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Biocompatible and sustainable DC stimulation of cells and tissue based on highly swollen polymer electrodes

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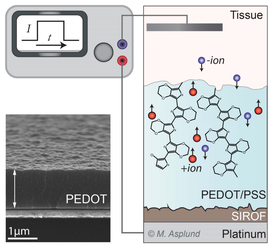
INTRODUCTION

Electrical field (EF) stimulation can direct the migration of certain cells in culture, so-called electrotaxis. Similarly, it is likely that EFs applied in tissue could control cell migration by exploiting this inherent electrotactic mechanism. The lack of suitable electrode materials for EF stimulation has nevertheless limited the therapeutic use.



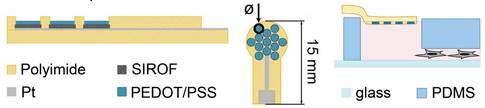
Concept: *Thick films of PEDOT/PSS exchange ions with the surrounding electrolyte in response to polarization. This can be used to generate biocompatible EFs in tissue.*

While electrostimulation with alternating signals can be supported by a broad variety of electrode materials, guiding EFs requires direct current (DC), which typically is associated with electrode corrosion and pH changes.

Aim: To show that we can address this, by extending metal electrodes with conducting polymers. A highly swollen network of PEDOT/PSS, stabilized on a sputtered iridium oxide (SIROF) substrate, can inject ionic charge into biological media via a reversible and non-toxic mechanism, maintaining stable ionic EFs over hours[1].

METHOD

Electrodes: Electrodes were prepared in a clean-room manufacturing process. Polyimide was used as top and bottom insulation, and SIROF electrodes were deposited by reactive sputtering onto a platinum base layer. The total electrode area of 0.2 cm² was divided into several smaller circles to reduce stress in films. Thick layers of PEDOT/PSS were coated onto SIROF by electrodeposition from an aqueous solution of the monomer and NaPSS salt.

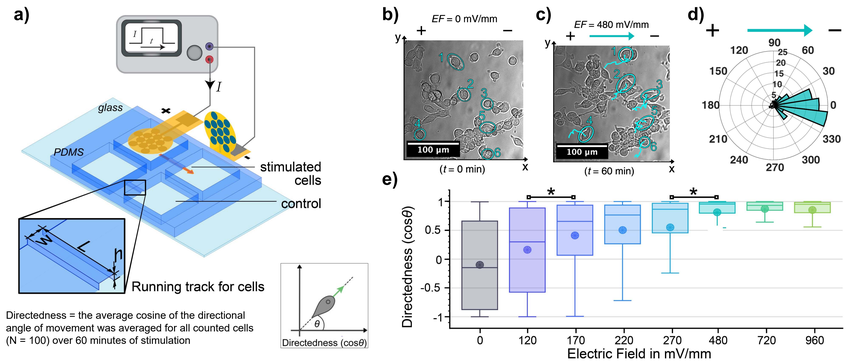


Materials: *Cross section (left) and top view (center) of the polyimide based electrode substrate. Cross section of the micro-fluidic "running tracks" designed for the biological experiments.*

Biological experiments: Migration of two types of cells, a prostate cancer cell line (MAT-LyLu) and immortalized human keratinocytes, was analysed with & without stimulation using phase contrast time-lapse Microscopy.

RESULTS

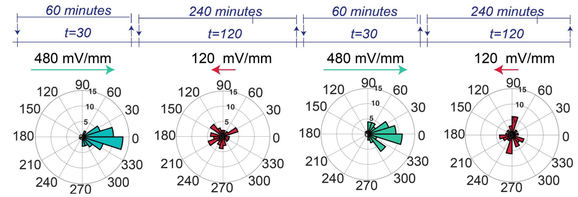
Experiment MAT-LyLu: Cells were cultured in "running tracks" (cross-section 0.1 x 0.3 mm²) with electrodes placed in contact with the culture medium on either side. A stable current was driven through the system to generate field strengths in the range between 0 to 960 mV/mm, each sustained over 1 hour. Electrotaxis was quantified through the average directedness* of the cells during this time. It was clear that a directional effect was seen even at the lowest field strength (EF of 120 mV/mm), with increasing directedness at higher fields. Full effect (maximum directional response) was reached first at 480 mV/mm. Stimulation could be maintained at least over several hours.



Electrotaxis, MAT-LyLu*: a) experimental outline and definition of "directedness", b) cells at t = 0, before stimulation was applied, and c) the same cells as outlined in green after 60 min. of stimulation. d) overview of directionality of all tracked cells after 60 min at 480 mV/mm. e) overview of directionality of the cells when stimulated at different field strength.*

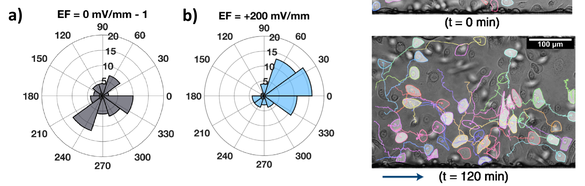
In order to extend the stimulation from hours to days, a concept must be established which allows electrode recharging. This is possible by stimulating the cells with a pulse of opposite polarity. In the positive phase, cells are stimulated above response threshold, and in the negative phase, below. This way, the net charge is zero while the net effect on cell directionality is maintained.

Experiment keratinocytes:
Immortalized human keratinocytes (skin cells important for wound healing) were cultured in the "running tracks" and their response thresholds were determined in similar manner as for the MAT-LyLu's. Cells in a control channel (without stimulation) migrated randomly, while stimulated cells showed clear directional response already at 100 mV/mm, and reached full directedness at 200 mV/mm.



Charge balanced stimulation: *From left to right, showing the directionality of the same group of cells stimulated with a sequence of 1 h above threshold, 4 h below threshold, repeated twice. Blue lines indicate the time the pulse was maintained.*

Directional migration of keratinocytes:
(a) *Without stimulation keratinocytes in culture migrate randomly, while at 200 mV/mm, the majority of cells migrate in direction of the EF* **(b)**, *here shown in an experiment over 2 h.* Position of individual cells *before* **(c)** *and after* ***(d)*** *stimulation*



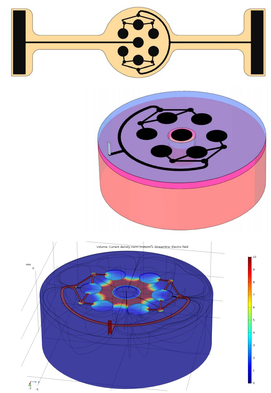
CONCLUSIONS

- We here show how biocompatible EF stimulation and DC can be accomplished using highly swollen PEDOT/PSS. By fine-tuning the material and optimizing stimulation parameters, a non-toxic and sustainable concept for DC stimulation was demonstrated, with promising perspectives for future clinical applications.
- The electrodes operate using the small ions available in abundance in the biological environment, resulting in a DC concept that is biocompatible. Thus, there is no need for salt-bridges or similar buffering layers, but the stimulation can be applied directly to the cultured cells.
- The combination of SIROF and PEDOT/PSS was particularly efficient for EF stimulation. SIROF contributed as adhesion layer, strongly binding the polymer to the base metallization[2].
- Over the longest stimulation sessions (3 h or more at 480 mV/mm) we furthermore observed that SIROF actively contributed to charge transfer, beyond the capability of the PEDOT-film alone.

OUTLOOK

One possible therapeutic application where the polymer electrode concept could become valuable is in the treatment of large and complicated skin wounds. Preliminary data on human keratinocytes shows, that electrotaxis can be established already at an EF of 200 mV/mm, which the electrodes can sustain over hours before recharge is even required.

In future work we will explore this possibility, developing electrodes with maximized charge injection and suitable for use on skin. First prototypes of such a system are currently in development.



Prototype for wound dressing: Design sketch and simulated field distribution

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