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Technology for Bioelectronic Medicine

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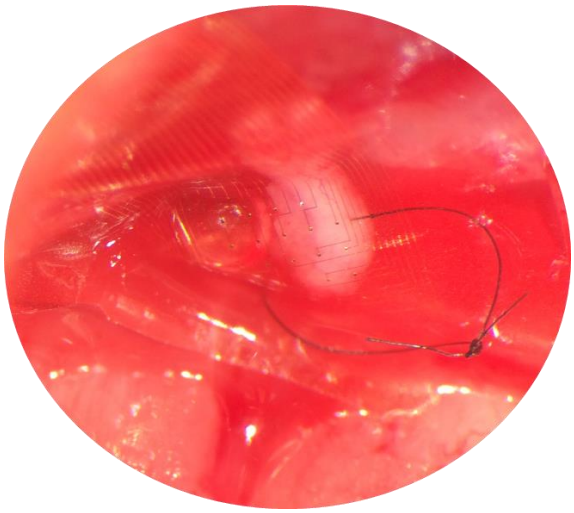
Bioelectronic medicine provides a new means of addressing disease via the electrical stimulation of tissues: Deep brain stimulation, for example, has shown exceptional promise in the treatment of neurological and neuropsychiatric disorders, while stimulation of peripheral nerves is being explored to treat autoimmune disorders. To bring these technologies to patients at scale, however, significant challenges remain to be addressed. Key among these is our ability to establish stable and efficient interfaces between electronics and the human body. I will show examples of how this can be achieved using new electronic materials and devices engineered to communicate with the body and evolve with it.



This figure shows a flexible implant sutured on a nerve

Modeling the cell-electrode interface with the help of simultaneous intra- and extracellular signals: an extension to electro-porating nano-electrodes

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Studying the interface between electrogenic cells, like neurons and cardiomyocites, and the electrode to which they are coupled is key to understand extracellular measurements of cell activity. There is a half-century long history of attempts to model the cell-electrode system by means of electrical circuit elements.[1,2,3] Many such approaches incorporated ion-channel dynamics into their model parameters to model the cells' intracellular potential, making the computations overly complex and unstable from a numerical regression perspective. With modern recording methods it is possible to simultaneously measure intra- and extracellular potentials with great signal-to-noise ratio, leaving only the interface between cell and electrode to be modeled.[4] We demonstrate a modified circuit for the interface which captures all the extracellularly measured signal shape characteristics and show that previous models are inherently flawed. Furthermore, our micro-electrodes are augmented with nano-pillars (NEA)[4] that can cause spontaneous and/or stimulated electroporation of the cell membrane, allowing us to switch between extra- and intracellular recording. We further enhanced the circuit model to capture the poration of the membrane. This complete model allows the precise study of cell-membrane healing dynamics. We anticipate such quantitative and fundamental understanding of the cell-electrode junction to pave the way for novel, tailored and task-specific nano-engineered electrode surfaces.

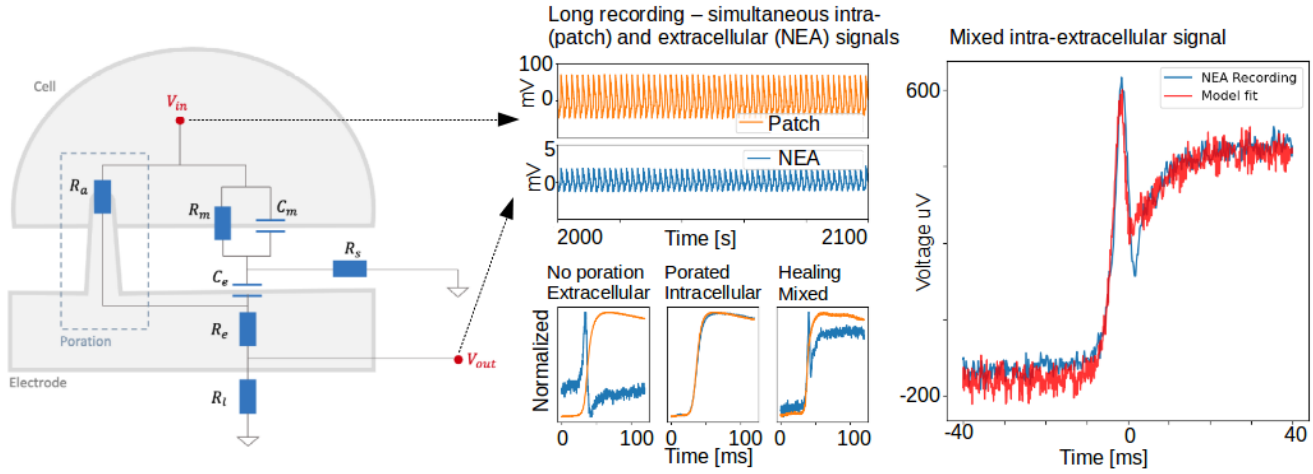

Figure 1: The proposed cell-electrode circuit (left) not only fits recordings better than previous models, it can capture poration events caused by nano-pillars. We can record both signals simultaneously (center) in this case from a cardiomyocite. The electrode-side recording (blue) exhibits a rich variety of signals – purely extracellular, intracellular (when the membrane is porated), and a mix of both intra- and extracellular when the membrane heals and closes up. We use the intracellular signal provided by patch-clamp to reproduce the electrode recording (right) and thus evaluate our model. The model successfully captures the entire variety of electrode-side signals.

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Advancing peripheral nerve interfaces

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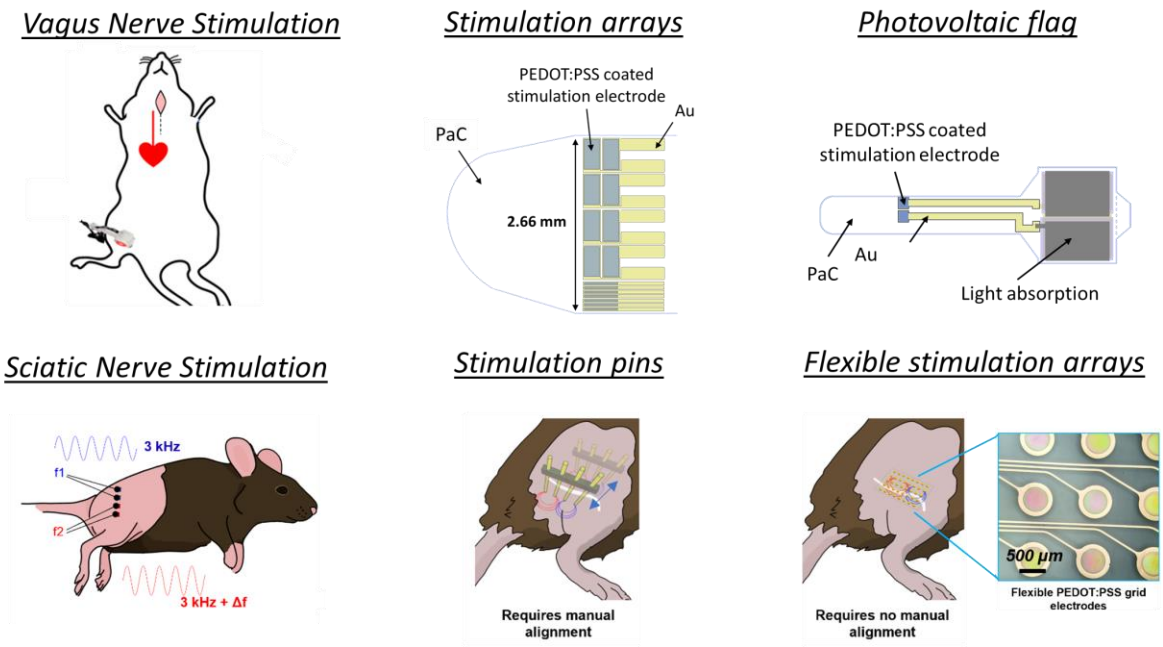
The informational density and relative accessibility of the peripheral nervous system make it an attractive site for therapeutic intervention. Although electrode-based electrophysiological interfaces for peripheral nerves have been under development for over half a century, achieving spatial specificity and minimally invasive devices remains challenging. The value in tackling this challenge lies in the possibility to treat disorders such as, for example, chronic pain, overactive bladder, depression, and epilepsy[1]. We aim to improve peripheral nerve interfaces through the use of wired flexible electrode arrays to optimize reduced invasiveness stimulation approaches. Examples include the use of the conformal arrays to intelligently design wireless optoelectronic devices for vagus nerve stimulation (Figure 1, upper panel) and to provide scannable electrode grids for focal stimulation from the surface of the skin through temporal interference (TI, lower panel)[2,3]. The material systems utilized are non-toxic and allow for intimate interaction with the nerve where implantable devices are of interest as well as conformal skin contact for surface arrays. The advancement of these methods not only contributes to the understanding of signaling pathways and disease treatment through peripheral nerve stimulation, but also holds clinical translation potential.



Ultrathin, flexible devices for peripheral nerve stimulation. Wired electrode arrays (upper, middle) are utilized to find optimal patterns and parameter for vagus nerve stimulation. This information is used to inform and design wireless optoelectronic stimulation devices (upper, right). Temporal interference (TI, lower left) is explored as a completely non-invasive neuromodulation method for the peripheral nervous system. Rudimentary pins (lower, middle) are used to test various conditions while conformable arrays (lower, right) allow optimal skin contact and parameter scanning to optimize stimulation.

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Large-scale organic electronics for epilepsy

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Analysis of neurophysiological signals is a cornerstone of epilepsy diagnosis and therapy. Deriving more usable information from neurophysiologic methods by increasing the scale and precision of recordings could benefit care of these patients, but current approaches to such monitoring are hampered by a trade-off of resolution and invasiveness. Our approach is to design and develop organic electronics that permit large-scale acquisition and manipulation of brain activity patterns to the resolution of individual neurons. Leveraging the conformability and volumetric capacitance of these materials, we have created devices that permit high spatiotemporal resolution interaction with *in vivo* neural networks. These devices enabled us to identify novel network properties in epilepsy models that are amenable to targeted therapeutic interventions. We have also been able to investigate pediatric epilepsies, an area in which investigation is typically limited due to the small size and fragility of the developing brain. Such studies have set the foundation for translation of these devices in human subjects, where we have characterized cortical microcircuits in patients undergoing neurosurgical procedures. Our results highlight the potential for organic electronics to safely enhance our ability to acquire, interpret, and modify neural signals, with the goal of facilitating discovery of biomarkers and therapies for patients with epilepsy and other neurological disorders.

Non-invasive Deep-brain Stimulation with Temporal Interference for the Suppression of Epileptic Biomarkers in Humans

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Neurostimulation applied from Deep Brain Stimulation (DBS) electrodes is a primary form of therapeutic intervention in patients suffering from surgically-intractable drug-resistant epilepsy, most notably in forms of mesio-temporal lobe epilepsy (MTLE). Inhibitory DBS stimulation to suppress seizures and associated epileptogenic biomarkers is performed with high-frequency stimulation (HFS), typically between 100–165Hz, targeting the Mesio-temporal lobe (MTL) where observed changes in brain rhythms, specifically in the hippocampus, include alterations in high-frequency oscillations (HFOs), namely increases in ripples and reductions in pathological Fast Ripples (FRs), and decreases in pathological interictal spikes (IRs). In the work here, we demonstrate the use of Temporal Interference stimulation to provide a non-invasive focal DBS (130 Hz) of the of the MTL, specifically the hippocampus, which increases physiological ripples, and decreases the number of FRs and IRs in both mouse models of epilepsy and in human patients. Similarly, we show the inability of traditional transcranial stimulation (TCS) to provide similar HFS results. The method could potentially revolutionize how DBS, certainly in epilepsy as results show the excellent penetration of TI into the human hippocampus compared to TCS.

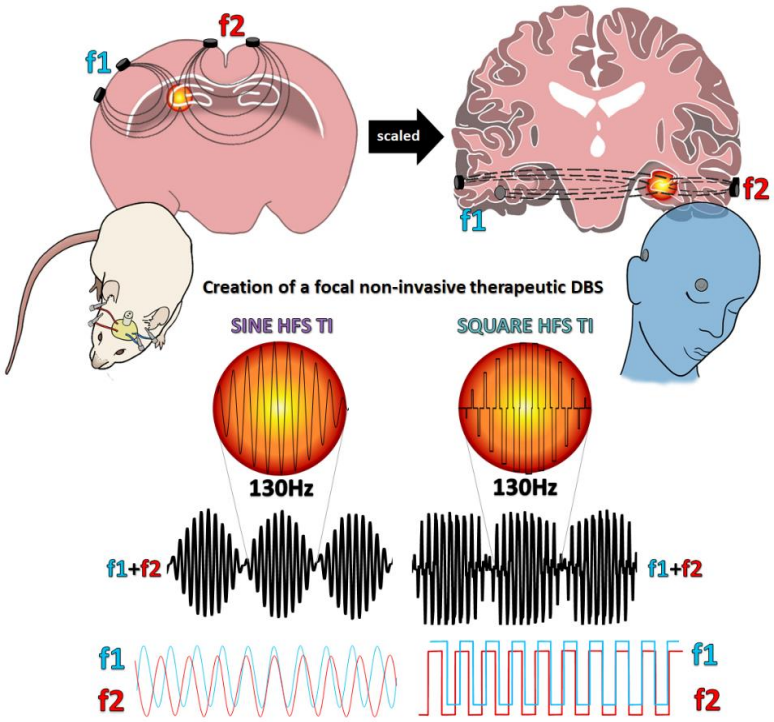

Figure 1: Forms of Temporal interference and ability to scale to larger subjects. Topical electrodes (2 pairs) were placed on the cortex of mice and transcutaneous on human cadavers and finally on human patients in order to investigate a minimally invasive DBS via TI. The target was to focally reach one side of the hippocampus. Coordinates of the 2 pairs were calculated to reach the target and to create a focal non-invasive stimulation. We demonstrated in this work that this stimulation, first tested on animals is totally compatible and scaled to human subjects. We include here the effect of the TI stimulation in both sine waves (as originally developed) and show the innovation of PWM TI (square waves) as a potentially more effective form of TI in the suppression of epileptogenic biomarkers.

Development of Tissue Engineered Bioelectronic Implants for the Nervous System

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Stable, long-term interfaces between implanted devices and the nervous system remain a challenge. One of the major issues inhibiting long-term recordings is the lack of integration between an implant and surrounding tissue.[1] Tissue engineered implants provide a solution to this problem through their superior integrative capacity.[2] Here, we discuss the design, development, and testing of a tissue engineered implant with an integrated microscale PEDOT:PSS-based recording system for monitoring the bioelectrical signaling.

We aimed to build a device that contains a series of discrete recording sites, based on previous designs,[3] within a tissue engineered gel (Fig, 1a-c). We initially examined the integrative capacity of these implants by placing them into the brains of rats (Fig. 1d). We also performed chronic electromyographic recordings from the musculature of rats (Fig. 1e). These results indicate excellent potential for integration of these implants into the body and for recording at the implantation site.

In this study, we have developed a tissue engineered neural implant to promote integration with surrounding tissue. Our next step will be to incorporate cells into these devices and move towards long-term recordings in the brain. Overall, we have generated a new type of neural implant, utilizing tissue engineering and bioelectronic principles, to enhance regeneration at the implantation site and enable long-term recordings of the nervous system.

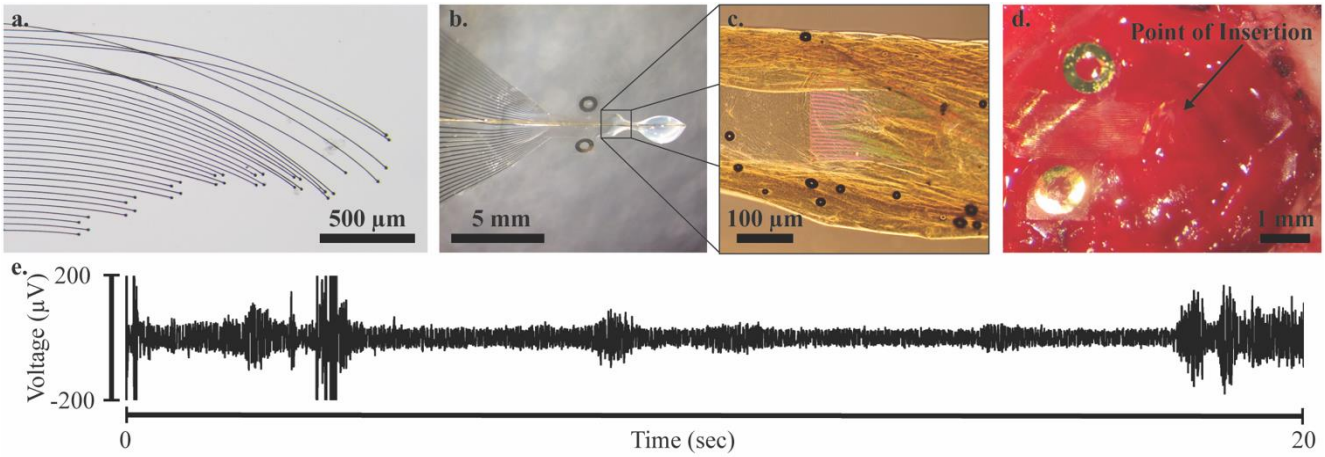

Figure 2. a. Optical image of microfabricated device for recording neural signals. Image shows individually articulated neural recording wires that each possess a cross-section of ~8 x 4 µm, terminated in conductive polymer-coated gold pads. **b.** Image of implant with tissue engineered probe body. **c.** Inset showing embedded device wires within tissue engineered gel. **d.** Image of tissue engineered, bioelectronic implant inserted into the sensory cortex of a rat. **e.** Electromyographic time trace recorded 24 hours after implantation of tissue engineered neural probe from dorsal musculature of rat.

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Design and application of high-density neuroelectronic interface for extracellular recording and stimulation of activities of primary auditory neurons cultured in-vitro

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Despite notable advances in recent decades in the field of cochlear implants (CI), the neuroanatomical gap has so far proved to be an insurmountable obstacle to further progress. Controlled growth and alignment of spiral ganglion neurons (SGN) stand out as one of the possible approaches to solving this problem, as previous studies have shown that the use of chips with a morphologically optimized surface structure containing micropillars can affect both growth and alignment of SGNs [1-2]. However, success in applying this approach requires a better understanding of behavior of SGNs in these conditions. For this purpose, we designed the neuroelectronic interface consisting of an operational amplifier, printed circuit boards (PCB) and silicon-based high-density electrode array with capability of simultaneously recording and stimulating 64 electrodes. Several in-vitro experiments in which the chips, previously coated with poly-L-ornithine and laminin, have been used as a substrate for growth of neonatal SGNs extracted from rat pups, were conducted. Bursts of spontaneous action potentials (AP) were recorded and neurophysiological footprint specific to SGNs was found as proof of presence and functionality of SGNs. Recording sensitivity and amplitude dependence with respect to distance between the electrodes were quantified after the single signal source detection was conducted. Cultures were successfully stimulated with different biphasic pulses of various amplitudes and phase durations. Acquired data were analyzed and visualized in custom MATLAB application. The results are discussed considering the potential use of the high-density stimulation-recording interface combined with graphene-based substrates and its application in cochlear implants.

Acknowledgments: "This research was partially supported under the project STIM – REI, Contract Number: KK.01.1.1.01.0003, a project funded by the European Union through the European Regional Development Fund – the Operational Programme Competitiveness and Cohesion 2014-2020 (KK.01.1.1.01)." BP has been fully supported by the "Young researchers' career development project – training of doctoral students" of the Croatian Science Foundation funded by the European Union from the European Social Fund.

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Towards an Ultrasonically Powered Efficient Multichannel Neurostimulator Implant

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During the last few decades, electrical neural stimulators have successfully been employed as a means of treatment for a wide range of neurological disorders. By targeting the peripheral and central nervous systems, electrical neurostimulators activate/inhibit neural activity by manipulating the stimulus-induced electric field arising at the targeted area through diverse electrode configurations. Aiming at reducing the overall size of implanted stimulator systems, these are being designed to be wirelessly powered and batteryless. Technologies for wireless power transfer to implants are mainly based on inductive coupling and, more recently, ultrasonic waves.

To increase the power efficiency and thereby minimize the power consumption of the implant, we have previously proposed a stimulation technique that alters the electric field at the tissue by delivering charge in small packets in a very rapid manner (e.g. 1 Mpps). This charge is consequently accumulated by the tissue's integrating nature.[1] This approach removes the need to ensure continuous accurate control of the stimulus current amplitude, resulting in power savings. To the same end, in an ultrasonically powered system, unnecessary power-conversion blocks can be eliminated, as the incoming ultrasonic wave is harvested, converted into an electrical signal, rectified, and then directly used for stimulation.[2]

Inspired by the above, this work focuses on providing a discrete-component multi-channel neural stimulator, powered wirelessly through ultrasound (US), to activate and inhibit neural activity. The use of mostly commercially available discrete components will ensure reproducibility by other research labs and help provide a platform technology that can be used as an experimental tool in a variety of applications.[3] The US pressure wave obtained at the receiving US transducer will be converted into electrical energy and used both for powering the system as well as to shape the charge packets of the eventual stimulus pulse. In this manner, the need for DC-DC up-conversion, typically needed for neuromodulation, will be eliminated.[4] A charge-balancing technique, matching the fast pulse repetition rates (PRR) required for inhibition of neural activity (typically ≥ 5 kHz), will ensure the safety of the implant, while the injected charge will be constantly monitored and adapted for safe and efficacious activation/inhibition.

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Real-time sensing in vascularized Organ-Chip models

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Organ-chips are microfluidic cell culture models that can recapitulate the complex functions of human organs *in vitro*[1]. We recently have shown how different Organ-Chips can be connected to accurately predict whole human body responses of drug uptake, distribution, and excretion as well as show drug responses in specific organs[2]. The improved mimic of the human in vivo environment makes this approach promising for drug development. However, the technology can also enable unpreceded monitoring of interactions between functional physiological units. We created a system of coupled Organ-Chips of the neurovascular unit, the blood vessels in the brain, and could with the help of off-line metabolomics and proteomics evaluation study previously unknown metabolic interactions[3]. To further improve mechanistic understanding of drug-tissue and tissue-tissue interactions, real-time assessment is key. In electroactive cells, mainly neurons and cardiomyocytes, we can monitor of extracellular field potentials for long-term, non-invasive assessment.

Moreover, for all barrier models, including endothelial and epithelial models, we can evaluate resistance across the cell layer is an essential metric. We have integrated barrier measurements (transendothelial resistance measurements) in our recent pluripotent cell-derived blood-brain-barrier (BBB) chip[4], and have now developed improved temporal resolution for bioelectric BBB monitoring[5]. We further monitored drug actions on beating frequency and blood-vessel integrity using metal electrode arrays in a Heart-chip model[6]. Our current models have limitations in detection sensitivity of bioelectric signals and long-term electrode-cell interaction. We have, therefore, taken a new approach for generating a new generation of organic electrochemical transistors (OECTs) for electroactive Organ-chips. We are using conducting polymer blends and chemical conjugation of cell-specific cues to combine high electronic/ionic conductivity, with a functional OECT/cell interface and long-time operation stability in physiological conditions.

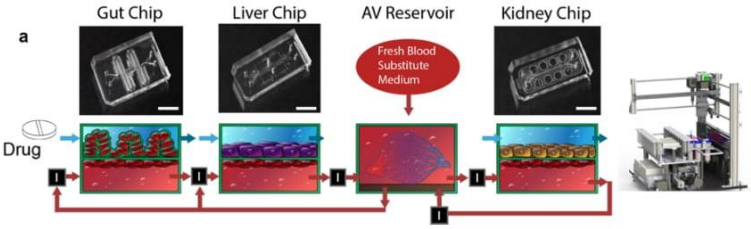

Fig. A linked 4 Organ Chip system for drug uptake, metabolism and excretion studies. "I" illustrates fluid transfer with the instrument to the right.[2]

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Screen Printed, Non-invasive Electrophysiology Probes for the Mouse Model

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Skin-compliant bioelectronics are finding many uses in human healthcare for long-term monitoring of physiological parameters. Their emergence can be attributed to advances in large-area manufacturing, such as screen printing, which enable incorporation of functional material into soft, flexible substrates which readily interface with the body. There is now an opportunity for using similar flexible sensors to interface with animals. Preclinical research utilising transgenic mouse models relies on electrophysiology monitoring, and has been instrumental in advancing our understanding of cardiovascular, muscular, and neurological disorders. However, current monitoring tools for mice are rigid and require invasive implantation. For example, the gold-standard for electrocardiogram (ECG) collection in free-moving mice requires surgical implantation of a device weighing up to 20% the animal's body weight, and demands a 2-week recovery period.

This work investigates flexible, on-skin sensors for mice, which could refine experimental design by progressing a non-invasive alternative for recording electrophysiology. We have designed three screen printed ECG probes. Two were fabricated by printing Ag/AgCl onto a 25 µm thick polyester substrate, attached to mouse skin using either conductive electrophysiology paste or an adhesive polyurethane (PU) backing; while a third variation was printed on temporary tattoo paper (**Fig 1**). Skin-contact impedance assessment showed at frequencies relevant to electrophysiology conductive paste electrodes exhibited substantially greater admittance (**Fig 2**). Models created using frequency sweep data suggest this is the result of a resistive connection facilitated by the paste, contrasting with the capacitive connection modelled in the other probes. Furthermore, assessment of ECG signals collected from unconscious mice found all probe designs capable of acquiring signals with discernible waveform features (P-Q-R-S-J), with PU backed electrodes showing the greatest signal-to-noise-ratio (37–39.2 dB), outperformed only by the implanted electrode gold-standard (39.9–42.3 dB) (**Fig 3**). This research indicates flexible, capacitively coupled electrodes may offer a viable means of recording ECG in mice. However, future experiments must investigate sensor durability when attached to free-moving animals, and explore solutions to challenges including fur regrowth and grooming behaviour.

Fig 1. Schematics and images of screen printed ECG electrodes on mouse skin

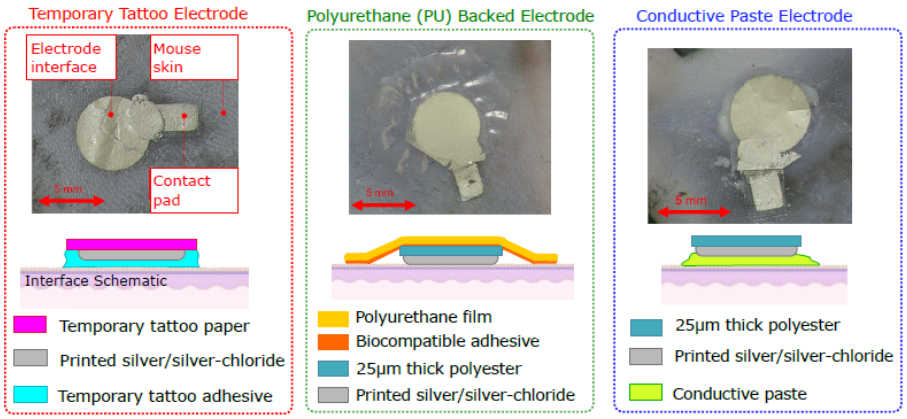


Fig 2. Probe impedance charicterisation and modelling

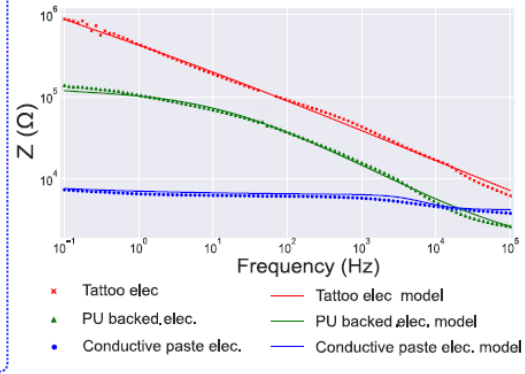
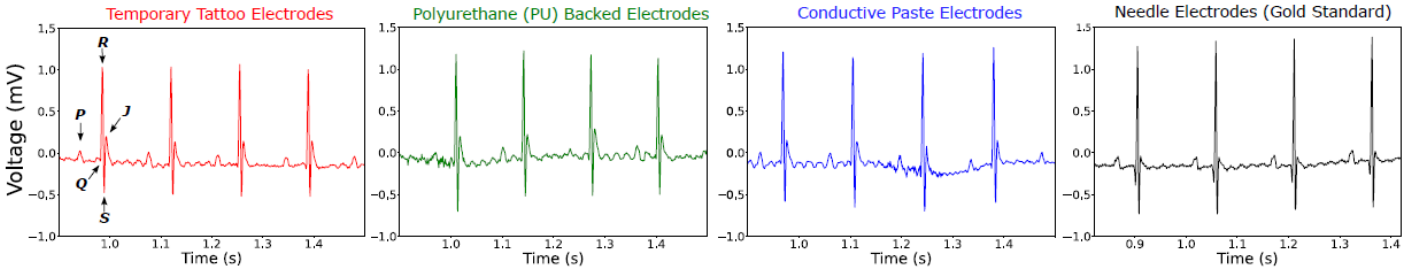


Fig 3. Mouse electrocardiograms acquired using printed interfaces



Drug Delivery towards *in vivo* Brain Tumors using Chemotherapeutic Ion Pumps

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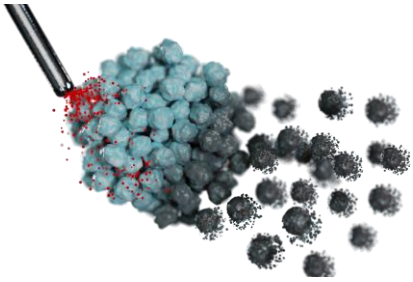
Poor delivery and systemic toxicity of many chemotherapeutic agents limit their therapeutic success in cancer treatment. Local chemotherapy approaches offer a new path to efficiently interfere with cancer growth and reduce tumor size, especially in the case of brain tumors.
We present miniature devices for iontronic drug delivery able to administer chemotherapeutics via electric control with high spatiotemporal precision.[1] Incorporated in these devices are anionic hyperbranched polyglycerol membranes (AHPGs), forming an ion selective matrix of multiple fixed negative charges.[2] Through this polymeric ion exchange membrane, drugs electromigrate in an electric field towards a target of choice.
These bioelectronic devices, called chemotherapeutic ion pumps (chemoIPs) used for the delivery of chemotherapeutics and their performance were characterized and tested in different brain tumor models with increasing complexity (cell culture and different *in vivo* models). Treatment efficiency is analyzed based on cell death, tumor suppression and drug distribution.
AHPG ion exchange membranes enable drug delivery with pmol*min^{-1} delivery precision at currents in the nano-ampere range. The further application of this electrical and temporal control was shown in brain tumor cell culture, triggering the disintegration of targeted tumor spheroids among chemoIP treatment. Gem furthermore triggers cellular effects suitable for the application in the brain: it effectively kills brain tumor cells and is at the same time harmless to neurons and astrocytes. Additionally, we show preliminary results indicating that chemoIP treatment significantly induces cell death in *in vivo* brain tumors.
The here exemplified electrically-driven drug delivery via chemoIPs is a drug administration method that can serve as basis for further implant development, which has the potential to increase the efficacy of chemotherapy due to highly-targeted and locally-controlled drug delivery.



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Hybrid fabrication of multimodal intracranial implants for electrophysiology and local drug delivery

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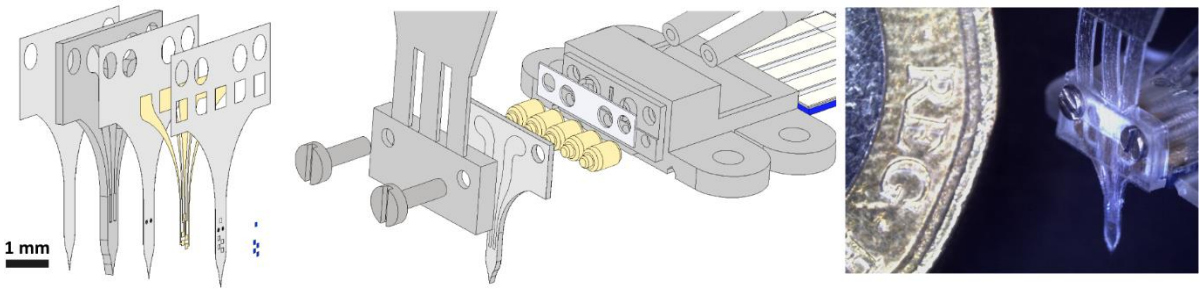
New fabrication approaches for mechanically flexible materials hold the key to advancing the applications of bioelectronics in fundamental neuroscience and the clinic. By combining the high precision of microfabrication of a thin-film bioelectronic array with the versatility of additive manufacturing of microfluidic systems, we are showing a new, straight-forward approach for the fabrication of intracranial probes capable of multichannel local field recordings and convection-enhanced drug delivery. The process makes use of state-of-the-art parylene-based device architecture and a commercial VAT polymerizable elastomer, combining to produce a flexible implant. The mechanical and electrical properties of the implant are characterised, and its function is validated in an in vivo rodent model. We show that the implant can pharmaceutically modulate neuronal activity in the hippocampus through local drug delivery, while simultaneously recording local field potentials by its electrodes. Chronic implantation tests show good implant stability and minimal tissue response one-week post-implantation. Our work shows the potential of hybrid neuronal probes combining different manufacturing technologies – lithography-based printing and thin-film microfabrication – and paves the way for a new approach to multimodal probes combining the capabilities of both.



The figure shows an explosion schemata of the hybrid fabricated device, its heterogeneous integration, and a microscopic picture of the final device with a one-pound coin for scale.

The Dark Side of the Spine: Using Flexible Bioelectronics to Interface with the Spinal Cord

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Implantable bioelectronic devices for diagnosing and treating disease are emerging as a prominent component of modern healthcare. Within this field of therapy, the spinal cord offers an interesting target as the primary bi-directional information highway between the brain and the rest of the body. However, there remains several technical and clinical barriers within the development of new tools to interface with the central nervous system (CNS). Overcoming these barriers could improve the lives of people suffering from conditions such as Parkinson's[1], chronic pain[2], and paralysis[3] as well enabling better neuroscientific research, diagnostics, and prognostics.

In this work, we present thin, flexible, and shape adaptive implants based on the conductive polymer PEDOT:PSS which can be used to interface with the CNS. The devices are fabricated from biocompatible materials such as parylene-C/silicone and use conductive metals and polymers to stimulate and sense the tracks of the spinal cord. These devices are fabricated using scalable manufacturing techniques to create conformable interfaces up to 100 times thinner than commercially available spinal cord devices. This allows larger coverage than previously possible, whilst minimizing surgical risk during implantation. To validate the surgical implantation of these devices they have been tested within a human cadaver model, with utility-based studies explored in vivo.
After showing our technology can be used as a minimally invasive interface for conventional spinal cord stimulation[4], we are now aiming to elucidate the 'dark' side of the spinal cord, where the majority of motor information lies. Taking these recording devices 360° around the spinal cord, has proven to be a fascinating tool in describing the spatial and temporal arrangements of spinal networks and may offer therapeutic benefits in both prognostics and SCI therapy.

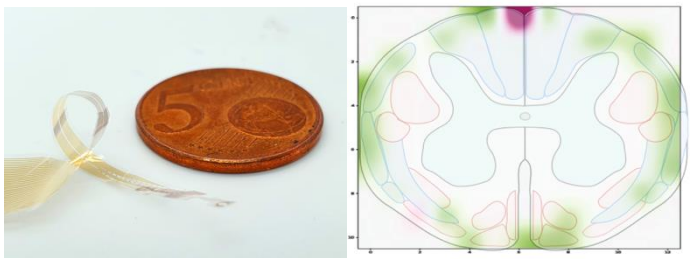

Figure 1: Left, a 360-degree spinal device. Right, representative spatiotemporal data derived from a rodent spinal cord.

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Development of semiconducting polymers for organic bioelectronics

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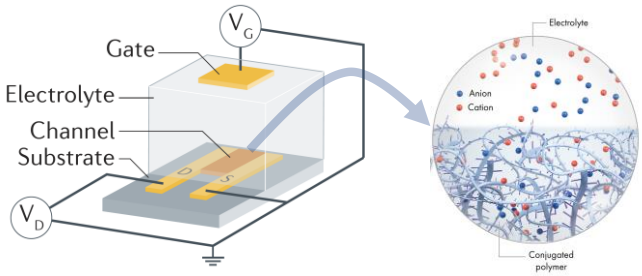
Organic electrochemical transistors (OECTs) have been shown to be promising devices for amplification of electrical signals and selective sensing of ions and biologically important molecules in an aqueous environment, and thus have potential to be utilised in bioelectronic applications. The sensitivity, selectivity and intensity of the response of this device is determined by the organic semiconducting polymer employed as the active layer. This work presents the design of new organic semiconducting materials which demonstrate good OECT performance, through operation in accumulation mode, with high transconductance and low operating voltage.

We discuss here the design, synthesis and performance of novel intrinsic semiconducting polymers for efficient accumulation mode OECT devices. Key aspects such as ion and charge transport in the bulk semiconductor and operational voltage and stability of the devices are addressed in order to elucidate important structure-property relationships. A range of new semiconducting polymers, designed to exhibit facile electrochemical doping of either holes or electrons, facilitate ion penetration and migration, as well as have aqueous compatibility are reported. Optimisation of a series of polymer parameters including electrochemical doping, charge carrier mobility and capacitance are discussed.



a) Schematic representation of an OECT with source (S), drain (D) and gate (G) electrodes (top),b) ionic charge movement into open microstructure of an OECT

Multifunctional Conducting Polymer Scaffolds for Human Stem Cell Cultures

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Stem cell research has benefitted enormously from a variety of different materials used to create scaffolds to grow stem cells in 3D, however, only few studies report the development of structures that are able to recapitulate 3D tissue-like environments and exhibit multifunctional properties (i.e. electrical and optical). Three-dimensional organic bioelectronic devices proposed to bridge the dimensionality mismatch between 2D/static electronics and 3D/dynamic biology, comprising a versatile platform for hosting and monitoring cells. These devices take advantage of the soft mechanical properties, and mixed conduction properties of conjugated polymers (CPs) and enabled the realization of highly biomimetic, electro-active interfaces. Here we show the development of 3D, multifunctional polymer composite structures that exhibit good electrical conductivity, photo-sensitivity and mechanical properties compatible with human tissue. Water-based solution mixtures of PEDOT:PSS, polythiophene and a polyethylene glycol-based crosslinker are freeze dried and 3D scaffolds with an average pores size of 50 μm are realized. These structures exhibit lower Young's modulus and higher water swelling capacity compared with pure PEDOT:PSS scaffolds cross-linked with GOPS. Scaffold slices with different thicknesses ranging between 100 μm - 400 μm are attached on flat ITO substrates and their (photo) electrochemical properties are evaluated with electrochemical impedance spectroscopy (EIS) and photo-amperometry. These multifunctional platforms are used to host 3D human adipose derived stem cells (hADCs) and to monitor their proliferation in situ with both EIS and fluorescence microscopy. Differentiation of the 3D hADCs cultures to neurons is attempted via chemical, electrical and light stimulation by leveraging the scaffold's multifunctional properties.

Heater integrated in a lab-on-chip device

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The increasing need to reduce the cost and time consumption of laboratory testing leads to a need of integrating all the necessary tools into the smallest systems, which directly translates into fewer materials. One current problem in testing is that several biological reactions such as LAMP (loop mediated isothermal amplification) or PCR need a heating step.

We present as a solution for this problem an integrated heating system on a lab-on-chip device (LOC). Our heater is UV-imprinted on top of a polymer foil based microfluidic chip where DNA amplification by LAMP and multiplex DNA detection is implemented (see **Figure 1 left**). The polymer chips are produced in our unique Roll-to-Roll (R2R) UV-NIL pilot line for high throughput production of microfluidic systems.[1,2] The heater consists of a structure with v-lines (10µm wide, 15µm deep and 220µm of distance between lines) that are filled with silver ink, which as a metal is an electrical conductor. The heating layers production consists in a 8 step process: cleaning of the substrate plasma treatment to activate the surface for the following imprinting, another plasma treatment for activation for the capillary based filling process with silver ink, heating during 30 min at 120°C in a heating plate for ink drying, solvent evaporation in a vacuum oven for 3 hours at 120°C, and finally, the sample is sintered for homogenization of ink particles. During the heating experiments, they are subjected to a voltage power, which generates a current depending on the layer resistance. This electrical energy is transformed to thermal energy reproducing the physical effect of Joule heating. The experimental temperatures have been controlled by an IR Thermal Camera for the heating layer, and a thermometer connected to a thermocouple for the in-chip liquid, finding a difference of maximum 5°C between both. The stability of the samples can be assured over prolonged heating (60°C over 40 min, requirements for LAMP reaction) and even at higher range temperatures (100-200ºC). By other hand, a screen printing approach of different heating structures have been considered using a two layer printing process of a carbon thermal ink over a silver ink heating structure (see **Figure 1 right**).

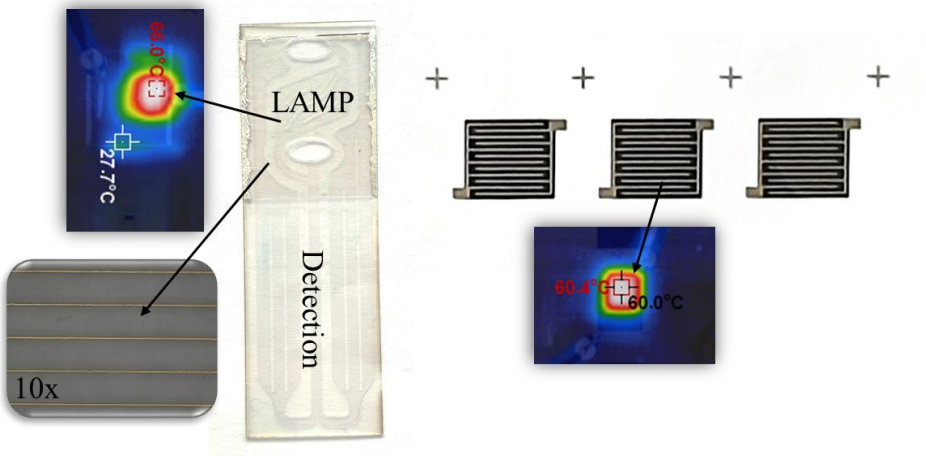

***Figure 1**. On the left, a LOC device with an integrated heater is shown, with the thermal image obtained by the IR camera during the experiments and a microscope picture of the filled v-lines. Indicated the position for LAMP and detection on chip. On the right, a sample of one of the heating structures considered for screen-printing with its thermal image is presented.*

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Technology for interacting with the brain the way the brain interacts with itself

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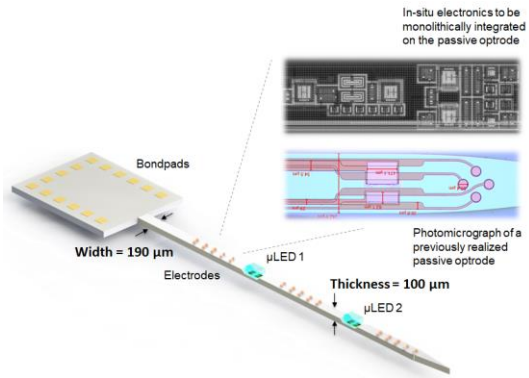
In order to better understand the brain and better treat brain disorders, it needs to be neuromodulated by means of 'brain-like' waveforms[1]. Moreover, these waveforms need to be applied in a smart way, based on feedback and in a closed-loop fashion. This requires sensing technology, not only for reading the electro-chemical signaling of the brain itself but also of other physiological parameters. Additionally, this feedback needs to be self-learning so it can learn to recognize the (personal) brain activity and connectivity characteristics that characterize a symptom and the intensity of a symptom. It then selects an optimal stimulation design to normalize the symptom by increasing or decreasing connectivity to change the network structure. Finally, it can predict symptoms to prevent relapses of chronic disease states[1].

The above needs are still far away from the state of the art. State-of-the-art neuromodulation is almost exclusively done using tonic rectangular pulses, at a single stimulation site, often not based on any form of feedback from the brain itself, and never self-learning. State-of-the-art technology for neural recording is not able to record the infraslow waves that modulate and thereby synchronize the more local brain activity, and, as it is either acquired from passive electrode arrays or from CMOS-based probes, is not able to reveal the brain's small-world emergent network behavior[2].

Innovative technology for both neuroscience (leading to a better understanding of the brain) and neuromodulation (leading to better treatment of brain disorders) should thus: 1. cover large parts of the brain for recording (reading) and stimulation (writing) and thus make use of flexible, stretchable arrays; 2. be minimally invasive; 3. excite or inhibit multiple neurons in various regions of the brain accurately (viz. with high spatiotemporal resolution), 4. with precisely controlled degrees of synchronicity amongst recorded or stimulated neural elements, 5. with more 'brain-like' stimulation patterns, such as noise, burst, infraslow waves, preferably using neuromorphic devices and self-learning interfaces (see figure), 6. by means of electrical, optogenetic, or other (e.g. ultrasound) neuromodulation (see figure); 7. record from, and stimulate, larger populations of neurons or assemblies than was hitherto possible; 8. do so in a 'brain-like' fashion that reduces data, but preserves information for self-learning and closed-loop control; and 9. last ideally forever, and thus be adaptive, upgradable, biocompatible, and biostable.

This talk will address how these 'bioelectronic medicines' can do this, what they will look like, and which future microfabrication and circuit and system developments are needed to make them a reality.



The layout of a microfabricated active optrode for neuroscientific research that allows for multi-site optogenetic neuromodulation and wide-bandwidth electrical recording. Credits: Ronaldo da Ponte, TU Delft. Not yet published.

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Functional organic neuromorphics biointerfaces

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The interface between biological cells and non-biological materials has profound influences on cellular activities, chronic tissue responses, and ultimately the success of medical implants and bioelectronic devices. The optimal coupling between cells and materials is mainly based on surface interaction, electrical communication and sensing[1].
In the last years, many efforts have been devoted to engineer materials to recapitulate both the environment (i.e., dimensionality, curvature, dynamicity)[2] and the functionalities (i.e., long and short term synaptic plasticity)[3] of the neuronal tissue to ensure a better integration of the bioelectronic platform and cells. In this scenario, resembling the operation and the composition of the neuronal membrane might be beneficial to reconstitute synaptic proteins' arrangement (i.e. synaptic receptors) and electronic functionalities to further optimize the communication between neuronal cells and *in vitro* bioelectronic platforms[2].
Here, we explore how organic neuromorphic devices and supported lipid bilayers (SLBs) can recapitulate short and long term plasticity in biohybrid synapses.
Through the neurotransmitters' oxidation (i.e. catecholamines) first, we were capable of modulating the synaptic potentiation and ultimately the coupling with biological cells to form a functional synapse.
and artificial membranes we were capable of tuning the response of a neuromorphic platform to modulate its conductance over time. Then, we functionalized the organic neuromorphic transistor with a supported lipid bilayer and investigated how the PEDOT:PSS – artificial membrane interface can affect the ion flow and thus the device's conductance over time and the short term plasticity of the biohybrid synapse.
In turn, this could represent a first step toward *in vitro* adaptive neurohybrid interfaces to engineering neuronal networks with biomimetic structural and functional connections at synaptic level.

Implantable iontronic nerve-cuff therapy for peripheral pain

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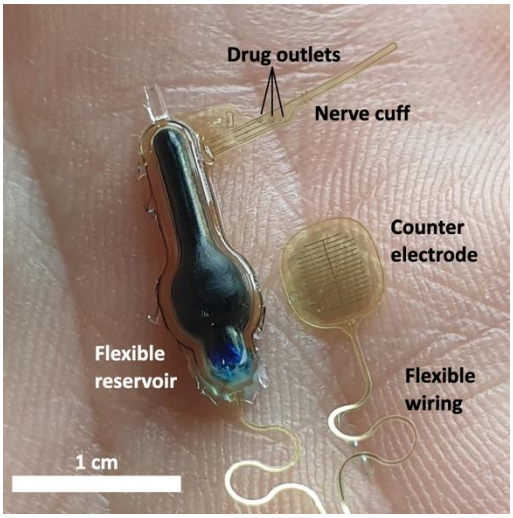
Over the past decade, our group has developed so-called iontronic drug delivery, most notably exemplified by the organic electronic ion pump (OEIP).[1] OEIPs and other such iontronics convert electronic addressing signals into the electrophoretic transport of charged substances (*e.g.*, analgesic molecules, neurotransmitters) from a liquid or gel reservoir through thin organic polymer films out to a target region (*e.g.*, tissue, cells) – without requiring liquid flow.

Following our successful demonstration of pain therapy by delivery of the neurotransmitter GABA to the spinal cord in rats[2], we have been pursuing fully implantable devices for applications in peripheral pain. We strongly believe that approaching pain applications first in the periphery provides a much more straightforward path for translation to the clinic and helping patients in need.

In this presentation, I will detail our efforts in this direction. Specifically, I will discuss our development of a nerve-cuff ion pump (including customized materials[3] and processes[4] for implant stability), our *in vitro* demonstrations of delivery of the pharmaceutical analgesic bupivacaine, our *in vivo* "well-being tests" to ascertain minimum side-effects of the implantation, and of course our progress with *in vivo* demonstrations of peripheral pain therapy. I'll conclude with our path forward and lessons-learned in these first stages of translation to the clinic.



Example of the implantable nerve-cuff ion pump designed for implant in the thigh (sciatic nerve). The flexible reservoir comprises 3D-printed/molded features to encapsulate the driving electrode and drug solution (the dark color here is a dye to indicate potential leakage). The nerve-cuff itself features three parallel outlets to maximize delivery to the nerve (which would be positioned directly above the outlets).

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Textile organic biosensors for advanced wearable healthcare

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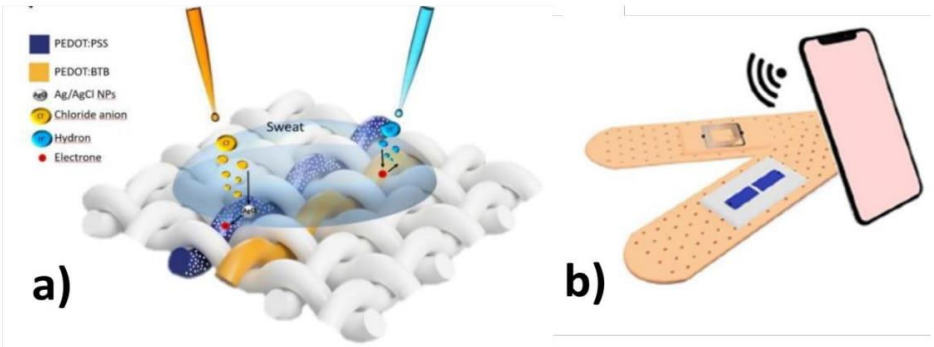
Real-time and non-invasive monitoring of physiological and biological parameters by means of wearable bio-sensors holds great promise for next-generation technologies in personalised healthcare. However, such emerging applications pose several constraints to conventional sensors, as conformability, robustness and simple architecture stand out as essential requirements. It has been recently demonstrated that by combining specific and sensible materials integrated directly into textiles it is possible to successfully design, realise, and develop new ideas for textile chemical and physical sensors. Here, the main recent findings on textile human-sweat and wound sensors, pressure and X-ray sensors are presented.
As chemical sensors, selective OECT-based sensors (requiring low operating voltage (<1V) and providing intrinsic signal amplification) are discussed for the detection of relevant analytes in sweat (dopamine, adrenaline, glucose, lactic acid, etc)., thanks to a selective functionalization of the active layer based on the conducting polymer PEDOT:PSS (poly(3,4-ethylenedioxythiophene) poly(styrene sulfonate). Smart band-aid devices are presented, able to detect in real time the moisture level and pH value of a bed wound, allowing the remote monitoring of the healing process of severe and chronic wounds. Finally, physical sensors will be discussed, used to monitor the pressure distribution for rehabilitation, workplace safety, or sport tracking, together with a novel wearable fully-textile device able to measure the incident X-ray dose for medical or security applications.



a) Single thread textile sensors for the selective detection of Cl- and pH in sweat; b) wound-moisture sensor with remote readout

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Transitioning From the Outside to Inside: Reducing Power and Redesigning Sensors to Move from Wearables to Insertable/Implantable Systems

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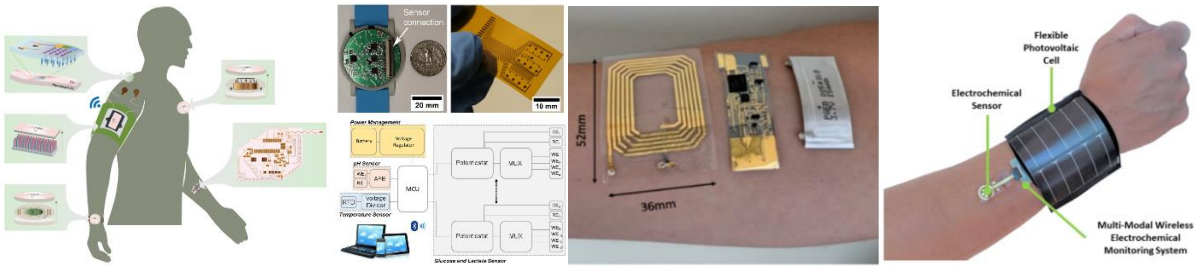
To date, wearable health monitors for sensing electrical, photonic, and mechanical bio-signals have matured into viable commercial technologies; furthermore, the necessary technologies for realizing wearable electrochemical sensing are just beginning translation into demonstrable integrated systems. With the National Science Foundation's Center for Advanced Self-Powered Systems of Integrated Sensors and Technologies (ASSIST), we have developed and tested integrated multimodal systems for the deployment of both electrochemical and photonic sensing on a wearable platform. The integrated multimodal systems include the necessary potentiostats and optoelectronics instrumentation to operate a suite of sensors (see Figure for examples) [1-3]. As these systems mature, we have started the design transition to insertable/implantable systems. Whereas biopotential and biophotonic signals can be suitably recorded with completely passive wearables, biochemical signals have limited availability using non-invasive methods. In this presentation, I will introduce the importance of transitioning to insertable/implantable for contemporaneously collecting both biochemical and biophysical signals, discuss the challenges associated with integrating biochemical sensors into insertables/implantables, and present the necessary design, fabrication, and performance changes necessary to realize bioelectronic for insertable/implantable monitoring of human health and/or performance. First, I will present our systems for low-power, and potentially self-powered, electrochemical measurements. Nest, I will present our system for decoupling the sensing *wetware* and bioelectronic hardware for insertables. Lastly, I will conclude by presenting opportunities for future integration of both transdermal biofluid sample collection and transdermal sensing probes to evaluate the interstitial space and connect biochemical and biophotonic measures of health and performance.



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Sustainable soft electronic and robotic systems

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We are increasingly supported by and depend on a wide range of electronic and robotic appliances, with an ever more intimate integration of the digital and biological spheres. These advances however often negatively impact our ecosystem, with growing demands on energy, contributions to greenhouse gas emissions and environmental pollution. Mitigating these adverse effects is amongst the grand challenges of our society and at the forefront of materials research. The currently emerging forms of soft, biologically inspired electronics and robotics have the unique potential of becoming not only like their natural antitypes in performance and capabilities, but also in terms of their ecological footprint.

This talk introduces materials and methods or soft systems that facilitate a broad range of applications, from transient wearable electronics to metabolizable soft robots. These biogel-based embodiments are highly stretchable, are able to heal and are resistant to dehydration. Our forms of soft electronics and robots are designed for prolonged operation in ambient conditions without fatigue, but fully degrade after use through biological triggers. Electronic skins provide sensory feedback such as pressure, strain, temperature and humidity sensing. Recent advances in 3D printing of biodegradable hydrogels enables omnidirectional soft robots with multifaceted sensing abilities (Figure 1). Pushing the boundaries further, design concepts for fast actuation in soft robotics systems, from exploiting mechanical instabilities to leveraging magnetic interactions on the millimeter to centimeter scale are introduced. Applications range from safe machine-assisted working environments to using soft materials in environmentally friendly cooling systems that exploit the giant elastocaloric crystallization effect.

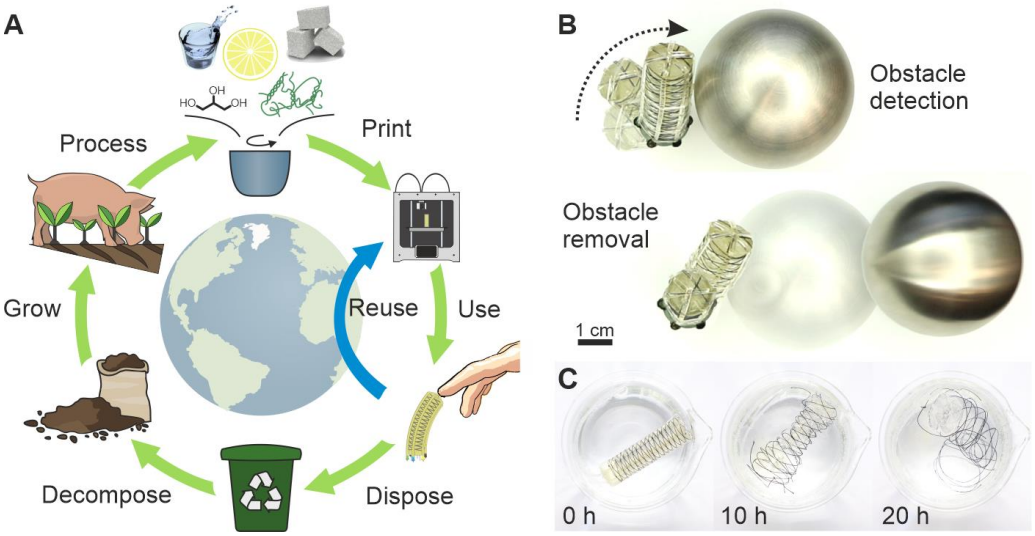

Figure 1: (A) Sustainability in materials for soft electronics and robotics. (B) Soft, sensor-clad multi degree of freedom actuator capable of detecting and removing obstacles.

Glycosaminoglycan-PEDOT conductive hydrogels for iPSC-derived cardiomyocyte implantation and electrical coupling

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Recent advances in the production of human cardiomyocyte surrogates, particularly those derived from induced-pluripotent stem cells (iPSC-cardiomyocytes), represented a game changer in the field of cardiac cell therapy, showing potential reduction in cardiomyocyte apoptosis and infarct size in pre-clinical infarct models. Although iPSC-cardiomyocyte closely resemble the physiology and function of mature cardiomyocytes, their electromechanical activity is still rudimentary, leading to absent cardiac coupling with the heart tissue and/or graft-induced arrythmia in pre-clinical studies. Previously, conductive materials have been used to attempt electrical coupling. Although there is no evidence yet for electrical coupling with cardiomyocytes, these have shown to interact electrically with the myocardial tissue. Also, their limited mechanical performance, biocompatibility and electrical stability need to be addressed to make these materials a feasible clinical approach for cardiac repair. We have aimed to achieve cardiomyocyte electrical coupling using conductive hydrogels as scaffolds for iPSC-cardiomyocyte implantation. A series of glycosaminoglycan (GAG)-PEDOT hydrogels have been fabricated using imine-based crosslinking. Results showed that GAG-PEDOT biomaterials inherit the mechanical properties of GAGs, while PEDOT confers optical and electric properties to the material. The hydrogels precursors are soluble in aqueous solutions, which render them injectable, and gel once implanted into tissues within minutes after mixing. This also makes them suitable for 3D printing, using a gelatin supporting matrix to extend the time of printing. Gelation kinetics and stiffness of the hydrogels can be controlled with polymer concentration, with higher concentrations resulting in short gelation time and higher stiffness. Electrochemical studies showed that GAG-PEDOT biomaterials exhibit both ionic and electric conductivity, in which ionic conductivity is the predominant in the hydrogel. Electrical conductivity on non-conductive hydrogels was not detected. Compared to non-conductive hydrogels, the impedance and resistivity of conductive hydrogels is significantly lower. The conductivity of GAG-PEDOT hydrogels in culture conditions is stable at least 12 days. The biomaterial showed to preserve the viability of iPSC-CM both seeded as monolayers and encapsulated within the hydrogel matrix. Similarly, we observed preserved cardiac tissue quality and structure after intramyocardial injection of the hydrogel, demonstrating biocompatibility. Electrostimulation of cardiomyocytes was successfully achieved, reaching synchronous pacing in all cardiomyocytes, with no arrhythmic events observed. Therefore, these results demonstrate the potential of GAG-PEDOT hydrogels for cardiomyocyte implantation and electrical coupling with the host myocardium, which is currently being under investigation.

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Portable device for heart failure biomarkers home monitoring

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The medical progress of the last century has led to a chronicisation of formerly fatal acute diseases. Thus, life expectancy is constantly increasing, but life expectancy in good health tends to stagnate. This evolution reflects the progression of chronic diseases and their prevalence.

Biomarkers and diagnostic tests are key elements in the identification of a disease or a pathogenic process, in the prediction and monitoring of patients during their treatment, and in the measurement of their response to a therapy. It is estimated that 70% of medical decisions require a biological act, such as a blood test. However, these tests are limited by their response time, from the taking of the sample to the return of the result, and by a cost that has stabilized at a relatively high level, incompatible with a generalization of coverage that would make it possible to rebalance progress in terms of quality of life versus longevity. This makes them incompatible with urgent decision making and regular patient follow-up, whether in private medical practices, at home, in rural areas, etc.

Omini uses electrochemical sensor technology to develop portable blood analysis devices for home monitoring of heart failure (HF) patients. Indeed, heart failure is the only cardiovascular disease whose incidence is increasing every year. Currently 26 million patients worldwide, out of the 423 million with cardiovascular disease, are suffering from the heart failure.[1] To tackle the problem of HF patients monitoring, Omini is developing the first affordable system for home monitoring that contains:

- Single-use strip with 4 sensors capable of detecting key HF biomarkers: sodium, potassium, creatinine and NT-pro-BNP
- Connected strip reader
- Online platform to send the results immediately to a healthcare professional.[2]

Our team has already developed the proof of concept for each of the four biomarkers. We are able to simultaneously measure the physical concentration of each biomarker in a whole blood human sample using Omini system.

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2. https://www.ominilabs.com/

Biowires of Cable Bacteria

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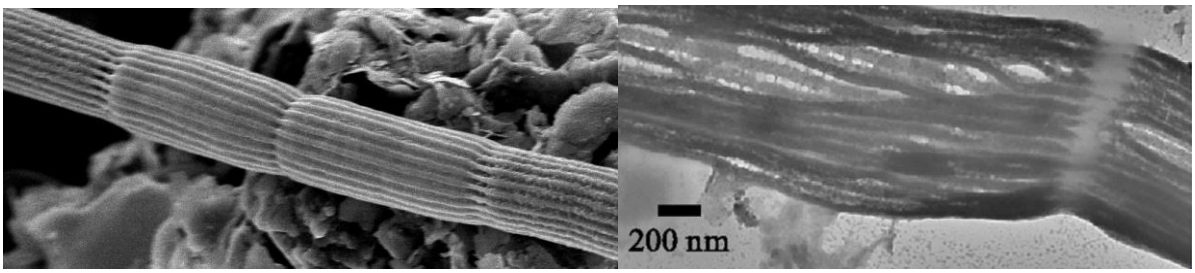
Cable bacteria are filamentous bacteria that can conduct electrons from one end to the other. They are typically centimeters long filaments with thousands of cells in a chain and 15-73 parallel wires running uninterrupted within common periplasmic channels [1] [2]. They live by oxidizing sulfide in anoxic aquatic sediments and conduct the derived electrons to oxygen at the sediment surface [1] [2]. Recent studies have found the conductivity of cable bacteria wires to match the very best synthetic organic conductors [3]. The exceptional integration of electron conductors and biochemical processes evolved in cable bacteria through millions of years readily suggest applicability in bioelectronics including biosensors and tissue-electronics interfaces. Both PilA, additional proteins, metals and carbohydrates seem essential for the function of cable bacteria biowires but the assemblage and the actual mode of electron transport in is yet to be settled. [2,4].



Cable bacteria biowires. Left: Four intact cells of a cable bacterium with periplasmic wires running uninterrupted beneath the distinct ridges of the outer envelope. Right:Wires stripped from membranes and cytoplasm retain structure, conductivity, and electrocatalytic properties.

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Carbon Nanotube Uptake in Photosynthetic Bacteria for Near-infrared Imaging and Enhancing Bioelectricity Generation in Living Photovoltaics

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The distinctive properties of single-walled carbon nanotubes (SWCNTs) have inspired the development of many novel applications in the field of cell nanobiotechnology. However, studies thus far have not yet explored the effect of SWCNT functionalization on transport across the cell walls of prokaryotes. We explore the uptake of SWCNTs in Gram-negative cyanobacteria and demonstrate a passive length-dependent and selective internalization of SWCNTs decorated with positively charged biomolecules. Notably, we show that lysozyme-coated SWCNTs (LSZ-SWCNTs) spontaneously penetrate the cell walls of both unicellular Synechocystis sp. PCC 6803 and filamentous Nostoc sp. strains, with measured SWCNT adsorption and internalization rate constants of kads = (1.26 ± 0.02) x 106 s-1 M-1 and kin = (1.466 ± 0.011) x 10-4 s-1, respectively, for the wildtype Synechocystis cells. A custom-built, spinning-disc confocal microscope was used to image the distinct near-infrared (NIR) SWCNT fluorescence within the autofluorescent cells, revealing a highly inhomogeneous distribution of SWCNTs. Real-time NIR monitoring of cell growth and division further reveal that the SWCNTs are inherited by daughter cells. Moreover, these nanobionic living cells showed retained photosynthetic activity and an improved photo-exoelectrogenicity (up to ~15-fold enhancement) when incorporated into bioelectrochemical devices.

Protamine-Controlled Reversible DNA Packaging: A Molecular Glue

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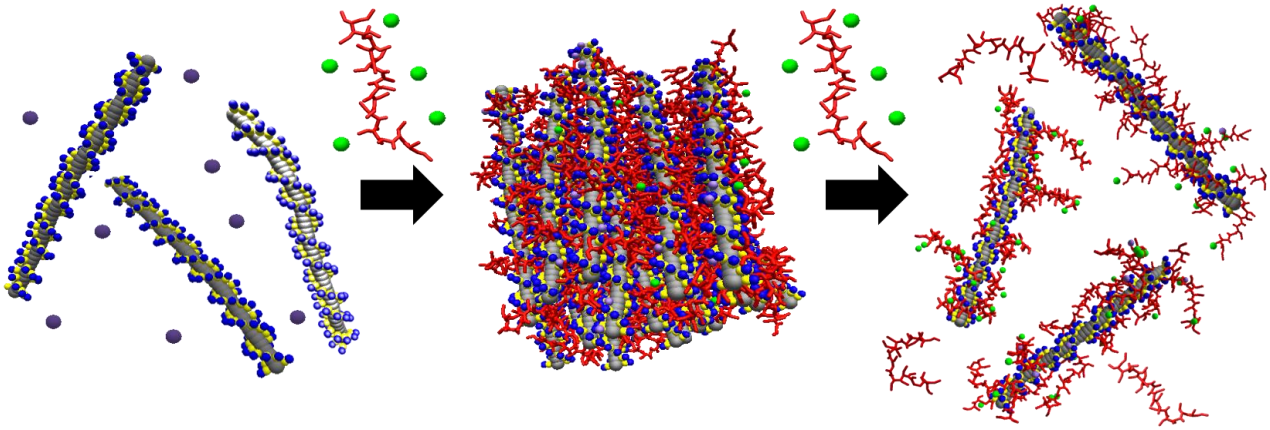
While DNA is one of the longest and the stiffest molecules in nature and is negatively charged, they are strongly condensed in a tiny space of cell nuclei. DNA undergo precise cycles of even stronger condensation and de-condensation during cell division or in sperm cells. Packaging paternal genome into tiny sperm nuclei during spermatogenesis requires 10^6-fold compaction of DNA, corresponding to a 10-20 times higher compaction than in somatic cells. Understanding and simulating the molecular-level principles underlying such fascinating and dynamic processes would not only bring us one step closer to the origin of life but also have applications in various other fields such as medicine, materials, and energy. However, while protamine, a small arginine-rich basic protein, is known to participate in such a high level of compaction, the precise mechanism at play is still unclear. In a series of our work,[1-2] effective pair potential calculation and large-scale molecular dynamics simulation using a simple idealized model incorporating solely electrostatic and steric interactions clearly demonstrate a reversible control on DNA condensates formation by varying the protamine-to-DNA ratio. Microscopic states and condensate structures occurring in semi-dilute solutions of short DNA fragments are in good agreement with experimental phase diagram and cryoTEM observations. The reversible microscopic mechanisms induced by protamination modulation should bring valuable information to improve a mechanistic understanding of early and intermediate stages of spermatogenesis where an interplay between condensation and liquid-liquid phase separation triggered by protamine expression and post-translational regulation might occur. Moreover, recent vaccines to prevent virus infections and cancers using protamine as a packaging and de-packaging agent might be fine-tuned for improved efficiency using protamination control.



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Designing New Bioinspired 3D Nanostructure for Biological Applications

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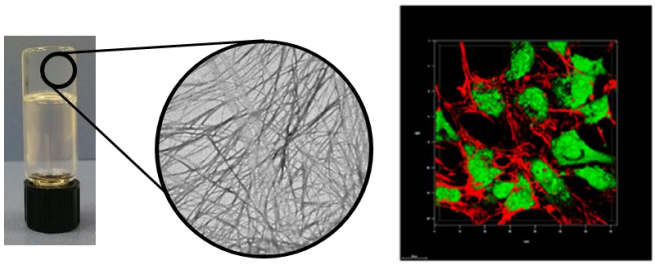
Molecular self-assembly is a key direction in current nanotechnology-based materials science. In this approach, the physical properties of the formed assemblies are directed by the inherent characteristics of the specific building blocks used. Molecular co-assembly at varied stoichiometry substantially increases the structural and functional diversity of the formed assemblies, thus allowing tuning of both their architecture as well as their physical properties.

In particular, building blocks of short peptides and amino acids can form ordered assemblies such as nanotubes, nanospheres and 3D-hydrogels. These assemblies were shown to have unique mechanical, optical, piezoelectric and semiconductive properties. Yet, the control over the physical properties of the structure has remained challenging. For example, controlling nanotube length in solution is difficult, due to the inherent sequential self-assembly mechanism. Another example is the control of 3D-hydrogel scaffold's physical properties, including mechanical strength, degradation profile and injectability, which are important for various applications.

Here, in line with polymer chemistry paradigms, we applied a supramolecular polymer co-assembly methodology to modulate the physical properties of peptide nanotubes and hydrogel scaffolds. Applying a co-assembly approach on hydrogel forming peptides resulted in a synergistic modulation of the mechanical properties, forming extraordinary rigid hydrogels.[1-2] Furthermore, we utilized the hydrogel's aromatic pocket's ability to encage O_2 and significantly limit the O_2 diffusion and penetration through the hydrogel. The O_2-hypersensitive enzyme [FeFe]-hydrogenase was encapsulated in the hydrogel to maintain the enzymatic activity, which holds promising potential for utilizing hydrogen gas for sustainable energy applications.[3]

This work provides a conceptual framework for the utilization of self-assembly and co-assembly strategies to push the limits of nanostructures physical properties obtained through self-assembly.



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Hard-Cation-Soft-Anion Ionic Liquids for PEDOT:PSS Conductivity Enhancement

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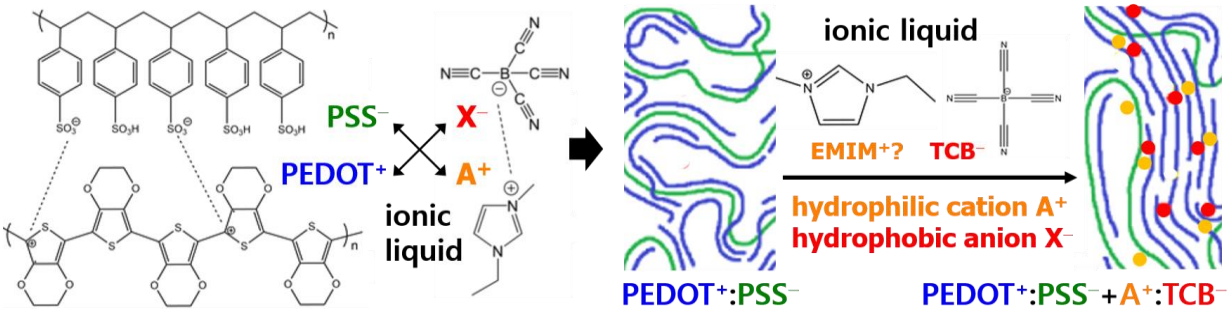
Water-processable mixtures of positively-charged poly-3,4-ethylenedioxythiophene (PEDOT) and negatively-charged polystyrenesulfonate (PSS) have received great attention as a flexible, stretchable, conformable, lightweight, transparent, and low-cost organic (semi)conductor and electrochemical transistor, which can be used for applications such as organic LED, solar cell, thermoelectric genera-tor, self-powered implantable sensor and actuator, and artificial skin. They form 10-to-30-nm granular domains, where conducting-but-hydrophobic PEDOT-rich regions are surrounded by hydrophilic-but-insulating PSS-rich regions, hindering formation of large conducting PEDOT domains. It makes PEDOT:PSS water-soluble, thermally stable, and environmentally benign, but poor in conductivity. Adding polar organic solvents, acids, or organic/inorganic salts to the PEDOT:PSS solution has enhanced the conductivity by 2-3 orders of magnitude. Recently, remarkable 5,000-fold improvement of conductivity has been achieved by mixing proper ionic liquids (ILs) in a PEDOT:PSS solution of deionized water and polar organic solvents.[1-2] In a series of free energy calculations using density functional theory and molecular dynamics simulations[2-6] based on the classic "hard soft acid (cation) base (anion)" principle, we have demonstrated the followings: (1) ion exchange between $PEDOT^+$:PSS^- and A^+:X^- ILs would help $PEDOT^+$ to decouple from PSS^- and to grow into large-scale conducting domains of π-stacked $PEDOT^+$ decorated by IL anions X^-; (2) the most spontaneous decoupling between *hydrophobic/soft* $PEDOT^+$ and *hydrophilic/hard* PSS^- would be induced by strong interaction with *hydrophobic/soft* anions X^- and *hydrophilic/hard* cations A^+, respectively; and (3) the most efficient IL anions X^- remaining in the PEDOT domain after the ion exchange would sustain the highest amount of charge carriers uniformly distributed along the PEDOT backbone to further enhance the conductivity.



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Characterization of Novel Highly Stable Conductive Polymer Composite PEDOT:DBSA for Bioelectronic Applications

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Organic bioelectronics represents a potential revolution in medicine as it holds the promise of personalised treatment with reduced side effects compared to conventional pharmaceutical drugs. Conducting polymers have emerged as an excellent tool for the construction of bioelectronic devices, due to their unique properties. Among them, poly (3, 4-ethylenedioxythiophene) doped with poly(styrenesulfonate) (PEDOT:PSS) has become increasingly selected as a material of choice for applications in this field thanks to its superior properties. Nevertheless, recent research[1] has shown its biocompatibility, one of the key parameters for bioelectronics, is only limited. Thus, there is an effort to provide material with improved properties compared to PEDOT:PSS, with a special focus on biocompatibility. This study addresses such demand for PEDOT:PSS substitution and presents polymer material consisting of PEDOT doped with dodecylbenzenesulfonic acid (DBSA) as a promising material for bioelectronic transistor applications.

The properties of novel material composite PEDOT:DBSA were characterised and further optimized to meet all the requirements imposed on materials for bioelectronics. The special attention was focused mainly on the characteristics of essential importance for such a field, especially biocompatibility, stability in aqueous media and electrical properties. Thus, the modification of PEDOT:DBSA thin film using cross-linker GOPS was studied to improve its long-term stability and sulphuric acid post-treatment was applied to enhance its conductivity. Thin films of such optimized material showed complete resistance against delamination and redispersion while in an aqueous environment. The biocompatibility of such material is significantly improved compared with PEDOT:PSS, as MTT assay revealed almost twice as high relative viability of cells grown on PEDOT:DBSA compared with PEDOT:PSS. The electrical conductivity of the proposed material was found to be of the same order as that of PEDOT:PSS. Its applicability in bioelectronics was proved using a model transistor device containing PEDOT:DBSA as an active layer, which showed behaviour typical for a p-channel transistor working in the depletion mode. The μC* value determined for such material revealed sufficient electrical properties for bioelectronic transistor applications, comparable or even better compared with other organic mixed conductors used in such field. This indicates that PEDOT:DBSA is a potential substitution of PEDOT:PSS and has a great potential for use in bioelectronic applications.

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Where we are now in Bioelectronics and the difficult route to reach “Green”

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Through its appealing avenues of processing the component devices at room temperature and from low-cost precursor materials, organic electronics has a tremendous potential for the development of products able to achieve the goals of production sustainability as well as environmental and human friendliness for electronics.

In an effort to stave off the e-waste growth, the presenter and his research group went further down the path opened by organic electronics research and investigated a large number of biomaterials as substrates, dielectrics, semiconductors and smoothening layers for the fabrication of organic field effect transistors, integrated circuits and organic solar cells. The presentation will focus on the highlights of our recent research, especially with respect to materials investigated, devices fabricated and the immense potential for follow up research:

- Flexible natural and biodegradable substrates
- Natural dielectrics
- Bio-origin, H-bonded semiconductors in the families of indigos, anthraquinones and acridones
- Bio-degradation protocols for organic semiconductors

These highlights will be placed in the context of the mountain that one has to climb in order to reach the coveted “green” connotation for electronics, sensors and integrated circuits:

- Biocompatibility issue
- Biodegradability issue
- Compostability issue
- Cost of production / energy expanded in production issue
- Materials choice issue (carbon foot print)
- Toxicity and the environmental impact of the synthetic avenue for component materials

The potential of follow-up research in the green electronics field is immense, with large area electronics fabrication, biomedical implants, bio-sensing and smart labeling, representing only the tip of the iceberg of many more immediate possibilities of high interest for our group. Natural and nature-inspired materials have the unrivalled capability to create “safe-first” electronic markets for human and environment, with minimal or even neutral carbon footprint.

Electroactive Nanocomposites from Naturally Derived Materials

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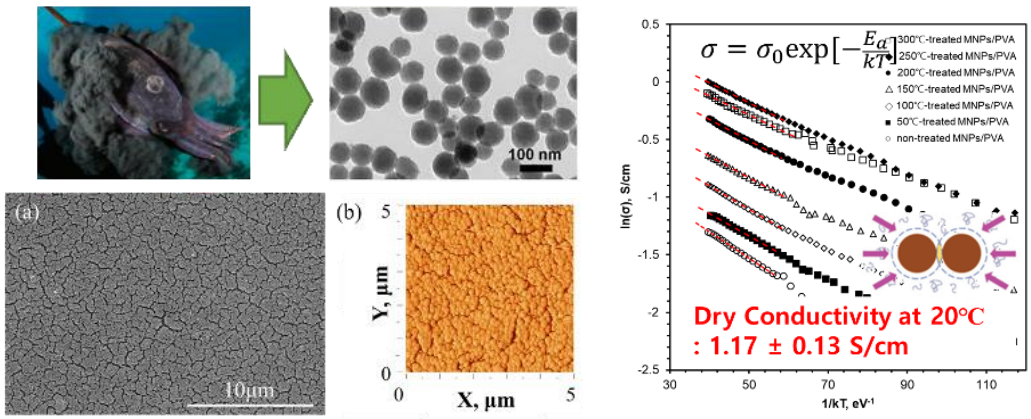
Natural systems utilize multifunctional biocomposites by a bottom-up assembly of nanomaterials for creating hierarchical and multiphasic structures. Inspired by nature, we introduce multifunctional nanocomposites from naturally derived materials including conductive melanin-like polydopamine (PDA). While PDA has barely been used in bioelectronics because of its low electrical conductivity and poor material functionalities, here we present that natural melanin nanoparticles are structured to possess finely tunable electrochemical conductivities, optical reflectivity, and casting shape stability with inherent biocompatibility. Furthermore, a process of creating electrically conductive PDA has been recently developed in our laboratory and thus we want to present their unique functional features in the applications of biosensor and bionic interface. These melanin-like PDA composites can be used as essential functional materials in emerging bioelectronic applications such as biotic-abiotic interfaces, edible sensors and actuators, and eco-green electronics.



(Left) Natural melanin nanoparticles and their nanostructured film assemblies.
(Right) Electrical conductivities of the films formed by depletion force assisted tight packing of natural melanin nanoparticles

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Single-entity detection and monitoring using high-frequency nanocapacitor arrays

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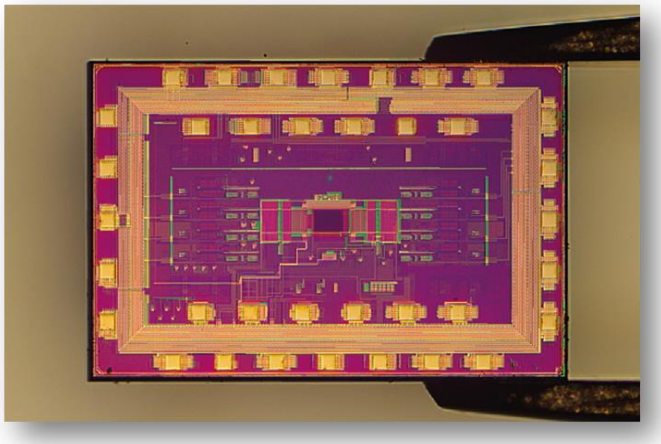
CMOS-based nanocapacitor arrays allow locally probing the impedance of an electrolyte in real time and with sub-micron spatial resolution. At sufficiently high frequencies the electric field penetrates beyond the electrical double layer caused by screening ions, allowing a form of electrochemical imaging of micron-sized synthetic and biological entities.[1,2] For nanoscale analytes, on the other hand, the response takes the form of discrete, step-like changes in impedance upon binding to the surface of an electrode.[3] Here we illustrate these capabilities by monitoring in real time the formation of a lipid bilayer from the fusion of lipid vesicles.[4] Several nanoscale vesicles are detected as they impinge upon the surface of each individual electrode and gradually coat its surface. Even though the impedance signal at each of the 2^{16} electrodes is stochastic in nature, the total response exhibits the smooth behavior expected for the formation of a macroscopic lipid bilayer. This work is a collaboration with NXP Semiconductors.



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A BRAIN-ON-CHIP PLATFORM TO STUDY THE OPTIMAL PARAMETERS OF FOCUSED ULTRASOUND NEUROMODULATION

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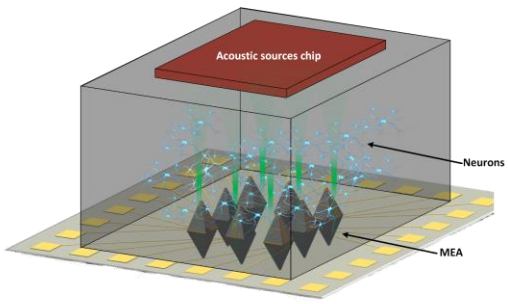
Brain stimulation techniques are essential tools to address various neurological conditions. Currently, approved neuromodulation techniques are either invasive, requiring patients to undergo risky surgery, or non-invasive but suffering from low spatial resolution. Focused ultrasound stimulation (FUS) is a promising modality for non-invasive brain stimulation which was shown capable of eliciting and suppressing neural activity with comparable resolution to invasive modalities.[1-2] However, the role of the modulation parameters in FUS is still heavily debated due to the limited performance of commercial FUS transducers and their inadequate pairing with typical in vivo neuronal recording technologies. Differences in experimental setups, in parameters used to characterize the ultrasound, and in parameter estimation have led to conflicting conclusions, and make prior studies difficult to compare.[3] There is therefore the need for a platform that can reliably correlate ultrasound stimulation parameters and their effect on neuronal tissues.

Organ-on-chip (OoC), specifically brain-on-chip (BoC), is an emerging technology which may address this need. OoC utilizes microfabrication techniques to create devices that mimic human physiology in vitro in an organ-specific context to help develop drugs and treatments.[4] In this context, we aim at incorporating three-dimensional tissue constructs (such as organoids and neural networks) on a micro-electrode array (MEA) to monitor neuronal electrical activity in the presence of ultrasound stimuli. One major technological challenge to develop such BoC platform lies in the ultrasound transducer. To understand the effect of ultrasound on neuronal substrates, the ultrasound transducer has to be able to deliver focused stimulation with single-neuron resolution.
This work presents the development of an ultrasonic piezoelectric transducer compatible with a BoC platform on a wafer-scale. Finite element modeling was used to explore different ways to improve the spatial resolution and acoustic pressure of the transducer. These findings were incorporated into the design and fabrication of the transducer and BoC platform using wafer-level micromachining techniques. While still a work in progress, the resulting platform is expected to push forward the understanding of the biophysical mechanisms of ultrasound neuromodulation.



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Light stimulation of neurons on organic photocapacitors induces action potentials with millisecond precision

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Nongenetic optical control of neurons is a powerful technique to study and manipulate the function of the nervous system. Herein we have benchmarked the performance of organic electrolytic photocapacitors (OEPCs) at the level of single mammalian cells. These optoelectronic devices use nontoxic organic pigments that form a planar semiconductor on top of ITO and act as an extracellular stimulation electrode driven by deep red light.

Light stimulation and signal propagation require close contacts between cell membranes and pigments. We could biochemically prove cell viability and show with SEM imaging that cell culture cell lines adhere to the surface, neurons establish physiological networks and exhibit neurite outgrowth.

Our electrophysiological recordings show that millisecond light-stimulation of OEPCs shifted heterologous expressed voltage-gated K^+ channel activation by ~ 30 mV. We further demonstrate a time-dependent increase in voltage-gated channel conductivity in response to OEPC stimulation and compared our experimental findings with a mathematical model of this bioelectronic-cell system.

In a further step we cultured primary hippocampal neurons on OEPCs and found that millisecond optical stimuli trigger repetitive action potentials in these neurons. Our findings demonstrate that OEPC devices enable the manipulation of neuronal signaling activities with high precision. OEPCs can therefore be integrated into novel *in vitro* electrophysiology protocols, and the findings can inspire new *in vivo* applications for the regeneration of axonal sprouting in damaged neuronal tissues.

Conjugated Polymer Based Nanoparticles as Smart Photoactive Bio-Interfaces

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Conjugated polymers are emerging as optimal candidates for bio-interfaces, because they are highly biocompatible and they can combine the chemical and mechanical advantages of organic materials with the peculiar optoelectronic properties of semiconductors. In form of nanoparticles (NPs), they can be internalized inside cells and provide a remarkable spatial resolution. They can be prepared in water based solution and in sterile condition, suitable for biological applications. They show high brightness and they are mainly employed for in vivo and in vitro imaging or for drug delivery applications. Only very recently they started to be used as photo-transducers in order to optically modulate living cells activity with reversibility, high selectivity and spatio-temporal resolution, avoiding optogenetic tools. Some recent works have demonstrated their reliability in the modulation of cellular metabolism and even animal behavior.[1,2]

We report on the fabrication and the optoelectronic characterization of biocompatible, low band-gap semiconducting polymer nanoparticles, based on poly[2,6-(4,4-bis-(2-ethylhexyl)-4H-cyclopenta[2,1-b;3,4-b]dithiophene)-alt-4,7(2,1,3-benzothiadiazole)] (PCPDTBT), a polymer extensively studied in bulk heterojunction solar cells as electron donor material. The absorption and emission spectra are located in the far red region of the visible spectrum and in the near infrared (NIR), respectively; at these wavelengths the auto fluorescence of biological matter is lower and the tissue penetration is higher, therefore these NPs are strongly appealing for potential in vivo applications. We synthetized the NPs, through the miniemulsion method, starting from an amphiphilic rod-coil block copolymer composed of PCPDTBT as the rigid block, while the coil segment consists in a short chain of poly-4-vinylpyridine. The latter is hydrophilic and therefore allows to fabricate NPs based on a hydrophobic polymer avoiding the need of surfactant. We also synthetized NPs starting from the pristine PCPDTBT adding a surfactant, polyvinyl alcohol, to investigate the effects of the presence of a surfactant on the optoelectronic properties of the material. NPs were prepared in water, they can be lyophilized and they show excellent colloidal stability. The nano-dimensions of the polymer beads are confirmed by dynamic light scattering measurements and scanning electron microscopy images. Upon illumination with visible light in the correspondence of the absorption peak, NPs dispersed in water generate a photocurrent signal, and the latter has a larger amplitude in case of surfactant free NPs. Also the photoinduced absorption spectroscopy measurements, performed on NPs dispersed in water, show that, in case of surfactant free NPs, the amplitude of the signal attributed to charged states is higher.

Secondary line cell models (Human Embryonic Kidney cells, HEK-293) have been treated with NPs. Both the NPs with and without the surfactant internalize within the cell cytosol, without affecting proliferation. The functional interaction between cells and polymer beads upon optical excitation has been studied by fluorescence imaging experiments. In case of surfactant free NPs, the photo-electrochemical activity generates intracellular Reactive Oxygen Species (ROS), at non toxic levels.

ROS regulate different biological functions as signal transduction, blood pressure modulation and metabolism regulation; alteration in their concentration might lead to pathological conditions as autoimmune, cardiovascular and neurodegenerative diseases. Therefore, the capability of polymer NPs to optically modulate ROS balance by on-demand illumination might open the path for studying biological processes with a minimally invasive procedure and with unprecedented spatiotemporal resolution, laying the foundation for developing novel therapeutic approaches.

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The Reactions of Organic Mixed Conductors with Oxygen

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The majority of organic mixed ionic electronic conductors (OMIECs) have over potentials for oxygen reduction reactions (ORRs) that lie within their usual operational range in aqueous electrolytes.[1–3] However, such investigations have been limited to p-type OMIECs with only one report published recently for electron conducting n-types.[4] While p-type OMIECs undergo ORR in their non-conducting state, for n-type OMIECs, the potential window for electrochemical doping and ORR is the same. The ORR for n-type devices is, therefore, crucial as it leads to unwanted side reactions, current loss and chemical species formed that might interfere with device operation and stability. In this work, we evaluate the reactions of the most used n-type OMIECs in bioelectronics with oxygen and benchmark their oxygen reactivity with respect to their chemical structure, energetics, kinetics, and reaction mechanism. For the device level translatability of these fundamental studies, we use these materials in organic electrochemical transistors (OECTs) and study the impact of ORR on device performance. Our findings reveal the often under-looked interactions of n-type materials with oxygen and suggest routes to control it. Optimization of ORR based on these guidelines allows to design n-type materials for high performance fuel cell anodes and enzymatic metabolite sensing electrodes.

In-operando and *in-vivo polymerization of trimers for neuromorphic and bioelectronic systems*

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Thiophene-based trimers is a new class of organic electronic molecular units, which enable the organization and polymerization of conjugated structures within living biological systems and inside operating electronic devices and systems[1-3]. The trimers can be equipped with side groups that targets and promotes coupling to specific surfaces and (bio-)chemical cues making self-organization and -assembly of organic bioelectronics possible in a novel manner. The chemical and physical fundamentals of the trimers, the route of polymerization, and their performance while operating in neuromorphic and *in vivo*-manufactured bioelectronics will be reported. Specifically, neuromorphic systems based on organic electrochemical transistors including the trimers will be described along with bioelectronic systems formed inside living plants, cells and animal models. Our findings promise for radically new ways of forming bioelectronic systems in living systems, which mimicks the structures and functions of the signaling of biology.

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Controlling morphology, adhesion and electrochromic behaviour of PEDOT films through molecular design and processing

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The structure-based tuneability of the electronic and optical properties of conjugated polymers has enabled their application across a range of fields, including energy harvesting and optoelectronics. However, in the context of biological sensing, the use of conjugated polymers has thus far incorporated little specificity in terms of covalent modification.

Developing robust, highly selective, biologically compatible sensing platforms is of critical importance because the measurement of analyte concentrations in biological samples is crucial for the management or detection of many diseases.[1] Currently, many devices which function for this purpose are complex, multi-component systems. However, directed synthetic strategy with conjugated polymers enables the fine-tuning of analyte specificity, electroactive or optical functionality, and physical and morphological properties, within a single multi-purpose material. In addition, these entirely organic systems offer affordability, biocompatibility, and simple design and fabrication.

I will present my work on novel covalently-modified PEDOT polymers.[3,4] The introduction of a 15-crown-5 moiety is shown to enhance a diverse range of characteristics in comparison to regular PEDOT, including: adhesion to ITO substrates, physical and electrochemical integrity, film uniformity, and spectroelectrochemical properties.[5] Furthermore, we find that the long-term electrochromic behaviour of PEDOT-Crown is altered in the presence of its chelating ion, sodium, compared to unmodified and analyte-free controls, which may open the door to its application as a sensor material.[6] The adaptable nature of the synthetic pathway also unlocks access to range of potential sensor materials, featuring differently modified PEDOTs specific to other biomarkers, a space we have begun to explore, including the fabrication of molecularly imprinted conjugated materials with distinct morphological and electrochemical properties.

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Ionic communication for implantable bioelectronics

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Implanted bioelectronic devices require data transmission through tissue, but ionic conductivity and inhomogeneity of this medium complicate conventional communication approaches. Here, we introduce ionic communication (IC) that leverages ions in biologic tissue to propagate MHz-range signals. We demonstrate that IC operates by generating and sensing stored electrical potential energy within polarizable media in a frequency-dependent manner. We determined the geometric properties that govern IC transmission depth and controlled transmission radius to permit multi-line parallel communication. We integrated IC with advanced neural interfaces to create a fully implantable device capable of acquisition and non-invasive transmission of neurophysiologic data from freely moving rats over a period of weeks. IC enabled a stable, efficient link with implanted components, and had communication efficiency (data rate/power consumption) several orders of magnitude above other approaches employed with implantable bioelectronics. We used IC for real-time transmission of multi-channel local field potential (LFP) and neural spiking data, with data quality sufficient for clustering of individual neuronal action potentials. IC creates a high-speed, low-power link between implanted and external electronics with the potential to enhance the safety and efficiency of a wide range of bioelectronic devices.

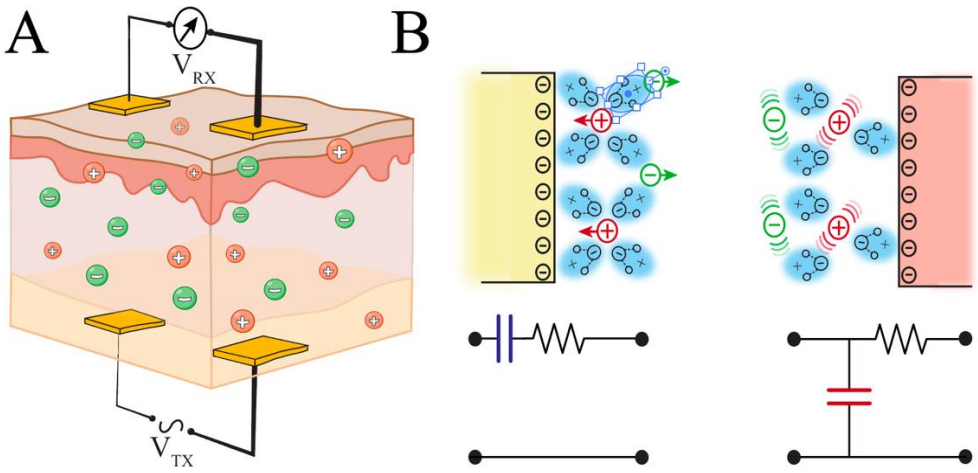

Figure 1: Configuration and characterization of IC.

A. Cross sectional schematic illustration of an IC device consisting of an implanted transmitter (TX) electrode pair inside biological tissue with ionic charge carriers (anions (green) and cations (red)), and a receiver electrode pair (RX) on the surface of the tissue. V_{TX} denotes the transmitter signal while V_{RX} represents the measured voltage from the RX outside of tissue.

B. Schematic of EDL capacitance (yellow; top left) at electrode/electrolyte interface and its corresponding simplified series RC model (bottom left). Schematic of capacitance resulting from media polarizability (red; top right) and its parallel RC model (bottom right).

Distant stimulation and tissue ablation with high-intensity nanosecond electric pulses

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A recently discovered unique feature of nanosecond electric pulses (nsEP) is the so-called 'bipolar cancellation" of biological effects.[1] Bipolar nsEP are less effective than a single phase of the same pulse at stimulation and at cell membrane disruption by electroporation (Fig. 1A). The bipolar cancellation phenomenon comes in sheer contrast to conventional electroporation, where bioeffects are proportional to the time duration when the pulse voltage exceeds a critical level. Bipolar cancellation of neurostimulation and of electroporation may be caused by different mechanisms and show different dependence on the electric field parameters.

We have recently introduced a novel paradigm of non-invasive, selective electrostimulation and electroporation of deep targets, remotely from electrodes.[2,3] The biological efficiency of bipolar nsEP can be restored by the superposition of two properly shaped and synchronized bipolar nsEP into a unipolar pulse, i.e., by "**can**cellation of **can**cellation" (CANCAN). Hence, the highest biological response will be evoked locally within nsEP superposition area, which can be remote from stimulation electrodes (Figs. 1B and 2). It is important to emphasize that CANCAN relies on a change in pulse shape from bipolar into unipolar, not on an increase in the pulse amplitude or duration. We will report how the CANCAN effect depends on various nsEP parameters. The ongoing work is focused on achieving the CANCAN effect on a larger scale, potentially suitable for clinical applications.

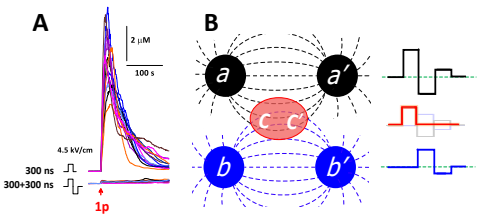

Fig 1. An example of bipolar cancellation (A) and a schematic of remote stimulation by the superposition of two bipolar nsEP into a unipolar waveform. (**A**): Ca^{2+} transients in CHO cells evoked by a single 300-ns monopolar pulse (top), or a 300+300 ns bipolar pulse (bottom), both at 4.5 kV/cm. (**B**): two pairs of electrodes (***a-a'*** and ***b-b'***) deliver bipolar pulses such as shown in the right panel. The superposition of bipolar pulses in ***c-c'*** area forms a biologically-effective unipolar pulse remotely.

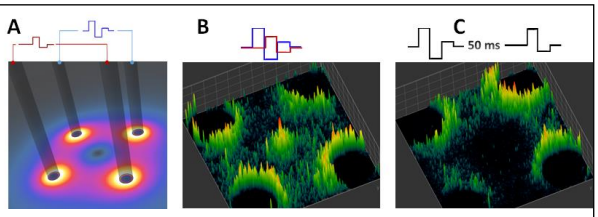

Fig 2. Stimulation targeting by CANCAN effect. (A): A quadrupole electrode array installed orthogonal to a cell monolayer. (**B)** and (**C**): Images of YO-PRO-1 dye uptake in cells following CANCAN (**B**) and control (**C**) exposures. This dye becomes fluorescent when it enters electroporated cells. Black circles at the corners are the electrodes' footprints. In C, bipolar pulses were separated by a 50-ms interval to prevent their superposition into a unipolar waveform.

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Numerical modeling for stimulation device screening

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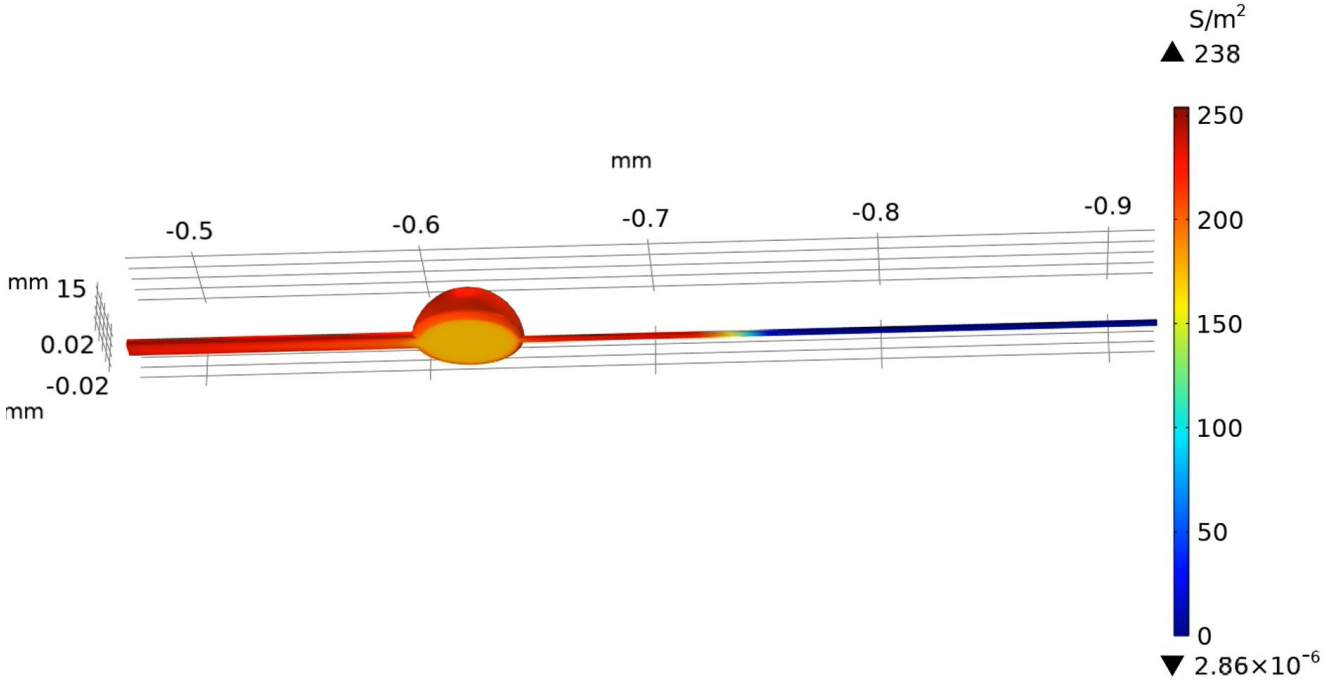
Minimal invasiveness and size are among the most important requirements for electrical neurostimulation devices. Additionally, wireless power and communication with the device is highly preferred to wired or battery-powered operation. However, device downsizing typically results in lower charge delivery performance of the stimulators, due to the limitations in charge generation and interface impedance[1]. Thus, effectiveness level of the stimulation device on your model of choice is not easy to guess, which can result in large number of unsuccessful in-vivo experiments. Today's state of the art in available computing power and software development enables integration of fully numerical 3D models of both the stimulation device and the stimulation target. Such models could speed up the device development and reduce the costs involved with repetitive *in-vivo* experiments. We have developed a numerical device model coupled with the single neuron to study the suitability of our devices for single cell stimulation. Based on the model's results, we will present important guidelines and requirements for the successful stimulation of single cells. Finally, we will show how our optimization toolset can be extrapolated and applied to a more general class of bioelectronic interfaces.



Na channel conductivity during the firing of the action potential along the axon of a model neuron stimulated by a model organic electrolytic photocapacitor.

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Organic semiconductive polymer nanoparticles for neural photostimulation

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Central nervous system disorders can lead to a wide range of impairing conditions, including blindness, paralysis or Parkison's disease. At the terminal phase and after failure of drug therapies, retinal/brain implants represent a unique countermeasure to rescue neural activity, ultimately improving the patient's quality of life.

Nanotechnologies for the light-driven neuronal activation have been proven to interrogate specific brain circuits and compensate for nervous system pathologies in which neuronal degeneration has induced a specific lack of function. Among these, organic semiconducting polymers have been proved capable of effective bio interfacing and neural photostimulation[1,2].

By exploiting a novel chemistry,[3] we synthetize a library of semiconductive polymer nanoparticles. Thanks to the functionalized polyethylene glycol (PEG) corona, these nanoparticles are stable in physiological environment and can be readily functionalisable with biorecognition elements for specific targeting. Moreover, this library of polymers covers a broad wavelength spectrum ranging from UV to NIR. Enhanced stability, specific cell targeting, and broader wavelength coverage pave the way for a more effective and versatile photostimulation tool.

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Flexible interdigitated electrode for selective stimulation of small fibers in humans

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Small fiber neuropathy (SFN) is a difficult to diagnose pathology caused by a severe loss of free-endings of unmyelinated sensory nerves (small fibers) that produces a range of symptoms, including difficult-to-treat-pain.[1,2] SFN occurs across a number of different diseases, being diabetes mellitus the most common.[2] Current approaches to diagnosis are based on skin biopsy or elicited responses evoked by selective small-fiber stimulation by radiant or contact heat.[3] However, skin biopsies are invasive and costly, and heat stimulation may cause inadvertent burns on skin. Development of safe and non-invasive devices that are capable of selective stimulation of small-fibers seems key for monitoring the evolution of SFN over time in patients.

Based on the interdigitated configuration proposed in the work of Leandri et al,[4] we developed a flexible and adhesive electrode capable of selectively stimulating small-fibers. The conformability of the device provides comfortable, reliable and stable electrical connection to skin. Furthermore, a PEDOT:PSS coating enhances the electrical impedance between electrode and skin, reducing by half the voltage required to circulate an epidermal electrical current.

We assessed the selectivity of the interdigitated device by comparing the response reaction time with an unselective gel electrode in the upper and lower limbs. Results show that responses elicited using the interdigitated electrode are slower, as it exclusively activates unmyelinated fibers. Moreover, the device was tested on healthy volunteers who have undergone a local application of capsaicin cream procedure, making the small fibers temporarily shrink to reproduce SFN symptoms.[5] Pre- and post-treatment electrical stimulation of the affected area shows that they remain unresponsive after application of the cream.

The improvements shown in this research permit the use of a wider range of stimulating electrical currents using medical equipment, providing clinicians with more flexibility during the diagnostic phase of this pathology, and making the device safer for the patient. The simplicity and potential affordability of the device could pave a straightforward way into the clinics, allowing an earlier detection and treatment for SFN patients.

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An engineered heart tissue platform with integrated pacing microelectrodes

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Engineered heart tissue (EHT) models have demonstrated valuable potential to reproduce the (patho)physiology of human cardiac tissue *in vitro*[1]. Current platforms for culturing EHTs mostly provide static mechanical stimuli for tissue formation, by means of anchors with different geometries around which the tissue self-assembles[2]. Beside the mechanical support for tissue formation, other biomechanical as well as electrical stimuli are of great significance for faithful *in vitro* recapitulation of the human cardiac *in vivo* environment.[3,4] However, electrical stimulation is usually incorporated into the existing EHT platforms by manually immersing external electrodes into the wells with the tissues[3.4].

We developed a platform for culturing and stimulating EHTs with integrated pacing microelectrodes (Fig. 1a-c). A pair of microelectrodes for electrical stimulation of tissues was integrated within an elastic polymer-based EHT platform, composed of a microwell enclosing a pair of micropillars.[5] Using standard microfabrication techniques in combination with polymer deposition, metal sputtering, photo-lithographical patterning, and wafer bonding (Fig. 1d), we integrated TiN microelectrodes within our original EHT platform. The microelectrodes were positioned in proximity of the base of the micropillars (Fig. 1c), and designed to create an electric field perpendicular to the direction of tissue formation. Additionally, we designed and implemented a portable electronic circuit for tissue stimulation. The circuit generates rectangular bipolar pulses with amplitude in the range of 0-30 V peak-to-peak, and with tunable frequency and duty cycle. The whole system is configured as a multi-well plate suitable for high-throughput biological assays (Fig.1a). Preliminary electrical characterization of the microelectrodes as well as cell-viability experiments attest the suitability of the integrated microelectrodes to replace external pacing electrodes as well as the biocompatibility of the overall platform.

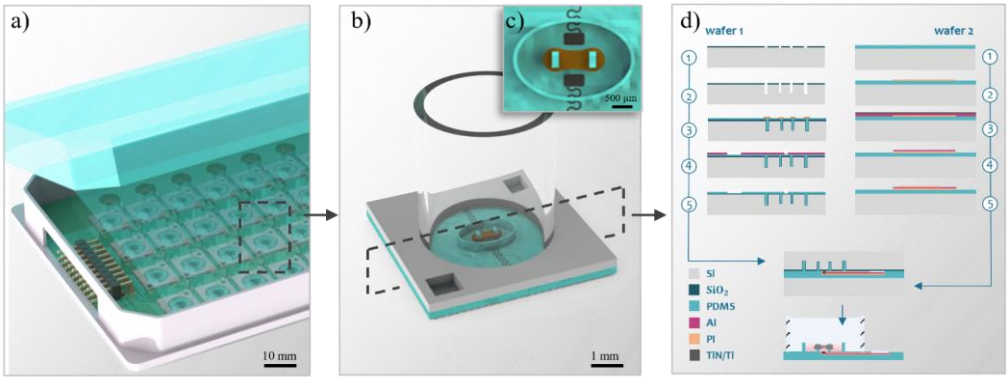

Figure 1: a) 3D model of the array of 32 EHT platforms in the multi-well plate format compatible with the external pacing electronic circuit; b) 3D model of the single EHT platform with c) close-up image of integrated TiN electrodes; d) Process flow for the microfabrication of the EHT platform.

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Developing 3D bioelectronic platforms for monitoring lung cell culture *in vitro*

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The respiratory epithelium is one of the first lines of physical and immunological defence in the body. However, a dysfunctional respiratory barrier may lead to chronic lung diseases and infection, which are among the leading causes of death worldwide.[1] This, coupled with the recent COVID pandemic, highlights the importance of having *in vitro* models which accurately reflect the complexity found *in vivo* for enhancing therapeutic delivery and treatment of respiratory conditions. Although over the past decades, advances have been made in developing 3D cell cultures, there remains a limited capacity in sensor technologies which can continuously and non-invasively monitor these systems.[2] Additionally, Air Liquid Interfaced (ALI) cultures pose further challenges such as the necessity of measuring in the air, rather than submerging the system in an electrolyte for electrode operation. To address this issue, the work presented highlights the fabrication and characterisation of a novel conducting polymer scaffold system, with tuneable chemical and physical properties. This 3D sensing system demonstrates the ability to not only house multiple cell types in a biomimetic Extracellular Matrix (ECM) environment, but continuously and non-invasively measure cell growth, epithelial barrier formation and toxicology responses.

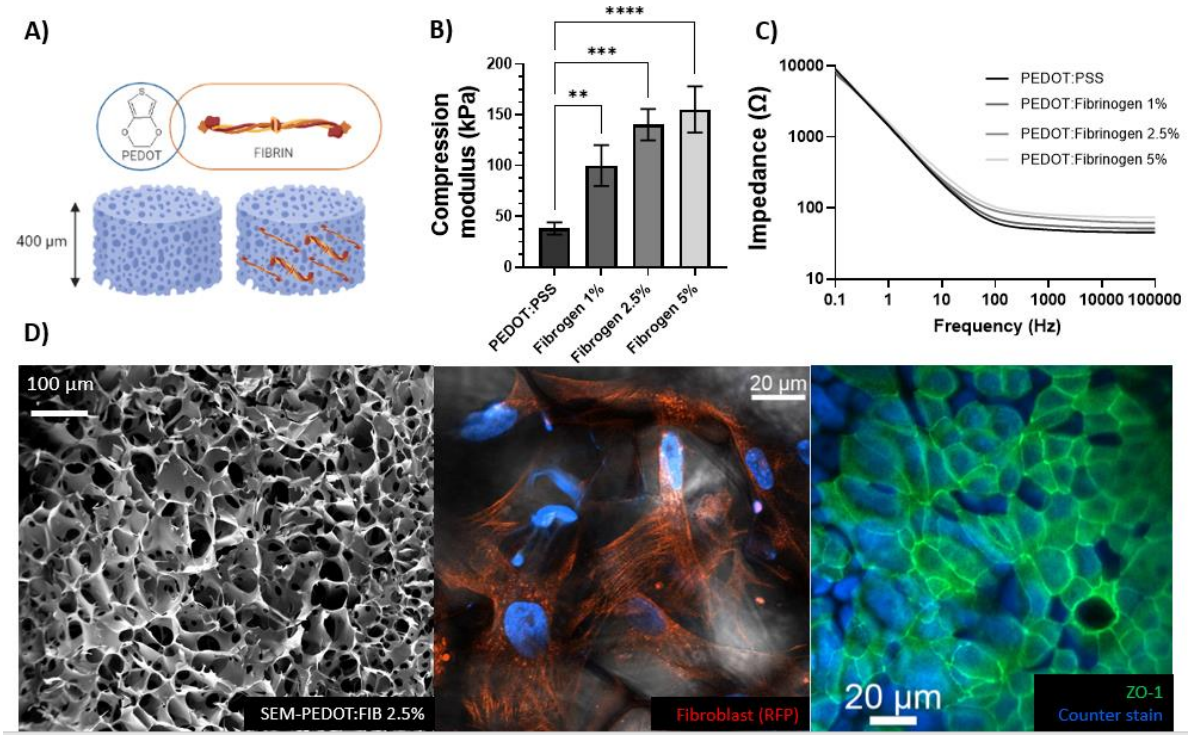

Figure 1 – Schematic representation (A) and characterisation (B-D) of the conducting composite polymer scaffold system, which is physical tuneable (B) and can be monitored electrically (C). SEM and immunofluorescent images demonstrate the capability of housing and monitoring multiple respiratory cell types (Red = transfected RFP fibroblasts; green = tight junction protein ZO-1 of CALU-3 cell line; Blue = DAPI).

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Interaction of nanomaterials with somatic cells towards successful biomedical application

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The rapidly evolving field of nanomedicine refers to application of nanomaterials in preclinical or clinical level. However, even though the nanomedicine output in science increasing enormously, nano-medicinal products in clinical medicine are still very rare because of the safety, regulatory and ethical issues. When speaking about the successful journey of the nanoproduct into the real application starting from controlled synthesis and precise physico-chemical characterization thereof, the next step concerns a complex and careful in vitro cytotoxicity/biocompatibility testing. In this lecture I will present last journeys of nanoparticles based on iron oxide and carbon derivatives which were prepared in our Catrin Institute focusing mainly on in vitro testing. Interaction of nanomaterials within the somatic cells (see Figure 1) on cellular and subcellular level represents an important prerequisite for their future application in diagnostics and therapy.[1-6]

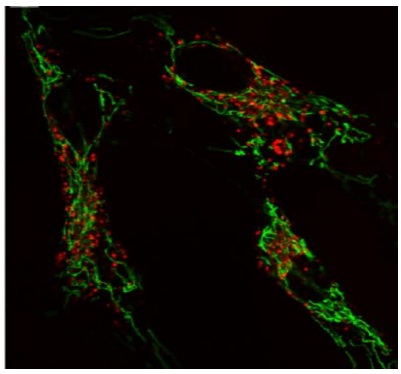

Figure 1: Fluorescence confocal imaging of stem cells after 24 hours of incubation with iron oxide nanoparticles labeled with Rhodamine dye (red spots). Notice: Green color is special dye for mitochondria labeling.

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PPIA and YWHAZ constitute a stable pair of reference genes during electrical stimulation in mesenchymal stem cells

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This work has been peer-reviewed and revisions have now been submitted to the Applied Sciences journal.

Mesenchymal stem cells (MSCs) are multipotent adult stem cells with great potential in regenerative medicine[1]. One method for stimulating proliferation and differentiation of MSCs is via electrical stimulation (ES)[2,3]. A valuable approach for evaluating the response of MSCs to ES is to assess changes in gene expression, relative to one or more reference genes. In a survey of 25 publications that used ES on cells, 70% selected GAPDH as the reference gene. We conducted a study to assess the suitability of six potential reference genes on an immortalized human MSC line following direct current ES at seeding densities of 5,000 and 10,000 cells/cm^2. We employed three methods to validate the most stable reference genes from qRT-PCR data. Our findings show that GAPDH and ACTB exhibit reduced stability when seeded at 5,000 cell/cm^2. In contrast, we found that the most stable genes across both plating densities and stimulation regimes were PPIA and YWHAZ. Thus, in ES gene expression studies in MSCs, we support the use of PPIA and YWHAZ as an optimal reference gene pair, and discourage the use of ACTB and GAPDH at lower seeding densities. However, it is strongly recommended that similar verification studies are carried out based on cell type and different ES conditions.

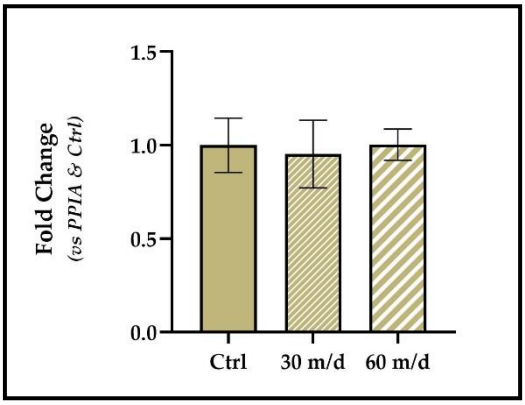

Figure 1: Expression of YWHAZ in primary human MSCs stimulated for 30 and 60 minutes per day (m/d) normalized to PPIA and the unstimulated control group. N=3 for each group.

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Portable electroanalytical Nucleic Acid Amplification Tests using paper, textile and PCB-based devices

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The SARS-CoV-2 pandemic has made it clear that inexpensive and point-of-care (POC) diagnostic tests are urgently needed to minimize an outbreak.[1] Electroanalytical DNA devices appear as one of the best options, since they combine the specify of the DNA with the sensitivity of electrochemical measurements.[2] Two obstacles have however been hampering their dissemination: 1) the use of expensive electrodes; 2) the lack of integration from sample-to-answer. To tackle these problems, we developed two electroanalytical nucleic acid amplification tests (NAATs). Both devices incorporate a self-assembled monolayer (SAM) on gold electrodes, an isothermal amplification step by recombinase polymerase amplification (RPA), bypassing the need of thermal cycling, and an amperometric readout relying on an enzyme-linked sandwich hybridization assay. As a proof of principle, we showed the analytical capability of both systems to detect the toxic microalgae *Ostreopsis* cf. *ovata*.

In the first device, we showed that mass-produced, industrial standard, printed circuit boards (PCBs) can be repurposed to act as near-zero cost electrode for SAM-based biosensing. The well-established and standardized PCB technology enables both their rapid and low-cost prototyping as well as scalable industrial mass-manufacturing.[3]

In the second device, we showed that off-the-shelf gold plasma-coated thread electrodes can be incorporated within paper to develop microfluidic paper-based electroanalytical devices.[4] Besides being disposable and portable, the use of paper substrates provides properties necessary for the different steps of NAATs such as fluid transport through capillary forces, filtration, adsorption, and reagent storage.[5] Our 3D microfluidic device integrates sample incubation, rinsing, and detection steps by using movable stacks of filter papers to allow time-sequenced reactions.

We think these two prototypes fabricated with PCBs, textile and paper will pave the way for numerous other advanced electroanalytical devices, allowing POC testing at a low cost.

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Printed Organic Electronic Sensors for Plant Health Monitoring

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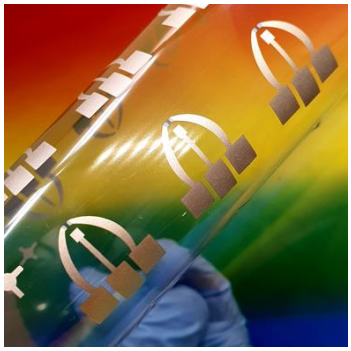
Low-cost, flexible, and lightweight electronic biosensors offer an exciting pathway to future products for environmental and plant health monitoring. Organic electrochemical transistors (OECTs) based on poly(3,4-ethylenedioxythiopene) polystyrene sulfonate (PEDOT:PSS) are particularly favorable sensing platforms due to their high sensitivity, low power requirements, and use of soft, biocompatible materials.[1,2] Furthermore, OECTs can be manufactured with scalable printing techniques, which makes them suitable for large-area electronics (LAE) applications.[3,4] This presentation will describe the development of fully printed, flexible OECTs that can accurately detect a suite of analytes (e.g., nutrients, pH, moisture) that are relevant to plant health. For example, these devices were used to accurately detect macronutrient concentrations in raw whole plant sap in real-time. The rheological and electronic properties of the OECTs were tuned by doping PEDOT:PSS channel with sugar alcohols, super absorbing polymers and thickening agents. These additives boosted the transconductance and sensitivity of the transistors, which led to a super-Nernstian response to the target analyte. By utilizing ion selective membranes (ISMs), we fabricated OECT-based ion sensors that demonstrate selectivity to the target ion against similar ions over five orders of magnitude in concentration and a limit of detection (LOD) as low as 10 µM.[5] The results confirm that lightweight, printed organic electronics are a suitable path to high-throughput, low-cost assessment of plant health, which could lead to massive efficiency gains in agriculture.



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Towards a miniaturized cuff implant for highly selective US neuromodulation of peripheral nerves

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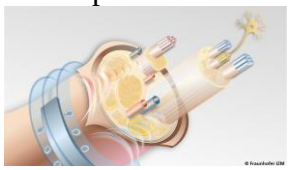
In an attempt to reduce the side effects caused by the chemically-based drugs used to treat neurological disorders, the field of bioelectronics has been focusing on the development of smart and reliable solutions that could, ideally, interact with the tissue at a resolution of individual cells.[1] Conventionally, electrically-based systems have been used.[2] However, increasing the resolution at which they interact with the body leads to the development of invasive electrode arrays, which can cause long-term side effects.[3] Another approach, based on acoustic waves, has recently emerged. Ultrasound (US) neuromodulation has been proven to be effective in modulating the response of peripheral nerves, in an *in-vivo* setup[4] and has the potential to achieve higher spatial selectivity.[5]

In this work, we aim to fabricate an implantable cuff for US neuromodulation, which would employ an array of US transducers to deliver focused US to specific nerve areas in a non-invasive manner. To this end, the potential of different US transducer arrays for peripheral nerve applications is evaluated, assessing the acoustic performances as well as ease of assembly and integration. More specifically, two of the most important parameters that affect neural excitation are the frequency and output pressure generated by the US transducers.[4] Conventional bulk PZT transducers can generate a wide range of output pressures but these are not small enough for this application. PZT-based arrays, integrated on CMOS have recently emerged, and will be part of this evaluation[6]. However, these have not yet been integrated on flexible substrates. On the other hand, micromachined US transducers (MUTs) have been gaining a lot of interest, particularly capacitive MUTs (CMUTs) which can operate at high frequencies, thus reducing the focal point significantly.[5] CMUTs can be fabricated on flexible substrates, using biocompatible materials, rendering them a very attractive candidate for the envisioned cuff. However, CMUTs usually feature lower Q factors compared to PZTs, hence the output pressure still has to be evaluated for neuromodulation. In addition, this work will also discuss important characteristics of the materials used for encapsulation, as these should ensure the required flexibility of the cuff without negatively affecting the acoustic performance of the transducers.



Concept of the US-based cuff implant for peripheral nerve stimulation.

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Multifunctional Neural Interfaces and Tapered Optical Fibers Technology

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As optical approaches to study the brain have seen a boost since the advent of optogenetics, technology development is facing the challenge of providing neuroscientists and clinicians with multimodal neural interfaces and paradigms to simultaneously gather multiple signals from the living brain.

After a review of recent work in the field, we will present the tapered optical fibers (TFs) technology [1], which combine the peculiar optical and photonic properties of narrowing waveguides to non-planar micro and nanofabrication methods to explore unconventional approaches to interface with brain tissue. In this framework we will describe how the properties of the narrowing waveguide can be exploited for: (i) depth-selective detection of functional fluorescence, compatible with depth-resolved fluorescence lifetime photometry [2-4](ii) three-dimensional multi-point electrophysiology with simultaneous optical access to the same brain region, obtained by two-photon lithography on the highly curved surface of the taper, (iii) exploit the coupling of guided modes with plasmonic structures realized on the taper side, to realize implantable plasmonic neural interfaces based on light-matter interactions [6].

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Tumour treating fields effect on cell viability is determined by cell orientation and field direction.

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Engineering approaches for the treatment of cancer have gained significant interest in recent years. For brain tumours, these include the alternating current (AC) electric field therapy known as Tumour Treating Fields (TTFs)[1,2]. TTFs are believed to disrupt the mitotic spindle of tubulin during the formation of microtubules in cancerous cells. By inducing an AC electric field at specific frequencies, dipolar molecules in the cell polarise, ultimately leading to early metaphase exit and cellular death. During cytokinesis, cells dividing parallel to the direction of the electric fields experience large dielectrophoretic force at the division furrow thought to cause macromolecule movement and further cellular breakdown. There is sufficient evidence that electric fields can be used to align and pattern cells, but to date only computational models exploring TTFs and their dielectrophoretic effects have been reported with no clear experimental or in vitro/vivo validation.

Here, we show by using photolithographic patterning and protein attaching arrangements, that adherent glioblastoma stem-like cells can be organised into parallel, perpendicular and diagonal patterns with respect to the applied electric field. The indication of whether or not a cell can commit to cell division is shown by using live cell FUCCI (fluorescence ubiquitination cell cycle indicator) transduction where cells fluoresce green in G1 and red in S/G2/M cell cycle phases. This provides visual indication of the directionality effect of TTFs in terms of cell cycle disruption. In tandem, actin filament staining confirmed by Fast Fourier transforms represent the angle at which the effect of TTFs is greatest and weakest. This work not only provides insights into the mechanism of action of TTFs, but also design considerations for electrode placement. Thus, these indications offer insights for greater therapeutic benefit and recruitment of TTFs in practice as well as improved patient outcomes for treating Glioblastoma.

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Interfacing Colon Cancer Models with Smart Bioelectronics

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The generation of *in vitro* platforms as biosensors and/or capable of mimicking the *in vivo* situation as an alternative to animal models is necessary for medicine and drug development. Organic semiconductors are the perfect match for interfacing with biological microenvironments (*in vitro*) starting from the study of the simple cells (2D) to complex models like 3D cell cultures and organoids.[1] Here, we present smart routes of interfacing bioelectronics with cells in 2D and 3D, by giving additional capabilities to the sensors. In this sense, we have developed 2D thermos-responsive bioelectronic sensors by preparing hybrid copolymers made of the poly(3,4-ethylenedioxythiophene) poly(styrene sulfonate) (PEDOT:PSS) and poly(N-isopropylacrylamide) (pNIPAAm), capable of performing label free, non-invasive cell capture, release, and simultaneous electrical monitoring of sw480 cells (colon adenocarcinoma cancer) (Figure 1A).[2] Regarding 3D interfaces, we have developed scaffolds made of the conducting PEDOT/hyaluronic acid (HA) and collagen (COL) to give an extra biomimetic capability to the 3D cell cultures. Interestingly, these scaffolds successfully support growth of 3D cell cultures of sw480 cells. When integrated with electrodes, they further allow real-time electrical monitoring of cell growth and proliferation. Upon the addition of the flavonoid morin, cell apoptosis and death was monitored by electrochemical impedance spectroscopy and optical immunostaining. , demonstrating the promise of these scaffolds for cancer cell progression modelling. (Figure 1B) Moreover, their integration into microfluidic devices offers optical transparency, miniaturization, and controlled media perfusion required in organ-on-chip models. Here, we present an innovative platform with integrated 3D scaffolds made of PEDOT:PSS for the simultaneous optical and electrochemical impedance spectroscopy (EIS) monitoring of sw480 cells migration inside microfluidics (Figure 1C). The generation of smart routes as the addition of thermo-responsive materials, biopolymers or integration of fluidics extent the capabilities of traditional bioelectronics field and opens the possibility of developing sensors for a multitude of applications.

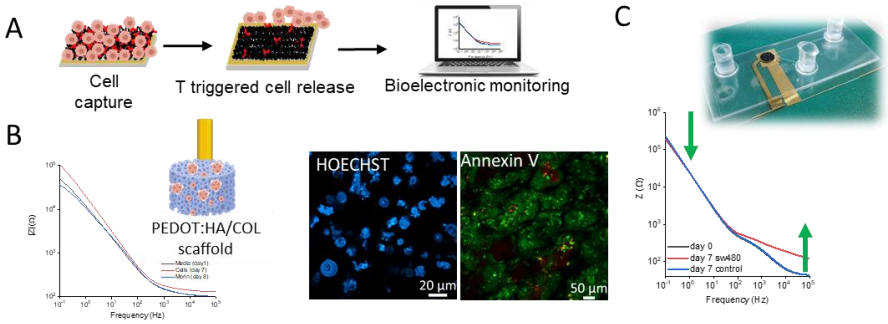

Figure 1: Smart bioelectronic interfaces with biology: A) Capture and release of sw480 cells by temperature actuation of the thermoresponsive copolymer PEDOT:PSS/pNIPAAm, B) 3D scaffolds made of PEDOT:HA and COL for interfacing with sw480s cells. After treatment with morin cells become apoptotic (HOESCHT, Annexin V staining), C) Integration of 3D scaffolds into microfluidic devices for the electrical monitoring of sw480 cells.

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Sensing at the zeptomolar concentration level with large area bioelectronic interfaces

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Nanosized interfaces have been the favoured route to single-molecule detections so far. However, because of the so-called *diffusion-barrier* issue, such *near-field* approaches, encompassing nano-wire transistors but also nanopores and many others, are, not able to detect at concentrations lower than nanomolar. Bioelectronic field-effect transistors endowed with l*arge-area* (mm^2 wide) detecting interfaces, are perceived as unsuited too, because the footprint of a single molecule is negligible when compared to a large detecting interface. *This is challenging as it would be like detecting a single droplet impacting on a kilometer-wide surface*. However, many different groups have published data proving that field-effect large-area biosensors can detect at limit-of-detection of femtomolar and below.

Moreover, these single-molecule large-area bioelectronic based technologies can involve small readers, are fast, easy to operate directly in the fluid to be analyzed, and the electronic outputs are already in a convenient digital form so that an easy transfer to app is possible. Moreover, they can be fabricated by cost-effective technologies including printing and other direct-writing processes. Hence, ultrasensitive large area bioelectronic sensors are likely to have a bright future in healthcare. This lecture will give an overview of this field, discussing device architectures, materials used, and target analytes that can be selectively detected as well as the sensing mechanisms.

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Ultra-sensitive detection of neurofilament light chain with a label-free electrolyte-gated organic field-effect transistor-based immunosensor

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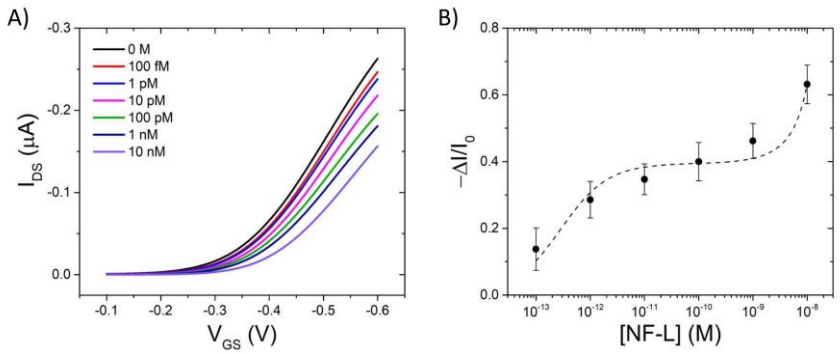
Organic electronic-based immunosensors, such as electrolyte-gated organic field-effect transistors (EGOFETs), are emerging as promising alternative strategies for the ultra-sensitive and label-free detection of biological analytes. Here, we report the first EGOFET-based immunosensor for the detection of neurofilament light chain (NF-L), a candidate biomarker for neurological disorders.

In the proposed architecture, the specific recognition of the biomarker is ensured by immobilizing anti-NF-L antibodies on the gate electrode surface, with a controlled and uniform orientation. The observed response of the biosensor, fitted to the Guggenheim-Anderson-De Boer (GAB) adsorption model, suggested the presence of two simultaneous events occurring at the gate surface, interpreted as the concomitant formation of a strongly adsorbed protein-antibody layer, and an additional layer corresponding to weak protein-protein interactions. Further morphological characterization of the gate surface supported this interpretation. Our EGOFET immunosensor demonstrated to selectively detect NF-L in a wide dynamic range of concentrations (100 fM-10 nM), providing a rapid, reproducible, and label-free response, indicating its potential as a promising strategy for the detection of NF-L in neurological disorders such as Parkinson's disease or multiple sclerosis.



A) Transfer curves of the EGOFET-based biosensor upon exposure to increasing concentrations of NF-L. B) Biosensor dose curve $-\Delta I/I_0$ vs log[NF-L], dashed line is the fit to the GAB adsorption isotherm.

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Modular FET-Based Sensor for Organ-on-Chip Platforms

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Continuous monitoring of tissue microphysiology is a key enabling feature of the organ-on-chip (OoC) approach for *in vitro* drug screening and disease modeling. Integrated sensing units in OoCs are essential and highly demanded for the purpose. However, sensitive *in situ* & real-time measurements are not very common yet, due to the inherently small sizes of OoC devices, characteristics of commonly used materials, and external hardware setups needed to operate the sensing units. Here we propose a silicon/polymer hybrid OoC platform which combines the softness, optical transparency and biocompatibility aspects of polymers (PDMS) at the sensing area with the inherently superior electrical characteristics and ability to house active devices of silicon (see Figure 1). This platform is moreover versatile and modular, and it includes two different sensing units. The first unit consists of a floating-gate field-effect transistor (FG-FET), which is used to monitor changes in pH in the sensing area. The change in pH can be an indicator of certain disease phenotypes, as in the case of *e.g.* cortical spreading depression (CSD).[1] The floating gate has no terminals to apply voltage; rather, the FET's threshold voltage is regulated by a capacitively-coupled gate and changes in the charge concentration in close proximity to the extension of the floating gate, which can be thought as the sensing electrode. The second sensing unit exploits the extension of the floating gates as microelectrodes in order to monitor the action potential of electrically active cells, such as *e.g.* hiPSC-derived cortical neurons. In addition, the layout of the silicon/polymer chip and of the supporting, custom-designed printed circuit board are compatible with commercially available microelectrode array (MEA) measurement setups, which are commonly used by biologists. As future addition, sensing selectivity towards potassium ions will be integrated in the OoC platform to enable *in situ* measurements of different analytes on the same chip.

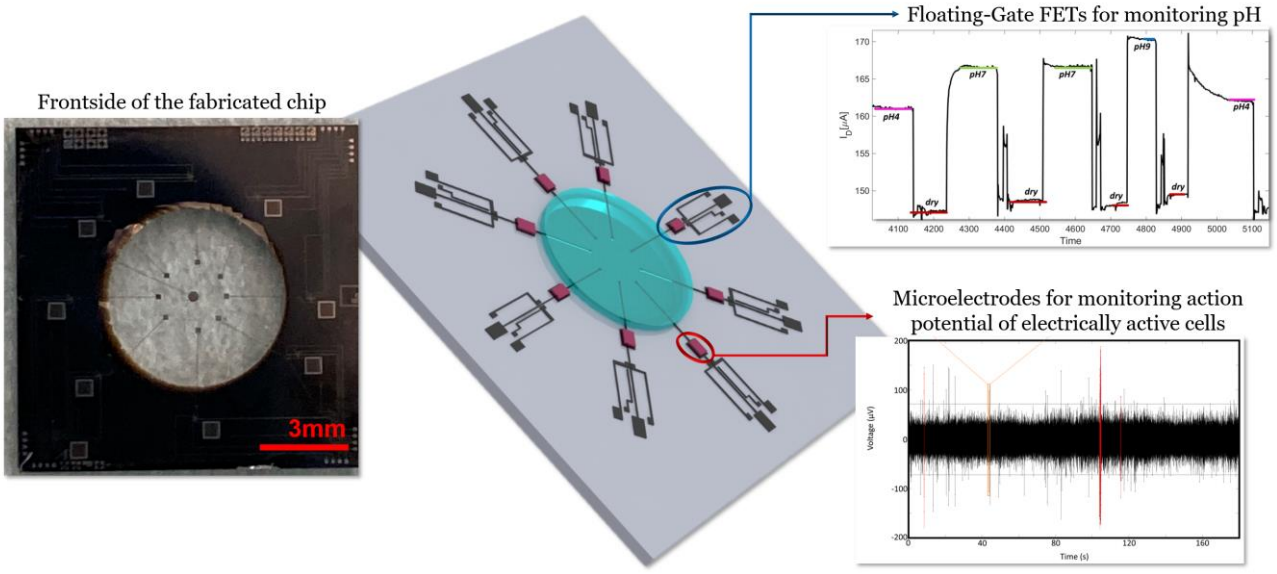

Figure 1. Modular sensing units integrated on the OoC platform. Picture of the fabricated silicon/polymer chip (left), schematic of the design and placement of sensing units (center), measurements solutal pH change (upper-right) and of action potential of hiPSC-derived cortical neurons (lower right).

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Engineering extracellular electron transfer in *Escherichia coli* for microbial electrochemical devices

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Microbes hold great potential for green electrochemical synthesis as well as bio-electricity generation from organic waste. The key to the successful development of microbial electrochemical devices lies with the ability of the microbes to transport electrons across their outer membranes. These devices thus often rely on the use of microbes known as exoelectrogens, which include bacteria that have evolved metabolic pathways that enable extracellular electron transfer (EET) to solid substrates for cellular respiration. While exoelectrogens demonstrate facilitated electron transfer with the electrodes of these devices, natural exoelectrogens are often restricted to utilizing a narrow range of substrates that limits their versatility for processing different organic wastes and electrosynthesizing a rich diversity of high-value chemicals. By contrast, non-exoelectrogenic microbes such as *Escherichia coli* that lack efficient EET pathways are host to rich metabolic reaction networks that can be more readily tailored for various applications using synthetic biology.

In this work, we biologically engineer *E. coli* for enhanced EET. To this end, we expressed different combinations of proteins from the natural exoelectrogen *S. oneidensis* MR-1, as well as *E. coli* native proteins to optimize EET of the bioengineered strain. The bioengineered strains show significant improvements in electron transfer rates, as confirmed through colorimetric reduction assays of soluble electron acceptors and electrochemical characterizations following electrode reduction. The improved EET demonstrated in this work paves the way for increasing the efficiency of existing *E. coli*-based electrochemical devices while opening the doors to new applications that benefit from the broad chemical repertoire of these microbes.

Optical tracking of ion dynamics in mixed conducting polymers

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Mixed conducting polymers have recently risen as a promising material choice for bioelectronic and biochemical devices due to their low impedance, soft mechanical properties, and ability to transduce ionic signals to electronic currents.[1] However, the current understanding of the electrochemical phase transition dynamics during ion gating remains limited. In this work, we address this gap by probing the electrochromic transition of the polymer backbone in a "moving front" experiment[2] with optical transmission microscopy with 1-ms temporal resolution and 400-nm spatial resolution. Furthermore, we utilize a prism and CCD to achieve time-resolved 1D-spectral, 1D-spatial imaging (**Figure 1a-c**). While the de-doping front near the electrolyte interface follows the expected distance squared *vs.* time relationship for ionic drift,[2] the front flattens as the ions reach the electrical contact (**Figure 1b**). We model this phenomenon using 1-dimensional drift-diffusion model which captures local potentials and electric fields within the polymer film. Contrary to previous work at much larger distances (several mm) and timescales (seconds to minutes),[2] we observe cation mobilities which are 2 orders of magnitude lower than previous models which ignore the contribution of ion diffusion. Finally, we use this technique to compare the electrochemical transitions of both doped and intrinsic mixed conducting polymers, revealing ionic *vs.* electronic bottlenecks. This work helps resolve the fundamental physics of electrochemical doping in mixed conducting polymers, providing guidance for optimizing the microstructure and chemistry for improved device performance.

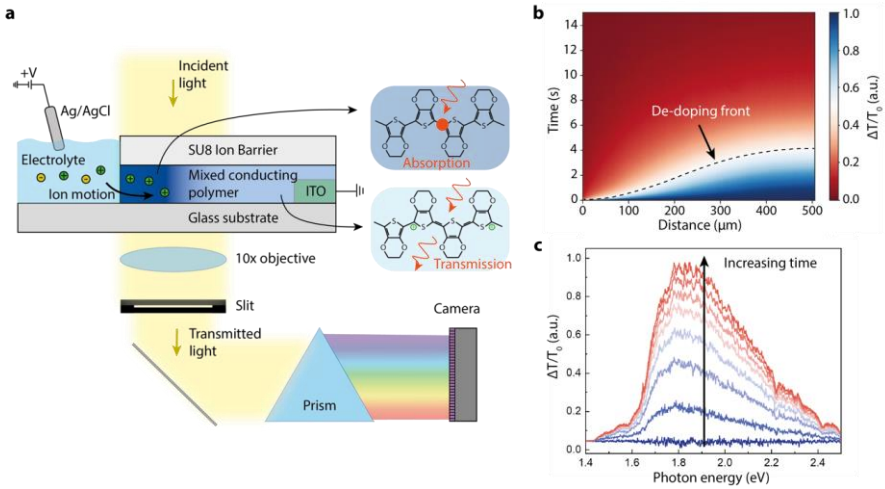

Figure 1. Optical transmission microscopy. a. Schematic of experimental setup for moving front experiments. **b.** Spatiotemporally resolved ion doping map. **c.** Time-resolved transmission spectra for PEDOT:PSS during moving front experiment.

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BIOEL 2022

Poster Presentations

Poster Sessions: Monday, March 14th & Tuesday, March 15th

(organized alphabetically by last name of presenting author)

MANUFACTURING OF FOIL-BASED MICROFLUIDIC CHIPS FOR NEURON CELL CULTURE AND AXON OUTGROWTH MONITORING

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The key technology within NextGenMicrofluidics is production of microfluidics-on-foil-substrates by imprinting, printing, and lamination processes, which we adapted for the demands of microfluidics. The technology is related to cell culture devices for pharmaceutical testing and the major aim is scaling up foil-based microfluidic devices for neuron cell culture applications and axon outgrowth monitoring. Usage of foil-based production method provides large area production, non-toxic modifiable surfaces, as well as biocompatibility and allows the combination of several devices, for example at the bottom of a 96-well microtiter plates. Additionally, for the characterization of the cell adhesion on the micropatterned surface, impedance measurement was chosen as an electrical characterization method. A roll-to-roll (R2R) based manufacturing process allows realizing smaller microfluidic channel sizes and additional functionalities such as electrodes. R2R-based manufacturing uses flexible substrates in production while increasing throughput, decreasing production cost, and simplifying substrate handling [1, 2]. Recently we have shown the applicability of roller-based nanoimprinting for manufacturing microfluidic chips used in point of care diagnostics [3]. For the first time, micro structuring, electrode printing and surface modification will be applied to the microfluidic chip in the same process where enables a scale-up of the chip manufacture. Axon outgrowth assays therefore become accessible to high throughput screening. The final product will have integrated sensors on the inlets/outlets and can be connected to an impedance measurement device. With that measurement, neuron cells growth will be inspected inside the channels. Consequently, the design of the chips was done and the batch production of the different parts of the device has been initiated.

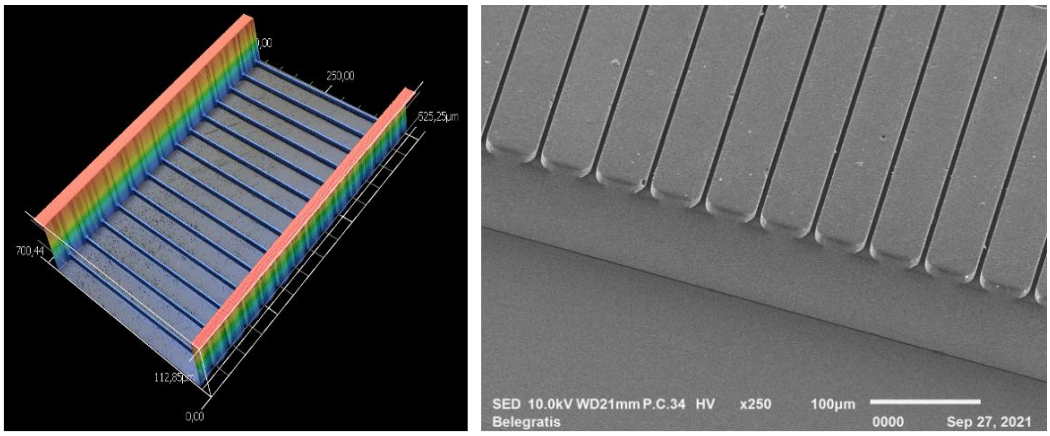

Figure 1: 3D image of the polymer shim that was used on R2R machine for imprinting process (left). The highest value of the colors is 112 µm. SEM image of the imprint (right). Scale bar is 100 µm.

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Photocurrent and Photovoltage Generation Dynamics at the Organic Semiconductor/Water Interface in p-Type Conjugated Polymers

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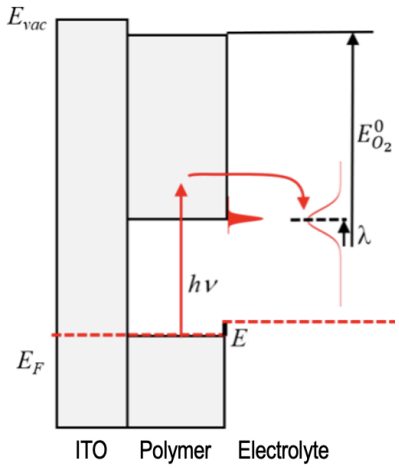
P-type organic semiconductors (OSCs), such as photoactive semiconducting polymers, are gaining interest as phototransducer capable to generate stimulating signals inside biological cells or tissue.[1] We are particularly interested in them as phototriggers of reactive oxygen species (ROS) due to their intrinsic conductivity and optical properties.[2] When used as bioactive photoelectrodes, the direct interface between the semiconductor and the electrolyte gives rise to different, competing electrochemical phenomena such as the photofaradaic or the photocapacitive processes, depending on whether the photogenerated charges get involved in redox processes or accumulate at the interface.[3,4] Understanding the different competing optoelectronic and optoelectrochemical phenomena arising from the direct interface between the OSC and the aqueous electrolyte is of central importance for the development of future devices. In this work, by comparing photocurrent and photovoltage transients in presence and absence of oxygen we demonstrate the crucial role of the oxygen reduction reaction and the generation of ROS species for such interfaces. By combining the findings with electrochemical impedance spectroscopy, we develop a simple equivalent circuit model to explain the large observed photovoltage and its build-up dynamics in OSC floating photocathodes in contact with electrolyte. Based on Butler-Volmer kinetics describing the photoreduction process, we obtain a quantitative description of the photovoltage transients and charging behaviour of p-type photoelectrodes. The findings are further combined with nanoscale morphological investigations and Kelvin-Probe Force Microscopy leading to a quantitative interfacial energy diagram such as depicted in the Figure.

This research is of particular relevance to understand wireless, optically triggered bioelectronic transduction as achieved with p-type OSC in the form of transducer patches or micro- and nanoparticles in contact with biological cells.



Photoelectrochemical mechanism scheme

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Pushing resolution limits in neural interfaces: downsizing of vOECTs

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Organic electrochemical transistor (OECT) is a device that has experienced significant advancement over the last few years. A multitude of applications was shown, ranging from neural recording[1] and biosensors[2,3] to neuromorphic devices.[4] When used as a recording device, the local amplification property of a transistor is advantageously utilized to improve the signal-to-noise ratio (SNR). Compared to conventional neural recording electrodes, the SNR of an OECT can be higher by 20 dB.[1] The recently introduced vertical OECT (vOECT) has shown significant increase in transconductance.[5] In this work, the vertical geometry is adapted to create devices both on rigid and flexible substrates with the goal to further scale the values of transconductance and bandwidth. We fabricated vOECTs with channel length of ≈ 400 nm, defined as a step etched in silicon dioxide. The PEDOT:ClO_4 channel was created by electropolymerization. Device characterization in KCl solution yielded very high values of transconductance reaching 80 mS.

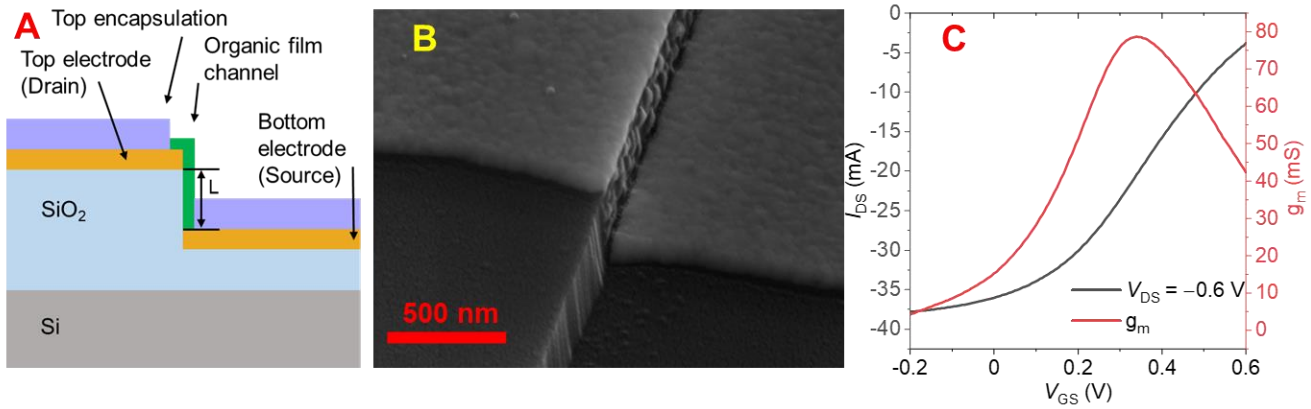

Figure: (A) Schematic of the fabricated vOECT showing channel length (L); (B) Gap between drain (left) and source (right) electrodes without the polymeric channel; (C) Transfer characteristics (black) and transconductance (red).

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Interfacing Neuronal Cells using Bioconjugate Organic Electrochemical Transistors for electrophysiological measurements

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Obtaining a good interface between biological systems such as complex neural cell networks and electrodes is essential for understanding their cellular mechanisms and electrophysiological properties. In contrast to metal and inorganic semiconductors, organic mixed ion-electron conductors (OMIECs) have superior properties to interface biological materials due to 1.) their mixed ionic and electronic conductivity and 2.) the possibility to tune elastic modulus, electronic properties, and combability with cells by chemically modifying their side chains.[1]

To sense changes in ionic concentrations, organic electrochemical transistors (OECTs) can be fabricated using OMIECs. In this transistor configuration, the OMIEC is used as the channel material connecting source and drain contact and an electrolyte connecting the gate and the channel. Injection of ions into the OMIEC channel change the doping (redox) state and thus also its conductivity. In contrast to alternative transistor configurations, ions penetrate into the bulk OMIEC channel, which leads to a higher amplification of the signal and higher transconductance.[2] High transconductance allows measuring small changes in ionic concentration and is an essential property to perform electrophysiological sensing. Polar glycolated polythiophenes such as the polymer p(g4T2-T) or p(g4T2-TT) have recently reported very high transconductance values and outperformed previous benchmark material such as Poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS).[3,4]

Here we introduce a new polar polythiophenes-based polymer p(g4OH2T-T). Compared to p(g4T2-T), 20% of the glycol side chains are functionalized with a hydroxyl group. OECTs fabricated using p(g4OH2T-T) can undergo 1000 on/off switching cycles and showed long-term stability in cell culture conditions at 37 deg. C in BPS. Simple salinizations can be performed with the hydroxyl groups. Modifications with (3-Aminopropyl)triethoxysilane (APTES) or a zwitterionic sulfobetaine silane (SBSi) enhance or hinder attachment of cells on the polymer film, respectively. It was possible to differentiate Lund Human Mesencephalic (LUHMES) neuronal cells on p(g4OH2T-T) modified with APTES. In contrast, modifications with SBSi hindered cell attachment on the polymer surface. SBSi modified P(g4OH2T-T) is not toxic to cells, meaning cells still grow and differentiate right next to it. This gives the opportunity to control where on p(g4OH2T-T) cells grow without the need of complex comparimetalized cell seeding chamber systems.

The presented method provides simple tools which can be used to measure electrophysiological activity of neurons for toxicological screenings and evaluating the maturity of differentiating stem cell derived neuros.

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PEDOT:PSS for OECT-based sensing of cytokines

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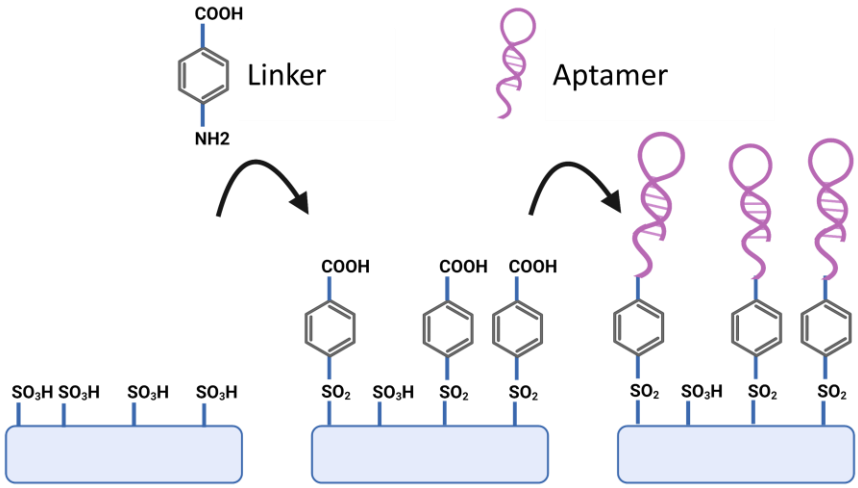
Poly(3,4-ethylenedioxythiophene) doped with polystyrene sulfonate (PEDOT:PSS) is the most common organic semiconductor in OECTs. The comparably high transconductance compared to OFETs, combined with easy circuit integration, makes them good candidates for biosensors. While metabolites and neurotransmitters have been successfully sensed with OECTs, only a few reports for cytokine detection based on OECTs have been reported – but none have used PEDOT:PSS for attachment of the recognition element.[1–3]

Here, we demonstrate a strategy using a small linker molecule to attach to the sulfonates on PSS, which then allows EDC/NHS coupling to bind a recognition element. In this study, we choose aptamers as the recognition elements to demonstrate the concept. Aptamers are synthetic oligonucleotides that exhibit high specificity, low production costs, good shelf-life, and can be modified for various immobilization strategies,[4] making this a versatile, cheap, up-scalable approach.



Functionalization strategy

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A flexible neural probe for drug delivery and chemical sensing in the brain

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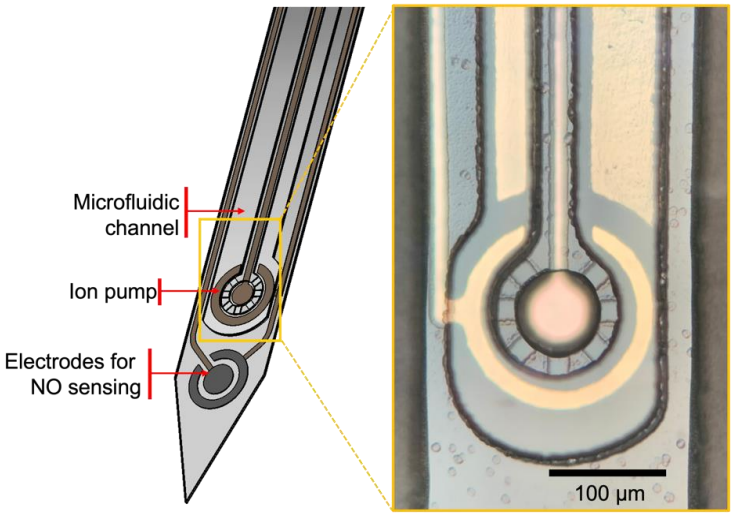
Various strategies have been developed and used to treat neuronal diseases/disorders. Among the novel techniques, the organic electronic ion-pump (OEIP) has received great attention as a promising therapeutic solution. Devices developed based on the OEIP have demonstrated the powerful fundamental ability of precise electrophoretic on-demand drug delivery into a limited region of interest with high temporal resolution. [1–3] Despite the superior performance as a drug delivery method, the combination with other electrophysiological tools, which could expand the potential, has been limited. Here we demonstrate advancement beyond these limitations through the development of a flexible neural probe incorporated with an OEIP and a chemical sensor. This device contains a reservoir for long-term drug delivery, integrated with and connected to the microfluidic channel that provides the solution of a charged substance (e.g., $GABA^+$) to an ion exchange membrane. To measure the nitric oxide (NO) concentration, which is known as a radical signalling molecule in the nervous system, in the brain in real-time, electrodes were placed near the ion pump outlet to function as an electrochemical sensor. The working electrode was electrodeposited with porous platinum and coated with a permselective membrane to improve the detection performance for NO. These functional elements were fabricated on a flexible, implantable neural probe to reduce the problems that may arise due to the mechanical mismatch between the probe and brain tissue. We expect that the combination of these two abilities in a single device facilitates the simultaneous monitoring of changes of a specific analyte and modulation of the neuronal activity chemically and on-demand.



Schematic of the proposed flexible neural probe integrated with an OEIP and a NO sensor (left). Photograph of a part of the fabricated device (right)

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Bidirectional thin-film peripheral nerve cuffs for high-resolution sensory and motor interfacing

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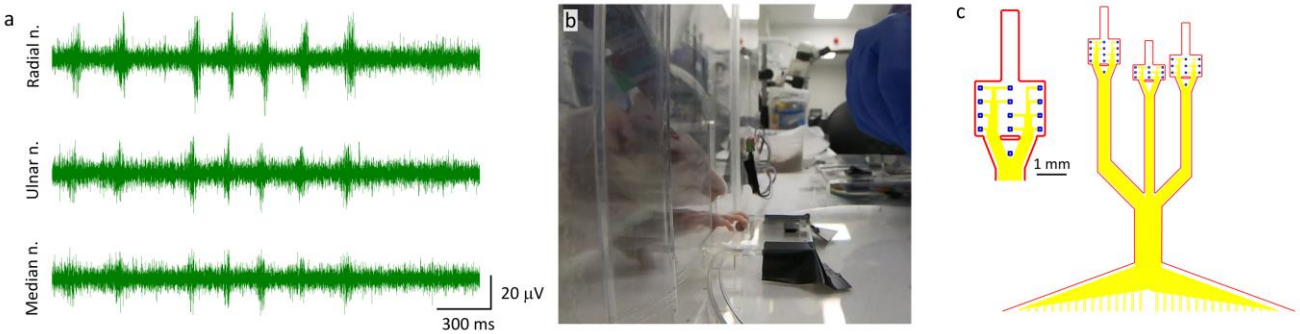
Peripheral nerve recording and stimulation interfaces can be used for the treatment of dysfunctional structures controlled by nerves.[1] While interfacing with sensorimotor nerves holds great potential for the restoration of movement and sensation in patients suffering from conditions such as spinal cord injury, nerve injury and amputations; there is currently a lack of peripheral nerve interfaces capable of providing a high enough recording/stimulating resolution to restore skilled function.[1]

Here, we present a peripheral nerve cuff interface capable of both stimulation and recording from sensorimotor peripheral nerves. The devices are microfabricated using thin-film technology, and are composed of high-density poly(ethylene dioxythiophene):poly(styrene sulfonate) (PEDOT:PSS)-based microelectrode arrays on parylene-C which can be rolled into cuffs for high resolution sub-nerve compartment recording and stimulation. We validate the ability to record nerve action potentials and stimulate muscle contraction of the thin-film cuffs through implantation into the ulnar, radial and median nerves controlling arm function in rat models. When chronically implanted for four weeks, we further show that the cuffs exhibit good long-term tissue compatibility when compared to currently available nerve cuff technologies. Finally, we demonstrate the translational potential of the thin-film nerve cuffs by recording sensorimotor nerve activity in chronically-implanted awake animals performing skilled behavioural grasping tasks.



Neural recordings from forearm nerves in awake animals performing a skilled grasping task using parylene thin-film cuff implantable interfaces. a) *Electroneurograms of nerve activity recorded with parylene thin-film cuffs 6 days post-implantation (0.4 - 2kHz bandpass filtered). Action potentials can be detected in radial, ulnar, and median nerves – responsible for sensorimotor control of arm and paw – while animal performs a grasping task.* ***b)*** *Image of rat performing task (reaching out, grasping and retrieving sugar pellet).* ***c)*** *Schematic of implantable parylene thin-film triple cuff (red – parylene-C outline, yellow – gold tracks, blue – PEDOT:PSS electrodes).*

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Towards home-based nucleic acid tests, using paper

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Diagnostics are an integral part of healthcare and play a key role in preventing communicable disease outbreaks. Antigen tests that rely on paper-based lateral flow technology are rapid and affordable, but cannot match the sensitivity of Nucleic Acid Amplification Tests (NAATs), as seen during the SARS-CoV-2 pandemic[1]. We still lack rapid and affordable, integrated NAATs for the point of care, and these tests are currently carried out at centralized laboratories at high cost. The reason is that NAATs are much more complex than antigen tests and must integrate more steps including DNA extraction, amplification, and detection. Sample preparation, remains a bottleneck here that hinders truly integrated solutions for bacterial samples. This is because current DNA extraction methods introduce inhibitors that require rinsing[2], or enzymes that require heat deactivation prior to downstream processes[3]. To solve this problem, we have shown that immobilized lytic enzymes on nitrocellulose can be used to separate the enzymes from the lysate[4]. The amount of DNA extracted from these enzymes is similar to that extracted by the same enzymes in solution, which allowed us to integrate this paper-based method of DNA extraction with amplification and detection suitable for point-of-care applications[5]. We demonstrated a sample-to-answer NAAT for the detection of Staphylococcus epidermidis, a gram positive opportunistic human pathogen bacterial species. The test uses Recombinase Polymerase Amplification (RPA) for amplification, and lateral flow dipsticks for detection of target DNA. Our work shows the possibility to perform NAATs from sample to answer, without any instruments by utilizing paper-based technology. Future development could make home-based DNA test kits a reality.

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Modulation of cytoskeletal systems with intense nanosecond pulsed electric field

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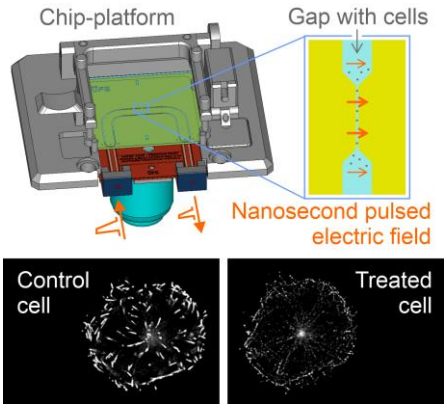
Ultrashort duration intense pulsed electric field (PEF) represents a unique tool to modulate the function of biological systems with potential applications in bionanotechnology and biomedical therapies. However, an integrative understanding of PEF effect from atoms to cells and beyond is missing for the rational development of any potential biomedical/ bionanotechnological method based on PEF.
We present our bottom-up approach for the understanding of PEF effects on biomolecular building blocks of cytoskeleton from *in silico*, through in *vitro* and up to *in vivo* approaches. At first, we show our insights of nanosecond PEF effect on tubulin and microtubule in molecular dynamics simulations[1,2]. We leveraged these insights in interpreting our findings on nanosecond PEF ability to modulate tubulin conformation to control self-assembly of tubulin to microtubules in vitro[3]. Then we demonstrate how the marriage between chip microfabrication technology and advanced microscopy brought us tools to observe effects of nanosecond PEF on cytoskeleton network *in vivo* (in cells)[4,5].



This figure shows a nanosecond chip platform integrated to a microscope. The chip platform enables real-time observation of live cells with super-resolution microscopy while delivering intense nanosecond pulsed electric field. We demonstrated that nsPEF remodels the cytoskeleton via observation of fluorescently labeled microtubule end-binding proteins in rat basophilic cell line. Figure is adopted from reference [4].

Acknowledgements: We acknowledge Czech Science Foundation (GAČR), project nos. GA18-23597S and GA20-06873X.

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Novel diketopyrrolopyrrole derivatives as the active material for organic electrochemical transistors

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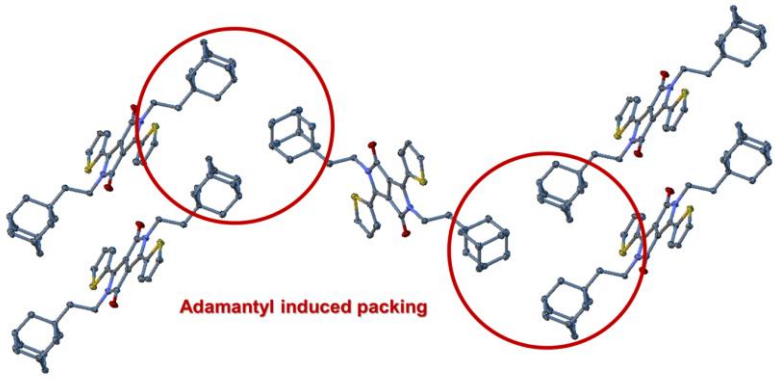
The diketopyrrolopyrrole (DPP) molecule, systematically named 2,5-dihydropyrrolo[4,3-*c*]pyrrolo-1,4-dione, belongs to the major organic high-performance pigments, which have already found several significant applications in the fields of organic electronics.[1] Its structure offers an inexhaustible range of possible structural derivatizations, whereas one of the most important is the nucleophilic substitution of various alkyl chains on the nitrogen heteroatoms of the DPP core. This modification using e.g. hydrophilic side chains allows the preparation of an efficient and biocompatible *p*-type mixed ion-electron conductor usable for organic electrochemical transistors (OECTs).[2]

Our research group published the DPP modification by incorporation of bulky ethyladamantyl chains on nitrogen heteroatoms, which has brought the unique, hitherto unprecedented influence of alkyl side chains to the molecules' arrangement.[3] The resulting derivative formed herringbone aggregations leading to smaller π-π stacking distances between the conjugated cores, which resulted in ambipolar characteristics of this derivative with excellent electron mobility of 0.2 $cm^2 \cdot V^{-1} \cdot s^{-1}$, which is an excellent value for such modified derivatives.

The current trend, which moves the importance of DPPs to an even higher level, is their application in OECTs. This is made possible by the incorporation of ethylene glycol fragments into the DPP molecule and the subsequent preparation of conjugated polymers based on such modified DPPs, as described by Krauss et al.[4] They further found that the higher the ethylene glycol substituent content in the DPP-based polymer (beyond 40 wt%), the significantly higher the performance of the material in the OECT device. This kind of relatively facile derivatization opens a window of the DPP-based conjugated polymers utilization as the active materials of OECTs. Therefore, the DPP molecule is a very attractive building block for the construction of devices in the field of bioelectronics.

Acknowledgement: The authors thank for their financial support the Austrian Agency for International Cooperation in Education and Research (OEAD-GmbH, WTZ, CZ01/2020, 8J20AT025).



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Inferring the spatial resolution of optogenetically or electrically stimulated retinal ganglion cell neurons

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Decades of extensive studies into the field of electrical retinal stimulation have led to various approaches on stimulation protocols[1,2], novel prostheses prototypes[3,4] and materials for prosthetic devices fabrication[5]. More recently, a new technique, the optogenetic therapy emerged as a possible approach to vision restoration[6]. In this study, we compare the spatial resolution achieved through direct electrical stimulation and optogenetic stimulation in adult rod degenerated mice expressing the blue light sensitive opsin channel rhodopsin (rd10 - ChR2).

Retinal ganglion cell (RGC) activity from different retinal samples was recorded by means of a high-density CMOS microelectrode array. Sinusoidal electrical stimuli with a frequency of 40 Hz were applied at various spatial frequencies. Light ("optogenetic") stimulation consisted also of similar spatial patterns delivered at 460 nm wavelength and with a 10 and 2.5 Hz switching frequency, respectively.

A population level analysis of the spiking activity of RGCs was performed and the spatial resolution achieved through the stimulation protocols in these two cases was inferred from a support vector machine classifier.

Our preliminary results indicate that for the protocols tested, optogenetic stimulation leads to considerably better discrimination of artificial grating stimuli.

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Plastic waste-derived activated carbon/PEDOT:PSS composite for EDLC flexible supercapacitor electrodes

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Plastic waste piling on landfills is a well-known growing problem. Worldwide estimation of landfilled plastic waste for 2018 is 2.6×10^6 tons.[1] In this work we provide a way of processing and utilizing common plastic waste (PE, PP, PES, PS, PA) by applying a low-cost simple two-step procedure: hydrothermal conversion followed by pyrolytic-chemical activation. Different activated carbon materials are presented and used as an electrode materials for supercapacitors. Activated carbon obtained by pyrolyzed plastic offers good electrochemical performance (up to 354 $F\cdot g^{-1}$ at 1 $A\cdot g^{-1}$), stable lifetime (85 % retention after 150 000 cycles) and extremely large surface area (S_{BET} = 1335 $m^2\cdot g^{-1}$). Subsequent utilization of selected polyamide-derived carbon (C-PA) is showed by a preparation of foldable hybrid PEDOT:PSS/C-PA supercapacitor electrodes. Hybrid electrodes show good electrochemical performance (38 $F\cdot g^{-1}$ in symmetric supercapacitor cell) and very good bending properties (99 % retention after 500 bending cycles (180°)) which predetermines them as bendable supercapacitors. This work shows promising approach of utilizing common plastic waste for supercapacitor electrodes and bendable supercapacitor devices.

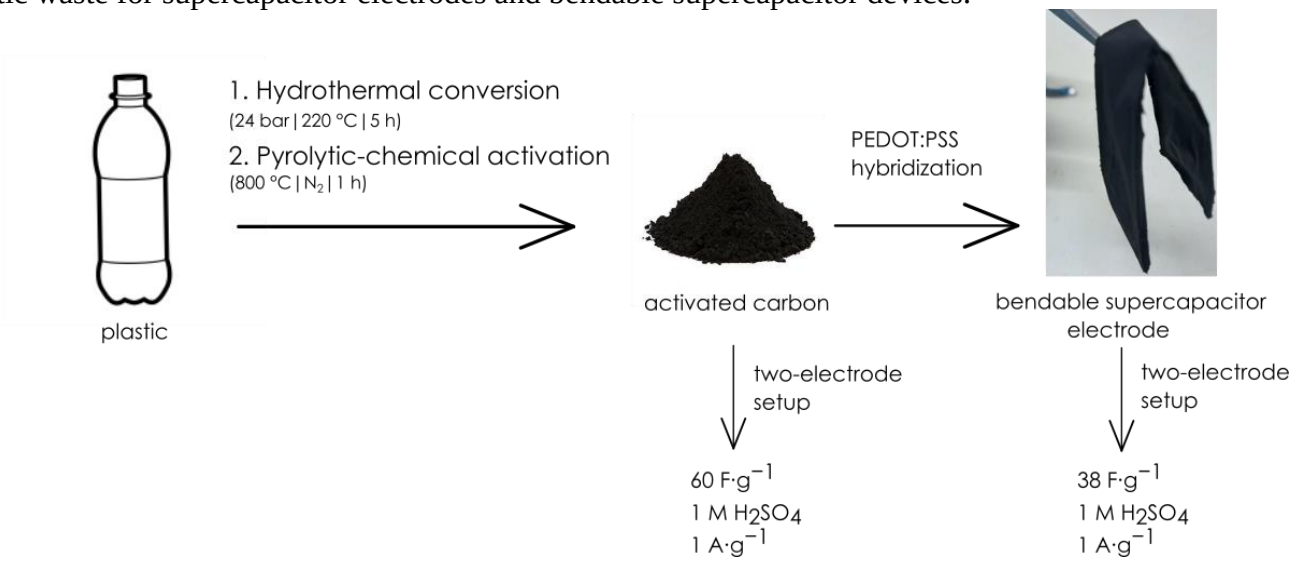

Figure: Graphical depiction of procedure how to utilize common plastic waste

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Direct measurement of oxygen reduction reactions at neurostimulation electrodes

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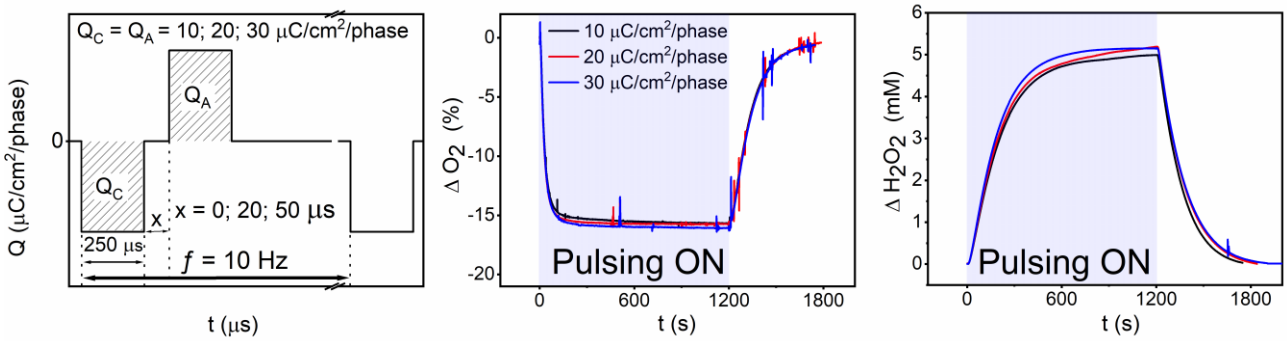
During electrical neural stimulation, charge-balanced biphasic current pulses are typically used to trigger action potentials in excitable tissues. Cathodic pulses depolarize cells, evoking action potentials, meanwhile subsequent anodic pulses are meant to reverse the products of possible electrochemical processes back to their original form. Balanced biphasic current pulses are thus generally considered to be safe for chronic applications.[1] However recently it has been found, that not all reactions that could possibly happen at the electrode-tissue interface during the stimulation are perfectly reversible. Products of these reactions can be possibly dangerous to the tissue or act as signalling molecules. The identity and the scale of such reactions still remains an unexplored area.[2]

Our work examines oxygen reduction reactions (ORRs) at the electrodes made out of commonly used materials used for neural stimulation electrodes (platinum, gold, tungsten, nichrome, iridium oxide, titanium, titanium nitride, and PEDOT:PSS). Oxygen can be reduced either to water or hydrogen peroxide by 4- or 2-electron process. Both reactions can significantly reduce the quantity of dissolved oxygen near the electrode, creating hypoxic conditions harmful to neurons. Peroxide, meanwhile, can induce toxic reactions or act as a signalling molecule. We have examined the amount of reduced oxygen and produced peroxide by various biphasic stimulation protocols using amperometric sensors (Clark electrodes) and compared electrocatalytic activities of studied materials.

Main finding is that typical charge-balanced biphasic pulse protocols do lead to irreversible ORRs. Some electrode materials induce highly hypoxic conditions near electrode surface, others additionally produce an accumulation of hydrogen peroxide into the mM range.



Example of charge-balanced cathodic-leading current pulses and subsequent amperometric sensor response 200 µm above the electrode surface. Gold electrode was used for this demonstration.

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Electrical Imaging of non-electrogenic Cells Using Adhesion Noise for the Evaluation of Epithelial Mesenchymal Transition

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Objective. Cell adhesion noise (CAN) spectroscopy constitutes a potent tool to investigate cellular behavior in health and disease probing the contact properties of non-electrogenic cells in a non-invasive way.[1,2] Biological processes like body formation during development and pathological processes like cancer require the acquisition of migratory and invasive properties. There is evidence that the gain of this feature implies changes in cell adhesion and phenotype through the process known as epithelial mesenchymal transition (EMT).[3,4] In cancer, the number of cells undergoing EMT is associated with the patient's prognosis.[5] At the time, the estimation of cells undergoing EMT relies on complex molecular biology techniques based on the presence of several proteins in the tumoral cells.[6] In this work, we intend to set up a fast and cheap protocol to allow for estimating the proportion of cells undergoing EMT in a 2D culture of cancer cells by using electrical imaging. Devices with high electrode density called microelectrode arrays (MEA) enable spatiotemporal studies of cellular behavior at subcellular resolution.[7]
Approach. For the initial experiments, the epithelial colorectal cancer cell line HT-29 is cultivated on the MEA compromising thousands of sensors at a pitch of ~10 µm. The power spectral density (PSD) of the voltage noise in the cell junction is measured at frequencies between 1 kHz and 450 kHz. Data processing is accomplished via customized Python scripts. The noise levels of the cell adhesion are recorded before, during and after EMT initiation. We also evaluate cell proliferation on the MEA using the Cell Counting Kit CCK8 and viability by fluorescence staining with Hoechst and Propidium Iodide. Brightfield and Alexa Fluor 488 Phalloidin images are taken to correlate between both the electrical and fluorescence images.
Main results. While sensor areas without cells showed a low noise level, areas with attached cells resulted in high voltage noise. The electrical imaging of non-electrogenic cells showed a high accordance with brightfield images via microscopy. The chips coated with Poly-L-lysine allowed for a reliable cell attachment to the surface for several days. Importantly, the assays do not have a detrimental effect on cell proliferation or viability.
Significance. Being able to electrically image non-electrogenic cells at subcellular resolution makes CAN spectroscopy a powerful tool to study cellular behavior in a non-invasive, label-free, fast and cheap manner in comparison with the actual molecular biology approaches.

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I

Ion-beam reactive deposition of nitrides for bioelectronics: high-conductivity stoichiometric titanium nitride microelectrodes, and AlN passivation layers

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Bioelectronic devices such as neural stimulation and recording devices require stable low-impedance electrode interfaces. Various forms of nitridated titanium are used in biointerface applications due to robustness and biological inertness. Herein we fabricated stoichiometric TiN thin films using a dual Kaufman ion-beam source setup for ion beam sputtering (IBS) enhanced by ion-beam assisted deposition technique (IBAD). These layers are remarkable compared to established forms of TiN due to high degree of crystallinity, excellent electrical conductivity, and relative optical transparency. We characterized electrochemical properties in terms of TiN microelectrodes impedance, capacitance, and biphasic current pulsing. Such prepared TiN compares favorably with commercial TiN, and stands out for good conductivity and low faradaicity. Using the same sputtering setup, we have developed AlN as a promising passivation insulation material for bioelectronics.

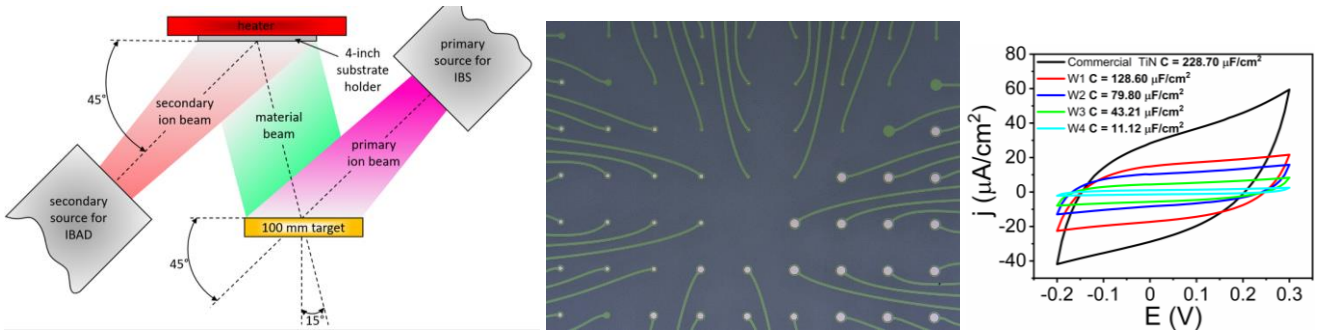

Figure 1. *(Left)Schematic of deposition instrument equipped with two Kaufman ion-beam sources; (center) TiN microelectrode array passivated with parylene-C; (right) Cyclic voltammograms of ion-beam sputtered TiN compared with state-of-the-art commercial TiN. Our TiN maintains at least two-times lower resistivity while maintaining competitive capacitance.*

Contactless nanoscale motion control for noninvasive neuron interface guidance and signal recording

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Develop dynamic micro/nano-electrodes with controllable motion could benefit both cellular guiding and sensing for minimally invasive bio-electronic transduction study.[1] Motion control in either orientation or movement from micro/nanoscale and to molecular scale has demonstrated promising potential in cell interface for precise medicine.[2,3] However, directional shaping/moving in micro/nano-electrodes is yet to be met, especially in a contact free mode to allow noninvasive and targetable cell manipulation.[4]

Aims to achieve selective and contactless cell interface guidance. Furthermore, to sense the resulted electrical signal change noninvasively and transduce related signal inter/intracellularly in real-time. We designed to fabricate light-driven micro/nano-pillar arrays with conductive nanoparticles, as shown in the Figure 1. Here, we present our recent discovery in intensity dependent re-shaping (low intensity) and moving (high intensity) of micro/nanopillar arrays. Both re-shaping and moving were achieved by single wavelength laser (555 nm) stimulation on azobenzene-based polymer (poly(disperse red 1 methacrylate), pDR1m) pillar arrays. We proposed viscoelastic (dynamic) region after time dependent photo-deformation analysis. Pillars' re-shaping and moving direction could be simply adjusted by light polarization within this region.

Based on previous work of using pDR1m pillars to regulate cytoskeleton structure,[5] we propose this device could provide a precise stimulation on cell interface and record resulted bioelectric signal in real-time, which could particularly benefit neuron interface study to allow individual axon regulation.[6,7]

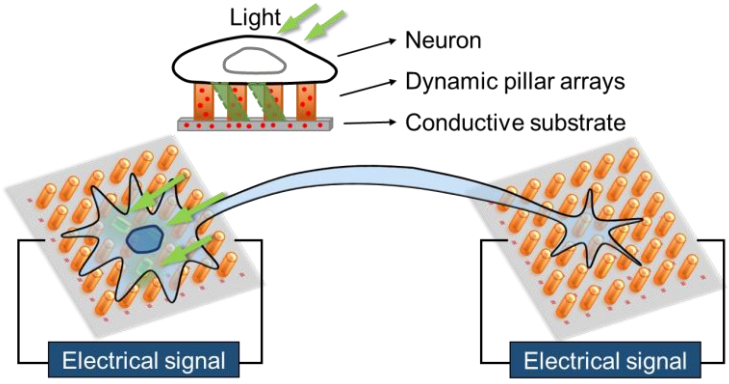

Figure 1. Illustration of proposed light-driven pillar arrays on a conductive matrix for neuron stimulation and bioelectrical signal recording/transduction.

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Protection of Oxygen Sensitive Enzymes by Peptide Hydrogel

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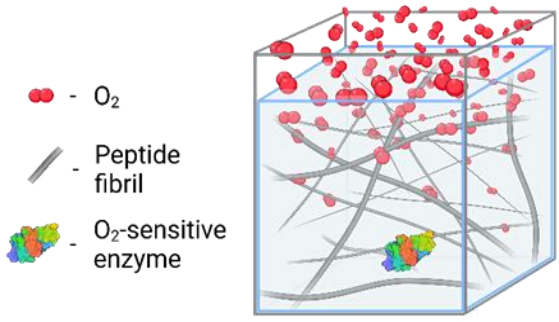
Molecular oxygen (O2) is a highly reactive oxidizing agent and is harmful to many biological and industrial systems. Although O_2 often interacts via metals or reducing agents, a binding mechanism involving an organic supramolecular structure has not been described to date. In this work, the prominent dipeptide hydrogelator Fluorenylmethyloxycarbonyl-diphenylalanine, is shown to encage O_2, and significantly limit its diffusion and penetration through the hydrogel. Molecular dynamics simulations suggested that the O_2 binding mechanism is governed by pockets formed between the aromatic rings in the supramolecular structure of the gel, which bind O_2 through hydrophobic interactions. This phenomenon is harnessed to maintain the activity of the O_2-hypersensitive-enzyme [FeFe]-hydrogenase, which holds promising potential for utilizing hydrogen gas for sustainable energy applications. Hydrogenase encapsulation within the gel, allows hydrogen production following exposure to ambient O_2. This phenomenon may lead to utilization of this low molecular weight gelator in a wide range of O_2 sensitive applications.[1]



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Decoding the vagus nerve: strategies for identification of ECAPs and estimating nerve activation

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Motivation. Closed-loop bioelectronic medicine is becoming a promising strategy for treatment of chronic diseases [1]. Reliable detection of neural events is therefore essential, although extraction of neural events from peripheral nerves poses challenges that arise from the small diameter of the nerves, the low amplitude and high signal-to-noise ratio of the neurograms, and the non-stationary nature of these signals, among others. Despite ongoing research in neural decoding algorithms is evolving, there is no consensus on the best methodology to process and extract information from peripheral nerves.

Methods. Three strategies to detect evoked compound action potentials (ECAPs) from neurograms recorded at the subdiaphragmatic vagus nerve were compared. The first strategy used a temporal analysis by detecting events over a simple threshold ($V_{th} = 5\mu V$ as described in [2]), whereas the other two exploited the benefits of wavelet analysis for filtering and identifying events in non-stationary signals. For the wavelet strategies, each neurogram was divided into a reference signal comprising the region preceding the stimulation artefact providing a good representation of the background noise and basal neural activity, and an evoked signal comprising the region after the stimulation artefact. The first wavelet-based strategy consisted of a continuous wavelet transformation (CWT) using orthogonal Daubechies wavelets of order 3 (Db3) at the scales corresponding to the length of the ECAPs (1.5 to 3.2ms), Then, a modified hard-thresholding wavelet de-noising technique from the original method proposed by Donoho was employed [3], which essentially applies a threshold to the coefficients at each decomposition level before transforming them back into the original domain. In our work, the maximum coefficient obtained from the CWT of the reference period on each neurogram was selected as the threshold for identifying the ECAPs on the subsequent evoked signal. The second wavelet-based strategy consisted of a de-noising multiresolution analysis (MRA) of the signal using discrete wavelet transformation (DWT) as proposed by Diedrich [4]. Orthogonal Db3 using 7 levels of decomposition were again selected. In this case, the maximum amplitude of the reconstructed reference signal was selected as the threshold to identify the evoked activity int the evoked region.

Results. From the strength-duration curves obtained using the three strategies it was observed that the rheobase currents from all fiber types reached the smallest value in the MRA method, followed by the CWT and the fixed temporal threshold (the rheobase current for C-fibers was *0.596mA, 0.596mA* and *0.596mA,* respectively). This result was expected as most of the background noise was removed in the wavelet-based methods whereas ECAPs masked in noise were also possibly removed by setting a fixed temporal threshold. Interestingly a pulse width of 0.5ms caused the recruitment of the highest number of fibres for the same amplitude, which suggests that this pulse width is optimal for fiber recruitment.

Conclusion. The methodology and results of this study contribute to the use of a generalised reliable algorithm and signal analysis strategy to decode neural recordings that will ultimately enable to extract real-time information about the state of the nerves and the related organs. We particularly highlight diabetes as a high-impact chronic disease that could significantly benefit from the exploitation closed-loop neuromodulation of the metabolic dysfunction for its diagnosis and treatment.

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Oxygen plasma etching of nano particle to develop microneedle electrodes for plant bioelectronics measurements

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Sensing technologies are the basic perception layer of the so called "Agriculture 4.0", a novel and fast-growing agricultural revolution playing a promising role in enhancing sustainable farming[1]. In this context, there is a need for more efficient, continuous, and real-time in-planta sensing technologies, enabling an early-stage stress detection and thus an early-solving intervention leading to an eco-sustainable yield increase[2].
Among the recent technological advances in in-planta sensing technologies, microneedles (MNs) electrodes represent a newly applied concept, extremely useful to decrease invasiveness and at the same time increase sensitivity of sensing measurements[3]. In particular, such electrodes can be applied to real-time monitor plant water status using electrical impedance spectroscopy (EIS). Here, we show the fabrication of these electrodes using oxygen plasma etching of nano spheres on the top of a PEDOT:PSS layer, employing both rigid and flexible substrates. We demonstrate also the attachment of the electrodes to the leaf surface, comparing their performance with standard electrodes (e.g., needle electrodes, planar, as shown in Fig. 1.

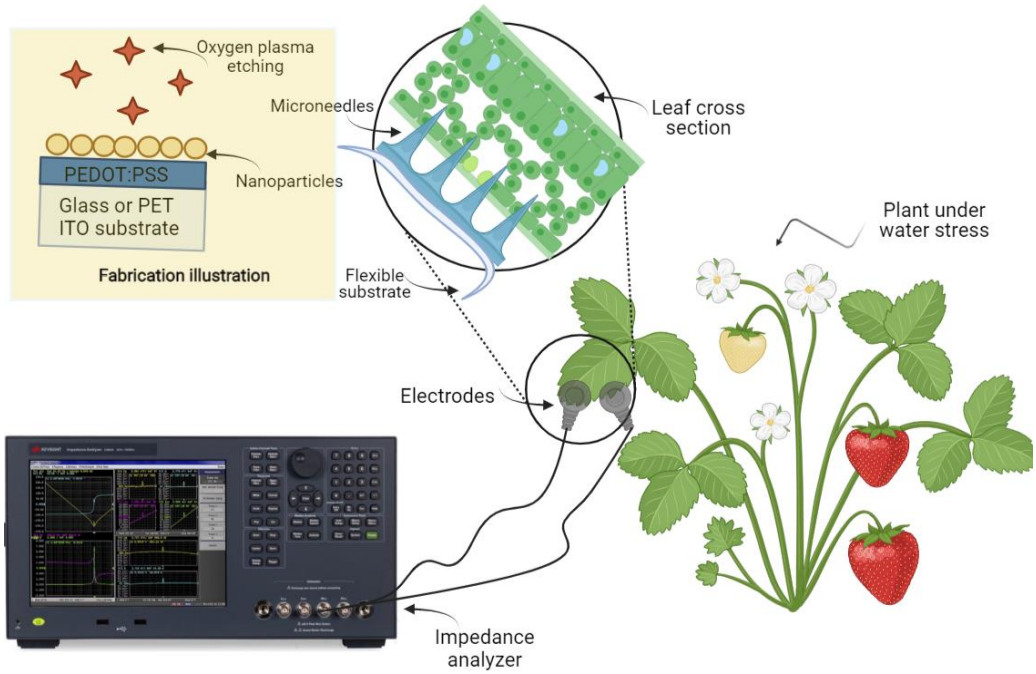

Fig. 1. An illustration of the microneedle electrodes fabrication process for bioimpedance analysis of water-stressed strawberries plants in a growth chamber.

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Iontronic Pumps as a new approach for local chemotherapy on the Chick Chorio Allantoic Membrane

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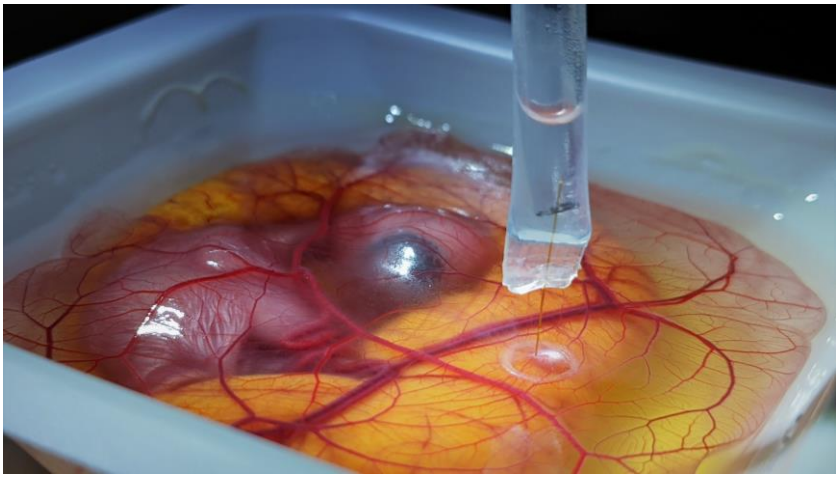
Patients diagnosed with Glioblastoma multiforme (GBM), the most aggressive brain tumor form, only have a poor survival rate of only 15 months, despite combined standard treatment of maximal safe resection, chemotherapy and radiotherapy. Moreover, various clinical attempts with novel chemotherapeutics did not result in increased life expectancy, as these drugs are often shielded by the blood-brain-barrier (BBB). Therefore, we developed a novel approach based on organic electronic ion pump (OEIP) to bypass the BBB and allow treatment with much more potent chemotherapeutics. The principle of this technology is based on electrophoresis, where electrically, spatiotemporal controlled delivery and dosing of chemotherapeutic drugs (Gemcitabine or GEM) via an ion-exchange (hyperbranched polyglycerols (HPGs)) is achieved with high precision. We have recently shown that apoptosis and disintegration of microtumors in vitro is induced by this chemo OEIP technology[1]. To investigate this promising bioelectronic device as a new chemo drug delivery method for brain tumor treatment in-vivo, we now established an 3R (reduce, replace, recycle) animal model. Herein, the Chick Embryo Chorioallantoic Membrane Assay (CAM) was used to cultivate vascularized GBM tumors that were treated with Iontronic Pumps filled with a chemotherapeutic drug (GEM). After electronically controlled treatment, we analyzed the tumor area histologically and immunohistochemically. In control conditions, indeed U87 GBM tumor cells generate rapidly growing and highly vascularized, solid tumors on CAM. Remarkable, treatment with Gemcitabine Iontronic Pump significantly interfered in tumor growth combined with apoptotic areas already within few days of treatment. Using this 3R cancer animal model I will discuss improved treatment of glioblastoma treatment with the Iontronic Pump.



[1] L. Waldherr et al, *Advanced Materials Technologies*, DOI 10.1002/admt.202001302

4D-Printable Sodium Polyacrylate Superabsorbent Polymer, A Material for Self-folding Cuff Electrodes to Interface Peripheral Nerves

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Cuff electrodes are often used to stimulate and record from peripheral nerves. Several implementations of such electrodes are equipped with a zip tie like closing mechanism or surgical threads to fix them on the nerves.[1,2] These approaches work well on larger nerves with diameters of several hundred micrometers. However, on smaller nerves in the range of 100 µm and below, the closing mechanisms are often too bulky, and the manual attachment increases the risk of rupturing the nerve. An alternative solution could be a cuff electrode that closes and wraps itself around the nerve, like a tiny grabbing hand. Since classical mechanical assemblies with joints, such as robotic hands, are stiff and difficult to fabricate, additive manufacturing with soft "smart" materials (4D-printing) represent an alternative option.[3] For example, hydrogels that swell in water can be suitable "smart" materials, which deform a device over time due to an external trigger.

Here, we present a 3D-printable high swelling hydrogel based on sodium polyacrylate for this application. The superabsorbent properties of sodium polyacrylate materials are already used in baby diapers or as fake snow.[4] Our printed structures show a swelling over 20 times their printed weight in water and maintain the swelling movement even against an externally applied pressure. A bilayer with a flexible, not swelling polymer can achieve directional folding. The swelling under load is an advantage to other reported smart hydrogels, which allows folding down to small radii of the printed bilayer structure in the size of the targeted nerves. We used the developed superabsorbent resin to print a tiny hand that folds after exposure to electrolyte solution. With integrated electrodes, the system forms a soft self-folding cuff electrode, which can grab, hold on to, stimulate, and record from a small peripheral nerve.

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Approaching Stimulation of Living Cells with an Organic Photosensor Utilizing a Polymer-Based Electrode

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We envision a future prosthetic device that would replace degenerated photoreceptors within the retina of vision impaired people. To enable a seamless interface between biological tissue and the intended vision sensor, we focus on small molecules based photovoltaic layer blend[1,2] and a polymer-based electrode which has mixed ionic and capacitive charge injection.[3] This polymer electrode consists of poly(3,4-ethylenedioxy-thiophene) blended with polystyrene sulfonate (PEDOT:PSS) polymer and a crosslinker for stability in the aqueous environment.[4] Such electrodes have already proven functionality in bioelectronic applications.[5,6] To bridge the surface polarity gap between the hydrophobic photoactive layer and the hydrophilic electrolyte, a double layer of different PEDOT:PSS formulations is required. Optionally, an ultrathin gold doping layer is inserted between the two PEDOT:PSS layers for enhanced conductivity.

To study the photocurrent of the organic photosensor injected into the electrolyte, we use an electrochemical setup that is equipped with an external light trigger. This mechanism has a transient nature, but yet the photocurrent response of the device can be traced by the electrochemical cell setup to study photo-capacitive and photo-faradaic processes.[7]

Moreover, we are interested in the effect of the organic photosensor stimulation on living cells. The stimulation of the living cell is achieved by the generated potential field which stimulates the cell membrane and originates from the electrolyte/electrode interface based on a capacitive mechanism. Therefore the patch clamp method is used to investigate photosensor stimulation on living Human Embryonic Kidney (HEK-293) cells, which are reported to be biocompatible with the PEDOT:PSS surface .[8]

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Cytotoxicity study of PEDOT based thin films

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The usage of organic semiconductors in the construction of bioelectronic devices represents a very promising alternative to metal electrode systems and traditional inorganic semiconductors. For these devices, the biocompatibility of the interface between the electronic element and living cells is a critical parameter. Poly (3,4-ethylenedioxythiophene): poly (styrene sulfonate) (PEDOT: PSS) is probably the most successful conductive polymer in terms of practical application. It has many unique properties, such as excellent optical transparency in the visible light range, high electrical conductivity, and good physical and chemical stability in air. However, the issue of long-term biocompatibility of PEDOT: PSS is not completely resolved. Therefore, new ways to improve biocompatibility are constantly being sought. DNA biofunctionalization, for example, seems promising.[3]

In our cytotoxicity study, we monitored and compared the cytotoxicity of PEDOT:PSS and PEDOT:DNA films deposited on a glass substrate on the NIH3T3 mouse fibroblast cell line. We focused on the morphological changes of cells after contact with the film observable in the microscope (see Fig. 1) and on their viability determined by flow cytometry and confocal microscopy. The obtained data show that the PEDOT:DNA combination represents a promising way to preserve the benefits of the polymer while increasing biocompatibility to living cells.

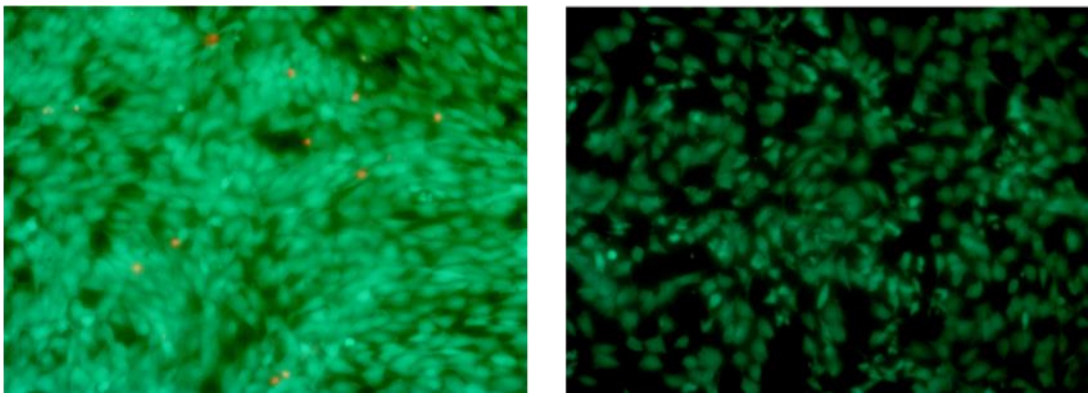

Figure 1. Fluorescence microscope images of PEDOT:PSS (left) and PEDOT:DNA (right); Note: green colour shows a calcein dye in cytosol of living cells while red spots represent a propidium iodide dye inside nuclei of dead cells.

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Role of interfacial layers in the performance of EGOFET-biosensors

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Electrolyte-gated organic field-effect transistors (EGOFETs) show promise for use in biosensors as there are naturally biocompatible and have a high amplification in their electrical signal [1]. It has been shown that the low voltage variations generated by the (I) binding of proteins or (II) electrically excitable cells can be recorded [2]. In the first case, the electrodes or semiconductors are functionalized with biorecognition layers to detect antigens in an analyte where the electrode is immersed [3]. The response in the EGOFETs signal is controlled by the variations at the electrolyte/semiconductor and the electrolyte/gate interface. With the antigen binding to their specific antibodies, the distributed capacitance and the charges at the interfaces are changing, leading to a change in the semiconductor's conductivity [4]. Likewise, Stern layers inside the electrical double layer at these interfaces affect the output characteristics.

Our studies determine these effects by parameter studies with numerical simulations. We show the effects of fixed charges, capacitive effects, and the influence of the ionic strength in the electrolyte solution. We built a finite-element model (FEM) of an EGOFET in the Nernst-Planck-Poisson and drift-diffusion frameworks. We display the transfer and output current-voltage characteristics for different semiconductor properties, ionic strength, and above all, the properties of the interfacial layers. We show the voltage profiles and the distribution of charges to reveal the formation of accumulation layers. With these studies, we provide further explanations of the behaviour of EGOFETs towards stable operation for a future in biosensing techniques.

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Natural Dielectrics for Bio-Organic Electronics: Waxes and Wax Components from Plant and Animal Origin

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The main focus of this work is the investigation of the dielectric layer, that is an essential component of field effect transistors. Common dielectrics used in high performance FET-devices include inorganic oxides such as Al_2O_3, Ta_2O_5 or HfO_2. These inorganic materials show great dielectric properties; however they require a thin passivating layer when interfaced with organic semiconductors. As thin capping layer materials, nine different natural waxes and wax components have been investigated: Beeswax, Carnauba wax, Lanolin, Montan Wax, Rice Bran wax, Shellac wax, Cholesterol and Hexadecyl Palmitate. These class of dielectrics show good film forming characteristics, high hydrophobicity, excellent dielectric behavior over a wide frequency range and good insulating properties in OFET devices operating at voltages as low as 10 mV. Such dielectric materials may find their applicability in implantable devices operating at low voltages required to interface the electronic components with the neural network of human brain for example.

Novel Soluble Nature-Inspired Riboflavin Derivatives: Side-chain Engineering and Material Characterisation

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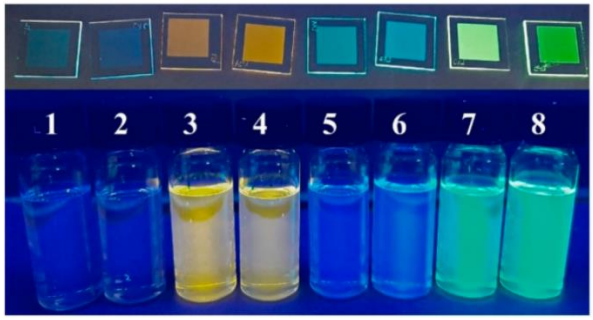
The family of flavins encompasses yellow π-conjugated organic pigments occurring in all the kingdoms of life. The natural source, riboflavin, capable of both one- and two-electron transfer processes, plays an essential role in enzymatic redox reactions in the cellular respiratory chain as a cofactor of flavoenzymes.

Both natural and artificial flavins share unique properties attributed to their molecular structure, defining the redox and optical properties. Moreover, considering their biocompatibility, non-toxicity, versatility, and sustainability, together with the potential for low-cost production, it is apparent that they are prospective materials for bioelectronic applications.

Our research group has recently studied the synthesizability of nature-inspired flavin derivatives resulting in the preparation of NH-free molecules with prolonged conjugation of the aromatic system, followed by a comprehensive material characterisation.[1] Preceding research served as a springboard for preparing N,N′-alkylated flavin molecules and studying the side-chain engineering for solubility and processability enhancement together with fine-tuning their physicochemical properties.

The study introduces a series of molecules synthesised by direct alkylation of earlier-published NH-free derivatives[1]. The reaction was performed with corresponding alkyl halides or tosylates, and after the purification, the materials were obtained in moderate yields. Afterwards, a complex material study was performed to evaluate the effect of linear butyl and bulky adamantylethyl side-chains. [2]

Thin films of flavins were prepared using a physical vapour deposition technique. The optical, electrical, electrochemical, and morphological measurements were performed to determine material properties. Subsequently, we evaluate the potential applications of these unique molecules in organic electronic devices. [2]



The emission of thin films and solutions of N,N′-alkylated flavins illuminated with a light of 366 nm.

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Wireless optoelectronic peripheral nerve stimulators

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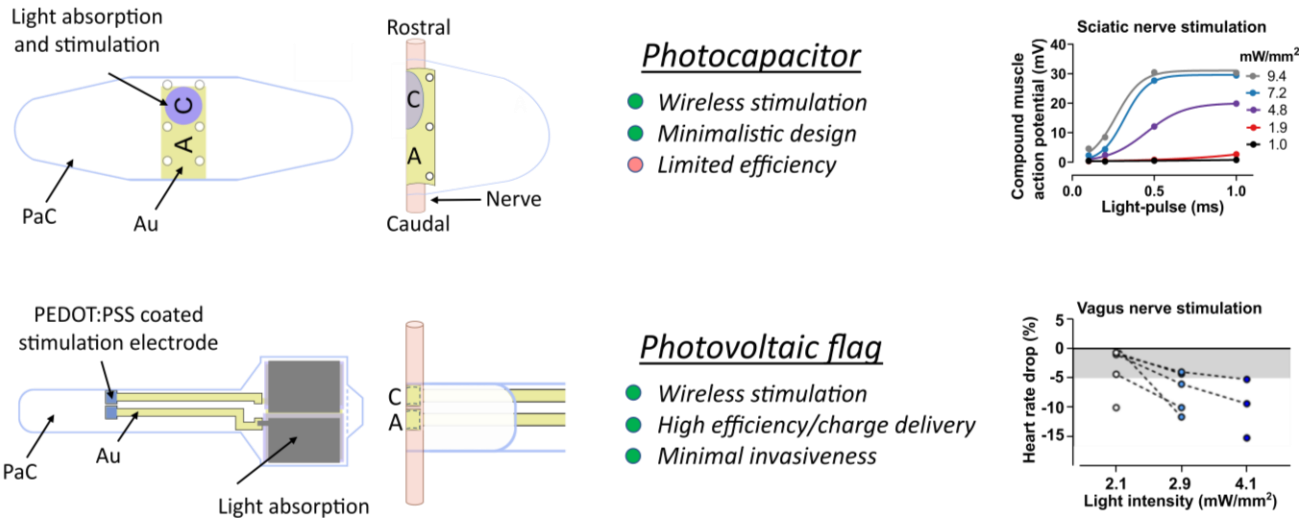
Peripheral nerve stimulation is used to treat a plethora of neurological conditions ranging from neuropathic pain over obstructive sleep apnea, epilepsy, and depression to autoimmune and inflammatory diseases. Implantable neuromodulation devices ought to be designed for robustness, reduced invasiveness, and ease of implantation. Here, we introduce wireless optoelectronic devices based on organic photovoltaic cells driven by tissue-penetrating deep-red light. The devices are fabricated on ultrathin parylene substrates to ensure good conformability around the nerves. Owing to variations in peripheral nerve sizes and their anatomical positions, two device concepts were developed to ensure robust neural interface. More minimalistic organic electrolytic photocapacitors (OEPCs) utilize photoactive pixels directly as stimulation electrodes and are thus optimal for large-diameter nerves implanted relatively close to the skin to ensure sufficient light absorption by the implant. On the other hand, a "photovoltaic flag" device composed of a photovoltaic driver connected to a pair of stimulation microelectrodes enables application for deeper-lying and smaller targets. We showcase the devices on two *in vivo* deployments: sciatic nerve in rats; and vagus nerve in mice. The low-volume, robustness, and biocompatibility of the photovoltaic devices facilitate wireless chronic stimulation of peripheral nerves and thus enable novel *in vivo* experiments.



Representative applications of wireless optoelectronic devices for photoelectric nerve stimulation.

Silverå Ejneby, M. et al. Chronic electrical stimulation of peripheral nerves via deep-red light transduced by an implanted organic photocapacitor. *Nat Biomed Eng* (2021). https://doi.org/10.1038/s41551-021-00817-7

Natural Dielectrics for Bio-Organic Electronics: Plant Resins from Coniferous Pinaceae Trees

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Organic electronics has an immense potential for the development of products that are both sustainable and environmentally friendly. In this poster presentation, a large list of natural origin dielectric materials belonging to the class of pinaceae trees are introduced and explored in the fabrication of field effect transistors *i.e.* black pine (*pinus nigra),* shore pine *(pinus contorta),* Rocky mountain fir *(abies lasiocarpa),* silver fir *(abies alba),* European spruce *(picea abies),* Atlas cedar *(cedrus atlantica),* larch *(larix decidua), Baltic amber (Pinus halepensis)* and the commercially available rosin. Apart for the outstanding dielectric and film forming properties presented by this poster, it is worth noting that the above mentioned pinaceae resins are inherently, biocompatible (even edible) and have well known antimicrobial and antifungal properties, with many of them used in traditional medicine to treat various illnesses or sores. Thus, this class of pinaceae resins may find in future applications in the branches of science where dielectric materials are part of bio-integrated electronics.

RuO_2 Supercapacitor-integrated Safe and Efficient Optoelectronic Biointerface

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Non-genetic optoelectronic biointerfaces show promise for neurostimulation owing to their wireless operation and high spatiotemporal resolution. Capacitive photovoltaic biointerfaces has attracted significant attention due to their safe charge injection mechanism that is based on electrode-electrolyte double layer capacitance. However, the limited double layer capacitance values of optoelectronic biointerfaces (on the order of 1 to 10 μF cm^{-2}) prevents achieving high charge injection densities that is needed for more light-sensitive neurostimulation. In this study, we integrate supercapacitor RuO_2 into an organic photovoltaic biointerface architecture to achieve efficient and safe neural photostimulation. Integration of RuO_2 to the return electrode of ZnO/P3HT:PCBM-based organic biointerface provides a high redox capacitance in parallel with return electrode double layer capacitance, which enhances the charge injection density more than 20-fold by increasing the time constant of the photoresponse.[1] We confirmed the reversibility and safety of the charge injection mechanism of RuO_2-integrated biointerfaces by electrochemical analyses of RuO_2 coating and intracellular oxidative stress measurements of primary neurons. Patch clamp recordings from hippocampal neurons cultured on RuO_2-based biointerfaces showed the opening of voltage-gated sodium channels via 445 nm, 5 ms pulsed-light, which leads to reproducible action potential firing at 5, 10, 20 Hz photostimulus frequencies. Solution-processed RuO_2-integration presented in this study shows promise for fabrication of low-cost, flexible, safe, and light-sensitive next-generation optoelectronic biointerfaces.

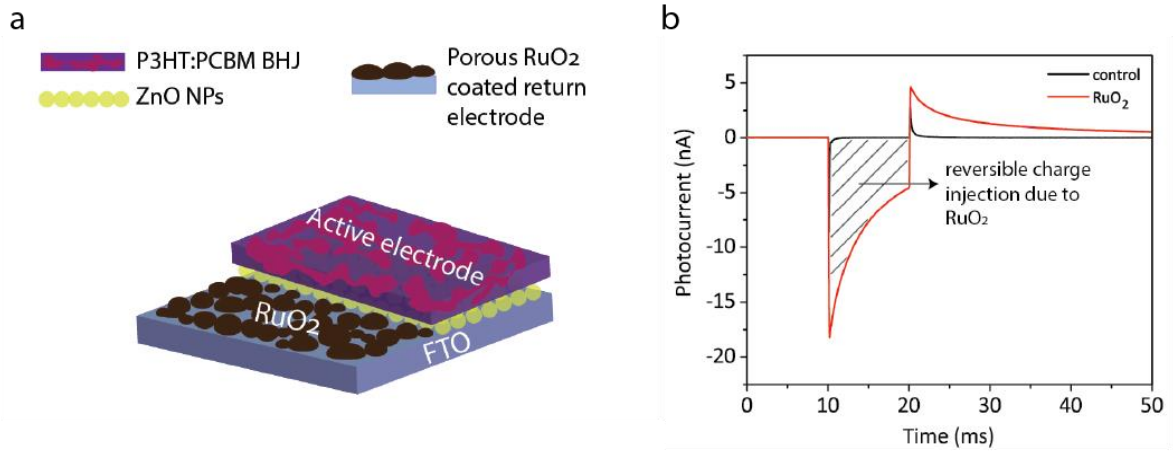

Figure 1: a) RuO_2-integrated solution-processed biointerface architecture. b) Surface photocurrent of control device without RuO_2 coating and RuO_2-integrated device measured with patch clamp setup, showing the additional reversible charge injection resulting from RuO_2 integration.

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The interplay of conjugation and metal coordination in tuning the electron transfer abilities of NTA-graphene based interfaces

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Complex protein-graphene interfaces, which are possible candidates for bio-electronic devices, such as biosensors, have been researched and developed in the last few years.[1,2] One of the basic aspects to design efficient bio-devices is the modification and optimization of the linker-metal interfaces, such as a self-assembly monolayer (SAM) of organic molecules interacting with a graphene monolayer. Previous research focused on SAM physisorbed on graphene, which leads to preservation of conductive properties of graphene, but at the same time the reversibility of the interaction can lead to the desorption of the molecules from the surface.[3,4] That is why chemisorption of SAM on graphene is now taken into consideration and the structures like the one shown in Figure 1 are studied. By mean of tight-binding density functional theory (TB-DFT), the aforementioned structures are optimized and their electronic properties analysed with the Quantum Espresso program. Three effects are considered: nature of the metal center, chain's saturation and surface coverage density. Overall, the work function shift is strongly dependent on the nature of the metal. Both cobalt and nickel ensure charge transfer, whereas strong charge recombination for copper inhibits it. In the presence of the metal-NTA complex chain saturation can slightly alter the work function shift and its molecular contribution for all interfaces but does not have a more significant influence on the charge transfer. Finally, the effect of surface coverage density influences all the parameters and outweighs the other two effects in creation of electronic properties of the interface.

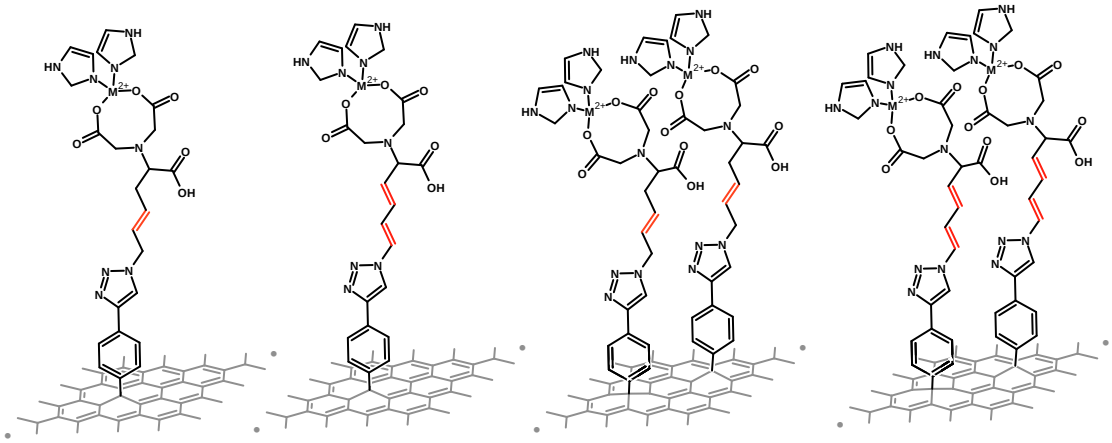

Figure 1 Scheme of discussed systems. M= Ni^{2+}, Co^{2+}, Cu^{2+}

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High Conductivity Melanin Film Supported by Depletion Force

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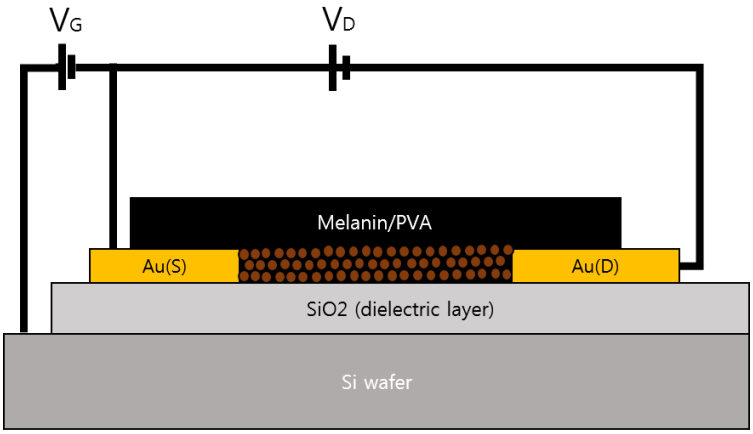
Melanin is a dark-brown pigment found in nature. Especially squid ink contains a large amount of melanin and is the main source from which melanin can be easily extracted. Melanin, due to its structure, has special optical properties, metal ion chelation, and electron-ion hybrid conductivity.[1] These properties imply that melanin has potential as a bio-electronic material. However, sufficient conductivity is required to use melanin nanoparticles as bio-electronic materials. It is also not easy to make a conductive path because the structure of melanin is spherical in shape with a diameter between 70 to 130 nm. Here we demonstrate a melanin coating supported by a depletion force and fabricate a melanin field effect transistor through it. The depletion force is generated by the interaction between poly(vinyl alcohol) and melanin nanoparticles, which causes melanin nanoparticles to aggregate.[2] Aggregated melanin nanoparticles facilitate electron transfer between melanin particles, helping thus overcome its low conductivity. The transistor operation proves the potential of melanin as a bio-electronic material.



Scheme of melanin transistor

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3D Printed Conductive Scaffolds for Self-Organized Bioelectronics via In-Situ Enzymatic Polymerization of Conjugated Oligomer-Based Hydrogels

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Hydrogel-based bioelectronics are attractive for communicating between medical devices and human neural tissues due to their similar mechanical properties. However, conventional hydrogel-based bioelectronics are lacking the electrical property which is to mimic the native tissue environment for transferring electricity needed for neuronal activities. Recently, our group has demonstrated enzyme-assisted polymerization of conjugated oligomers forming conductors within the structures of plants.[1]

Here we expand the application of this in situ enzymatic polymerization of conjugated oligomers by introducing in vitro platforms of conductive hydrogel-based bioelectronics using 3D bioprinting technology. Complex 3D structures of conductive hydrogel-based scaffolding were fabricated using 3D printing hydrogel inks loaded with the conjugated oligomers, followed by enzymatic polymerization in a physiological environment. Functionalized scaffolds were subsequently used for PC12 cell growth and controlled modulation of neuronal phenotype differentiation.

This work enables the fabrication of biocompatible, conductive, and customized 3D in vitro platform for studying neurological/cellular function with greater functionality than conventional 2D cell culture. And it paves the way for building self-organized electronics for modulating biological functions in vivo.

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Organic Microelectrode Array for In Vitro Drug Activity Studies of Neuronal Ion-channel Associated Diseases

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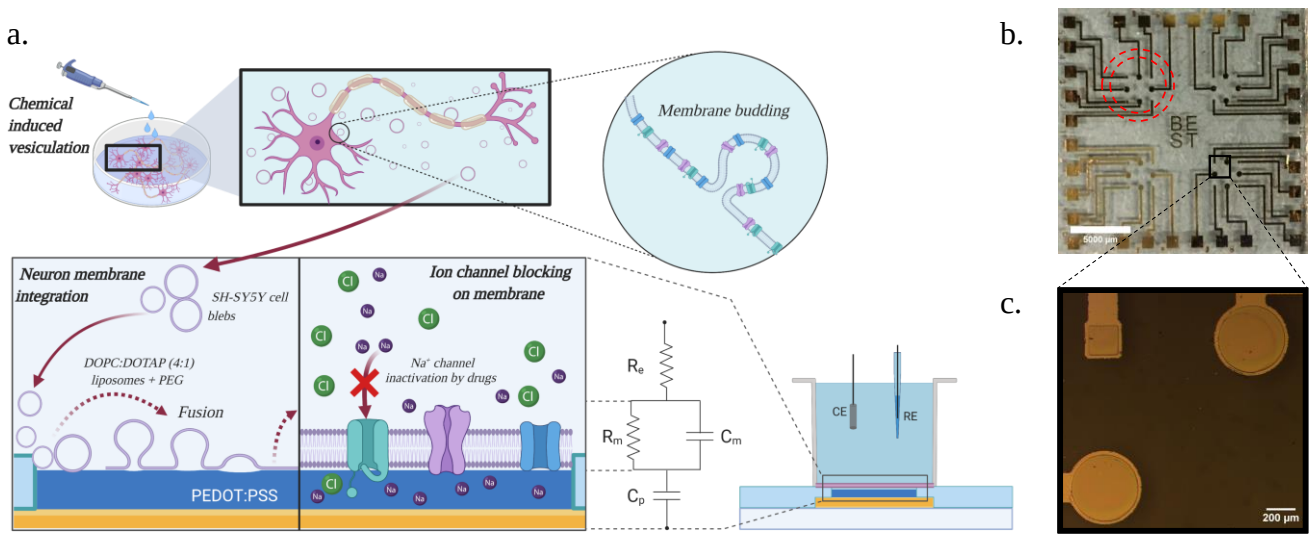
Neuronal Ion-channel protein is a crucial membrane component directly related to neuron electrical activities; disturbance of neuron signals and natural toxin effects caused Ion-channel dysfunctional diseases still burden the global healthcare system. The study of native ion channels from native membrane perspective provides a simplified and direct model to study the drug and toxin effects on targeted ion channels[1]. The microelectrodes were fabricated and coated with conducting polymer poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) as electrical layer.[2] The SH-SY5Y neuroblastoma membrane is successfully fused onto the organic microelectrode array and its membrane quality is characterized by electrochemical impedance spectroscopy (EIS). Furthermore, drug screening studies of lidocaine and verapamil on membranes were performed. The calcium-ion channel blocking activities related to verapamil were detected and analyzed by EIS. Also, the sodium-ion channel blocker,[3] lidocaine, was demonstrated to have both lipid disruption and ion-channel blocking activities. Our neuronal membrane-on-chip system provides a time-efficient and high-throughput platform for pharmacology study and drug discovery on native ion-channel activities.



The neuron membrane-on-chip system: (a) the process of isolation of neuron membrane vesicles, membrane integration on microelectrode, and ion-channel blocking test. (b) The PEDOT:PSS microelectrode array; (c) The zoom-in picture of the microelectrodes. (Unpublished)

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Investigating equilibrium binding models applied to EGOT-based protein biosensor

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Electrolyte Gated Organic Transistors (EGOTs) are rapidly emerging as one of the architectures of choice for label-free biosensing for their outstanding capability of amplification of small biological signals. EGOT family encompasses Organic Electrochemical Transistor (OECT) and Electrolyte Gated Organic Field Effect Transistors (EGOFET): they both operate in an aqueous environment, using an electrolyte containing the analyte as dielectric between the gate electrode and the organic (semi)conductor (OSC).[1] In order to use EGOTs as biosensors, one can immobilize a specific biorecognition element on the gate ; since gate and OSC are capacitively coupled, a change in the potential at the gate/electrolyte interface is transduced into modulation of the drain current.

In this work, we analyse the response of two potentiometric EGOT immunosensors, OECT and EGOFET, for the detection of pro-inflammatory cytokine Interleukin-6 (IL-6). To this end, a dose curve was constructed by plotting the change in the drain current normalized by the current value in the absence of the analyte, termed signal (S), as a function of the target analyte concentration. This dose curve can be described in terms of equilibrium binding models (isotherms), under the hypothesis of dynamic equilibrium between the biorecognition unit and the target analyte.[2]

Our study shows that the dose curve obtained by either EGOFET or OECT is better described by Frumkin isotherm, compared to the most typically used Langmuir and Hill isotherms. Frumkin isotherm provides a physical view of the competing phenomena at the gate/electrolyte interface[3], characterized by the increase of electrostatic repulsions between analyte molecules with the increasing surface coverage at the functionalized gate. Moreover, the corresponding analysis of free energy unifies the results obtained by both EGOT architectures, suggesting that the response is due to the binding events at the gate/electrolyte interface, and independent of the transduction mechanism. Therefore, EGOTs can be used not only as biosensors for analytical purposes but also as a tool for investigating fundamental aspects of biorecognition phenomena.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no. 813863.

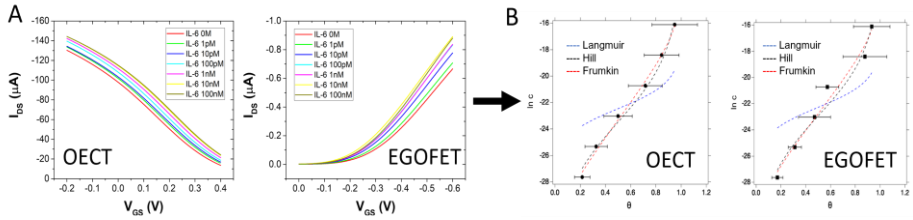

Fig1. A) Transfer characteristics for an OECT and an EGOFET upon analyte binding, at V_{DS} = -0.2 V. B) ln c vs θ plots for OECT and EGOFET, where *c* refers to IL-6 concentration and θ to surface coverage.

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Determination of ion exchange of organic semiconductors using fluorescence

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Organic electrochemical transistors (OECT) are used in a wide range of applications from energy-harvesting technologies to bioelectronic devices. The operation of these transistors is based on ion exchange between the environment and the organic semiconductor, and therefore on the mixed ionic-electronic conduction of conjugated polymers. Ion exchange thus plays a key role in the overall performance of the device and its sensitivity. This work deals with the possibility of studying ion exchange using two independent detection methods. The basis for the study of ion exchange is the electrical detection of positive ions (in the case of using a *p*-type organic semiconductor) using a modified OECT. The process of ion exchange is also monitored by a second independent method, namely the fluorescence of an ion-selective fluorescence probe. This combination of methods could contribute not only to a better understanding of ion exchange, but could also play a key role in material research in determining the volumetric capacitance of organic semiconductors. This knowledge could also help to elucidate the processes behind cell stimulation in stimulation platforms.

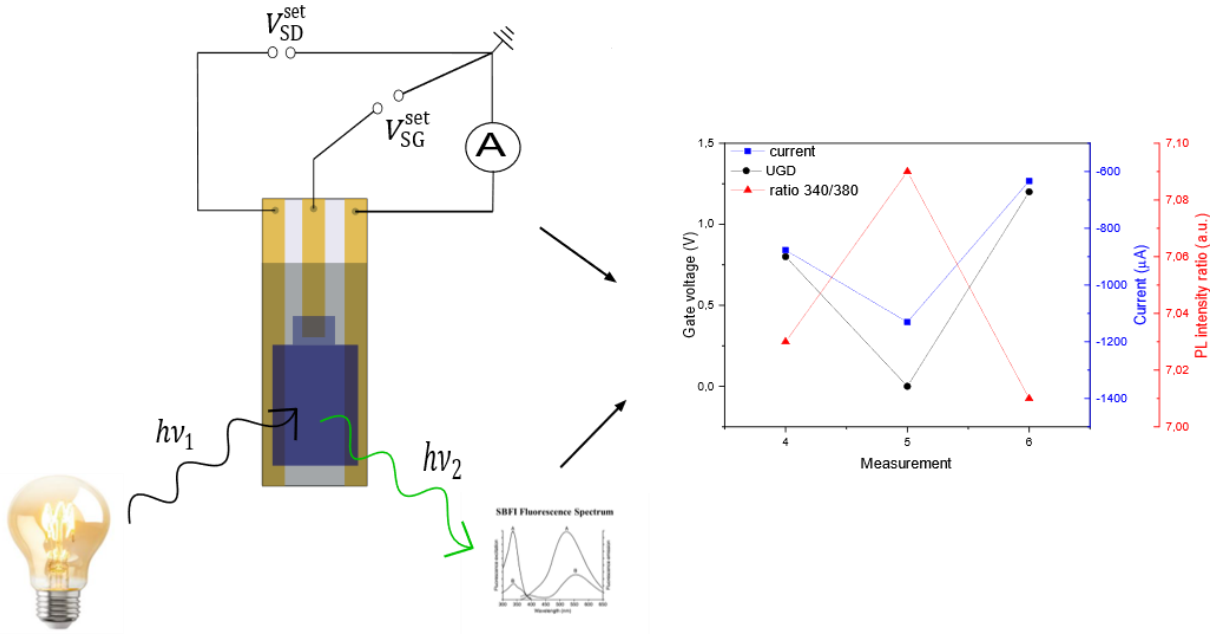

Fig. The layout of PEDOT:PSS OECT and the scheme of combination electrical and fluorescence detection. The graph shows the preliminary results of the measurement.

Microelectrode arrays for simultaneous electrophysiology and advanced brain imaging

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Advanced brain imaging techniques are crucial in neuroscience research as well as in clinical diagnosis. Optical microscopy enables the targeting of specific molecules in the brain and provides a high spatial resolution. On the other hand, spectroscopic techniques like magnetic resonance imaging (MRI) can access deep regions of the brain over a large area and identify their functions. Additionally, electrical readout through neural electrodes is indispensable in measuring their activity. It is important in several applications, for example, in mapping different functions to different regions of the brain. Clinically, it is utilised in studying the initiation and spreading of seizure like activities. Unfortunately, simultaneous brain imaging and electrophysiology is challenging. Firstly, conventional electrodes are non-transparent, thus inhibiting optical imaging. In case of MRI, this is further compounded by the possibility of heating caused by eddy currents in metal electrodes. Mismatch between the magnetic susceptibilities of common electrode materials and the surrounding tissue also results in significant artefacts due to loss of signal. In this regard, conducting polymer electrodes are prospective alternatives since their compositions are closer to biological tissues. PEDOT:PSS, one of the most widely used conducting polymers, has been used in neural electrodes to improve their signal to noise ratio. This can be achieved by virtue of the volumetric capacitance effect in the conducting polymer which significantly reduces the electrode impedance. Here, we present PEDOT:PSS based versatile transparent microelectrode arrays (MEA). The glass substrate-based MEAs are immune to laser-induced artefacts and enable simultaneous Ca^{2+} imaging with electrophysiology in vitro. They are also compatible with the state-of-the-art optical imaging as well as super resolution microscopy techniques. Further, in the form of conformable electrocorticography arrays, the PEDOT:PSS electrodes can be coupled with optical brain imaging techniques. They allow MRI along with simultaneous electrical interrogation through stimulation and recording.

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Thin Film ROS Pixel for Local Delivery of Hydroxyl Radicals

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Reactive oxygen species (ROS), such as hydroxyl radicals (OH•) or hydrogen peroxide (H_2O_2) play an essential role in certain biological processes in our body. At elevated concentration, though, ROS can cause oxidative stress in cells or even lead to cell death. However, this ROS-mediated cell death can be applied for anti-tumor therapy. Due to the high reactivity of ROS, the molecules' lifetime and transport distance in the human body are very limited and therefore, local production is essential. [1]

The Fenton reaction, where Fe^{2+} ions react with H_2O_2, is a well-known route to form OH•. In physiological conditions, though, sluggish kinetics and precipitation of iron hydroxides hinder the reaction. To circumvent these problems effort is expended on designing complex nanoparticles (NP) with heterogeneous Fenton and Fenton-like reagents. Still, NP systems suffer from reduced efficiency and aggregation. Moreover, due to the limited presence of endogenous H_2O_2 in the cell, only a limited amount of hydroxyl radicals can be formed.[2]

To evade these obstacles, we introduce a thin film device, which can be employed for local and targeted ROS generation. The device comprises a gold layer that electrochemically converts O_2 to H_2O_2 via $2e^-$ oxygen reduction. Concurrently, a thin chromium metal film, which acts as a sacrificial counter electrode, gets oxidized and dissolves. The dissolved chromium ions and H_2O_2 react in a Fenton-like way, resulting in hydroxyl radicals.

With this work, we present a novel platform for local ROS delivery with future potential for *in vitro* studies on cancer cells.

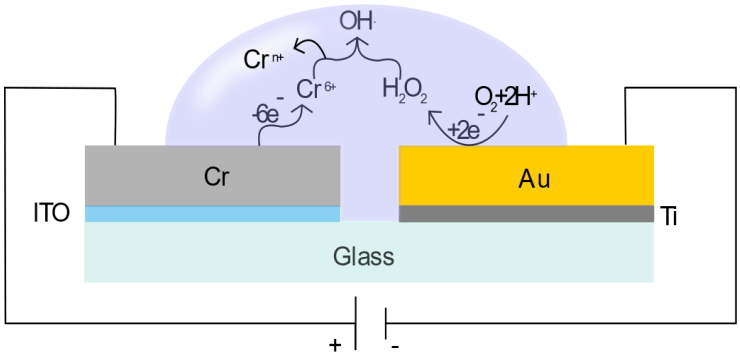

Fig. 1: Schematic presentation of thin film ROS pixel

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Ultrathin Indium tin oxide accumulation mode electrolyte-gated transistors for bioelectronics

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Electrochemical and electrolyte-gated transistor architectures have emerged as powerful components for bioelectronic sensors and biopotential recording devices. For amplification of weak electrophysiological signals, maximum transconductance, high cutoff frequencies, and large on/off ratios are key desired parameters. Organic conducting polymer devices have recently dominated the field, especially where flexible and conformable in vivo electronics are necessary. Herein we report flexible ultrathin amorphous indium tin oxide (ITO) electrolyte-gated transistors (EGTs). These accumulation-mode devices combine high transconductance, excellent on/off ratio, and fast modulation with excellent stability and the possibility of optically transparent layouts. While normally oxides are considered brittle, we obtain mechanically flexible and robust ITO layers by room temperature deposition of amorphous and ultrathin (30 nm) layers onto parylene substrates, which results in low strain. Devices survive bending and deformation tests. Based on their stability and performance, indium tin oxide EGTs represent a promising avenue for bioelectronic devices.

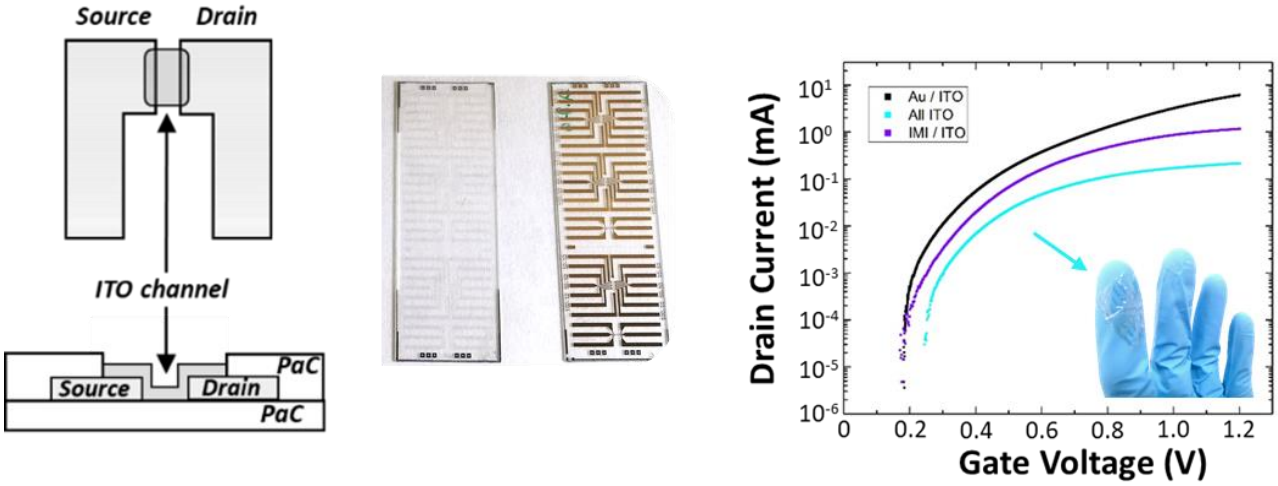

Figure 1. | ITO EGFETs can be fabricated on flexible ultrathin parylene C, and can have a high degree of optical transparency. They operate in accumulation mode with high n-type mobilities.

COX-2 binding fluorescent probe as cancer identification tool

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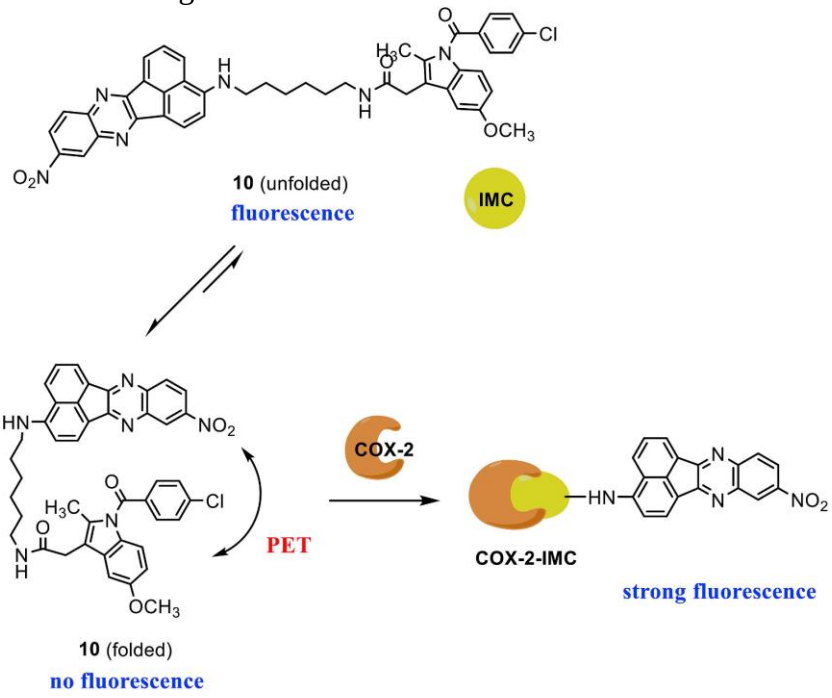
Cyclooxygenases are a family of enzymes consisting of the isoenzymes COX-1 and COX-2 that are important for prostaglandin biosynthesis during inflammatory reactions[1]. Moreover, COX-2 is overexpressed at all stages of the cancer[1], and therefore it can be used to identify cancer cells through the use of molecular fluorescent probes. Such probes can be made by combining known COX-2 specific inhibitors such as celecoxib or indomethacin[1] (IMC) with fluorophores. There are two types of fluorescent probes: "turn-on" - unbound ones show fluorescence, after binding with COX-2, the fluorescence is enhanced; and "off-on" which only fluorescence upon binding to enzyme[1]. The study investigated the mechanism of action of the ANQ-IMC-6 off-on fluorescent probe, consisting of the acenaphtho-1,2-b-quinoxaline (ANQ) fluorophore and an indomethacin inhibitor (IMC) connected with a hydrocarbon linker[2], which indicates the presence of COX- 2 in the Golgi apparatus. The model of the probe operation proposed in the literature assumes no fluorescence of the molecule in the bent conformation as a result of photoinduced electron transfer (PET) between ANQ and IMC, and after binding to the enzyme, the probe shows high fluorescence intensity[2]. The study optimized the molecule in the ground state and in the first excited state, and investigated the fluorescence properties with the use of molecular dynamics (MD) and quantum molecular mechanics (QM / MM) methods. The results of the study question the mechanism of fluorescence quenching in the folded ANQ-IMC-6 conformation associated with PET, proposed by Zhang et al in 2013[2], showing, among others, different electronic transitions. Understanding the mechanisms of probe fluorescence is important for the rational design of cancer cell identification tools.



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Digital detection of DNA *via* blockade impact electrochemistry

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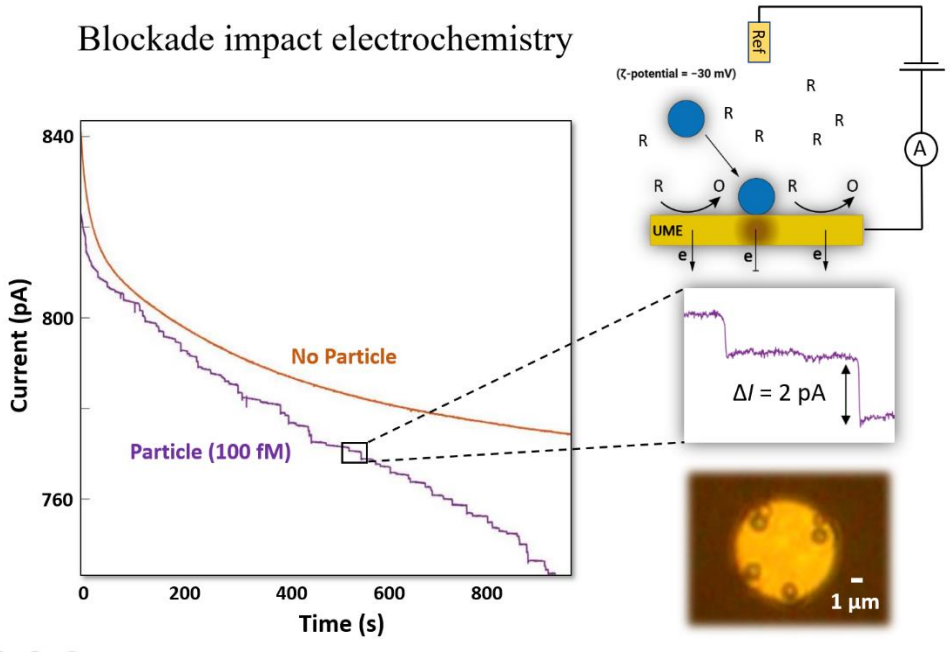
Electrochemical biosensors are an important class of solid-phase analytical methods for biomolecular detection. As a rule these 'macroscopic' methods require minimum amounts of analytes to transduce the specific biorecognition into a measurable signal. At ultralow concentrations, however, the average response upon binding of the analytes cannot be discriminated from the background response.[1] Single-entity electrochemistry (SEE) is an alternative overarching concept that aims to study and detect individual analytes. SEE implements miniaturized transducing element(s) for detecting individual events as discrete real-time signals.[2] More specifically, current blockade impact electrochemistry is a SEE sensor in which collisions of non-electroactive particles to a ultramicroelectrode (UME) surface can be sensed as discrete, step-like decreases in the current-time response.[3] Herein, we implement the concept of blockade impact electrochemistry to introduce a signal-off digital biosensor for the real-time detection of DNA oligonucleotides. In this sensing system, specifically anchored DNA-modified particles dissociate from the UME surface upon displacement of target oligonucleotides, leading to discrete signals in the current-time response. In the future, this sensor needs to be parallelized to overcome the limitations imposed by transport of biomolecules at ultralow concentrations, leading to a new sort of assay based on arrays of separately addressable single-entity detectors for digital biosensing.[4]



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Ionic electroactive polymer for organ-on-chip applications

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Organs-on-chip (OoCs) are microelectromechanical systems which recapitulate *in vitro* the *in vivo*-like microphysiological environment for cell culture to perform drug assessment and study diseases.[1] Integrating both actuation and sensing capabilities within OoC represents a crucial need for further development and acceptance by scientific community and pharmaceutical companies.[2] To this purpose, herein we propose the use of a specific type of ionic electroactive polymer, called ionic polymer metal composite (IPMC) and also known as "artificial muscle" in soft robotics.[3] IPMC is a very attractive transducer material for OoCs, since it is biocompatible, it works with sodium ions naturally present in standard cell culture media, it operates at low voltage, and it can deform to reach high strain values. As described in Fig. 1, we propose and demonstrate to use the IPMC as a strain sensor (left), as well as an actuator for mechanobiological studies (middle) and for integrated non-pneumatic pumping in OoC devices. The multi-purpose integration of ionic electroactive polymers could foster integrated electric sensing and actuation in OoCs, and thus their easier use and wider adoption by end users.

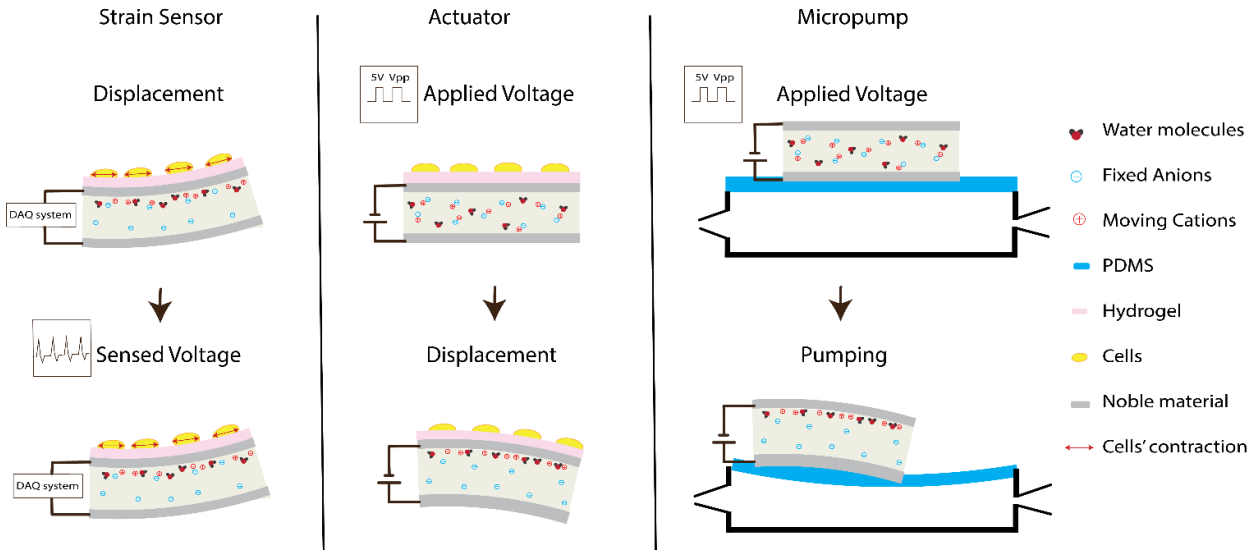

Figure 1: Sketch of IPMC-based organ-on-chip applications. In the strain sensor mode (left), upon IPMC bending induced by tissues, cations migrate, leading to a charge imbalance that can be read out as a voltage difference. In the actuator mode (middle), the applied voltage induces a migration of the cations, leading to the bending of the polymer for controlled mechanical stimulation on the cells culture. The actuation mode serves also as the basis for integrated pumping (right), whereby the ionic polymer is inserted into a soft membrane (PDMS) used to induce a pressure imbalance within a microfluidic chamber, and passive valves (of e.g. *nozzle diffuser type) convert the pressure imbalance into a unidirectional net fluid flow.*

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Electrophoretic devices for brain cancer therapy

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A fundamental limitation for the success of chemotherapy in brain cancer therapies is the blood-brain-barrier which significantly reduces the amount of cancer drugs which can be delivered into a tumour. [1] Bad vascularisation of solid brain tumours further complicates delivery of therapeutically relevant drug doses. [2]

Here, we present an implantable device which enables highly spatially selective delivery of charged drug molecules directly into brain tumours. Our device combines a microfluidic system for drug transport with embedded electrodes which enable electrophoretic transport of drug molecules into the target tissue. This allows delivery of chemotherapeutic agents without transport of bulk solvent preventing issues arising from intracranial pressure gradients. In order to overcome constraints originating from finite electrode capacities, we have investigated different electrochemical reactions, electrode designs and electric operation modes. We have thereby created a versatile drug delivery system, which is not limited to a specific drug and can easily be adapted to different cancer therapy schedules. The device is uniquely designed for cranial implantation ensuring minimal damage to healthy tissue when implanted. By employing stereolithographic 3D printing for the device fabrication, we allow easy modification of the design to adapt the device driven by individual tumour morphologies. While electrophoretic drug delivery was first described in the early 20th century and has been used since primarily for transdermal drug delivery, we believe that our approach is one of the first times this has been demonstrated for brain cancer therapy. Electrophoretic delivery will facilitate significantly higher drug concentrations in the tumour tissue than when systemically delivered while having minimal systemic impact.

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Gut-sensor axis: Semiconducting polymer-based sensors for the detection of diet-derived microbial metabolites

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In the digestive process of healthy individuals, toxic gaseous ammonia (NH3) molecules produced during the digestion of proteins are incorporated into urea molecules by the liver and are consequently excreted from the body via urine or feces. High ammonia concentrations excreted in breath, saliva, urine, colon and/or sweat can be an indication of underlying health problems such as chronic kidney disease (CKD), peptic ulcers, and acute liver failure (ALF).[1] To detect these ammonia molecules, ammonia sensors can be employed. Here we propose the use of a diode-based sensor fabricated on a glass substrate coated with an ITO electrode (anode) with a spin-coated semiconducting polymer layer (e.g. P3HT, PCBM) and aluminum counter electrodes (cathode) with a porous structure created by colloidal photolithography. Ammonia molecules can diffuse into the created nanopores after which the lone pair of amine gas molecules physically absorbed on the conducting polymer chain and thereby de-dope the p-type P3HT material resulting in a decreased hole concentration and a reduced current flow through the diodes.[2] In the beginning stages, the sensors will be designed to measure metabolites in simulated in-vitro gastrointestinal tract environment such as the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). After which the rigid glass elements of the sensor can be replaced using flexible and soft (biocompatible/edible) substrates in order to implement conformable ammonia sensors for skin or (in-vivo) gastrointestinal applications.

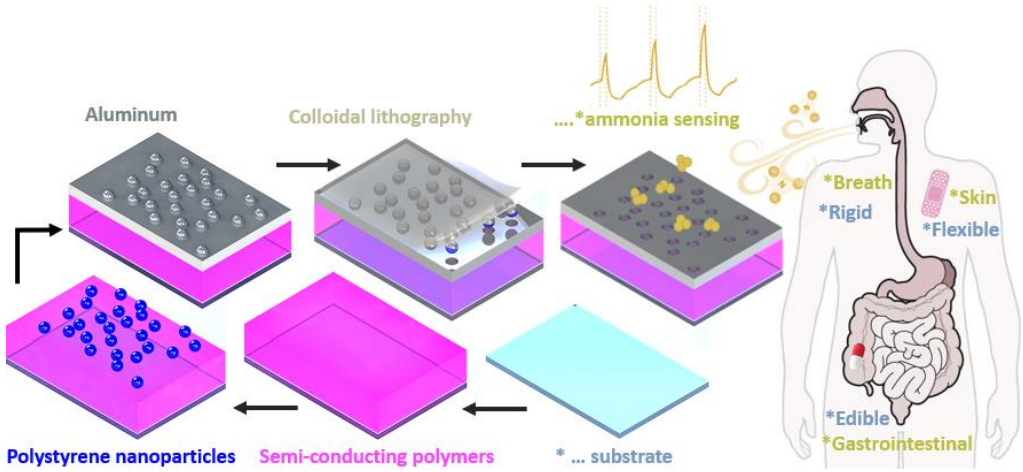

Figure 2 Fabrication and possible applications for the proposed ammonia sensor.

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Activation of regeneration-associated pathways in neurons following photocapacitive stimulation

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Neuronal stimulation exerts beneficial effects on restorative processes following many debilitating neurological disorders, such as stroke or brain trauma. Current drive to create lightweight and wireless devices for electrical stimulation aims to improve quality of life of patients. Organic photocapacitors (photocaps) charge rapidly upon illumination, which in turn creates an electric field that could be used for stimulation of the cells in their vicinity[1]. The aim of the study was to elicit molecular changes in stimulated neurons.

We cultivated primary neurons from postnatal rats on photocaps (round shape, 11 mm ø p-n layer of H_2PC-PTCDI, 30 mm ø back electrode of Au-ITO) until their maturation. Then, cells were stimulated by light pulses (5 ms pulse, 500 interpulse, 10 iterations, 5 s break – repeated for 30 min) with an LED red light source (10 mW/cm^2). Neuronal activation was assessed by c-fos immunoreactivity with fluorescent staining. Neurotrophin (BDNF, NGF, NT-3, NT-4) expression was analysed with real-time qRT-PCR.

Cells exposed to light pulses showed increased expression of c-fos protein compared to the controls without light stimulation. The signal intensity was lower than the one of positive controls exposed to glutamate. Three hours post-stimulation no significant change in the neurotrophin mRNA expression was detectable.

Increased translation of c-fos in light-stimulated cells grown on photocaps as compared to the non-stimulated group indicates a widespread activation of neuronal networks on molecular level. However, the intensity of c-fos signal did not reach the level expressed by a positive control, indicative of a room for further optimization of photocap size and stimulation parameters. Whether lack of change in the neurotrophin levels lies on the stimulation protocol, photocap design or intrinsic temporal dynamics of the gene transcription will be addressed by future experiments.

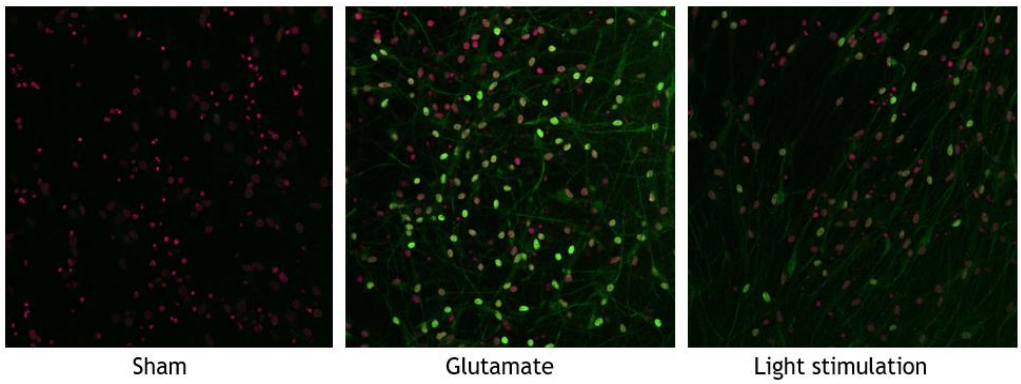

Fig. c-fos activation in cells stimulated by light compared to controls.

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3D FEM Model of Neuron Excitation Using an Organic Electrolytic Photocapacitor

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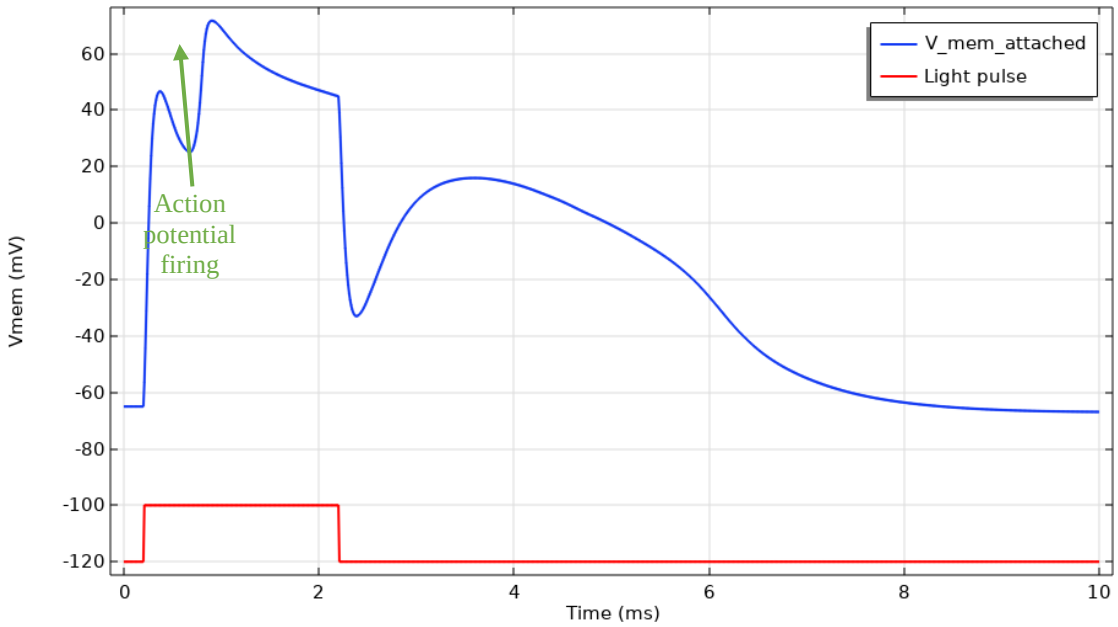
There are many parameters that can determine if a single neuron placed on top of the organic electrolytic photocapacitor (OEPC) can be successfully stimulated. We can divide those parameters into three categories: OEPC parameters such as maximum current density or voltage; interface parameters such as electrode geometry or neuron-electrode cleft distance; and neuron parameters characterized with neuron shape and ion channel conductivities. With so many variables it is crucial to understand the influence of each of those parameters to ultimately determine if the stimulation will be possible. For that purpose, we developed a complete 3D FEM model of capacitive photo-electrode and neuron in COMSOL Multiphysics. OEPC is characterized by its equivalent circuit model and contact electrical properties while neuron is modelled by solving Hodgkin–Huxley equations on the cell membrane with realistic ion channel distribution along the dendrites, axon and soma. Using our model, we can estimate the probability that the neuron is successfully stimulated in a particular experimental arrangement. Also, based on model predictions we can try to make improvements to the experimental setup in the most effective and viable way.



Membrane potential in one point of the attached membrane in time and the 2 ms stimulation pulse. Action potential firing can be seen on top of the stimulation artefact when the light is turned on and turned off.

Towards an Investigational Platform for a Multimodal Neuromodulation Approach

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Over the past decades, neuromodulation has been proven to be an effective treatment for several neurological disorders. Moreover, it continues to be a rapidly evolving field with a wide-ranging potential for biomedical applications. However, efficient and patient-specific targeted neuromodulation remains one of the biggest challenges for implantable devices.

Current studies explore the possibility of using multimodal neuromodulation techniques by combining electrical, thermal, optical, ultrasonic, and/or pharmacological methods to increase the specificity of therapies.[1] Moreover, it is hypothesized that by combining electrical and ultrasonic methods into a hybrid neuromodulation technique, the safety profiles and spatiotemporal resolution could potentially be increased.[2] Low-intensity focused ultrasound has the potential to alter the neural response in a wide range of neuronal targets, with an improved spatial resolution.[3,4] However, the most effective, reliable, and safe acoustic parameters are currently unknown, especially for the peripheral nervous system, due to the little understanding of the mechanisms that govern this method.[5]

In this study, we propose an investigational platform that will allow us to explore a variety of ultrasound parameters for a multimodal neuromodulation approach. The platform integrates a custom-adapted system for stimulation and neural recording, commercially available components for the ultrasound stimulation system, and an experimental control unit with a PC interface. The proposed setup facilitates the evaluation of the tested parameters during experiments on explanted nerve models. Here we will describe potential implementations of such a system and discuss challenges that can be faced during experiments on explanted nerves.

This work can be useful to increase our understanding of ultrasound neuromodulation on peripheral nerves and its benefits when integrated into a hybrid platform dedicated to multimodal neuromodulation

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Biohybrid plants with electronic roots via in-vivo polymerization of conjugated Oligomers

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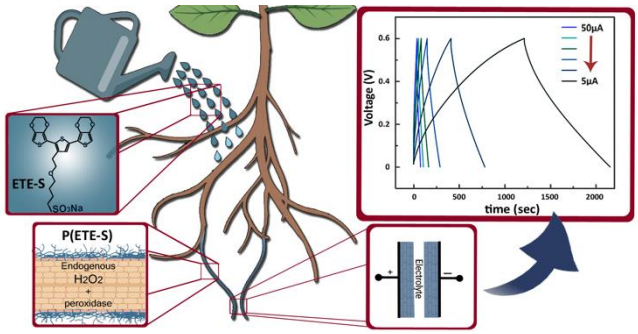
§

Over recent years there has been a growing interest in developing plant-based biohybrid technological systems by integrating smart materials and devices into plant structures[123]. The plants use the energy coming from the sun to power their development and growth. They are well suited to sense and adapt to various environmental stimuli, and they can self-repair via tissue regeneration. All these advantages of plants augmented with the versatile characteristics of organic conductive polymers enabled us the development of plant-biohybrid systems.

In our recent work we demonstrate electronic functionalization of intact plants via in vivo polymerization of conjugated polymers for long term integration of electronics into plants structure. The biocatalytic machinery of the plants cell wall was leveraged to seamlessly integrate conductors with mixed ionic-electronic conductivity along the plants root system. Cell wall peroxidases catalysed ETE-S polymerization while the plant tissue served as a template, organizing the polymer in a favourable manner. The conductivity of the resulting p(ETE-S) roots reached the order of 10 S/cm, and it remains stable over the course of 4 weeks while the roots continue to grow. The p(ETE-S) roots were used to build supercapacitors that outperform previous plant-biohybrid charge storage demonstrations. Plants were not affected by the electronic functionalization but adapted to this new hybrid state by developing a more complex root system. Biohybrid plants with electronic roots pave the way for autonomous systems with potential applications in energy, sensing and robotics.



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SYNTHESIS AND ELECTRONIC PROPERTIES OF ZNO FILMS BY A BIO-INSPIRED APPROACH

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The affinity of peptides to specific substrates, together with their versatility, and modularity, make them promising agents for the synthesis and functionalization of inorganic materials. Indeed, previous works in our group showed that semiconductors' surface work function can be modulated by peptides' functionalization in a sequence-dependent manner. Furthermore, it was shown that peptides can be used to facilitate the deposition of a continuous film of inorganic material. To further interrogate peptide-surface interactions, I present in this work studies of peptide interactions with different facets of ZnO. I show differences in the binding interactions of two peptides on the different facets of ZnO that in turn result in changes in the surface electronic properties. Furthermore, I demonstrate a direct correlation between the extent of peptide folding on the different ZnO facets and the level of modulation of the electronic properties. I further show that the sequence of the peptide is important for peptide mediated synthesis of ZnO thin films, mainly in promoting adhesion to the surface (allowing for improved surface coverage) and controlling the nanocrystalline morphology. The findings of this work highlight the dual role of peptides in the fabrication of films and controlling their elelctronic propeorites, making peptides attractive additive for the preparation of electronic materials under green conditions.

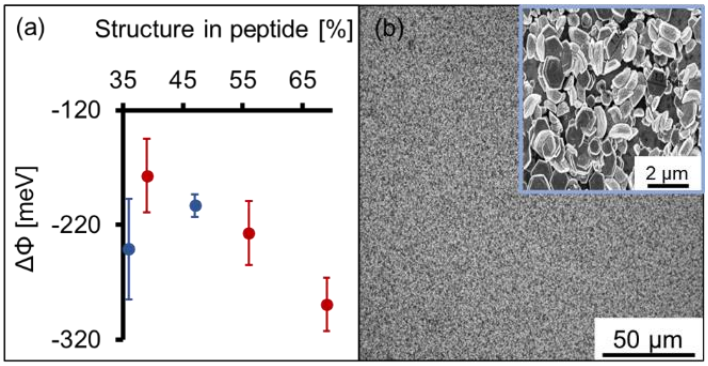

Figure 3: (a) Changes in the work function of monocrystaline ZnO dictated by peptide folding. (b) SEM image showing the formation of a homogeneous and crystaline ZnO film facilitated by peptides.

3D microelectrodes for tissue electrophysiology in Organs-on-Chip

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Despite the extensive research conducted on comprehending neurodegenerative conditions, such as Alzheimer's and Parkinson's diseases, the mechanisms governing their onset and eventual progression are still widely unidentified. This can be mainly attributed to the lack of reliable and accurate *in-vitro* models which can be utilized for performing various physiological studies to understand disease mechanisms and to test the efficacy of drugs.[1] Moreover, discrepancies in the genetic make-up between animal models and humans along with ethical concerns call for a transition from 2D cell cultures to 3D *in-vitro* models.[1] Recent technological advancements have led to the development of Organ-on-Chip (OoC) technologies which aim to accurately mimic the physiology and micro-environment of different human tissues on miniaturized, micro-engineered platforms. Neurons continuously communicate with each other and with other non-neuronal cells by sending electrical and chemical signals. In order to understand neural communication during the onset and progression of neural diseases, it is necessary to develop a Brain-on-Chip (BoC) device which is capable of monitoring and measuring the electrical activity of the nerve cells. Additionally, it is essential to provide a platform where nerve cells can communicate with each other in all spatial dimensions, in order to realistically recapitulate their *in-vivo* behavior.

Currently, 2D Microelectrode Arrays (MEAs) are commonly employed to measure the electrical activity of electroactive neural cells in high-density platforms. There have been several successful attempts in fabricating high-throughput 3D BoC devices with integrated titanium nitride (TiN) MEAs on standard glass[1] or silicon substrates,[2] as shown in Figure 1 (a). With additional complete optical access in fully transparent BoC substrates, the interactions between nerve cells can be extensively studied both electrically and optically during their selective activation and silencing, which in turn provides further insight into the pattern of migraines.[3] Therefore, in this work, it is proposed to fabricate a BoC device with 3D TiN MEAs encapsulated with silicon nitride on truncated pyramids, which are embedded in a transparent membrane supported by a silicon frame. Additionally, we aim to explore and study the behavior of nerve cells on rigid transparent membranes (such as silicon nitride) as well as on a soft transparent membrane (such as polymers, like PDMS).

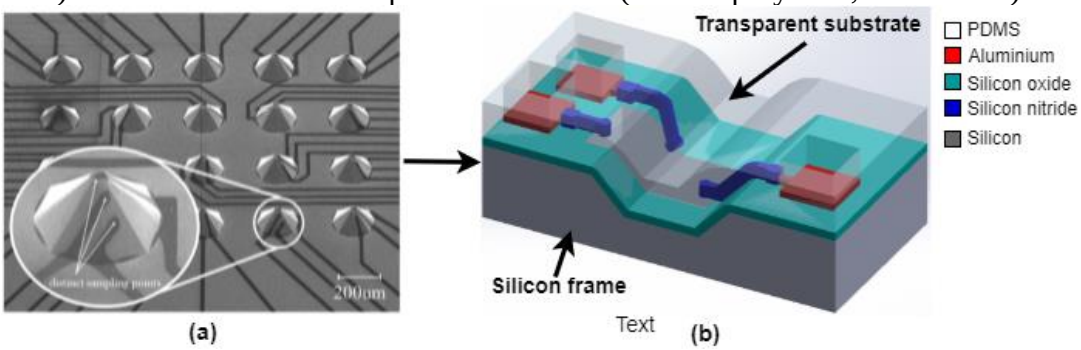

Figure 1: (a) *SEM image of 3D MEAs on truncated silicon pyramids of 85 µm height;*[2] ***(b)*** *Sketch of the proposed 3D MEAs embedded in a transparent substrate for Brain-On-Chip applications.*

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Living Photovoltaics based on Recombinant Expression of MtrA Decaheme in Photosynthetic Bacteria

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At the center of microbial bioelectricity applications lies the critical need to express foreign heme proteins that are capable of redirecting the electron flux of the cell's metabolism. This study presents a genetic construct for the heterologous expression of the periplasmatic decaheme MtrA c-type cytochrome from S. oneidensis MR-1, a dissimilatory metal-reducing exoelectrogen, inside the light-harvesting bacterium, Synechocystis sp. PCC 6803. Protein expression was verified through western-blotting and immunostaining, and oxygen evolution, optical density, and absorption measurements confirm sustained cell activity and viability under the tested expression conditions. Furthermore, the bioengineered cells show enhanced mediated exoelectrogenicity, as confirmed through a colorimetric iron assay and electrochemical measurements. Compared to wildtype cells on graphite electrodes, the bioengineered cells show a 2-fold increased extracellular electron transfer. The increased capacitance obtained under illumination and suppressed photocurrents in the presence of the photosynthetic inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) suggest increased extraction of photosynthetically derived electrons from the recombinant cells. The improved bioelectricity transport across the outer membranes, as achieved through the heterologous heme expression inside cyanobacteria, presents new opportunities for re-wiring the metabolisms of light-harvesting microbes.

Ultrathin organic microsupercapacitors for implantable and wearable electronics

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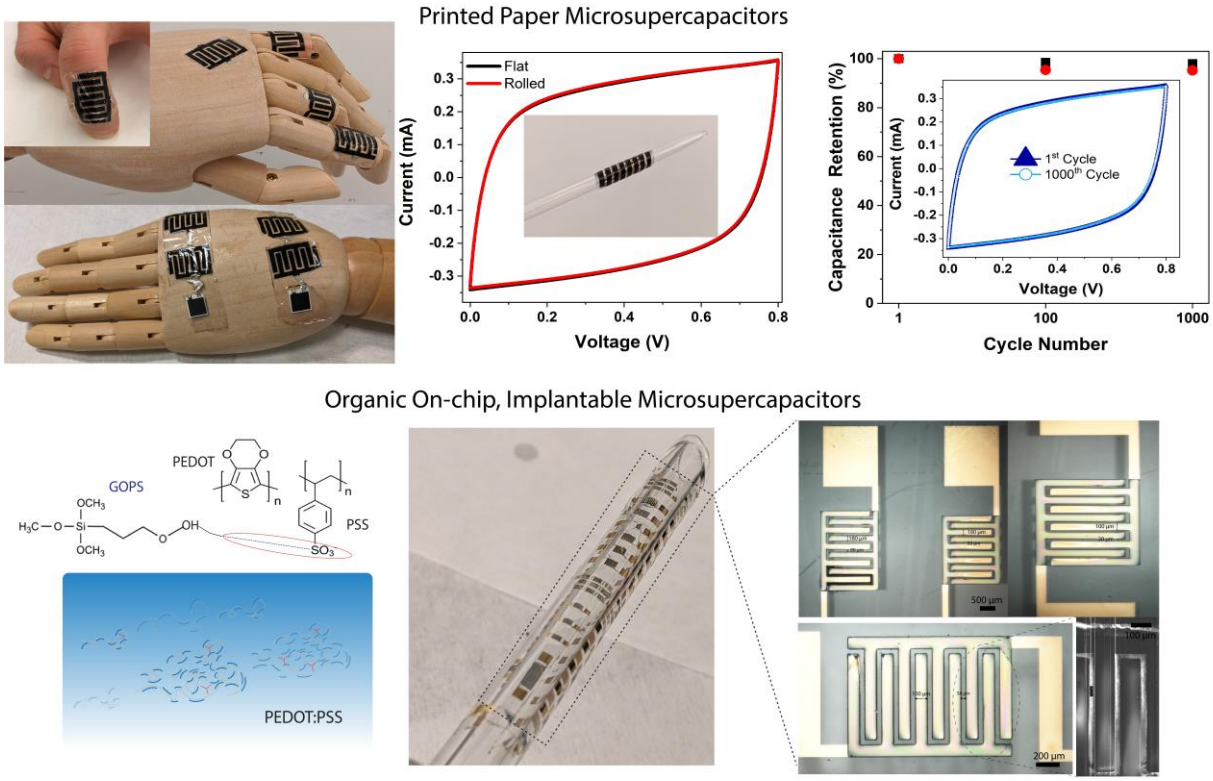
Ultrathin energy storage and harvesting are rapidly developing for medical applications and wearable electronics. Powering implantable electronics requires thin and ultra-flexible energy storage devices, which have to be removed after completing their action. A combination of ultrathin substrates (Parylene C) and conductive polymers is promising to provide biocompatibility for organic batteries and supercapacitors. Paper electrodes[1] provide mechanically robust, nanoporous networks for high energy density devices. Printing/coating methods are proven to achieve all printed symmetric microsupercapacitors (μSCs) on ultrathin parylene C substrates, where both electrode and gel electrolyte are based on the cheap and abundant biopolymer, cellulose[2]. The procedure of making paper electrodes allows an overall device thickness of around 10 μm, which can provide conformal device architecture. Another aspect of achieving on chip devices is using lithography, where active materials can be deposited using solution processing, high performance nanomaterials can be patterned as interdigitated electrodes. An ultrathin packaging material encapsulates μSC that fulfills implantable medical devices requirements. In our study, we employ finite element analysis to calculate the stress and strain for μSCs under different bending conditions to mimic the experimental bending radius, which is low as 2.5 mm. These organic μSCs show long-term operation capability (90% of capacitance retention after 10^4 cycles) and capacitance retention of 98% is achieved after 1000 bending cycles. Achieving such devices improves the current technology for implantable energy storage devices and realizes organic power packages and flexible low-power electronics.



Ultrathin organic supercapacitors for electronic skin and implantable electronics

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Utilizing Circular Dichroism to Study Excitons in Photoactive Semiconductors for Bio-Optoelectronics

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Semiconducting organic molecules are revolutionizing the electronic world by providing new and easy adjustable materials for various opto-electronic and bio-electronic applications.[1] Benefiting from recent developments, neurostimulating prosthetics to restore light sensitivity to the degenerated retina are finally conceivable. The functionality of this concept has already been demonstrated using a small molecular squaraine dye (SQIB) as active material.[2] For such optoelectronic sensing, however, it is essential to acquire a fundamental understanding of the underlying processes and mechanisms such as the generation and nature of the molecular excitons.[2,3] *N*-alkyl anilino squarines have been shown to exhibit strong excitonic intermolecular interactions, without the SQIB-typical formation of a self-assembled crystalline micro- or nanotextured morphology.[3,4] Coulombic coupled molecular aggregates with short intermolecular distances leading to short-range intermolecular charge transfer (ICT) interactions combined with Frenkel excitons provide a striking feature of these *n*-alkylated squaraine dyes.[4,5]

Here, we investigate the structure-correlated excitonic properties in photoactive thin-film semiconductors for bio-optoelectronics using non-chiral *n*BSQ, *n*OSQ and two new structurally correlated chiral anilino squaraines. Functionalisation with enantiomerically pure citronellyl-derived residues ensures the analogy to the corresponding *n*-alkylated compounds and additionally offers the investigation by Mueller matrix polarimetry[6] due to an emerging excitonic chiral dichroism (CD).[7] This allows a systematic and fundamental study of the structure and chirality correlated excitonic properties and provides the first part of a quantitative understanding of this feature.

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The effect of gold roughness on its biochemical functionalization and organic electrochemical transistor based sensor performance

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Gold is the most widely used electrode material in electrochemical biosensors due to its high electrical conductivity, biocompatibility, and well-established surface chemistry. Yet, robust chemical functionalization of the gold surface to anchor biorecognition units remains a challenge. Here, we reveal the effect of gold surface roughness on the quality of the biofunctional layer and, thereof, sensor performance. We deposited gold on three electrode substrates with different roughness values, i.e., borosilicate glass, polyimide, and polyethylene terephthalate. We characterized the quality of the gold films and the orientation of the chemically self-assembled monolayer (SAM) on these films using X-ray diffraction, atomic force microscopy, electrochemical impedance spectroscopy, and X-ray photoelectron spectroscopy. Through the SAM, we built a biorecognition unit for SARS-CoV-2 spike protein and used these electrodes as the gate contact of an organic electrochemical transistor (OECT). The sensor performance, evaluated in terms of reproducibility, stability, and sensitivity varied with respect to gold electrode surface roughness. Our work provides design guidelines for the development of high performance electrochemical biosensors, where the quality of the sensing electrode surface has the governing role.

A bioelectronic transmembrane model of the gut for mechanistical studies on host-microbiome interactions in humans

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Unarguably, the human gut microbiome plays a significant role in the development of non-communicable diseases like diabetes mellitus or inflammatory bowel disease (IBD).[1] Increasing evidence is linking dietary fibre and unabsorbed carbohydrates as a common source for anaerobic bacterial fermentation. This process generates mainly the bacterial short chain fatty acids (SCFAs) acetate, propionate and butyrate.[1] Current studies demonstrate health promoting effects of SCFAs for example by protecting barrier integrity in rodent IBD models mediated via GPR41/43 and 109a receptors.[2] However complete mechanisms of actions of SCFA in humans are not well understood. Reasons for that are limitations in studying the human gut non-invasively as well as the availability of surgical biopsies. Thus, mechanistical studies on host-microbiome interactions are mainly conducted in simplistic 2D *in vitro* models or in animals for *in vivo* effects. Although these models enable valuable high-throughput screenings and studying cause-effects relationships in pre-clinical phases, transferability to humans is challenging. Obviously, physiology of animals and humans is different and 2D cell signalling is influenced by a lack of the native tissue environment.[3] Thus, with the recently developed bioelectronic transmembrane device we are aiming for to generate improved predictions on host-microbiome interactions in humans.[4] By using an electroactive scaffold (PEDOT:PSS) that promotes cell hosting by its soft tissue-like structure and functions as an inline sensor for monitoring non-invasively cell activity in real-time.[4] Moreover, the Transwell structure of the device promotes the forming of a functional barrier of the human gut of differentiated gut cells *in vitro*. Currently, studies on molecular studies of SCFAs as well as the neuropeptides vasoactive intestinal peptide (VIP) and galanin used for IBD management are tested on Transwell inserts for bridging the transfer to the bioelectronic transmembrane device. (***Figure 1***) The phenotype of the bioelectronic transmembrane gut model is continuously improved by integrating bacteria (e.g. *Faecalibacterium prausnitzii*) as well as an oxygen control tool.

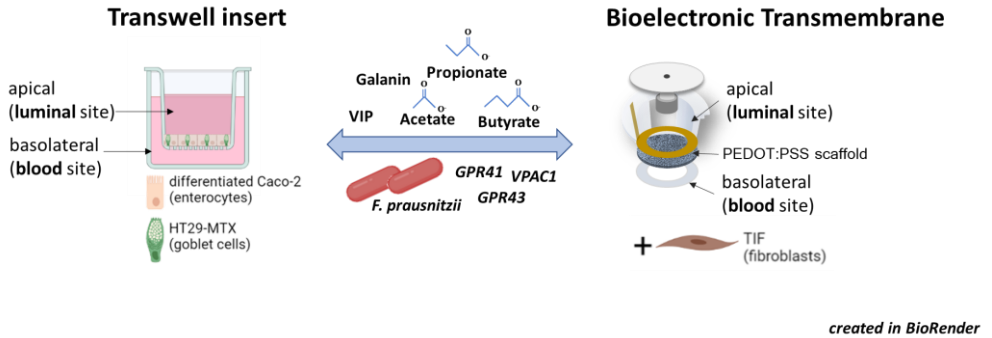

***Figure 1**: Comparing epithelial gut barrier cultured in 2D Transwell inserts and the Bioelectronic Transmembrane for studying host-microbiome interactions in vitro*

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Nanoscale Electrical Characterisation of Functional Electrolyte-Gated Organic Field-Effect Transistor by in-Liquid Scanning Dielectric Microscopy

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Electrolyte-Gated Organic Field-Effect Transistors (EGOFETs) are at the centre stage for transistor-based biosensing and bioelectronic applications.[1] EGOFETs provide two sensitive and efficient biosensing interfaces: gate/electrolyte and semiconductor/electrolyte. The nanoscale biorecognition events modify the interfacial properties, which changes the device response. So, the nanoscale behaviour is generally deciphered and understood based on the EGOFET output and transfer characteristics. However, this approach does not provide direct access to the interface, limiting the extent of information extracted. It thus demands the development of techniques to characterise these interfaces at the nanoscale under operating conditions.

Towards this objective, we have adapted in-Liquid Scanning Dielectric Microscopy (in-Liquid SDM) to access nanoscale electrical properties (conductivity and interfacial capacitance) of a functional EGOFET.[2] The local electrostatic force is recorded at each pixel in the device, and its variation with gate voltage is correlated with the macroscale current-voltage transfer characteristics. Our method can reveal minute electrical heterogeneities attributed to different phases and materials at the semiconductor/electrolyte interface. In addition, the onset of pinch-off and high gate voltage effects are investigated as EGOFET transitions from linear to saturation regime. Overall, In-Liquid SDM provides vast information about EGOFETs under operation and thus enable substantial optimisation of devices and their interfaces. Lastly, in-Liquid SDM is versatile and can be easily translated to the other systems of electrolyte-gated transistors.

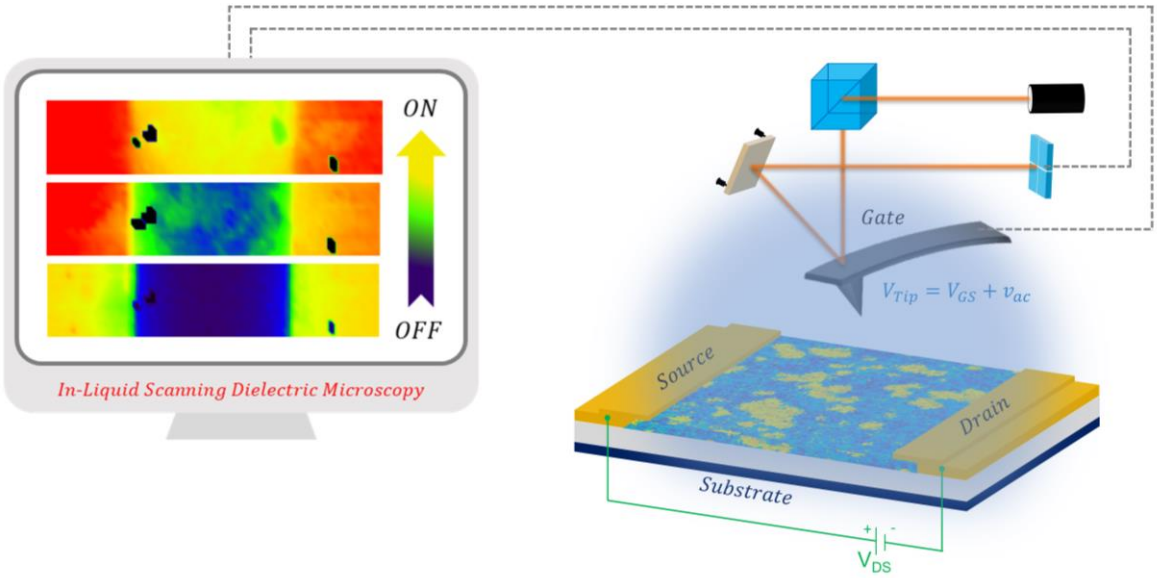

Figure: Illustration of In-Liquid Scanning Dielectric Microscopy on Operating EGOFET

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Energy Efficiency of Electrical Stimulation Pulses in Computational Models

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The development of brain-computer interfaces and neuroprosthetic bioelectronic devices require implantable stimulators with hundreds to thousands of output channels[1]. These devices have a limited power budget due to the wireless power link. Therefore, optimized power efficiency is crucial to facilitate as many channels as possible. Conventional electrical stimulation methods are not power-efficient, especially in multi-channel configuration. Previous work has shown that the conventional rectangular shape of the stimulation pulses is not energy optimal[2]. However, the coupling between circuit implementation and energy efficiency of non-rectangular pulses has not been addressed yet. This work explores the potential benefits of using non-rectangular pulses in cortical stimulation for reducing the required activation energy and aims to incorporate circuit implications in the evaluation.

For this, we use biophysically realistic single-cell models of cortical neurons[3] in the NEURON v8.0 simulation software[4]. We apply monophasic extracellular stimulation pulses to these models using a point-source electrode for different configurations changing electrode location, pulse shape, and pulse duration.

The models confirm that threshold current depends on the temporal properties of the stimulus, shown in fig. 1. The associated activation energy of the pulses is calculated using[2]:

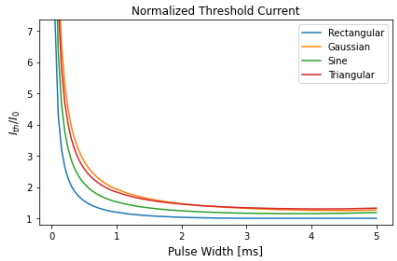
$$E = \int P(t)dt \propto \int_0^{PW} I^2(t)dt \qquad (1)$$

The resulting energy-duration curves are depicted in fig. 2. At shape-specific optimal pulse duration, a decrease in activation energy of approximately 9% can be observed for the non-rectangular pulses. However, (1) assumes a fully adaptive (adiabatic) voltage supply to generate the current pulses. In most stimulator systems, the stimulation current is created from a constant voltage supply. The activation energy is recalculated using:

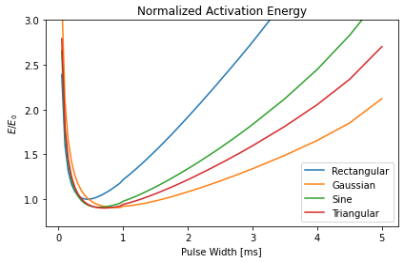
$$E \propto I_{th} \int_0^{PW} I(t)dt \qquad (2)$$

Here, the voltage supply is assumed to be constant and proportional to I_{th} during the pulse. Fig. 3 shows the resulting energy-duration curves. In this case, the rectangular pulses are the most energy-efficient. This result shows the importance of incorporating circuit implementation when comparing pulse shapes and indicates that rectangular pulses can be more efficient when using a constant voltage supply.

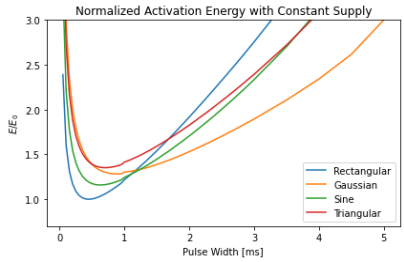
Future work includes more in-depth energy analysis and translation of the results to stimulator circuits optimized for activation energy.



4: Strength-Duration curves for different pulse shapes



5: Energy-Duration curves calculated with fully adaptive supply voltage



6: Energy-Duration curves calculated with constant supply voltage

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Molecular photoswitches for biological applications

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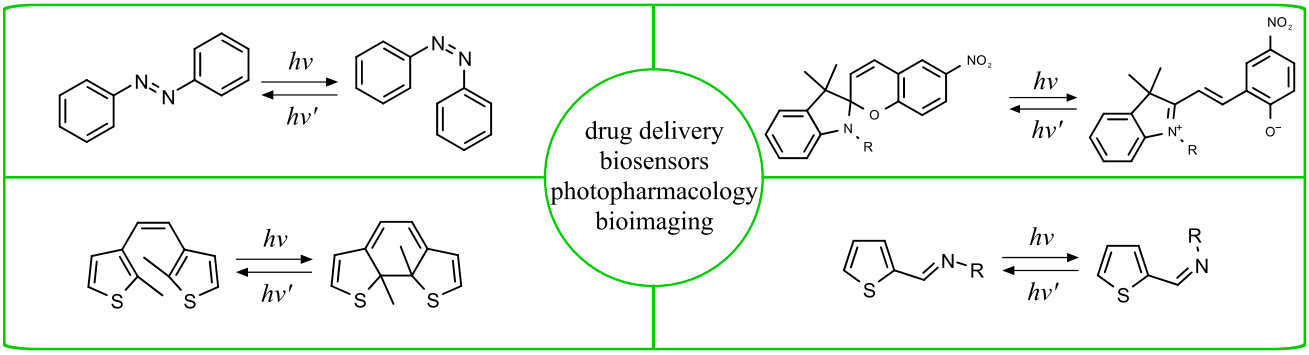
Light as a noninvasive stimulus can be considered as ideal candidate for biological applications thanks to the high spatiotemporal resolution and precise remote on demand regulation of wavelength and intensity. In recent years, significant development in the drug delivery systems, bioimaging, photopharmacology or on-demand cell adhesion have been made with utilization of the molecular photoswitchable materials.[1]

Todorov et al. synthesized and investigated azobenzene-containing hemorphin-4 chemosensor. *E/Z* isomers of the resulting compound exhibit different spectral and electrochemical properties depending on the solvent polarity. Further 6-Hz seizure and maximal electroshock tests showed 100% protection indicating strong potential for *in-vivo* anticonvulsant activity.[2]

Hati et al. constructed adaptable nanoplasmonic biosensor based on photoisomerizable spiropyran-merocyanine covalently attached onto gold triangular nanoprisms via self-assembled monolayers; it can be used for an ultrasensitive, highly specific, and programmable detection of both nucleic acids and proteins. Moreover, this sensing approach can be applied to detect bladder cancer biomarkers from different human biofluids like plasma or urine. Therefore, this label-free, optical-based technology has strong potential to expand clinical diagnostics through noninvasive "liquid biopsies".[3]

Our group synthesized and characterized novel and fast *E/Z* phenylene-thienyl imine and diimine photoswitches, which exhibit T-type photochromic properties. The phenyl and thiophene rings in the *Z* form are T-shaped oriented to minimise the orbital repulsions between the respected rings. Strong bathochromic shifts of the emissions were also estimated where this trend increases with the polarity of the used solvent topped with 10 000 cm^{-1} Stokes shift in methanol.



Brief overview of molecular photoswitches for biological applications

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