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In vivo evaluation of a microneedle-based miniature syringe for intradermal drug delivery

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Abstract A microfabrication process for miniature syringes is described. The MEMS syringes consist of a silicon plate with an array of hollow out-of-plane needles and a flexible poly-dimethylsiloxane (PDMS) reservoir attached to the back of the plate. The PDMS reservoir can be filled with a drug solution or microparticle suspension which is delivered into the skin simply by the pressure of a finger pushing on the miniature syringe. The efficiency of such a syringe for delivering a suspension of microparticles into skin tissue and a radiolabelled protein (albumin) solution into live mice is reported. Such microneedle devices could be used for the intradermal delivery of vaccination agents or for the systemic delivery of highly effective drugs.

Keywords Albumin · Drug delivery · MEMS syringe · Microneedles · Protein delivery · Skin

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1 Introduction

The parenteral route of drug administration via either subcutaneous (s.c.), intramuscular (i.m.) and intravenous (i.v.) injections is crucial when rapid drug absorption is needed (for example in emergency situations when a patient is unconscious or unable to accept oral medication) and in situations when drugs are destroyed, inactivated or poorly absorbed following oral administration. Drug inactivation is a major concern for the newer macromolecular drugs developed in the recent biotechnology revolution, which include peptides, proteins and viral components. For the parenteral delivery of easily inactivated drugs, there is generally negative patient acceptance primarily associated with pain and inconvenience. To get around this perception, developments since the 1990's have focused on "needle-free" injection systems (Stoeber 2008). Like typical parenteral injections, they can deliver drugs locally into the different layers of the skin, both as acute or slow release depot systems, where the drug either resides or further distributes through the blood capillaries in the skin to the circulatory system for peripheral and central action.

"Needle-free" injection systems are in fact not needle-free, but based on arrays of very small microneedles that can only be seen under the microscope. They are, however, not perceived as needles by patients as they neither induce pain nor result in any bleeding. The needles are in general only a few hundred micrometers long and thus will not reach the sensory nerves in the dermis (see Fig. 1). The microneedle systems have other advantages too. First, as there is no handling of needles involved, the training of the person using these systems is minimal, disposal is simple, and success of drug dosing is expected to be high. Also, the drug reservoir can be built-in and provide a long-term storage function, for example in the form of an oily drug

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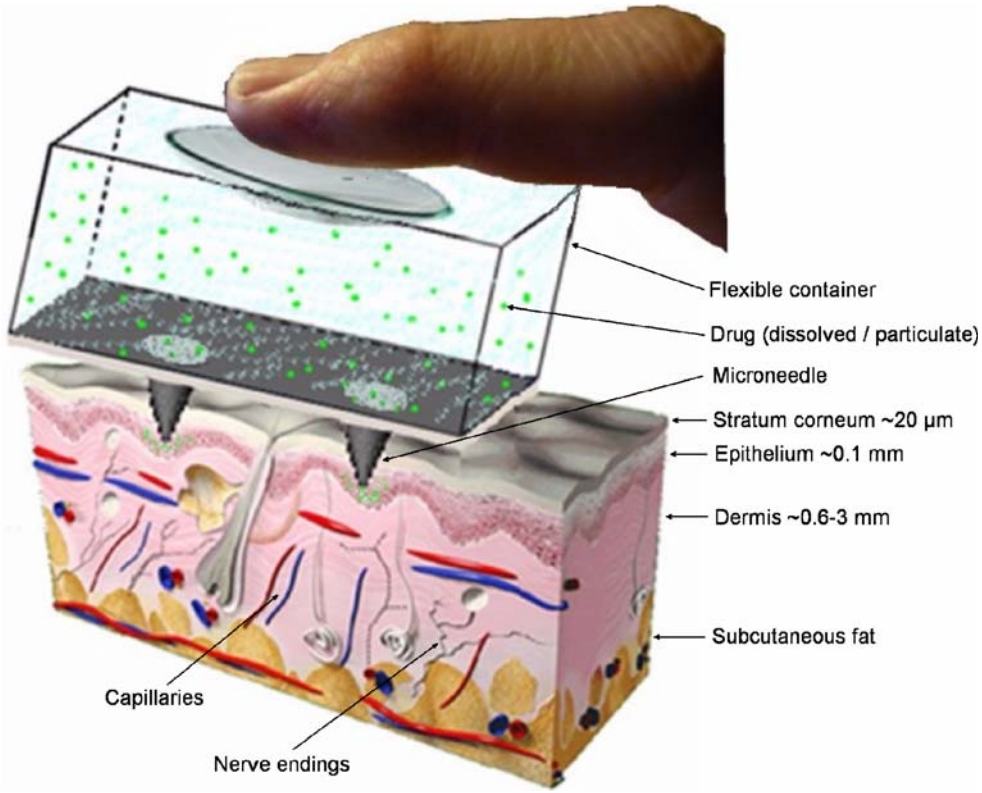

Fig. 1 Concept of a miniature syringe (not to scale). Pressing a finger on the deformable drug reservoir drives the needles into the skin and injects the drug suspension or solution into the epidermal skin layer

suspension. Such systems are especially suited to applications where only small amounts of drug are needed in the dermis, such as in vaccinations. Performed with conventional needles, such intradermal injections of for example anthrax recombinant protective antigen vaccines and influenza vaccines have been shown to be at least as effective as deeper intramuscular delivery (Alarcon et al. 2007; Mikszta et al. 2006).

The development of transdermal microneedles has resulted in solid needle devices typically used for skin perforation (Henry et al. 1998) and in true microsyringe devices for the delivery of drugs through a hollow needle (reviewed in (Stoeber 2008)). In this work, we are integrating an array of hollow, out-of-plane silicon needles (Stoeber and Liepmann 2005) into a functioning miniature syringe that could be used as a device to deliver a vaccination dose in areas with poor medical infrastructure. To deliver vaccination agents, the antigen has to be delivered directly into the dermal or epidermal areas of the skin, where the immune-reactive cells reside. We accomplish this targeted intradermal delivery with 200 µm long microneedles that we have previously used to deliver a small molecular weight lipophilic compound (methyl nicotinate) into humans (Sivamani et al. 2005). That delivery method was completely painless. The aim of this work is to deposit two different agents as model substances for application in vaccination delivery. One of the agents is the 67 kDa protein albumin as a model for large protein antigens. The other agent consists of approximately 1 µm microspheres as a model for particles to deliver antigens by controlled-release over extended time periods. Here, we demonstrate that the microneedle arrays connected to a newly developed deformable reservoir (Fig. 1) are able to deposit the model compounds into the anatomically desirable skin structures.

2 Fabrication of the miniature syringe

The two main components of the syringe design in Fig. 1 are a plate with an array of microneedles and a flexible reservoir. Silicon was chosen as the material for the microneedles, because it is easily available and the processing tools are well developed. In addition, as shown earlier, the silicon microneedles chosen for this device can withstand the typical forces associated with drug injection without breaking (Stoeber and Liepmann 2005). Polydimethylsiloxane (PDMS) was chosen as the material for the reservoir (Sia and Whitesides 2003) because it is flexible, simple to process and has the advantage of allowing easy integration with the silicon needles.

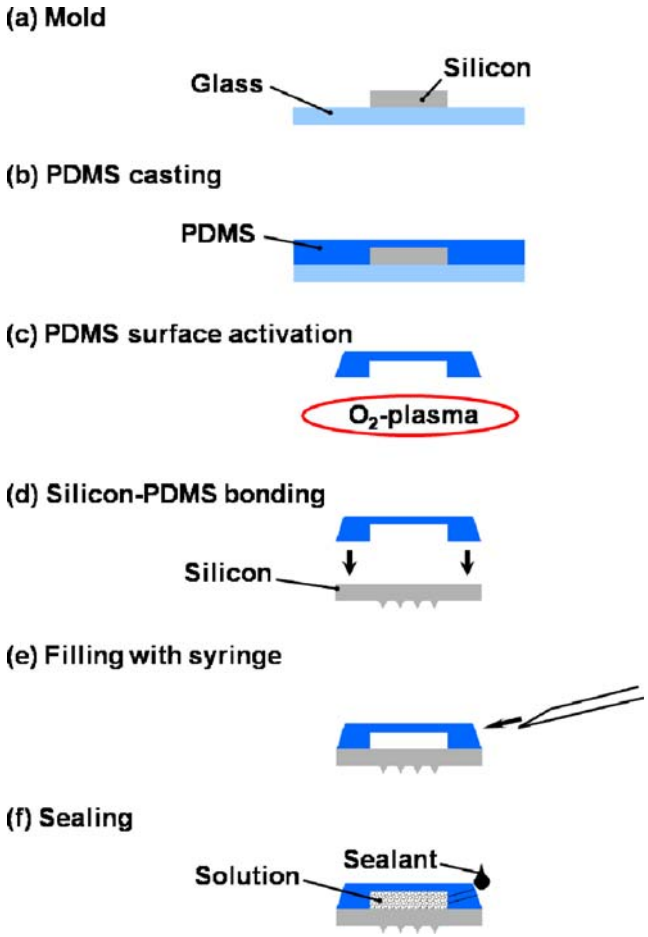

Fig. 2 Process flow for wafer-level PDMS reservoir fabrication and device integration. The silicon mold is prepared (**a**) and PDMS is poured onto the mold and cured (**b**). After peeling off and cutting to shape, the PDMS surface is activated using an oxygen plasma (**c**), allowing the bonding of PDMS and silicon substrate (**d**). After filling with a conventional syringe (**e**), the container is resealed (**f**)

The silicon needles were formed through a 2-mask MEMS process (Stoeber and Liepmann 2005). The 40 µm wide lumens were generated in a DRIE step through the silicon substrate from the back. The etch process was stopped on a silicon dioxide layer on the front side of the wafer without breaking through the substrate. This allowed photolithographic mask definition on the front side for subsequent etching of the needles. This occurred through under-etching of the circular mask with isotropic etching techniques, while the sidewall of the needle lumen was protected by a silicon nitride passivation layer. Offsetting the center lines of the etch masks for the lumens and the needles allowed us to form needles with sharp tips on the shaft circumferences. This design permits a density of 600 needles/cm^2 for shaft lengths of 200 µm. The needle density can be increased by choosing etch techniques with higher vertical than lateral etch rates.

The flexible container for the drug suspension was fabricated from PDMS as outlined in Fig. 2. The mold consisted of a glass base and elevated silicon blocks defining the drug compartments. These quadratic silicon blocks were 0.5 mm high, and had an edge length of 5 mm, corresponding to a volume of 12.5 µL. Ten parts of SYLGARD 184 Silicone Elastomer and one part of SYLGARD 184 Curing Agent were mixed well, then poured on the silicon mold (Fig. 2(b)). The PDMS was cured at 80 °C to accelerate the curing process.

After hardening, the PDMS was peeled off the silicon mold and cut to a size of 1 cm^2 to match the dimensions of the silicon chip. The bond surface of the PDMS was then activated in oxygen plasma at 500 mTorr and 30 Watts for 10 s in a Technics PE plasma etching tool. Following the surface treatment with oxygen plasma, the PDMS bonded irreversibly to the back of the silicon microneedle array when the two materials were brought into contact (Fig. 2(c, d)). The assembled MEMS syringes were then filled with the protein solution or microspheres suspension through the PDMS container, using a conventional 28 Gauge stainless steel needle (Fig. 2(e)). The needle insertion hole was subsequently sealed (Fig. 2(f)). While the syringe prototypes were sealed using fast curing epoxy for convenience, a more durable seal can be achieved using much slower curing PDMS. For the prototype devices, the sealing material was located in regions that did not undergo strong deformation to ensure that the epoxy seals were sufficiently robust.

A prototype of the MEMS syringe is shown in Fig. 3. Here, the 200 µm long, 2×4 needles are spaced 1 mm apart.

3 Experimental methods

Human serum albumin (HSA, Sigma), a large 67 kDa protein, was radioactively labelled to evaluate the filling and ejection efficiency of the MEMS syringe as well as to

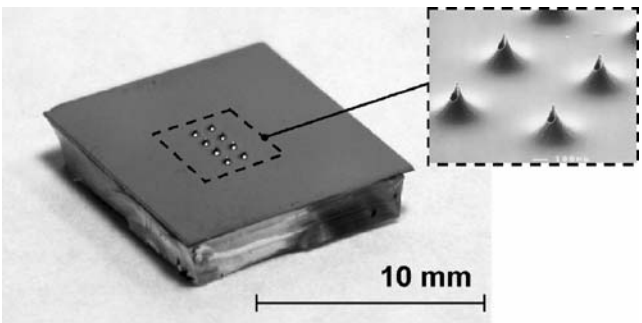

Fig. 3 A MEMS syringe with 8 silicon microneedles and a PDMS drug container

quantify the amount of protein delivered during *in vivo* experiments. The albumin was radiolabelled with the gamma-emitting radioisotope technetium-99m (^{99m}Tc) by reducing ^{99m}Tc-pertechnetate with Sn(II), using a method similar to that described by Wang et al. (Wang et al. 2007). For this purpose, a freshly prepared solution of 0.1 mg of $SnCl_2$, 1 μL of sodium potassium tartrate and 0.5 mL of 0.01 N HCl (all from Sigma) was added to 0.5 mL of an aqueous solution containing 0.1 mg of albumin. Next, 370 MBq of sodium pertechnetate ($Na^{99m}TcO_4$; kindly provided by Vancouver General Hospital) in 250 μL of saline was added to the HSA and incubated for 10 min at 37°C. The radiolabelled albumin was then concentrated using an Ultracel YM-30 column (Millipore Corp.) by centrifugation at 12,000 g for 10 min. The average labelling efficiency was 87.3±3.2%, as determined by thin layer chromatography (TLC) on black TEC-Control strips (Cat# 150-005; Biodex, Shirley, NY, USA), using saline as the mobile phase and developing in a phosphor imager (Cyclone storage phosphor imager with 20×25 cm^2 phosphor screen, Perkin Elmer, Waltham, MA, USA). The analysis was carried out using OptiQuest software. The activity of a known volume of the albumin solution was measured using a CRC-15 dose calibrator (Capintec, Ramsey, NJ, U.S.A.) for calibration purposes, to associate a volume of solution to a measured dose of radioactivity.

Several MEMS syringes with 4 or 6 microneedles were prepared as described above and filled with the radioactive albumin solution. The solution was then ejected through the needles onto an absorbent tissue. Only a slight fluid loss occurred through the needles due to capillary action in the tissue while initially placing the syringe (needles facing down) on the tissue. Likewise, no self sustained flow of solution from the needles occurred after release of the syringe. To evaluate the filling and ejection efficiency of the syringes, we measured the activity of the tissue and the remaining solution inside the syringe after ejection. A repeat sequence of filling and ejection was performed for each device.

A MEMS syringe with 8 microneedles was filled with a model aqueous suspension containing a 0.1% (v/v) mixture of 0.93 μm diameter blue polystyrene microspheres (Bangs Laboratories, Inc.) and 0.7 μm diameter fluorescent (fluorescein) polystyrene microspheres (Polysciences, Inc.). The miniature syringe was then pressed with a finger onto a 5 mm thick piece of fresh chicken breast. The finger pressure was sufficient to inject the suspension into this light-colored sample tissue, where the blue particles could be easily identified. The injection depth of the fluorescent particles was determined using a Zeiss 510 UV/Vis confocal microscope.

The injection efficiency of the MEMS syringes with 6 microneedles was also tested using injection of radiolabelled albumin solution into C57Bl6 mice (Animal Care Centre, University of British Columbia, Vancouver, BC, Canada). For this purpose, the lower backs of the mice were shaved and complete hair removal was accomplished using a depilatory cream (Nair) applied for 5 min. The activity of the filled MEMS syringes was measured and the syringes were pressed against the shaved area for 60 sec with a force of approximately 2 N. The injection location was then cleaned with several wet laboratory wipes to remove potential spills from the skin surface. The activities of the used syringe and each individual wipe were measured. After 1 to 1.5 h, the mice were sacrificed and the activity of individual organs was determined.

Table 1 Amounts of radiolabelled albumin solution filled into MEMS syringes as well as amounts ejected, as evaluated from activity measurements

Syringe number	Number of needles	Experiment	Fill volume [μL]	Ejected volume [μL]	Filling efficiency [%]	Ejection efficiency [%]
1	4	1	8.8	7.7	70.5	87.3
1	4	2	7.8	6.7	62.7	85.7
2	6	1	11.1	9.9	88.7	88.9
2	6	2	7.7	6.8	61.9	88.3
3	6	1	11.3	10.2	90.5	90.1
3	6	2	7.8	6.7	62.7	85.7
4	4	1	11.4	10.0	91.5	87.1
4	4	2	11.2	9.0	89.6	80.0
Average±S.D.			**9.7±1.8**	**8.4±1.5**	**77.3±14.0**	**86.6±3.1**
Coefficient of variation [%]			18.1	18.5	18.1	3.5

4 Results and discussion

4.1 MEMS syringe performance

Table 1 shows the amounts of fluid added to the reservoirs of 4 different syringes as well as the amounts of ejected fluid, as determined from activity measurements. Table 1 also shows the filling efficiency, equal to the total amount of fluid in the filled syringe divided by the nominal volume of the reservoir, 12.5 μL. The ejection efficiency was determined as the ejected volume divided by the volume filled into the syringe.

An average of 9.6 μL of fluid was added to the syringe reservoir, corresponding to a filling efficiency of 77.3% for the MEMS syringe. The ejected volume produced a very similar coefficient of variation: 18.5% and 18.1%, respectively, while the coefficient of variation of the ejection efficiency was much lower at 3.5%. This means that the amount of fluid ejected from the syringe depended directly on the amount of fluid added to the syringe. The number of needles (4 or 6) did not significantly affect syringe performance for these *in vitro* tests.

While the mold for the square reservoir was easy to fabricate, it may be useful to optimize its shape to improve filling and fluid ejection. A round shape may be advantageous, since no difficult-to-fill corners would exist inside the hydrophobic PDMS reservoir. In addition, the wall thickness and/or stiffness of the reservoir might be reduced to allow even lower finger pressures to provide complete liquid ejection. Further attention should also be paid to the method of filling the miniature syringe. Injecting the drug solution or suspension through the PDMS material directly into the reservoir with a needle worked well, since the disappearance of the air bubble could be directly observed through the transparent material. However, this method required that the injection hole be sealed with an epoxy adhesive. In addition, long term storage would require the microneedles to be covered with a protective sheet, a wax layer, or something similar that could be easily removed before use.

4.2 Injection of a microsphere suspension into sample tissue

Slight leakage occurred during injection into the chicken breast sample tissue, most likely because the tissue cannot absorb large amounts of liquid through only 8 injection sites. However, no liquid was drawn back into the syringe when the pressure was released. The photographic picture of the chicken skin surface in Fig. 4(a) shows a typical example of the 8 injection marks dyed by the blue microspheres in the suspension. Using image analysis, the blue pixels for each injection area were counted and compared (Fig. 4(b)). Overall, a coefficient of variation of 83% was found between the eight areas, indicating high variability for microneedle injection under identical conditions with identical needles. This high variability is likely associated with local variations in tissue properties such as absorbance and stiffness. It is expected, however, that the total injected amount can be well defined by using sufficiently large arrays of microneedles. The heterogeneity between individual injection sites is generally not crucial for applications such as drug or vaccine delivery, where only the total amount delivered is essential. The drug or antigen would still be able to diffuse into the immediate surrounding tissue. The same is true for microparticles that deliver drugs by controlled release. Once the drug has escaped the microparticle, further action is dependent on diffusion. Similar variations in microneedle delivery patterns have been found with other transdermal delivery methods such as the one by Wu et al. (Wu et al. 2008). These investigators used arrays of solid needles to pierce the skin, which increased percutaneous diffusion of calcein into the puncture wounds by a factor of 10,000 to 100,000.

The series of confocal microscope images in Fig. 4(c) shows the fluorescent microspheres inside the chicken tissue at one injection site, in sections parallel to the injection surface. The suspension's low particle concentration allowed imaging of the deep tissue layers without obstruction by an excessive amount of fluorescent particles close to the surface. The maximum fluorescence and, thus, the maximum particle concentration occurred about 20 μm underneath the surface. A strong fluorescence signal was still detected at a depth of 70 μm. This suggests that the suspension of microparticles was successfully delivered to the target depth for epidermal drug delivery using a MEMS syringe. Wu et al. also demonstrated that they could deposit fluorescent rhodamine down to a depth of 80 μm using 150 μm large solid microneedles and subsequent dye diffusion from a gel applied to the skin (Wu et al. 2008). The flexibility of the tissue likely prevented the injected material from reaching a depth closer to the actual length of the microneedles. It is, thus, important to choose a microneedle length about 100 μm longer than the target depth of deposition.

The successful deposition of microparticles into the tissue of an animal suggests that using a miniature syringe might be appropriate as a depot for the long-term delivery of highly active central-acting drugs, e.g., peptides; for the delivery of controlled release microspheres for the treatment of chronic local diseases; or for local interaction with cells of the immune system.

4.3 Protein injection into mouse skin

The intradermal injection of large proteins into the dermis is useful for interaction with immune cells and

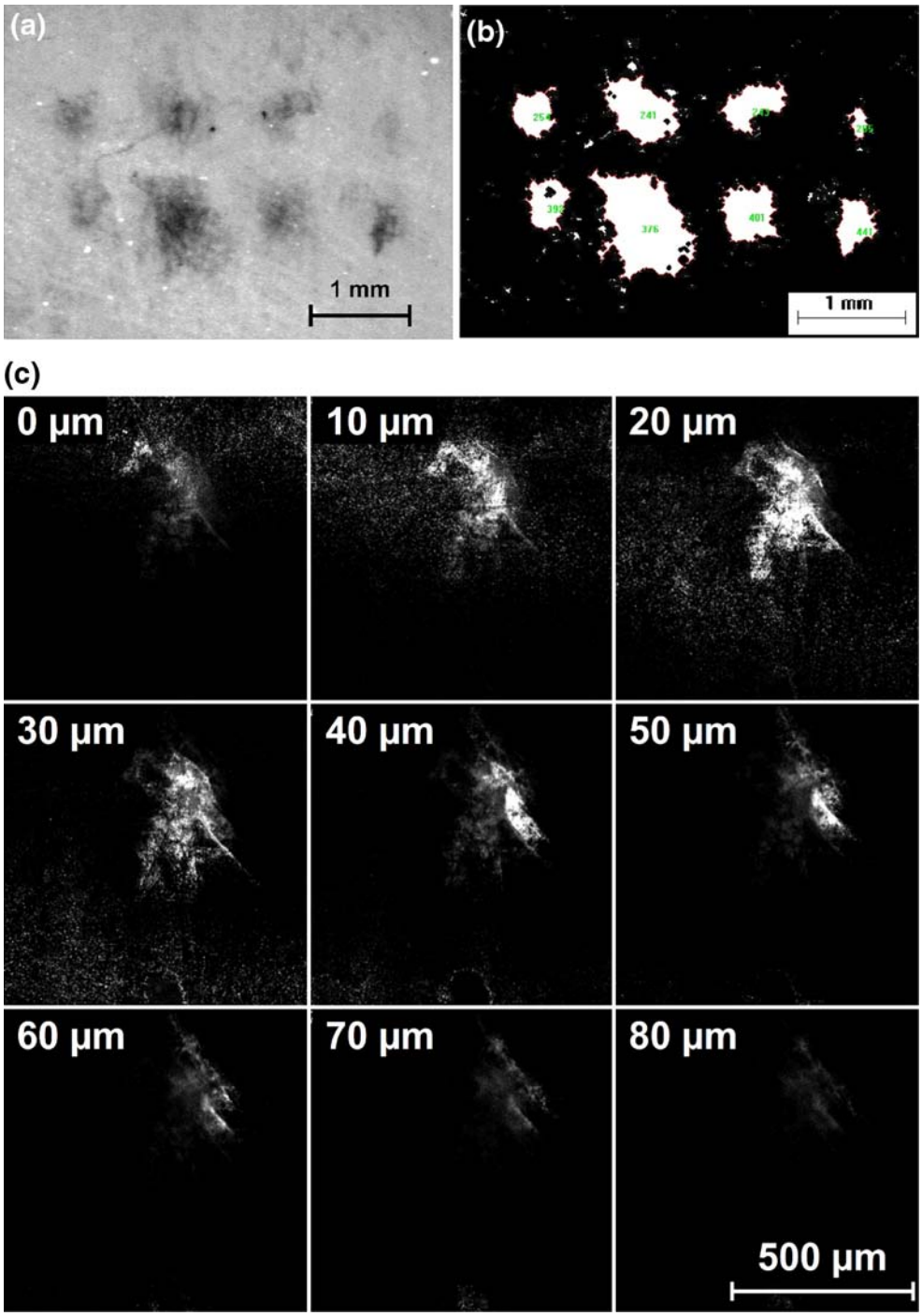

Fig. 4 (**a**) Injection marks on the surface of chicken breast left by blue 0.93 µm large polystyrene microspheres ejected from a MEMS syringe with an array of 2 x4 needles. (**b**) Image analysis of the blue injection areas. (**c**) Confocal microscopic images of 0.7 µm large fluorescent polystyrene microspheres at different depths in chicken tissue after injection with a MEMS syringe

leads to highly effective vaccination and venom immunotherapy (Lambert and Laurent 2008; Mikszta et al. 2006). In humans, ovalbumin is often used as a prototype agent for the induction of an immune response. We tested microneedle injection of the similarly sized human serum albumin in mice to verify possible penetration of the stratum corneum, deposition in the dermis, and possible (but undesired) movement through the capillaries into the blood circulation. For this purpose, the radiolabelled albumin solution was injected into 4 different mice using 4 different MEMS syringes, each with 6 microneedles. The ejection efficiency reported in Table 2 describes the volume of solution ejected from the syringe divided by the volume added to the syringe, as before. The ejection efficiency in these experiments was lower than for the experiments discussed in section 4.1, where water was

Table 2 Amounts of radiolabelled albumin solution filled into MEMS syringes as well as amounts ejected and injected into the skin of mice as evaluated using activity measurements

Mouse #	Fill volume [μL]	Ejected volume [μL]	Volume in the skin [μL]	Ejection efficiency [%]	Relative skin uptake [%]
1	9.3	5.5	2.6	59.5	47.4
2	9.1	2.5	0.5	27.2	18.4
3	9.0	2.8	1.6	30.9	58.3
4	5.7	1.8	0.4	31.9	20.0
Average±S.D.	**8.3±1.7**	**3.2±1.6**	**1.3±1.1**	**37.4±14.9**	**36.0±19.9**
Coefficient of variation [%]	20.7	51.7	84.4	39.8	55.3

ejected onto tissue with a much lower flow resistance compared to live animal experiments. The volume in the skin corresponded to the relative amount of solution recovered from the skin, as determined by activity measurements, and it indicated the amount of solution that reached the target area. The large coefficient of variation for the injected volume indicates either high variability in the injection procedure or a high variability of the local skin properties. The relative skin uptake corresponded to the volume in the skin divided by the ejected volume and indicated the fraction of ejected volume that reached the target site. This quantity was rather small, corresponding to leakage of fluid between the needle chip and the skin, and may be related to the limited absorption capability of the skin or to the high tissue flexibility at the injection site. Using a different injection location, such as the thigh, might have been preferable since that would have resulted in a more defined and stronger backpressure. In addition, the relative skin uptake having a lower coefficient of variation than the absolute volume found in the skin may indicate that the injected volume depends on the injection procedure.

After sacrifice, 1.2 h following ^{99m}Tc-albumin injection, most murine organs were removed to perform a full biodistribution analysis. Only four organs were radioactive above background levels: blood (0.16±0.08% of the injected activity), liver (0.09±0.05%), kidneys (0.17±0.09%) and muscles (0.48±0.31%). Since this activity totals less than 1%, it is likely that it comes from the ^{99m}Tc-pertechnetate, used to radiolabel the albumin. Despite removing most of it during the centrifugation step concentrating the radiolabelled albumin, it is difficult to completely remove the last few percent. Pertechnetate is a highly water-soluble molecule of small molecular weight (163 Da), that diffuses easily from the injection site to reach the blood stream and distribute throughout the body (Zuckier et al. 2004).

5 Conclusions

We presented a fabrication process for microneedle-based miniature syringes. Syringe prototypes were fabricated using MEMS technology, including arrays of 200 μm long silicon needles with lumen diameters of 40 μm and a flexible, fluid-filled reservoir. Finger pressure on the syringe's flexible reservoir was sufficient to eject the liquid from the syringe. A suspension of particles was delivered through the microneedles into sample tissue. The micron-sized particles were delivered at an appropriate depth for human skin, corresponding to a location below the stratum corneum and above the nerve endings. Using the presented miniature syringe for intradermal drug injections is, thus, expected to be painless. The device was also successful in directly depositing a radiolabelled albumin solution into the skin of mice. A significant amount of the radioactivity could not be removed even with vigorous wiping with a wet tissue, indicating successful dermal protein delivery.

The results from the injection experiments indicate that the MEMS syringe is potentially a useful biomedical tool for the delivery of vaccines and protein drugs, but also for controlled release drug delivery systems such as microspheres. The experimental data, however, also showed significant variability in the delivered volumes. Redesigning the device's geometry will likely reduce this variability. In particular, the size, shape and flexibility of the syringe reservoir may be changed to optimize the ejection and the injection efficiencies of the syringe.

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