeper penetration, most probably due to the presence of a thick and dense collagen and elastin fibre network associated with the reticular dermis. Consequently, the subsequent microjets were dispersed sideways, forming a disc like shape in the dermis (Fig. 3). This caused a visibly swollen area of the skin in both ex vivo human skin and in vivo rat skin. These observations suggest that the initial barrier is the stratum corneum, with a threshold velocity of the microjet to breach this barrier. After breaching the stratum corneum, subsequent

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microjets penetrate further into the skin. This progress stops at the reticular dermis, forming the ultimate barrier for repetitive jets. Previous findings on microneedle penetration also suggests that the collagen-rich reticular dermis forms a barrier for microneedle penetration (Römgens et al. 2014). Moreover, previous studies with microjets showed a slightly deeper penetration with depths of 100-150 µm (Arora et al. 2007). The relatively superficial penetration of the microjets is beneficial for reducing the pain sensation of jet injection, since fewer nerve endings present in the dermis will be stimulated. However, in the case of repetitively applied microjets, the capacity of the superficial layer to store the injected volume may be limited and the clearance rate from the skin into the circulation may prove to be insufficient.

The delivery efficiency into agarose gel penetrating an epidermal layer was approximately 96% (Fig. 5). However, this efficiency drastically decreased when multiple microjets were injected into full-thickness human skin samples and was independent of the amount of microjets. This low efficiency into full-thickness skin confirmed previous observations (Stachowiak et al. 2009). The large difference in efficiency may be due to several causes. One of the causes may arise from the natural pretension of the skin in vivo, which is mainly present in the dermal layer, and is lost once the sample is excised from the surrounding tissue, resulting in shrinkage of the skin sample. However, when the epidermis is subsequently enzymatically separated from the dermal layer, it expands again. Thus it could be inferred that the presence of the dermal layer influences the compressive state of the epidermis. In the absence of pretension, the skin can bend to absorb the energy of the microjet, which may result in the low delivery efficiency in full-thickness skin samples. Indeed, penetration of the skin with a needle was previously reported to be dependent on the pretension of the skin (Butz et al. 2012). Therefore, the state of pretension may represent the different behaviour of epidermal and full-thickness samples. Secondly, the lack of clearance from the skin into the circulation in this ex vivo model may cause the accumulation of the injected solution into a fluid depot. This incompressible fluid depot can form a barrier for subsequent microjets to be delivered into the skin, which may result in a decreased efficiency. In addition, it might be suggested that the efficiency can be increased by increasing the eroding force of the microjet, thereby increase the penetration depth of subsequent microjets.

Despite the low delivery efficiency in ex vivo human skin, results demonstrate that a drug, in this case insulin, could enter the systemic circulation after being delivered with a microjet injection device and induce a decrease in the blood glucose level of the rats (Fig. 7). However, also in vivo, where an active circulation is present, a swollen area was observed. Apparently, the clearance rate in the rat skin is too

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low to transport the repetitively injected fluid away from the injection site. In order to use the microjet injection system as a wearable device for chronic diseases, the formation of a superficial fluid depot should be avoided, since it might cause irritation of the skin and influences the delivery efficiency. Lowering the ejection frequency of the microjets may prevent the formation of such a deposit. To facilitate the delivery of a required daily dose when using a lower frequency, a microjet injector could contain multiples nozzles delivering the same dose over a larger area. However, this still does not guarantee a higher delivery efficiency and should be further investigated.

In the current study, experiments were performed on ex vivo human and porcine skin and in vivo rat skin. The skin of these species exhibit different structural and mechanical properties, which should be considered when interpreting the findings. Results revealed an easier penetration of porcine epidermis compared to human epidermis. Consequently, the velocity threshold to penetrate the epidermis as determined for porcine skin (Fig. 2) may be different for human skin, since their penetration properties seem to be different. To be closer to its application, all other ex vivo experiments were performed on human skin samples. Moreover, rat skin is thinner compared to porcine and human skin. Consequently, drugs may easier be absorbed into the systemic circulation of rats compared to humans. By contrast, the capacity to temporary store a fluid may be limited in rat skin compared to human skin resulting in skin swelling and irritation. Therefore, the results of the in vivo experiments in rats cannot be translated to humans. However, the in vivo experiments did reveal that the function of the insulin was not affected by the high speed injection with the microjet system.

In addition, the hydration state of the skin may affect the penetration process of the microjet. The skin samples were kept well hydrated during the experiments, which has the advantage that the conditions were constant. However, the samples will not fully represent the in vivo state. Although, both demineralized water and saline water were used in this study, we did not observe any difference in jet formation, nor in jet penetration properties.

In conclusion, microjet delivery offers a possibility for a controlled and sustained delivery method, which may increase patient compliance. In the current study, we showed that a microjet injection device could repetitively delivery volumes in the nanolitre range into the human skin. These microjets penetrate up to the transition between the papillary and reticular dermis in human skin and were absorbed into the systemic circulation triggering a physiological response in rat. However, the obtained delivery efficiency into the human skin was poor and should be improved to make microjet injection a suitable delivery

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method. Moreover, the site of injection seemed irritated in rats, therefore, the level of skin irritation may be a critical aspect for a wearable drug delivery device to be successful.

Authors contributions

The research presented was designed and performed at Philips Research, Eindhoven, the Netherlands, by DRB, RK, MH, and MPBB, with the exception of a single series of experiments. AMR wrote the manuscript. DRB, DLB, CWJO, and MPBB critically revised the manuscript.

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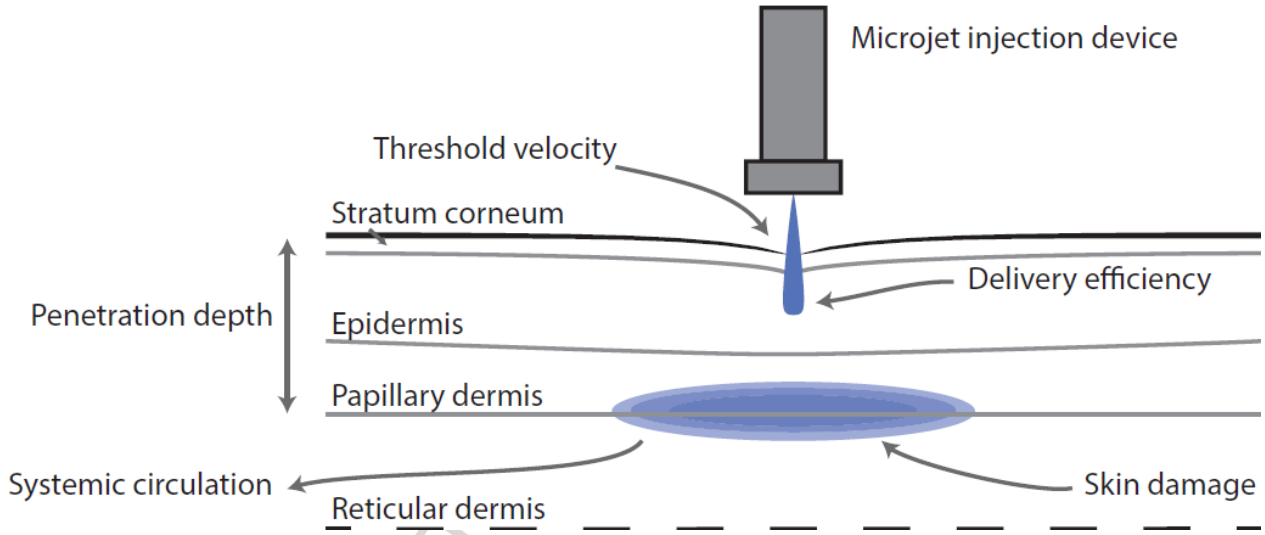

Graphical abstract

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