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An approach towards bronchoscopic-based gene therapy using electrical field accelerated plasmid droplets

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Abstract— **Idiopathic pulmonary fibrosis (IPF) is a devastating disease affecting the distal lung, due to failure of the alveolar epithelium to heal after micro-injuries, leading to inefficient gas exchange and resulting in death. Therapeutic options are very limited. A new therapeutic approach based on gene therapy restores the self-healing process within the lung in the experimental setup.**

A basic requirement of this therapy is the successful transduction of genes into the alveolar epithelium in the distal part of the lung, for which a new therapeutic instrument is required. In this paper we present the concept and first experimental results of a device which uses an electrical field to accelerate the charged droplets of plasmid suspension toward the tissue and which overcomes cell membrane with its impact energy. The aim is to develop a therapeutic device capable of being integrated into minimally invasive procedures such as bronchoscopy.

I. Introduction

Idiopathic pulmonary fibrosis (IPF) is a devastating disease affecting the distal lung. It is suggested that the failure of the alveolar epithelium to heal after micro-injuries, which triggers a complex biological processes, causing excess collagen deposition, leading to inefficient gas exchange leading to death. Current development looks at gene and drug delivery to the distal lung, with a focus on gene therapy for the treatment of IPF, using hepatocyte growth factor (HGF) for alveolar cell repair and regeneration to reduce fibrosis [1].

A major challenge in gene therapy is the delivery of the substances into living cells avoiding side effects. The cell membrane offers a powerful barrier to protect the interior of the cells from any intruders. For gene therapy this membrane has to be conquered effectively. There are different procedures for gene transfer, based on viral vectors and non-viral methods. Transduction uses viruses, which host the gene and introduce it as a part of their replication cycle [2]. However prominent local and systemic inflammatory and immunogenic response, leading to viral vector toxicity, restricts the clinical application of this system [3].

In contrast non-viral methods such as the use of short and intense electrical pulses (ranging from microseconds to milliseconds; kilovolts per centimetre) applied to cells or tissues, 'electroporation', offers a different mechanism for substances to enter the interior of cells. As a response to an electrical field, the cell membrane temporarily loses its semi-permeable properties, leading to ion exchange, the escape of metabolites and an increased uptake of drugs, molecular probes, and genes [4]. The feasibility of electroporation for sustained gene expression in vivo has been shown previously in various organs [5] including the lung [6]. Nevertheless the use of electroporation in therapeutic instruments seems to be limited. To our knowledge, there is currently only one device in development utilizing the electroporation effect to deliver anti-cancer drugs to intraluminal tissue for electro-chemotherapy [7].

Here we present the concept of a new device for a non-viral gene transfer to the distal lung tissue by the use of an electrospray process. Droplets containing a negatively charged plasmid are accelerated towards a positively charged electrode by an electrical field. In addition, the likely charged droplets are affected by Coulomb repulsion and experience an additionally accelerating force [8]. This interaction leads to the formation of very small sized droplets. The positive electrode nearby the targeted tissue guides a droplet bombardment towards the tissue, providing a high impact velocity for the collision with the cell membrane. The feasibility of this process for transfection has already been demonstrated by using water droplets [9], a plasmid suspension incorporating gold nanoparticles [10], as well as a pure plasmid suspension [10]. As both concepts described rely on the requirement of a counter electrode at or below the target plate, this set up tends to be less suitable for clinical practice.

For therapeutic application the delivery mechanism has to be transferred into an approved and commercially available instrument accompanied by a suitable procedure. Focusing on a therapeutic device which can be introduced into the lung through a bronchoscope, we elaborated a concept for an electrospray system. It enables the delivery of pure plasmid to distal lung tissue. Furthermore we realized a first, large scale prototype and proved the concept on cell culture and *ex-vivo* lung tissue of rat, using enhanced green fluorescence protein reporter gene (eGFP) for transfection.

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II. Device

A. Basics of Electrospray

Electrospraying is based on the migration of droplets emitted from an electrified meniscus towards a counter electrode [11]. Electrically charged droplets are accelerated due to the interaction with the electrical field and the Coulomb repulsion between the droplets. Additionally these forces will disrupt the droplets even more. Therefore very small droplets, travelling at high velocities can be obtained. In contrast to other aerosol generating systems, e.g. ultrasonic or pressure driven nebulizers, no mechanical movement of components or airflow is required.

B. Device concept

Transferring the electrospray process into a successful therapeutic device requires the integration within standard diagnostic or interventional procedures. For pulmonary examination this is a bronchoscope. We propose to introduce the electrospray device to be used within the working channel of such an endoscope, a concept using only a single port to access the targeted region; incorporating all relevant functional elements is fundamental. These elements are a minimum of two electrodes, for generating the electrical field, an acceleration stage where this field is applied and interacts with the fluid, and a fluid delivery mechanism, to provide the therapeutic dissolved substance or suspension.

The electrical field for acceleration is created by two electrodes, one formed by the outlet of the electrically conductive pipe, containing the liquid to be delivered, and a counter electrode, typically positioned below the targeted region. In recent publications [9, 10] the counter electrode was placed below the tissue. In contrast to these designs, our setup is using the targeted tissue itself as counter electrode.

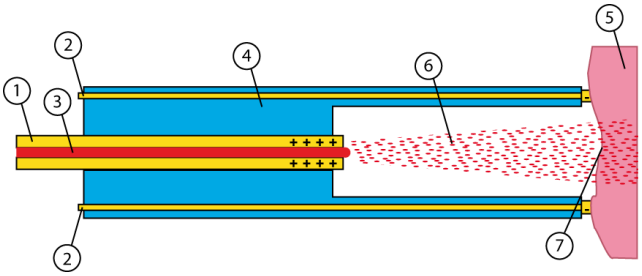

Figure 1: Schematic cross section through our device containing first electrode (1) in contact with the fluid (3), the second electrode (2), contacting the tissue (5), and a body construction (4), integrating both electrodes and forming a defined working distance for the electrosprayed (6) fluid to the targeted region (7).

As shown in Figure 1, a ground electrode (2) is used to ensure the ground potential at the tissue (5). The electrode is completely integrated into a body (4). The high voltage (negative polarity) to generate the electrical field is connected to the conductive pipe (1), which also delivers the fluid (3). An integrated cavity within the body provides a predefined working distance between the electrodes, and therefore, assuming constant electrical conditions within cavity, a defined electrical field for the electrospray process (6). Furthermore the cavity reduces the effect of changes in the surroundings, e.g. alternating airflow due to respiration, and we therefore expect a robust application.

C. Device realization

The main part of our device is the body with integrated electrodes. We chose additive manufacturing processes [12] to realize this construction using an Eden250™ 3D Printing System (Objet) to process a photopolymer (FullCure®850 VeroGray from Objet).

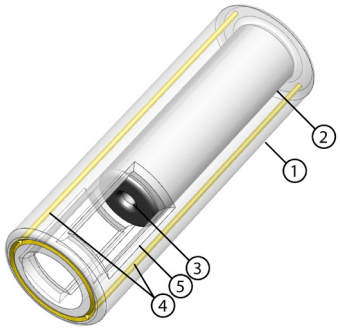

Figure 2: Sketch of the body, consisting of an outer part (1), inner part (2), collet for the conductive pipe (3), collets for the ground electrode (4) and observation window (5).

For improved experimental flexibility the body (Figure 2) consists of two pieces, an inner part (2), containing the collet (3) for the conductive pipe, and an outer part (1), containing collets (4) for the ground electrode. To assure a symmetrical electrical field we integrated two collets for the ground electrode. Furthermore we integrated two windows (5) to observe the electrospray process visually. The outer dimensions of the body are 30 mm length by 10 mm diameter. For the ground electrode connection we used stainless steel (1.4310, ∅300 µm). The ring shaped interface between the ground electrode and the tissue is realized using a conductive paint (Graphit 33, CRC Industries).

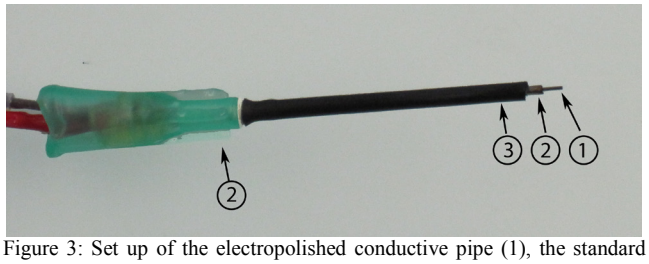

Figure 3: Set up of the electropolished conductive pipe (1), the standard needle (2), and the additional isolation (3)

Figure 3 shows the setup of the pipe arrangement: The conductive pipe (1) consists of a stainless steel tubing (SUS 316L, 28G tubing, o.∅ 360 µm, i.∅ 170 µm, ~50 mm length) inserted for stability purposes within a standard 21G needle (2). The edges of the pipe are deburred by an electropolishing procedure. The pipe is connected to the fluid reservoir (FEP tubing) and offers a high voltage electrical connection. Additionally the complete arrangement is insulated using heat shrink tubing (3).

D. System

The complete assembly creates a working distance from

the exit port of the pipe to the counter electrode of 8 mm.

For the delivery of the fluid, a precision syringe pump (cetoni neMESYS, with 500 µl glass syringe) is connected to the pipe, enabling delivery of a predefined volume at a predefined flow rate. A high voltage source (FuG HCP 35 - 6500 MOD, AIP Wild AG) was connected to the device to generate the electrical field. It can be used in pulsed or continuous mode.

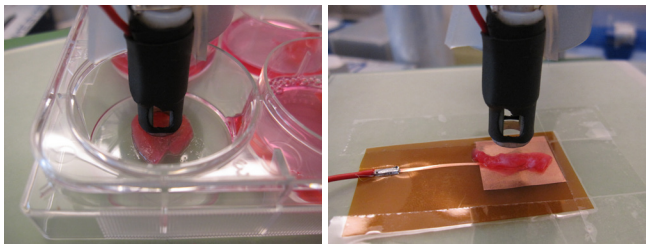

Figure 4: Application of the electrospray device on an explanted slice of lung tissue 1-3mm thick (Adult Fischer rat, F344) within the well plate (left) and with an additional external electrode (right).

III. Experiments

A. Experimental set up and procedure

We installed the device within a stand and placed the target (cell culture and lung tissue) on standard well plates. The well plate was placed on a height adjustable platform.

The height of the well plate was adjusted visually until the device was in contact with the target (cells or tissue slice). The voltage was then applied, followed by the syringe pump. To ensure complete fluid delivery there was a delay of 30 seconds before switching the power source off.

B. Cell culture

A549 cells (alveolar epithelial like cells) were grown to confluence in RPMI growth medium with 10% fetal bovine serum (FBS) in 24-well plates (15.6 mm in diameter). Before electrospray the growth medium was removed and electrospray was performed either in absence of medium or in presence of 100 µl of medium. For electrospraying 50 µg/ml enhanced green fluorescent protein (eGFP) reporter gene suspended in distilled water was used.

The current flow during the spray process was limited to 200 µA, while the applied voltage was set from 5.0 to 6.5 kV. Assuming a homogenous field distribution, this corresponds roughly to an electrical field in the range 0.56 to 0.81 kV/mm. At a flow rate of 100 µl/min we delivered 50 µL of plasmid suspension, corresponding to 2.5 µg of the plasmid. The cell cultures were subsequently incubated for 24 hours at 37 °C with 5% CO_2 and observed under a fluorescence microscope.

C. Explanted lung tissue

As proof of the concept on regular lung tissue, slices of explanted lung (Fischer rats, F344, thickness 1-3 mm) were used. The tissue was placed within a 6-well plate with DMEM growth medium with 10% FCS (Figure 4 left). Before applying the electrospray, the growth medium was removed, and only the tissue remained. For electrospraying 50 µg/ml enhanced green fluorescent protein (eGFP) reporter gene suspended in distilled water was used.

The current was limited to 200 µA, while a potential of 4.5 kV was applied. At flow rate of 100 µL/min a plasmid volume of 50 µL (2.5 µg plasmid) was delivered. The lung tissue was kept for 24 hours at 37 °C, with 5% CO_2 subsequently.

For comparison a second test was performed by applying an external electrode to the tissue, disabling the integrated ground electrode (Figure 4 right).

IV. Results

A. Cell Culture

Using a potential from 5 to 6.5 kV the transfection of eGFP (green fluorescent protein plasmid) DNA can be observed using a fluorescence microscope. Shown in Figure 5 the greenish spots represent single cells with transfected reporter gene. However, the transfection rate is quite poor, a transfection of GFP can clearly be observed. An improvement of transfection rate can be observed when increasing the potential.

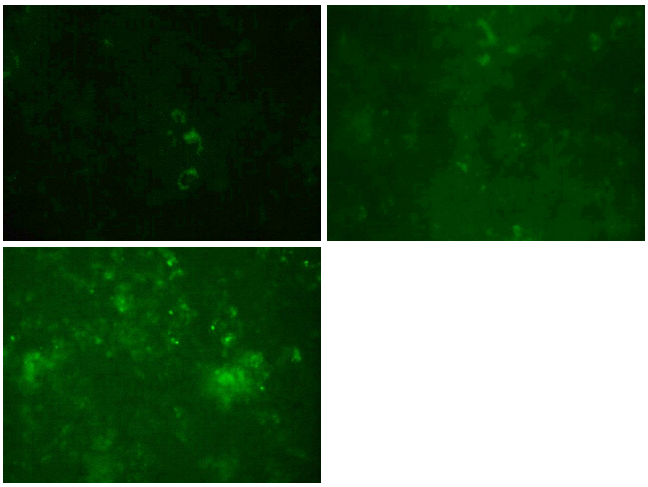

Figure 5: Fluorescence microscopic images of cell cultures, sprayed with GFP 5.0 kV (upper left), 5.5 kV (upper right) and 6.5 kV (lower left) after 24 hours incubation.

B. Ex-vivo lung tissue

Figure 6 (left) shows the fluorescence microscope images of the rat lung tissue, using an electrical potential of 4.5 kV. The greenish spots indicate the successful transfection of eGFP into the cells. The transfection rate is slightly higher as compared to the transfection of cell culture, even at lower applied potential. The fluorescence microscope image of the experiment with the external electrode is shown in Figure 6 (right).

To confirm the cell type transfected, a co-staining with surfactant protein C (SpC) antibody was performed. There were a number of double stained cells (eGFP: green SpC: red, co-stained: orange), in the tissue slice as shown in Figure 7. This proves that we are able to transduce the alveolar epithelial cells with this technique.

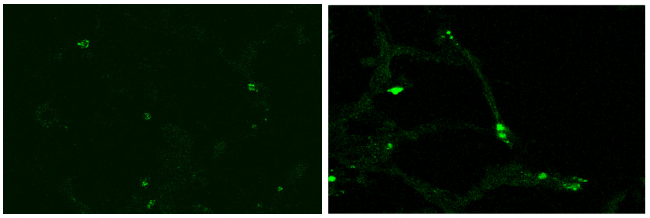

Figure 6: Fluorescence microscope images of lung tissue after 24 hours of incubation at 37 °C. GFP positive cells (green) can be observed using the device for electrospray (left) and using an additional external electrode (right).

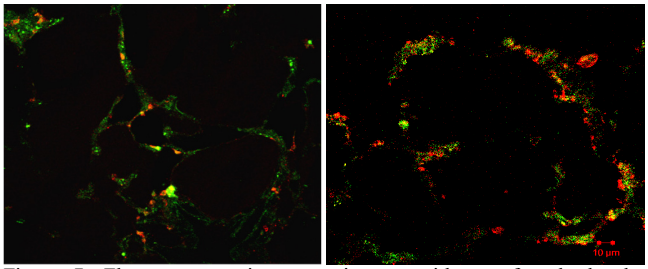

Figure 7: Fluorescence microscope images with transfected alveolar epithelial type II cells (orange), in contrast to non-transfected cells (red) and GFP (green) applied by our device (left) and with an external electrode (right).

V. Discussion

Utilizing the experimental device in both set-ups, cell culture and lung tissue, transfection could successfully be demonstrated. Although transfection was not homogenous and could be improved, it still proves the feasibility of the electrospray as a gene delivery concept without damaging cells.

The transfection rate tends to be higher in tissue than in cell culture experiments, even when using lower electrical fields. This effect needs be observed in more detail.

As regards the results of the lung tissue set-up, an enhanced transfection rate is observed when contacting the tissue with an external ground electrode. We assume that this phenomenon might be due to a lower conductivity at the electrode-tissue interface of our device, as well as to the lower conductivity of the painted ground electrode.

Besides the successful proof of concept, we investigated some technical issues to be considered in further development.

Firstly multiple use of the device results in a moistening of the body, leading to increased risk of electrical discharge. This may be solved by using hydrophobic material for the body or applying a hydrophobic coating to at least at the cavity of the body.

Secondly a continuous delivery of liquids to the target, leads to an accumulation at the bottom. This results in a change of the electrical field, which increases the risk of an electrical discharge. Furthermore droplets have to overcome this fluidic barrier. This will lead to a reduced impact velocity and a lower transfection rate. To overcome this issue either the amount of fluid to be delivered has to be reduced, assuring an identical delivered amount of eGFP by increasing the dilution rate. Alternatively, a redesign of the body-tissue interface, integrating microstructures for improved liquid removal, might improve this condition. Furthermore, this concept can be also adopted to be used for minimally invasive approach in other organ systems too.

VI. Conclusion

We successfully demonstrated the feasibility of our concept of transfecting an eGFP reporter gene to living cells in vitro and a lung tissue slice by electrospray. As the device requires only a single port access, the concept is suitable for application through an extended tubular device such as an endoscope, and it might therefore be an option for an intraluminal therapeutic instrument to deliver drugs, genes or chemotherapeutics. Using a bronchoscope it offers a novel and promising tool to treat idiopathic pulmonary fibrosis with an appropriate gene therapy.

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References

[1] A. Gazdhar, P. Fachinger, C. van Leer, J. Pierog, M. Gugger, R. Friis, R. A. Schmid, and T. Geiser, "Gene transfer of hepatocyte growth factor by electroporation reduces bleomycin-induced lung fibrosis," *Am J Physiol Lung Cell Mol Physiol,* vol. 292, pp. L529-36, Feb 2007.

[2] N. A. Wivel and J. M. Wilson, "Methods of Gene Delivery," *Hematology/Oncology Clinics of North America,* vol. 12, pp. 483-501, 1998.

[3] S. M. Albelda, R. Wiewrodt, and J. B. Zuckerman, "Gene therapy for lung disease: hype or hope?," *Ann Intern Med,* vol. 132, pp. 649-60, Apr 18 2000.

[4] T. Y. Tsong, "Electroporation of cell membranes," *Biophysical Journal,* vol. 60, pp. 297-306, August 1991 1991.

[5] R. Tavakoli, A. Gazdhar, J. Pierog, A. Bogdanova, M. Gugger, I. A. Pringle, D. R. Gill, S. C. Hyde, M. Genoni, and R. A. Schmid, "Electroporation-mediated interleukin-10 overexpression in skeletal muscle reduces acute rejection in rat cardiac allografts," *J Gene Med,* vol. 8, pp. 242-8, Feb 2006.

[6] A. Gazdhar, M. Bilici, J. Pierog, E. L. Ayuni, M. Gugger, A. Wetterwald, M. Cecchini, and R. A. Schmid, "In vivo electroporation and ubiquitin promoter--a protocol for sustained gene expression in the lung," *J Gene Med,* vol. 8, pp. 910-8, Jul 2006.

[7] D. Soden, M. Sadadcharam, J. Piggott, A. Morrissey, C. G. Collins, and G. C. O'Sullivan, "An endoscopic system for gene & drug delivery directly to intraluminal tissue," in *11th Mediterranean Conference on Medical and Biomedical Engineering and Computing 2007*. vol. 16, R. Magjarevic, Ed.: Springer Berlin Heidelberg, 2007, pp. 628-628.

[8] J. M. Grace and J. C. M. Marijnissen, "A review of liquid atomization by electrical means," *J. Aerosol Sci.,* vol. 25, pp. 1005-1019, 1994.

[9] Y. Okubo, K. Ikemoto, K. Koike, C. Tsutsui, I. Sakata, O. Takei, A. Adachi, and T. Sakai, "DNA Introduction into living cells by water droplet impact with an electrospray process," *Angewandte Chemie,* vol. 120, pp. 1451-1453, 2008.

[10] D.-R. Chen, C. Wendt, and D. Y. H. Pui, "A novel approach for introducing bio-materials into cells," *Journal of Nanoparticle Research,* vol. 2, pp. 133-139, 2000.

[11] O. Wilhelm, L. Madler, and S. E. Pratsinis, "Electrospray evaporation and deposition," *Journal of Aerosol Science,* vol. 34, pp. 815-836, Jul 2003.

[12] I. Gibson, D. W. Rosen, and B. Stucker, *Additive Manufacturing Technologies: Rapid Prototyping to Direct Digital Manufacturing*, 1st Edition ed.: Springer US, 2009.