Novel Devices for Drug and Gene Delivery

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***Abstract*—Mechanical standard application devices or methods for intra dermal drug delivery especially for DNA vaccination offer some disadvantages, which will be discussed. Reaching a certain skin layer is often difficult. With a cutting system and a tattoo based hollow needle device, two new technical approaches were developed to overcome several limits and allow an adequate and fast delivery of drugs at a certain depth. The integration of an electroporation electrode enhances the efficiency.**

***Index Terms*—drug delivery, vaccination cutting, electroporation, cannula, electrotattooing**

I. Introduction

Nearly 220 years ago, Edward Jenner vaccinated a boy with cowpox and later exposed him to smallpox. A procedure the boy luckily survived by an immune response acquired through the cowpox vaccination. But it took nearly another 200 years to eradicate smallpox completely [1].

Based on Jenners approach, familiar names like Pasteur, Koch, Chamberland or von Behring extended the knowledge of vaccination and the related mechanism [1]. Sabin and Salk developed the active immunization (against poliomyelitis) in 1954. In spite of the great advances in immunology, the war declared against diseases like malaria or tuberculosis by the WHO is not over yet [2], [3]. The research for sufficiently effective strategies against the HI-virus or Hepatitis C is still ongoing.

The most common vaccination method is still the intramuscular administration via a cannula, but oral, intradermal, transdermal or mucosal routes are also in use [4]-[7]. While in classical vaccination, the antigen (active immunization) or the antibodies (passive) were administered, DNA vaccines with genetically engineered DNA stimulate the body to produce their own antigen and afterwards the antibodies against specific proteins of the pathogen. The related immune response is different and more effective [8].

References to several methods which were considered for DNA vaccination can be found in literature. Compared to other regions especially the skin, the first barrier of our immune system offers significant advantages for the application of drugs, like the accessibility and the large number of lymphocytes or Langerhans cells [9].

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A literature review revealed the difficulties related to exact application depth, efficiency, pain and the application itself.

A combination and improvements of different methods lead to two novel vaccination devices.

II. Development/Design and Fabrication

Hence the focus is on the mechanical methods. Lipid carriers, cholera toxins, ultra sonic or similar strategies will not be mentioned. It is often difficult to penetrate the skin and reach a specific depth after penetration, due to the skins flexibility and the small heights of some of the layers.

A. Application Methods

Gene gun: Tungsten or gold particles were coated with a dried active agent and shot onto the skin. A direct transfection of Langerhans cells and keratinocytes is possible but the required preparation of the DNA is highly sophisticated and a lot of cells die from the impact [10], [4], [11].

Jet injection: The liquid active substance is accelerated and shot onto the skin in a two step process. First, the tissue is opened, then it is filled with the liquid at a lower velocity [12], [13], [11]. Indention depths range between 2 and 20 mm, while [13] reached depths merely attain 200 µm. The vaccination procedure is reported to be painful.

Cannulas: Cannulas allow a precise dosage of a vaccine, but injections of a DNA vaccine into the muscle are reported to be less effective than injections into the skin [14]. The "Mantoux Technique" for skin applications, where the cannula is moved into the skin at a very small angle and only to a small depth is difficult to perform. Single injections allow only a limited distribution [Gan08].

Micro-needles: Many different ways to use micro-needles to transfer the active substance into the skin were found. The permeability of the skin can be improved by simply opening the skin and applying the vaccine topical [15], [16]. Micro-needles with coatings or fillings carry the active substance directly [17]-[21]. Hollow micro-needles, made of silicon, metal or plastic allow a direct injection. Problems can occur with mechanical stability, complex manufacturing and deflection of the skin.

Tattooing: A tattooing machine consists of a drive (pull-type solenoid plungers or crank drive) and a package of needles that are somehow connected to the drive to move them. Older needles are manually soldered by the tattooist while modern systems like [22] use

single-use cartridges. Using a tattooing machine allows a rapid yet successful vaccination of a large area [23], [14]. The active substance is mostly dropped onto the skin and the needles punch it into it [24]. The repetition rate is around 100 Hz to achieve a high indention velocity. Initial experiments with tattoo ink have shown that it takes between five and seven repetitions to bring the dye to the desired depth.

Another problem reported in [25] is a major loss of active substance inside the tattoo cartridge. Up to 70 percent remained unused inside the cartridge and on the skin surface. Different results were obtained when using a permanent make-up or a tattoo cartridge due to needle geometry and cartridge shape.

Cutting/scratching: Using blades for smallpox vaccinations was common until the 1960s [26]. Ref. [27] used micro-needles with blunt tips in so called MEAs (micro enhancer arrays) to scratch several times above the skin to improve uptake of a vaccine. [28], [29] used micro-blades or electromagnetic radiation to open the skin followed by the application of an active substance in dermatology. The amount and the position of the substance is not defined, a large amount will remain on the device, on the surface and on all layers down to the target layer.

Electroporation: The supporting technique electroporation is used to improve cell permeability by creating temporary pores. An electric field between two or more electrodes with high pulse rates, a short pulse duration and often high voltages leads to this effect. These parameters were coupled to the electrode design [30]-[33]. One of the electrodes is mainly inserted deeply into the skin, which complicates the usage on humans. Subepidermal necrosis, scars and burnings are reported as side effects

The mentioned application methods show lacks in precision, efficiency or handling. New combinations of some of them promise better results. Electroporation enhances several of the listed methods.

B. Tattoo Based Approach

The first device is a combination of tattooing and the use of a cannula. The idea was to vaccinate a large area within the short time of 20 seconds with several injections (2000) at a shallow depth, compare to [34]. With each injection, a specific amount should be placed in the skin. The problem with skin deflection must be handled. Investigations of the dependency of skin deflection from indention speed have shown that the deflection can be reduced with increasing speed.

The newly developed vaccination device consists of three main units, a drive, a depth control and a fluidic system.

Drive: The requirements on the drive were moving the cannula into the skin with a frequency of about 100 Hz and a specific velocity profile. The high frequency grants a high indention velocity to reduce skin deflection and a specific asymmetric velocity profile provides a slower retraction speed to prolong the application time of the active substance. Usual drives for tattoo machines like pull-type solenoid plungers, crank drives or swash plate drive were not suitable. The requirements were fulfilled with a shifted or askew crank drive [35].

Depth control unit: The high indention velocity of the novel drive of 2.5 m/s is too slow to fully compensate skin deflection. According to the small target depth of about 150 µm, another approach for reaching it precisely was necessary. Ref. [36] used high frequent overlay, which couldn't be repeated by using piezo-electric actuators. Stretching the skin with different methods was one possibility but the results were hard to repeat and depended on the user.

The final design [37] is shown in Fig. 1. The idea was to limit the indention way of the cannula with a mechanical approach. The cannula can be moved into the skin much deeper than necessary, while an end stop limits the real indention depth. In the laboratory sample, the limit can be adjusted by turning the tip

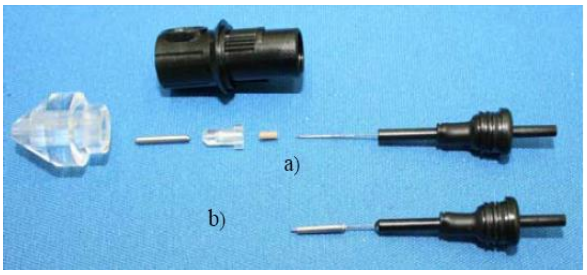

Figure 1. Depth control approach, a) flexible and b) fixed end stop.

Fluidic system: The most sophisticated subunit was the fluidic system. The system must transport the active substance from a reservoir into the skin at the same high frequency of 100 Hz while maintaining a steady dosage. Single use functionality was required for a medical product in order to avoid cleaning cycles for improved safety and handling.

The fluidic system consists of at least one cannula, a valve, a reservoir and tubes or pipes.

Several electromagnetic valves were tested but only a novel pinch valve was usable [38]. A pinch valve consists of a flexible tube that can be reversibly opened and closed as well as means to operate it. For small tubes, electromagnetic drives are typically used. The large size and low actuation frequency of less than 10 Hz of commercially available models prevented their usage.

The new method is shown in Fig. 2. The novel pinch valve consists of a tube, a valve body with cantilever, a pinch body and an abutment. The tube is closed when the pinch body is moved against the abutment. To operate the valve, the valve body has to be moved along a structured wall as can be seen in Fig. 2. The upper side of the pinch body "reads" the structure. If it reaches a higher level, the pressure inside the tube and the retraction forces of cantilever and tube open the tube and the fluid can be delivered.

The valve body was made of PMMA (poly-methyl meth-acrylate) on a micro milling machine (Medimill, Primacon) with endmills of 0.5 - 2.0 mm diameter. The PMMA allows easy machining, is transparent and can be glued with medical adhesives. The wall was also made of PMMA by turning and micro milling. The cannulas were

standard models from HSW (type Fineject), shortened on a wire EDM machine. They have been identified as the ones with the best properties in a previous investigation [25]. The tube of the early models with an inner diameter of 0.3 mm and an outer diameter of 0.7 mm was a commercially available silicon tube (Silicon HG, liquidscan).

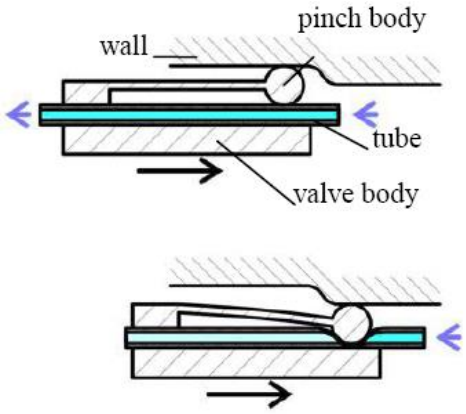

Figure 2. Principle of novel pinch valve.

All relevant parts were placed inside a modified tattoo cartridge (MT.Derm) as can be seen in Fig. 3. On the left side of the valve is the cannula, glued into the tube and fixed inside the valve body. The Drive directly transfers the movement to valve and cannula. A polymer spring applies the retraction movement to the valve body.

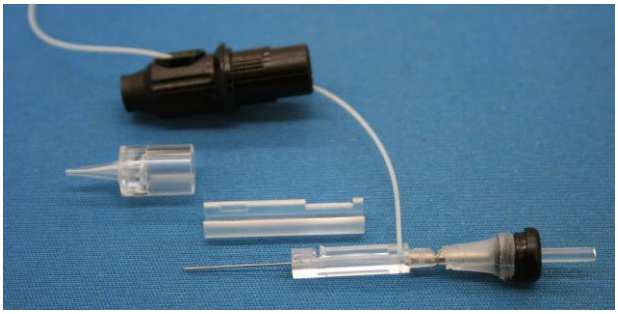

Figure 3. Modified tattoo cartridge.

The valve requires a specific pressure to be applied in order to operate. Active pumps like gear or piston pumps couldn't be used for a vaccination device due to contamination and cleaning problems. Different pressure units, like springs, linear drives or pneumatic pistons were discussed to apply a constant pressure on a reservoir. Only pneumatic pistons were identified to be useful. Their advantage is the constant force on the piston and the easy handling without any advanced control unit. The main disadvantage is the necessity of an additional energy source, which can lead to problems in less developed countries.

The reservoir and the valve system were divided into two parts for laboratory use to make sure that modifications can be done quickly. For real applications or clinical investigation sterile pre-filled single use cartridges would be used. All parts can be easily produced via the cost-effective injection molding process.

C. Cutting Approach

The second approach was called “immuno cutting”. Taking into account the observed problems related to the handling of skin deflection, this procedure was chosen with the smallpox vaccination in mind, where the vaccine was sometimes administered using a knife. First experiments testing the distribution of ink in shallow cuts in ex vivo pig skin revealed a rapid filling of the small channels.

The first model was a hand held device as shown in Fig. 4.

Figure 4. Hand-held cutting device [25].

It consisted of a surgical blade with means for liquid dosing, a slider with spring and micrometer screw for exact depth control, a housing and a cap with a slot for the blade.

The distance between cap and blade is the cutting depth. Cap and blade are made to be congruent. Preliminary studies have shown that a tight gap between slot and blade is vital. Otherwise the skin moves into the gap and the cutting depth increases. The cap was made of POM (ployoxometalate) by micro milling. The material of the housing was aluminum, with an anodizing step, the spring was stainless steel and the slider PEEK.

A stainless steel pipe was welded to the rear plane of the blade to ensure the transport of liquid substances into the channel. A silicone rubber tube was glued to the pipe. A Luer Lock connector and a syringe were placed at the other end of the tube. The necessary pressure was applied manually to the syringe piston.

Masks made of POM were micro-milled to achieve a constant vaccination area. All materials were chosen to be autoclavable, to fulfill medical regulations and prevent contaminations.

The final development was an automated device as shown in Fig. 5. It consists of three linear drives for a three axis movement, a blade cartridge holder with blade and fluidic system and a housing.

The first drive, a linear motor (LM 1247-020-01, Faulhaber) allows a fast acceleration of up to 175 m/s^2. The positioning movement perpendicular to the cutting movement has been implemented using a DC servo motor (1628 B, Faulhaber) with a fine thread spindle to achieve cutting distances down to 100 µm. For the positioning of the blade a small pneumatic actuator (Festo) was used.

A small cutting cartridge was manufactured by micro-milling a housing (PMMA) and inserting a part of a blade

cut using WEDM. The housing allows constant cutting depth of 0.3 / 0.5 / 0.8 mm for laboratory investigations. Two different fluidic systems were integrated. The first version was a small stainless steel pipe, welded or glued to the rear side of the blade. In the second version, a micro drill has been used to make a hole of 0.2 mm directly through the cutting edge. In both versions the upper end was connected to valve and pressure unit via a silicone rubber tube.

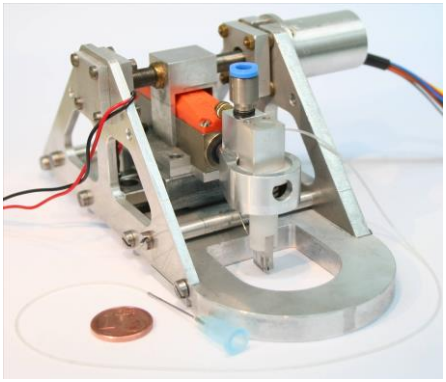

Figure 5. 3-axis fully automated cutting device.

All components were situated in a milled housing. The materials used were aluminum (housing), stainless steel (screws, spindle), brass (bearings) and POM (cartridge holder). The whole device was not autoclavable due to the drives, but major parts were created as single use. A drape could be used during clinical in-vivo investigations.

Movement and fluid dosage were controlled by a LabVIEW program. After starting the program a reference movement initialized the position. The next step was moving the cutting cartridge to the first cutting position. The blade was moved against the skin and the cutting started by a retraction movement of the linear motor. Active substances were poured into the cutting channel behind the blade during the cutting. At the end position, the dosing stopped, the cartridge was retracted from the skin and the remaining linear drives move the cartridge to the next cutting position. This procedure has been repeated according to the setup parameter.

D. Electroporation

Possible ways of integrating the electroporation as supporting technique into the drug delivery device were investigated. Due to the known risks and problems, a low voltage and small invasion of the device into the skin were assumed to be necessary.

The first approach was an array with two surface electrodes and a needle in between. FEM-simulations have shown problems with the range of the field if it is only applied between the side electrodes. Especially substances with low resistance on the surface lead to a short circuit. So the next step was to integrate the needle into the system. The electrical field has been applied between one or two side electrodes and the needle as counter electrode.

Initial experiments with mice [39] lead to another improvement. The actual size was too big for mice legs, so the whole assembly was miniaturized. Fig. 6 shows the final electrode version, fitted into a tattoo cartridge.

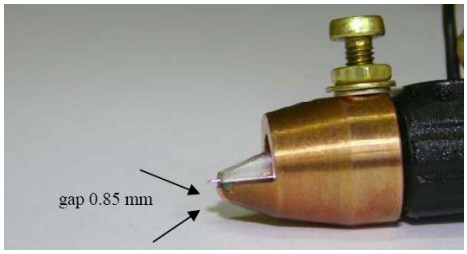

Figure 6. Electrotattooing cartridge.

III. Experiments and Results

Several tests and investigations have been made to determine the potential of the drug delivery devices and to optimize their mechanical properties.

A. Tattoo Based Approach

The emphasis of the underlying research project was on the drug delivery through a cannula, so it was investigated more deeply.

As mentioned earlier, a fast indention into the skin and a slow retraction speed are helpful for liquid dosing at a certain depth. Several common drives were investigated, to reach a high repetition rate and to fulfill the mentioned needs. All devices were located in front of a fast triangulation sensor (Opto NCDT ILD2220-20, Micro Epsilon). A high indention velocity of 2.5 m/s was measured for the novel drive, which is 2.5 times faster than the conventional.

Several investigations were made to examine the valves characteristics. The dosage limits, depending on activation time and applied pressure, the wear limit, the dependency from pipe / tube material and diameter and a variation of the viscosity of the liquid were investigated [40]. It was found, that a minimum amount of around 10 nl can be applied with each dosing step. The fatigue limit of valve and tube is somewhere above 10 million operations if the valve is operated with an external actuator and somewhere above of a million operations if operated via a structured wall [25], [40].

The main part of the modified valve, the improved tube is shown in Fig. 7. The new shape allows easier assembling and manufacturing. For this purpose an inexpensive manual molding technique for high temperature curing silicone rubbers for small series applications has been developed and qualified [40].

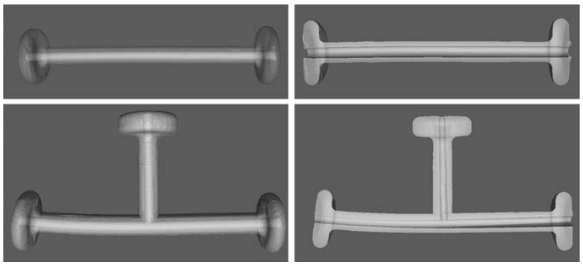

Figure 7. X-tomographic image of novel tube [39].

The reservoir was optimized in terms of leakage problems, dead volume and friction losses during application. Commercially available glass or plastic single use syringes have shown large variations of necessary pressing forces. A reservoir made of PMMA with a PTFE piston showed the best results [25]

Immunological tests have been performed with a novel cartridge and a standard tattoo machine (Cheyenne Hawk, MT. Derm). A single cannula has been used instead of nine needles. The whole application time was set to 20 seconds at an actuation frequency of 100 Hz. The pressure on the valve was set to 2 bar with a syringe pump. A luciferase expriming plasmid (in pVAX backbone, diluted in water) was used, that produces a luminous reaction with luciferin, measurable with a light sensitive CCD camera (IVIS system 100 (Xenogen, Hopkinton, USA)). Indention depth over all tested positions was set between 1.25 mm and 1.9 mm. It must be noted, that the application angle was not perpendicular to the skin as in standard DNA tattooing procedures. The bevel of a cannula of usually 11 ° [41], [42], requires an angular application. Otherwise the whole amount of active substance would be lost on the surface of the skin. The skin was stretched manually to reduce skin deflection.

Fig. 8 shows an image taken with the light sensitive camera 24 hours after vaccination. A smaller expression was detected during the cannula experiments (1-1), compared to standard tattooing (1-3). It was found, that a shallow application might lead to higher expression levels, but the application of Luciferin causes a surface reaction. The skin destruction from nine needles would be expected to be higher than from a single cannula. It can be assumed, that not all light was gathered by the camera, due to the smaller destruction and the hence closed surface. The smaller destruction might also lead to a smaller trauma and a smaller amount of other supporting cells. Antibody tests or histological investigations would give more insight.

Further investigations were needed to answer questions regarding application angle or depth, suitable amount of active substance or necessary number of cannulas. The usage of the new drive and the integration of the depth control or an electroporation electrode will improve the novel method significantly.

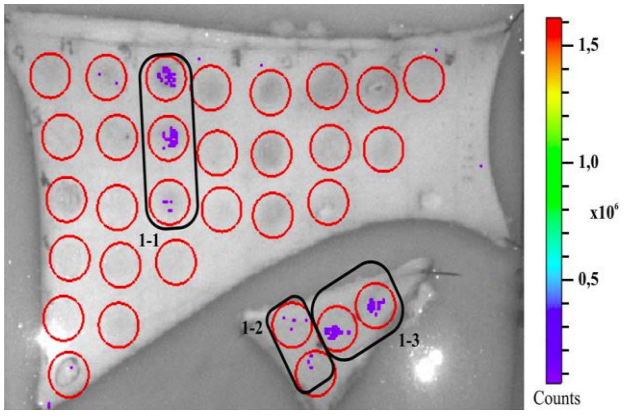

Figure 8. Image from light sensitive camera 1-1 novel vaccination cartridge, 1-3 standard cartridge for comparison [25].

B. Cutting Approach

The tests of the cutting approach were limited due to the relevance for the according research project.

After successful initial liquid distribution tests in cutting channels with black tattoo ink (MT.Derm) the main problems encountered concerned the precision with which a specific cutting depth could be achieved and the reliability of the fluid connection and its output at the blade.

Tests on ex vivo pig skin revealed that a narrow total gap between blade and housing in the range between 20 and 100 µm was necessary to obtain a constant cutting depth nearly independently from application pressure. While cutting in silicone rubber always leads to a constant depth, skin quickly creeps into the gap. The stretching of the skin appeared to be an improvement, but didn't prove to be sufficiently repeatable throughout the experiments. Stretching or high application pressure can also lead to variations of the height of the skin layers.

While with the manual cutting device the dosage of the active substance depends on the operator, the fully automated device allows a constant application pressure of the blade cartridge onto the skin and a constant amount of fluid in a cutting channel.

Immunological tests have yet been performed with the hand held device only. Experiments with PrV-NlA3 (Aujeszky) on in vivo pig skin were difficult to perform, due to the much higher thickness of the upper skin layers. The liquid dosage of the active substance was difficult, due to the early research state of the vaccination device. Nevertheless UL19 antigen expression and macrophages were noted after intracutanous delivery as shown in Fig. 9.

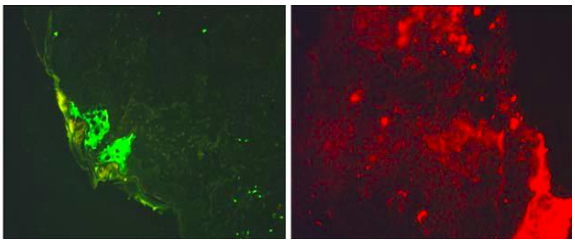

Figure 9. Antigen-UL19-expression (green), macrophages (red) [25].

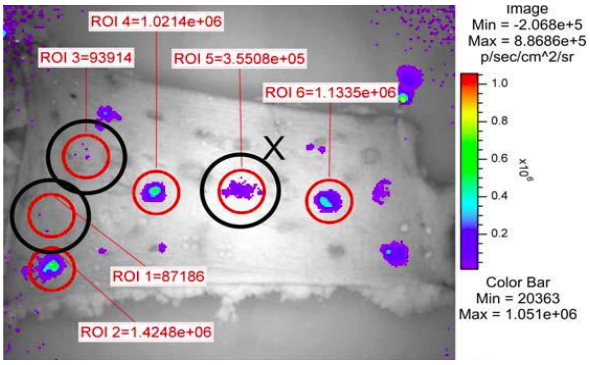

Figure 10. Image from light sensitive camera ROI 1, 3, 5 hand held cutting device, ROI 2, 4, 6 standard tattoo cartridge for comparison [25].

Tests with an early approach without fluid system were performed on ex-vivo human skin. An amount of 10 µl luciferase applied on the surface of the skin lead to the

result presented in Fig. 10. The positions ROI 1, 3, 5 were cutting positions, while position ROI 2, 4, 6 were reference points from DNA tattooing as described in [34]. It must be noted that at position 5 a different strategy (crosswise cutting) with more narrow cuts has been applied and the amount of luciferase was divided into two parts. Part two was administered during the procedure, while part one was applied on the skin beforehand.

It is assumed that these tests have shown the importance of an application into the cutting channel during the procedure. Otherwise a quick loss of active substance or depositing on wrong skin levels can be expected.

Further experiments have to identify the necessary amount of active substance, the required cutting density and depth. The potential of the new method, application of an active substance into the channels during the procedure, for e.g. skin regeneration [29] should also be considered. An integration of electrodes for electroporation is recommended.

C. Electroporation

For the investigation of the novel electroporation device a standard tattoo cartridge (MT.Derm) with nine needles has been modified and combined with standard tattoo machine (Aella, MT.Derm). The modified cartridge uses a distance of only 0.85 mm between the electrodes (needle and housing). This influences the setup parameter of the electroporation generator BTX ECM 830 (Harvard Apparatus). A voltage between only 60 V/cm and 1200 V/cm and pulse duration between 10 - 200 µs were suitable. According to [38] the tattooing parameters were set to 20 seconds, a frequency of 100 Hz and an indention depth of 1.0 mm.

A 66 percent higher gene expression has been measured, compared with tattooing without electroporation [39]. This result is lower than results with fork and plate electrodes. This has to be expected due to the much smaller electrical field caused by the smaller electrodes. The activation of the field was also not coupled to the electrode position (pulse frequency 0.5 – 3.7 Hz). Several pulses occurred when the needles were out of the skin.

Another big advantage is the lower pain and the possibility to integrate the electrodes into a single use device. The new combination is less invasive than common fork-plate electrodes. The low voltage prevents burnings or necrosis.

IV. Summary

In this paper two novel mechanical devices for intra-dermal drug delivery have been presented after pointing out the disadvantages of standard methods. The technical difficulties related to the development were shown.

The first approach, a tattoo based device, uses a cannula to deposit the vaccine at a certain depth. It consists of up to three subunits. The first subunit, the fluidic compartment, consists of the cannula, a valve, a reservoir with pressure unit and fluidic interconnectors. The novel pinch valve assures the synchronous dosage of the liquid. Small amounts in the lower nano liter range are possible, even at a high frequency. The second unit, the askew crank shaft drive, is a new development with a characteristic velocity profile. It allows a fast indention movement and a much slower retraction velocity. The last unit, the depth control, ensures the right application depth.

The second approach uses blades to open the skin at a shallow level. A micro drilled hole through the blade allows for the vaccine to be injected directly into the cutting channel during the cutting procedure. A fast and simple handheld device and a fully automated vaccination machine were built and tested.

Several mechanical tests have been made to identify the properties of the delivery devices. Initial tests on ex-vivo human skin and in-vivo pigskin with luciferase and PrV-NlA3 have shown the basic functionality for vaccination. The efficiency of the cannula based device can be increased by adding an electroporation tip, as shown with a modified tattoo cartridge. Similar results are expected for the cutting approach. Further investigations are going to improve the results significantly and have to show the exact efficiency of both methods.

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