4th Conference on Impedance-Based Cellular Assays

Edinburgh, 6th-8th June 2018

IBCA 2018 relies on the financial support of our sponsors. We gratefully acknowledge financial support by the companies and organizations listed below for providing the resources to organize this meeting. Thank you very much!!!

Welcome to the
4th Conference on Impedance-Based Cellular Assyas

Dear IBCA Participant,

On behalf of the organizing committee and our sponsors, we would like to welcome you in Edinburgh for the 4th Conference on Impedance-Based Cellular Assays. This year again the conference will cover all aspects of impedance sensing in 8 sessions with 40 talks spread over three days. It is good to see the IBCA community growing over the years, from ECIS user meetings back in 2009 to international conference with attendees coming from around the world.

The number of new exhibiting companies reflects this growing interest in the field and this should help to promote impedance-based cellular assays in both academia and the industry. It is fair to say that we will not be here together without Prof Ivar Giaever and Prof. Charlie Keese, and we would like to take this opportunity to acknowledge their remarkable scientific contribution that shaped a community.
I would also like to thank personally the founder of the IBCA meetings series, Prof. Joachim Wegener, for letting us host this year meeting, and who was again instrumental in gathering all the IBCA members and sponsors.

Finally, I hope you will take the opportunity to be in Edinburgh to discover the city and its wonderful history, as well as its surroundings. The conference centre is close to Holyrood Park within sight of Arthur's Seat, and this offers plenty of opportunity for beautiful walks. At the occasion of the conference dinner, we invite you to discover both the Old Town and the New Town of Edinburgh, that meet at the dinner location, the iconic Victorian hotel, The Balmoral.

We wish you a wonderful time in Edinburgh, and a very fruitful and stimulating conference.

Pierre,

On behalf of the organising committee:

Dr. Pierre Bagnaninchi (MRC Centre for Regenerative Medicine, University of Edinburgh)
Dr. Stewart Smith (Institute for Bioengineering, University of Edinburgh)
Prof. Joachim Wegener (Universitat Regensburg)

Wednesday 6th June

Session 1: Drug Testing and Signal Transduction		
09:00-09:20		**Welcome**
09:20-09:50	Talk 1-1	**Invited Talk: Charlie Keese** ECIS: Concept to Commercialization
09:50-10:10	Talk 1-2	**Judith Stolwijk** Increased Throughput in GPCR Screening using Impedance Assays: Inspiration from Organ Studies
10:10-10:30	Talk 1-3	**Katie Morgan** Chlorpromazine disrupts structural integrity of cell membranes in HepaRG cells and initiates a pro-inflammatory response
10:30-10:50	Talk 1-4	**Christian Kade** Time-resolved Response Profiles of GPCR Activation: Combining Two Independent Impedance-Based Approaches
11:00-11:20		*AM Tea and Coffee in the exhibition hall*
Session 2: Cell Death and Toxicity		
11:20-11:50	Talk 2-1	**Invited Talk:** Sioned Owen Electric Cell-Substrate Impedance Sensing (ECIS), a platform technology for cancer metastasis research and in search for anti-cancer compounds, a Wales wide approach
11:50-12:10	Talk 2-2	**Come Thieulent** Real-Time monitoring of Equid alphaherpesviruses infectivity in equine dermal cell based on impedance measurements: effects of aciclovir and ganciclovir treatments
12:10-12:30	Talk 2-3	**Fabian Bonetto** Discrimination between Normal and Cancerous Cells using ECIS and Artificial Intelligence Techniques.
12:20-14:00		*Lunch served in exhibition Hall*
14:00-14:20	Talk 2-4	**Patricia Ruas-Madiedo** Selection of probiotics and prebiotics against *Clostridium difficile* using an *in vitro* intestinal model based on impedance real time monitoring
14:20-14:40	Talk 2-5	**Pierre Pütz** Cells in Contact to Carbon Dots:A label-free, impedance-based and multidimensional approach

14:40-15:00	Talk 2-6	**Scott Boitano** Nanoparticle toxicity: cytotoxic and sub-cytotoxic measurements using xCELLigence real time cell analysis
15:00-15:30		*PM Tea and Coffee in the exhibition hall*
15:30-15:50	Talk 2.7	**Maria Zinkl** Monitoring the Toxicity of Bisphenol A using Multiple Impedance-Based Cellular Assays
15:50-16-10	Talk 2-8	**Krisztina Juhasz** Cell Monitoring using impedance and impedance spectra for high content toxicity and cell proliferation screening
16:10-16:30	Talk 2-9	**Vincent Senez** Impedance Analysis of Waterborne Parasite Infectivity
16:30-18:00		*Reception and Poster Session*

Thursday 7th June

Session 3: Novel Assays, Analysis and Technologies 1		
09:00-09:30	Talk 3-1	**Invited Talk: Andrea Robitzki** Impedance Spectroscopy in Microfluidic Devices for a Significant Enhanced Cell Monitoring in a Live Mode
09:30-09:50	Talk 3-2	**Yi-Ting Lai** Impedance Analysis of Adherent Cells Cultured on Various Sizes of Electrodes
09:50-10:10	Talk 3-3	**Michael Skiba** Impedance Analysis of Heterogeneous Cell Populations: Impact on Data Analysis and Modeling
10:10-10:30		AM Coffee Break
10:30-11:00	Talk 3-4	**Invited Talk: Dan spencer** Single cell impedance spectroscopy: theory and applications in healthcare
11:00-11:20	Talk 3-5	**Sven Ingebrandt** Transparent organic transistors for ECIS with single cell resolution
11:20-11:30		*Short Break*

Session 4: 3D Cell Culture and Tissue Models		
11:30-11:50	Talk 4-1	**Hancong Wu** Towards 3D impedance-based cellular assays with Electrical impedance tomography
11:50-12:10	Talk 4-2	**Charalampos Pitsalidis** Monitoring of 3D Cell Cultures Using Conducting Polymer Scaffolds
12:10-12:30	Talk 4-3	**Nadira Jamil** Flexible Microelectrode Array (Flex-MEA) Design for Micro-Bioimpedance Tomography of *Rhodococcus erythropolis*
12:30-14:00		*Lunch served at the John McIntyre restaurant*
Session 5: Migration, Wounding and Chemotaxis		
14:00-14:30	Talk 5-1	**Invited Talk: Laszlo Kohidai** Impedance-based analysis as a dedicated technique to characterize efficacy of novel antitumour compounds
14:30-14:50	Talk 5-2	**Rute Castelo Felix** Electric Cell-substrate Impedance Sensing (ECIS) as a method to test marine bioactive compounds in wound healing
14:50-15:10	Talk 5-3	**Marco Tarantola** Connectivity and reorganization of cardiomyocytes and fibroblasts in co-cultures
15:10-15:30	Talk 5-4	**Lisa Sauer** Impedance-based Characterization of pH-dependent Cell Behavior
15:30-16:00		*PM teas and Coffees served in the exhibition hall*
Session 6: Tissue Barrier and Filter Based Assays		
16:00-16:30	Talk 6-1	**Invited Talk: Prof. Peter Hordijk** The push and pull of endothelial integrity is balanced by Cdc42 and RhoB GTPases
16:30-16:50	Talk 6-2	**Ege Ozkaya** Investigating blue light illumination on human retinal pigment epithelial cell lines and its potential to model AMD in vitro
16:50-17:10	Talk 6-3	**Christina L. O'Neill** Chronic high glucose exposure induces cellular dysfunction in Endothelial Colony Forming Cells
19:30		**Conference Dinner at the Balmoral Hotel**

Friday 8th June

Session 7: Novel Assays, Analysis and Technologies 2		
09:00-09:30	Talk 7-1	**Invited Talk: Agnes Tixier-Mita** Impedance Sensing with Thin-Film-Transistors (TFT) Array Bio-sensors
09:30-09:50	Talk 7-2	**Simone Ruckdäschel** Expanding the information depth of impedance based assays by using piezoelectric growth substrates
09:50-10:10	Talk 7-3	**Sidahmed Abayzeed** Optical Imaging of Electrical Impedance using Surface Plasmon Resonance Sensors
10:10-10:40		*Am Tea and Coffee break*
10:40-11:10	Talk 7-4	**Invited Talk: Dr. Steffi Krause** Light-addressable potentiometric sensors for cell imaging applications
11:10-11:30	Talk 7-5	**Stefanie Michaelis** 'Instant ECIS': A concept for storing frozen cells on electrode surfaces for instant use
11:30-11:50	Talk 7-6	**Tse-Hua Tung** Optimized Multilevel Discrete Wavelet Transform Analysis for Mitochondrial Respiration Regulation of Human Mesenchymal Stem Cells
11:50-13:30		*Lunch served at Exhibition Hall*
Session 8: IBCA for Regenerative Medicine		
13:30-14:00	Talk 8-1	**Invited Talk: David Hay** Working toward the delivery of automated and real time reporting assays from pluripotent stem cells
14:00-14:30	Talk 8-2	**Invited Talk: Dr. Yama Abassi** Development of Real-Time Potency Assays for Cellular Therapies and Regenerative Medicine Using Impedance Technology
14:30-14:50	Talk 8-3	**Heinz-Georg Jahnke** A novel highly parallelized multimodal bioelectronic real time High Content Screening platform for hiPSC derived 2D and 3D cardiomyocyte cultures
14:50-15:20		*PM Tea and Coffee break*

15:20-15:50	Talk 8-4	**Invited Talk: Chun-Min Lo** Use of cross-linked polypeptide multilayer-coated electrodes to monitor osteogenic differentiation of human dental pulp stem cells
15:50-16:10	Talk 8-5	**Carol De Santis** Characterisation of *in-vitro* Cardiac Cell Models for Preclinical Assessment of Oncology Drug-Induced Cardiotoxicity
16:10-16:30		**Coffee and Farewell**

Invited Talk: ECIS: Concept to Commercialization

Charlie Keese
Applied biophysics, Troy, NJ, USA

Increased Throughput in GPCR Screening using Impedance Assays: Inspiration from Organ Studies

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G-Protein Coupled Receptors (GPCRs) belong to the most promising drug targets, as they are involved in a multitude of human diseases. In screenings for novel, effective GPCR ligands, studies on human cell cultures have nowadays largely replaced classical organ pharmacology. Especially, label-free impedance-based assays have evolved as a valuable tool to quantify the efficiency and potency of GPCR ligands, as the method is sensitive to all signaling pathways activated that evoke changes in cell morphology [1, 2]. This so-called holistic detection of the integrated response to receptor activation has many parallels to functional measurements on organ and tissue preparations.
Impedance assays are capable of quantifying GPCR activation in cellular systems with endogenous receptor expression non-invasively with a time resolution down to several milliseconds.
However, when considering the screening of large substance libraries, the costs for the disposable electrode arrays may become limiting. Moreover, with an increasing number of samples to be studied the available time resolution is reduced.

Inspired by protocols from organ pharmacology, we investigated a simple serial agonist addition assay that circumvents the above described limitations. We report on the possibility to establish a full concentration-response curve for a GPCR agonist using a single cell layer in an impedance assay. This significantly increases throughput and reduces the costs per assay, while the time resolution, necessary to detect fast and transient cell responses, is retained.

References:

[1] C.W. Scott and M.F. Peters, *Drug Discovery Today* 15 (2010) 704 – 716
[2] J.A. Stolwijk, K. Matrougui, C.W. Renken, M. Trebak, Pflügers Archiv 467 (2015) 2193 – 2218

Chlorpromazine disrupts structural integrity of cell membranes in HepaRG cells and initiates a pro-inflammatory response

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Chlorpromazine (CPZ) is a neuroleptic drug and a prototype compound used to study intrahepatic cholestasis. The exact mechanisms of CPZ induced cholestasis remain obscure. Traditionally, rat hepatocytes, or a sandwich culture of rat and human hepatocytes, have been the most commonly used models for studying CPZ toxicity in vitro. However, to better predict outcomes in pre-clinical trials where cholestasis may be an unwanted consequence, a human in vitro model, based on human HepaRG cells and electrical cell-substrate impedance sensing (ECIS) has been developed that is capable of real time non-invasive monitoring of CPZ toxicity. We used previously established concentrations of CPZ ranging from sub toxic, 25μM and 50μM, to toxic 100μM[1]. To assess this range of concentrations, we employed ECIS to measure viability and cell membrane interactions supported by the adaptive and detrimental processes activated within the cell. Key markers of selected molecular pathways were analyzed using qPCR (oxidative and defence response, apoptosis and inflammation pathways and markers of hepatocellular cholestasis and membrane transport).

There was a dose dependant response to CPZ treatment that involved pro-inflammatory pathways activation (TNF, IL-6), loss of cell-cell junctions and cellular adhesion. Despite significant expressions of CYP3A4 ($p<0.05$), membrane phospholipid transporter ABCB1 ($p<0.001$) and xenobiotic transporter ABCB4 ($p<0.01$), treatment with 50μM CPZ inhibited biliary acid exporter, ABCB11 ($p<0.01$). Furthermore, CPZ treatment in HepaRG cells induced an inflammatory response with activation of adaptive processes for cell survival (increased NRF2 and autophagy system activity), aiming to protect cells against proteotoxicity and apoptosis. The real time non-invasive monitoring by ECIS results mirrored accurately these events with regards to cell membrane changes and viability.

In conclusion, structural changes within the membrane caused by sub toxic and toxic doses of CPZ had an inhibitory effect on bile acid export leading to intrahepatic bile acid accumulation and cholestasis. ECIS revealed a unique pattern of CPZ toxicity that mirrored these molecular cellular events.

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Time-resolved Response Profiles of GPCR Activation: Combining Two Independent Impedance-Based Approaches

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The biggest class of cell surface receptors is the huge family of G protein-coupled receptors (GPCRs), which are responsible for many important physiological functions, such as the sense of taste, smell and vision or the regulation of blood pressure and immune system activity [1]. Given the enormous implication of GPCRs in cell physiology, it is not surprising that more than 40 % of all prescription pharmaceuticals on the market address GPCRs. Accordingly, there is an ever growing interest in assays and devices that are capable of reporting on new details of GPCR-drug interactions.

A promising device for monitoring cell-based assays in general and GPCR pharmacology in particular is the quartz crystal microbalance (QCM) technology, which has proven to be a powerful tool for monitoring changes in cell-substrate adhesion or cytomechanics. The core component is a quartz resonator sandwiched between two gold electrodes deposited on its surfaces that are used (i) to drive the resonant shear oscillation of the crystal and (ii) to read the oscillation parameters. Due to its piezoelectric nature the mechanical oscillation goes hand in hand with an electrical oscillation that can be probed by impedance analysis. We used the electrical impedance at an AC frequency close to the resonators' fundamental resonance frequency of about 5 MHz as a measure for changes within the cell mechanics. The QCM is a label-free, non-invasive and time-resolved technique with an adjustable time resolution down to tens of milliseconds [2].

When cells attach and spread on the quartz surface, the resonant oscillation is affected by the presence of the viscoelastic cell bodies and all mechanical changes within. Lately, the QCM has been successfully applied to study cell surface receptors and the associated signal transduction [3], so we took advantage of these findings by using the QCM technique as a monitoring tool to study GPCR activation. On the example of the human histamine H_1-receptor (hH1R) we could observe signal transduction mediated changes in cell mechanics by analyzing various parameters of the quartz oscillation. The observed changes in cell structure were robust enough to establish dose-response relationships and to run experiments in antagonism mode.

To further improve the information content, the QCM technique was combined with Electric Cell-Substrate Impedance Sensing (ECIS) in one experimental setup for simultaneous sensing of both, mechanical and morphological changes within cells that are exposed to the same stimulus. This combined ECIS-QCM approach may form the basis for more time-resolved response profiles and their subsequent analysis with respect to receptor pharmacology.

References:

[1] D. M. Rosenbaum; S. G. F. Rasmussen and B. K. Kobilka, *Nature* 459 (2009) 356–363.
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[3] J. Y. Chen; A. Shahid; M. P. Garcia; L. S. Penn and J. Xi, *Biosensors & Bioelectronics* 38 (2012) 375–381.

Invited Talk: TBA

Prof. Wen Jiang Title
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Real-Time monitoring of Equid alphaherpesviruses infectivity in equine dermal cell based on impedance measurements: effects of aciclovir and ganciclovir treatments

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Five herpesvirus have been reported to infect horses and 3 belong to the *Alphaherpesvirinae* subfamily (equine herpesvirus-1, EHV-4 and EHV-3). Among them, EHV-1 is the most pathogenic and causes respiratory disease in yougs horses, abortion in mares, neonatal death and neurologic damage, also call equine herpesvirus myeloencephalopathy disorders. EHV-4, also named the rhinopneumonia virus, is closely related to EHV-1 but mostly causes respiratory disease and only sporadic abortion and neonatal infection in horses. EHV-3 is distinct from previous viruses and is responsible for equine coital exanthema, characterized by formation of papules, pustules, ulcers and vesicles on the external genitalia of horses. The equine industry economical loss linked to EHV infection is significant, which warrants surveillance and prophylaxis. Currently, no antiviral molecule has a marketing authorization for equine species despite critically needs, warranting the development or adaptation of drugs that are effective against equine viruses.

The aims of this work were 1) to investigate the efficacy of Real-Time Cell Analysis (RTCA) system to monitor EHV infections in equine dermal cells 2) to assess the effectiveness of acyclovir (ACV) and ganciclovir (GCV) against EHV-1, EHV-4 and EHV-3 by RTCA system and 3) to investigate the capacity of RTCA system for the screening of compounds library in order to identified new inhibitors of these viruses. To confirm results achieved, monitoring of cell morphology by microscopy and quantification of viral loads were perform in parallel.
Antiviral effect of ACV or GCV against the EHV-1 KyD strain was evaluated by RTCA. Results showed that ACV and GCV prevented the impedance decrease induced by EHV-1 infection in a dose-dependent manner and EC_{50} measured were 9.88 ± 2.14 µg/ml and 0.62 ± 0.49 µg/ml, respectively. The ACV and GCV EC_{50} against EHV-4 were 17.38 ± 5.95 µg/ml and 2.79 ± 0.25 µg/ml, respectively. Against EHV-3, the ACV and GCV EC_{50} were 15.23 ± 2.85 µg/ml and 1.86 ± 0.46 µg/ml, respectively. All the data were confirm by qRT-PCR and microscopy observation. The screening of compounds library allowed identifying several molecules with an antiviral activity needing further investigation.
This study confirms the efficiency of RTCA to monitor cytopathic effect formation induced by equine alphaherpesviruses on E. Derm cells in real-time. In this model, GCV was shown to be the most effective against all viruses used to infect E. Derm cells. This technology aims to complement and support conventional methods used in the field of virology.

Discrimination between Normal and Cancerous Cells using ECIS and Artificial Intelligence Techniques.

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In this work we measure ECIS data for normal and cancerous cells for the migration, micromotion and wound healing phases of in-vitro culture. Based on an Artificial Intelligence (AI) technique (Machine Learning) we are able to discriminate (diagnose) between normal and cancerous cells.

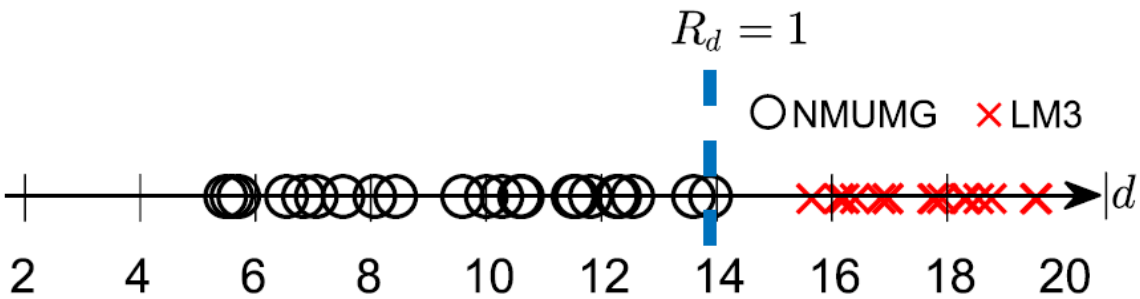
We developed a measurement protocol that consists of a set of a ECIS based techniques that allows us to characterize different cell lines.

We determined a set of parameters that are characteristic of normal and cancerous cells. These parameters allow to separate them in different populations. The parameters can be measured using the abovementioned protocol.

Based on the set of parameters that are characteristic of normal and cancerous cells we determined the more relevan tones. We the performed a Lineal Discriminant Analysis (LDA) to differenciate between normal and cancerous cells.

This proposed technique allows to discrminate effectively between normal and cancerous cells cultured in vitro with only 4.5% of false positives and 0% of false negatives. Standard techniques of discrimination have 20% of false positives and 20% of false negatives.
Keese [9].

The technique was applied to the cell lines NMuMG (normal) and LM3 (cancerous). Nevertheless given the generality of the chosen characteristic parameters the technique can be used for the study of other cell lines and also for primary culture. The results we obtained on the growth and confluence phases are consistent with the results obtained by Giaever and Keese with the WI38 (normal) and WI38-VA13 (transformed) cell lines.



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Selection of probiotics and prebiotics against *Clostridium difficile* using an *in vitro* intestinal model based on impedance real time monitoring

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Clostridium difficile is an opportunistic pathogen inhabiting the human gut and is the etiological agent causing *C. difficile* infections (CDI) currently linked to a dysbiosis of the intestinal microbiota due to antibiotic treatment. The incidence of CDI, which manifestation ranges from mild diarrhea to life-threatening conditions, is increasing not only in compromised subjects, but also in traditionally considered low-risk populations. In addition zoonotic and food transmissions are considered sources for *C. difficile* acquisition. Several virulence factors are related to CDI, the main ones being the production of toxins A and B involved in the clinical manifestation of the infection. The standard treatment for CDI is antibiotics but the recurrence rate of the disease is very high. This fact has attracted the interest to search for prevention/ therapeutic options to reduce the growth and/or toxicity of *C. difficile*; among others, probiotics, prebiotics, or a combination of both (synbiotics) constitute a promising approach[1].
Our group has developed a method for the real-time monitoring of the cytotoxicity of *C. difficile* upon human enterocytes (HT29 and Caco2 lines) using the label-free, impedance-based RTCA xCELLigence equipment (ACEA Bioscience Inc.) and following the morphological changes in the optical microscope LumaScope-600 Series (Etaluma) with time-lapsed imaging capture. Cytotoxic dose- and time-dependent effect of clostridial supernatants upon both cell lines was detected with the RTCA and different toxicological parameters (EC50 and LOAEL) were obtained from the sigmoid-curve fit of data; thus, we have proposed this method as a model for the screening of new anti-CDI agents[2]. Furthermore, this model was applied to search for probiotic candidates able to reduce the toxicity of the pathogen; among the twenty strains tested those belonging to *Bifidobacterium longum* and *B. breve* species were the most efficient reducing *C. difficile* toxicity, the RTCA-normalized cell index values being inversely correlated with the amount of the remnant toxin (A and B) presents in the clostridial supernatant. The strain *B. longum* IPLA20022 showed the highest anti-toxin activity by RTCA. An image analysis of HT29 monolayers, formed on the surface of 2-well μ-Slide cover slip (Ibidi GmbH), using the Leica TCS AOBS SP8 X CSLM (Leica Microsystems GmbH) was performed; monolayers treated with *C. difficile* toxigenic supernatant in the presence/ absence of the strain IPLA20022 showed that this probiotic candidate reduced the cellular rounding (due to the contraction of F-actin microstructure) and diminished the occurrence of apoptotic bodies. Besides, it seems that extracellular factors released by *B. longum* IPLA20022 are involved in its anti-toxin activity [3]. Finally, using the RTCA technology we have also demonstrated that the combination of specific bifidobacteria with prebiotics (certain fructo-oligosaccharides) is able to reduce the toxicity of *C.difficile* supernatants [4].

References:

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Cells in Contact to Carbon Dots: A label-free, impedance-based and multidimensional approach

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Carbon Dots (Cdots) represent a relatively new allotropic form of carbon with interesting material properties such as high quantum yield photo-luminescence and long-term photostability [1]. Cdots as model nanoparticles are only a few nanometers in size and can be synthesized via hydrothermal carbonization of starch and L-tryptophan. The nanoparticles exhibit moderate toxicity up to concentrations in the mg/mL range which was investigated for different cell lines using Electric Cell-Substrate Impedance Sensing (ECIS®) which allows for label-free and time-resolved monitoring of the cells. The results were confirmed by cross-checking against the resazurin-based viability assay PrestoBlue® which yielded similar results. Furthermore, the time-dependent cell response in presence of non-toxic Cdot concentrations in the incubation buffer was investigated in several in vitro scenarios during (i) attachment and spreading, (ii) proliferation and (iii) migration. Significant delays have been found in all phenotypic studies (i) to (iii) for increasing particle concentrations. Impedance-based measurements have additionally shown that Cdots in higher concentrations affect the regular beating of cardiomyocytes (Cor.At®), as the cells' ability to contract synchronously is heavily compromised for higher particle concentrations.

Cdots are en route to their application as labels in bioanalysis and even constitute a possible candidate for applications in photodynamic therapy. The nanoparticles produce reactive oxygen species upon irradiation at wavelengths from 330 - 380 nm [2] and can thus be used to destroy selected cells loaded with the particles. ECIS® studies revealed that Cdots exert phototoxic effects not only on cell monolayers of different cell lines but also on multicellular tumor spheroids (MCTS) when excited by near ultraviolet light. The method is therefore not limited to two-dimensional cell structures but can also be used to monitor three-dimensional tissue models.

These studies demonstrate the advantages of impedance-based methods to non-invasively monitor the impact of nanomaterials and other stimuli on living cells in real-time. The work with luminescent and phototoxic Cdots strongly underlines the value of label-free, non-optical readouts of cell behavior that is otherwise hard to study in such detail.

References:

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Nanoparticle toxicity: cytotoxic and sub-cytotoxic measurements using xCELLigence real time cell analysis

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Despite the clear benefits of nanoparticles (NPs) in industry and everyday products, the understanding of nanoparticle toxicity has lagged, in part due to the shear number of nanoparticles and their varied chemistries. We have used the impedance-based xCELLigence real time cellular analysis (RTCA) system as a high capacity screen to explore nanoparticle-induced cytotoxicity (i.e., cell death) on airway epithelial cells [1]. Because many of the compounds tested only showed cytotoxicity at relatively high concentrations, we used the RTCA to expand our nanotoxicity studies to explore the sub-cytotoxic effects of nanoparticles on airway epithelial cells. Following 24 hr exposure to hafnium oxide (HfO_2) concentrations that did not induce cytotoxic responses (i.e., < 2000 ppm), airway epithelial cells were exposed to ATP, a paracrine signaling molecule common to the airway, and their impedance response measured. We found a dose-dependent reduction in ATP signaling with HfO_2 concentrations as low as 50 ppm. Similar reductions in airway epithelial ATP signaling were observed in Ca^{2+} signaling experiments evaluated via digital imaging microscopy. Interestingly, cellular signaling toxicity experiments with non-nano sized HfO_2 suggested that the observed reduced signaling in airway epithelial cells was related to metals toxicity and not increased by nanomaterial chemistry.

In a second set of experiments with RTCA we assayed synergistic toxicity effects of nanoparticles used in semi-conductor industries (e.g., gallium arsenide (GaAs) indium arsenide (InAs)) and associated nanomaterial used in chemical mechanical planarization (CMP) -- silica (SiO_2), ceria (CeO_2) and alumina (Al_2O_3)). CMP processes are characterized by a high water demand and thus can generate high volumes of effluents containing inorganic oxide NPs as well as nanoscale As particulates. Although GaAs and InAs NPs generated similar characteristic kinetic patterns, GaAs displayed much higher toxicity on airway epithelial cells ($IC_{50} \approx 2.0$ mg/L) than InAs ($IC_{50} \approx 30$ mg/L). We found that much of the cytotoxicity from these NPs could be explained by the dissolution of highly toxic arsenic. Similar to the HfO_2 studies above; sub-cytotoxic concentrations reduced cellular response to ATP. In an effort to further understand nanoparticulate mixtures, we evaluated the toxicity of arsenite (As(III)) adsorbed onto cerium dioxide (CeO_2) NPs with RTCA. Application of 0.5 mg/L As(III) resulted in 81.3% reduction of cell index over 24 hrs when compared to cells treated with As(III)-free medium. However, when 0.5 mg/L As(III) was applied to the cells in the presence of CeO_2 NPs (250 mg/L), cell index was only reduced by 12.9% compared to As(III)-free medium. The CeO_2 NPs had a high capacity for As(III) adsorption (20.2 mg/g) in the bioassay medium; effectively reducing dissolved As(III) in the aqueous solution and resulting in reduced toxicity. We conclude that dissolved As(III) in the aqueous solution was the decisive factor controlling As(III) toxicity, and CeO_2 NPs effectively reduced available As(III) through absorption. These results indicate that the release nanoscale GaAs, InAs and associated particulates in CMP effluents should be controlled due to the hazardous potential to humans and the environment.

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Monitoring the Toxicity of Bisphenol A using Multiple Impedance-Based Cellular Assays

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Bisphenol A (BPA) is a synthetic organic chemical, that is heavily applied as an additive to produce clear and robust plastics and resins. It belongs to the mostly synthesized chemicals worldwide. Epoxy resins containing BPA are used to line water pipes and as coatings on the inside of many food and beverage cans. Because of its presence in food containers (even for baby food) concerns about its harmfulness have accumulated recently and led to new legislature in some European countries limiting its use. It has been included in the list of REACH chemicals which are monitored in detail for their harmfulness.

It was our intention to study the impact of BPA on cultured cells with a whole set of impedance-based assays focusing on different cell phenotypes like (i) cellular micromotion, (ii) cell migration, (iii) cell proliferation, (iv) toxicity in equilibrated cell layers. These studies were accompanied by well-established metabolic assays that report on metabolic activity (PrestoBlue) and the cells' oxygen consumption rate. These assays were used to test the hypothesis that BPA may affect cellular physiology differently dependent on the cellular state and that detailed toxicity profiles are more appropriate to describe a compound's toxic potential than just a single biochemical assay. Normal Rat Kidney (NRK) cells were used as model cell in all assays.

All impedance-based assays were performed according to state-of-the-art protocols taking advantage of different time resolutions and monitoring frequencies dependent on the details of the assay. Metabolic activity was studied by the commercial PrestoBlue assay that reports on the availability of the redox coenzymes NADH and NADPH, central products of catabolism. Oxygen consumption rates (OCR) were determined by monitoring the oxygen concentration beneath the cells using oxygen sensitive sensor foils and an imaging system provided by PreSens®. After dose-dependent experiments were performed, data analysis returned the EC_{50} values for each assay that are summarized in the radar plot below (Fig. 1).

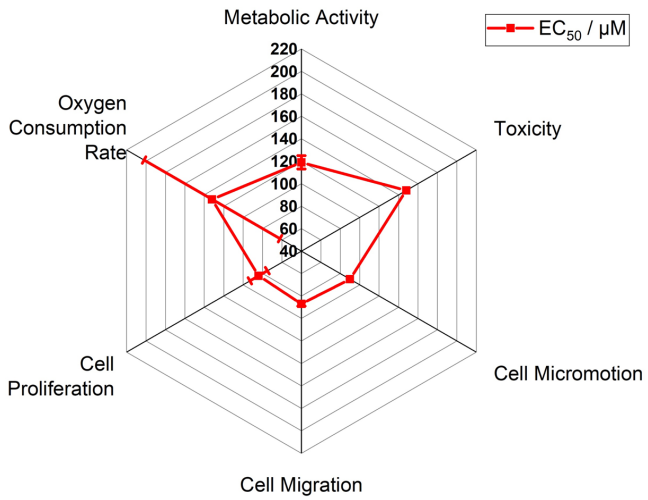

Fig. 1: Comparison of EC50 values, reporting on the impact of Bisphenol A on different cell phenotypes.

Cell Monitoring using impedance and impedance spectra for high content toxicity and cell proliferation screening

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Changes in the impedance time course of cell-covered electrodes are not only useful to monitor effects on cell contractility in a cardiomyocyte population but may also give profound insight in other cell phenotypes, such as changes in cell morphology, proliferation and toxicity, even over prolonged periods of time, giving a crucial advantage of this technique over standard cytotoxicity assays which are mostly endpoint assays. Moreover, impedance technology also allows monitoring changes in intracellular regulatory cascades or signal transduction, such as effect of drugs on G protein-coupled receptors (GPCRs). In all these applications, advanced information content is provided by using impedance spectra instead of single frequency recordings. Here, we describe the development and optimization of highly sensitive and reproducible cell-based assays for safety pharmacology, toxicity screens of adherent proliferating cells and hepatotoxicity.

Diverse cell types were monitored using a 96-well impedance screening instrument that reads impedance under physiological conditions (temperature, humidity and CO2) and enables short and long-term recordings without using an incubator. After initial proof of principle experiments in which we monitored cell proliferation and toxicity, we have further used impedance technology to investigate the effects of adjuvant chemotherapy for prevention of breast cancer tumor recurrence. The murine mammary carcinoma cells (H8N8 and H8N8 T3.2) were used and changes in impedance, and therefore confluency, were used as a measure of toxicity. Intrinsic (dose-dependent) effects of the standard clinical treatment regimen cyclophosphamide, doxorubicin and 5-fluouracil could be identified consistent with other methods of live cell analysis. Therefore, the utilized 96-well impedance system in combination with murine mammary carcinoma cells provides a novel tool for investigating therapy resistance of cancer cells in vitro. We also tackled the application of this technology to hepatotoxicity by investigating the hepatotoxic effects of paracetamol on monocyte-derived hepatocyte-like (MH) cells when exposed for 24 and 48 hours, and by applying different dosages. We were also able to monitor GPCR activation in various cell types by applying specific agonist in dose-response studies. Finally, we will show impedance spectra (0.1 to 100kHz) recorded in a 96-well format, applied to various cell types and targets.

In summary, our data and technical innovations strengthen the importance of testing compounds in assays complementary to established electrophysiology or biochemistry assays, in order to provide an all-inclusive safety and toxicity compound profile.

Impedance Analysis of Waterborne Parasite Infectivity

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Pathogenic microorganisms are widespread in the environment. To improve health and safety, it is of great importance to be able to perform on site, rapid but accurate analysis. Very sensitive techniques are mandatory since many pathogens can be harmful in very low concentration (i.e.: few infective agents). Lab-on-chip (LOC) technology is very well adapted to take on this challenge. Many quantification methods are well suited for integration into LOC **[1]**. The lookout for *Cryptosporidium*, one of the most common waterborne parasitic protozoans, outbreaks is a perfect case study to illustrate this technological challenge **[2]**. With the significant progress in microfabrication technologies, using MEMS to automate the detection process or decrease the detection limit further is now an attractive proposition. Several studies have tried to tackle this problem using various strategies such as impedance spectroscopy **[3]**, electrochemical biosensor **[4],** surface plasmon resonance **[5],** quartz microbalance **[6]**, cantilever based systems **[7]**, surface enhanced Raman spectroscopy **[8]** and flow cytometry **[9]** reaching sensitivities down to single oocyst/cyst level. As an important aside, it should be noted that detecting the parasite is insufficient to correctly assess the risk to which the population is exposed to when drinking contaminated water. Traditional in vivo infectivity assays are performed in neonatal-mouse **[10]** or on 2D in vitro cell culture. Determination of the viability of *Cryptosporidium* was achieved by electrorotation **[11]**. We here show that an electrical impedance-based device is able to get insights on Cryptosporidium development on a cell culture and to quantify sample infectivity. HCT-8 cells were grown to confluency on interdigitated microelectrode arrays during 76h and then infected by *Cryptosporidium parvum* during 60h.The impedimetric response was measured at frequencies ranging from 100Hz to 1MHz and a 7 min sampling period. As the infection progresses the impedance signal shows a reproducible distinct succession of peaks at 12h post infection (PI), 23h PI and 31h PI and local minima at 9h PI, 19h PI and 28hPI.

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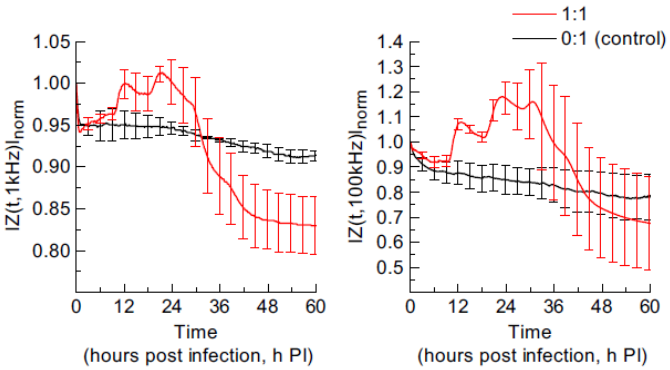

Figure 1: Real-time monitoring of Cryptosporidium infection of confluent HCT-8 cell layer during 60h (PI¼post infection).Time-course of the cell layer normalized impedance magnitude |Z(t,f)|norm is represented at 1 and 100kHz. Cells were infected with a ratio of 1 viable sporozoite per cultivated cell (ratio1:1) or with sporozoites inactivated by a freezing-and-thawing step (0:1ratio, negative control).Values are mean 7 SD, n¼3.

Impedance Spectroscopy in Microfluidic Devices for a Significant Enhanced Cell Monitoring in a Live Mode

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Currently the demands on chemical drug screening is focusing to a greater extend the integration of compound separation, purification, and analysis in smart microfluidic lab-on-chip devices. Therefore, novel microfluidic chips consist of microchannels, microreactors, electrophoresis fields but currently also integrated microelectrode arrays for an electrochemical monitoring of compound bioactivity and efficacy on viable target cells. In this presentation some aspects will be presented and discussed concerning micro-free flow electrophoretic separation of organic solvents from compound solution [1], and bioanalysis on selected cell models in a live mode [2]. Impedance spectroscopy will be combined with further biophysical measurement methods for a simultaneous multiparametric and multimodal real time recording of e.g. human induced pluripotent stem cell derived neuronal networks, human embryonic kidney cells expressing target molecules like G-protein coupled receptors or ion channels responding on active pharmaceutical ingredients. The most important aspect for impedance spectroscopy of viable cells in microfluidic devices i.e. included microelectrode compartments is the cellular adhesion. Thus a genetically designed human embryonic kidney cell line HEK-A expressing the neuropeptide Y1 receptor shows now stronger adhesive properties ideal for a disposition on the electrodes under flow condition on microfluidic chips. In that case, the recorded impedance signal related to Y1R agonist/Y1 receptor interaction and internalization is much stronger or higher than measured in a steady-state microelectrode array. The distribution of the compound as well as the more homogenous current field distribution over the electrodes in microfluidic lab-on-chip devices could be one reason for better and stable impedance measurements on viable cells [2]. But not only compound screening could be carried out using cell based bioimpedance spectroscopy but also cell and tissue characterization especially cells derived from human induced pluripotent stem cells. A complete stem cell differentiation to mature neuronal networks could be monitored on microelectrode arrays in a multiparametric live mode via impedance spectroscopy recording the morphological and physiological alterations [3] and by action potential recording detecting the electrophysiology [4]. All these studies revealed that such optimized and improved microelectrode arrays or microfluidic, bioanalytic lab on chip setups finally release comprehensive sensitive physiological information of various cell types via impedance spectroscopy.

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Impedance Analysis of Adherent Cells Cultured on Various Sizes of Electrodes

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In cell-based impedance assays, the measured impedance is dependent on the attached cells, the surface area of sensing electrode, and the constriction resistance between sensing and counter electrodes. To optimize the sensitivity of impedance measurement it is challenging to choose a suitable size of sensing electrodes. In this study, to figure out the optimal size of sensing electrode, HUVECs and MDA-MB-231 cells were cultured and measured on gold film electrodes with four different diameters (25, 50, 100, and 250 μm) and electrode wells containing ten circular 250 μm diameter active electrodes. In frequency scan measurement, HUVEC-covered electrodes were measured at frequencies from 25 Hz to 60 kHz and impedance data were compared with those obtained from cell-free electrodes. Our data showed that as the diameter of electrode decreased, the optimal frequency for measurement shifted from low frequency to high frequency region. In addition, the signal-to-noise ratio (SNR) demonstrated biphasic feature and the largest SNR of resistance was observed when 250 μm diameter of sensing electrodes were used. Interestingly, these phenomena were also observed for MDA-MB-231 cells which was unable to grow into a confluent monolayer. These results indicate the sensitivity of impedance measurement strongly depend on both electrode size and measuring frequency. In general, the 250 μm diameter of sensing electrode is the optimal selection for monitoring cell behavior of adherent cells.

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Impedance Analysis of Heterogeneous Cell Populations: Impact on Data Analysis and Modeling

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ECIS uses impedance readings to monitor changes in cell morphology within a confluent cell layer adherently grown on gold-film electrodes. Besides analyzing the impedance raw data, the *ECIS model* provides a more quantitative and structural description of the morphological changes provided that the impedance is recorded along an extended frequency range. Applying the non-redundant ECIS model breaks down the frequency-dependent impedance into three cell-related integral parameters: α (resistance in the cell-electrode junction), R_b (resistance of the cell-cell-contacts) and C_m (capacitance of the cell membranes) [1]. The ECIS model assumes a homogeneous cell population with circular cells of the same radius, which obviously does not mirror reality: individual cells within the same cell layer differ in size, height, dielectric properties of the membrane due to different amounts of membrane proteins, state of the cell cycle or metabolic status - to mention just a few sources for cell individualism. The impact of this heterogeneity on the applicability of the ECIS model and how it affects the results is yet unknown.

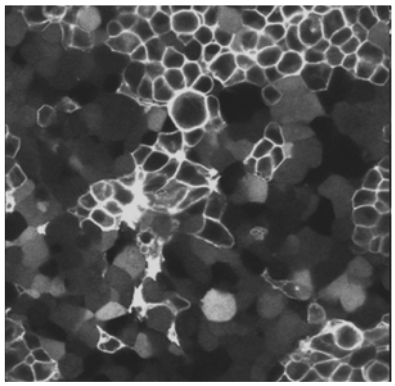

Fig 1: Two different HEK clones in a 1:1 ratio are shown. One of the clones expresses a chimeric cytoplasmic protein with an ECFP label, the second produces a chimeric membrane protein with an EYFP label.

We approached this problem experimentally by studying well-defined mixtures of two different cell types that are similar enough to form undisturbed monolayers on the electrode. These mixtures consist of (i) two different epithelial cell lines expressing a distinctly different barrier function or (ii) wild type cells mixed with their transfected offspring. Systematically different ratios of the individual cell species in the mixed monolayers were prepared by mixing well-defined cell suspensions of either cell type. The various cell mixtures were studied with respect to their spreading kinetics, differentiation, signal transduction and ECIS-parameters R_b, α and C_m.

The experimental approach was complemented by simulating the impedance data of electrodes covered with heterogeneous cell populations in all ratios. The model predicts the experimental situation surprisingly well in most cases and identifies the limits of the ECIS models for non-uniform cell layers.

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Invited Talk: Single cell impedance spectroscopy: theory and applications in healthcare

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Transparent organic transistors for ECIS with single cell resolution

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In recent years, we developed a technique using ion-sensitive field-effect transistors (ISFETs) for Electrical Cell-substrate Impedance Sensing (ECIS) and coined the term **F**ield-**E**ffect **T**ransistor **C**ell-substrate **I**mpedance **S**ensing (**FETCIS**) for this technique [1]. In our first works we utilized silicon ISFET arrays. However, such devices are very tedious to fabricate and resulting chips are opaque. Nevertheless, we applied the ISFETs in practical cell assays, where apoptosis can be followed down to a single cell level.
Organic thin film transistors (OTFT) are another version of FET devices, which gained a lot of attention in the field of biosensing in recent years. Their versatility, easy fabrication and their extremely high biocompatibility make those devices a new and exciting alternative for cell sensing applications. We developed arrays of electrochemically-gated OTFTs (OECTs) out of poly(3,4-ethylenedioxythiophene) doped with polystyrene sulfonate (PEDOT:PSS). OECTs offer a completely novel gating mechanism compared to the classical, purely capacitive coupling of cells to silicon ISFETs. We realized a wafer-scale fabrication protocol for OECTs on silicon and glass substrates. Devices exhibited excellent sensor characteristics with transconductance values exceeding those of our silicon ISFETs. Firstly we published recordings from electronically-active cardiac myocytes with OECTs [2]. Here we present FETCIS assays with individual HEK 293 cells with OECTs (Fig. 1).

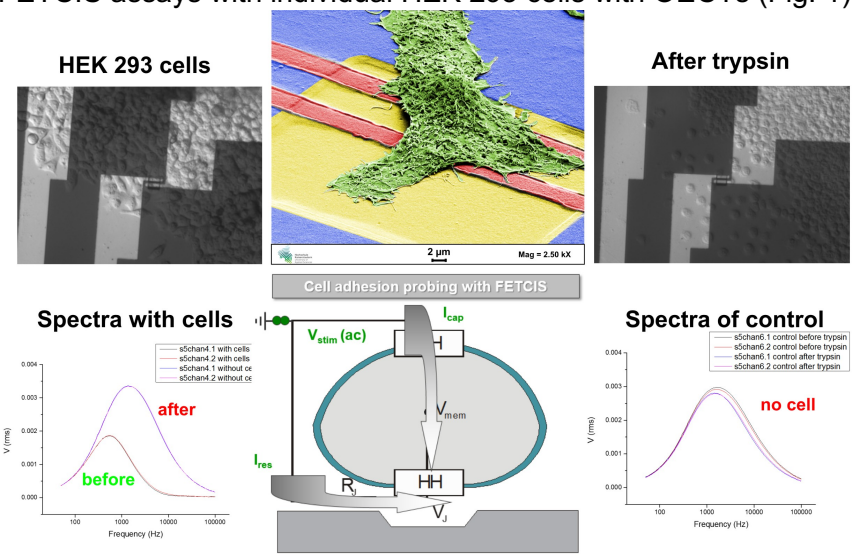

Figure 1: Example for FETCIS with HEK 293 cells on our OECT devices. The adhesion of individual cells is clearly imposing a low pass characteristics in FETCIS very similar to our earlier studies with silicon ISFETs.

In future the OECTs could be produced such cheap that disposable sensors embedded in standard plastic cell culture dishes would be possible. This could be a very interesting alternative to the classical sensor types in ECIS.

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Towards 3D impedance-based cellular assays with Electrical impedance tomography

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3D cellular assays are desirable tools to predict the cellular responses of 3D cell culture with enhanced cell-cell and cell-matrix interactions that can be used to better mimic the behaviour of cells growing in the natural environment [1]. However, traditional biochemical techniques optimized for 2D assays do not perform well for assessing 3D culture. Most of them lack penetration depth or can only provide a lumped value for the whole tissue instead of a visual image giving an insight into the spatial distribution. In this study, we demonstrate the potential of electrical impedance tomography (EIT) to overcome these limitations through real-time and non-destructive monitoring of the size and the viability of a multicellular spheroid.
We showed that we could reconstruct the conductivity distribution of spheroids inside the miniature sensor in real-time and label-free with EIT [2]. In order to evaluate the cell viability, 1ml culture medium with 2% Triton X-100 was filled to the sensor and the MCF-7 breast cancer cell spheroid was placed into the solution. Because of the chemical insult, the membrane protein of the cells was solubilised and their conductivity increased. Fig.1 shows the EIT images of the conductivity variation of the spheroid. The increase of the spheroid conductivity at different time-points can be used as an indicator of cell mortality, and this was validated against biochemical assays. The conductivity of the spheroid from 20 minutes to 30 minutes is similar, so it can be concluded that the cell death happens in the first 20 minutes after adding the spheroid into the solution. This study highlights the simplicity of EIT measurement and the high temporal resolution of its imaging data, showing the potential of EIT in the applications of drug-screening and tissue engineering using 3D cell culture.

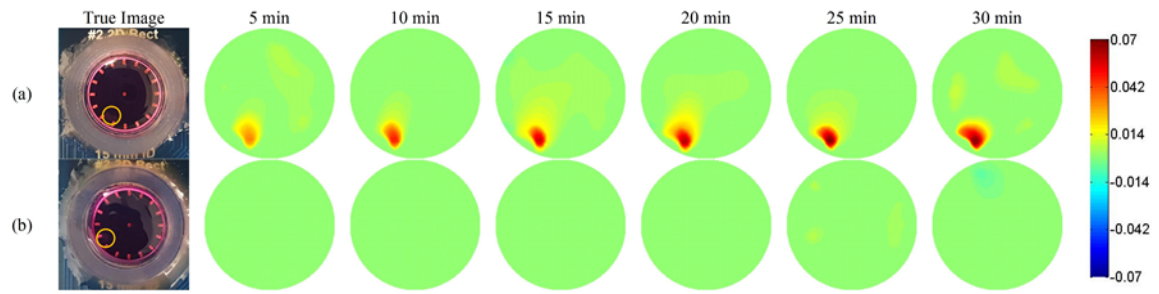

Fig.1 Reconstructed images for the spheroids in (a) 2% Triton X-100 solution and (b) culture medium (control).

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Monitoring of 3D Cell Cultures Using Conducting Polymer Scaffolds

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Tissue generation is considered physiologically relevant when conducted in three-dimensional (3D) spaces that resemble the physical architectures of tissues. Advances in 3D cell culture materials and techniques which aide accurately mimicking *in vivo* systems to study biological phenomena, have fostered the development of organ and tissue models. While 3D tissues can be generated, technology that can accurately assess the functionality of these complex models in a high-throughput and dynamic manner is not well compatible with the biological tissue. We herein report a live-cell monitoring platform based on 3D conducting polymer scaffolds consisting of poly(3,4-ethylene dioxythiophene):poly(styrenesulfonate) (PEDOT:PSS).[1] The conducting scaffolds are used concurrently as a biocompatible host to support 3D cell cultures as well as an electrode to electrically probe cell growth. The inclusion of cells within the pores of the scaffold was found to significantly affect the impedance of the electrically-conducting polymer network. Furthermore, by tuning the composition parameters and the microstructural properties of the fabricated scaffolds we were able to regulate the device performance as well as the cell behavior. The proposed platform tested with various cell types including fibroblasts and epithelial cells, represents a nondestructive and label-free in-situ cell-based toxicity screening platform, paving the way towards next generation in vitro toxicology assays.

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Flexible Microelectrode Array (Flex-MEA) Design for Micro-Bioimpedance Tomography of *Rhodococcus erythropolis*

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Electrical impedance tomography (EIT) is a non-invasive imaging technique that maps variation in conductivity in a sample, in two or three dimensions [1]. This technique has been successfully used in many clinical applications, for example, acute stroke differentiation [2], detection of bleeding due to traumatic brain injury [3], and detection of bacterial infection on a surgical site [4]. The capability of EIT to spatially map biological processes also enables it to be used to monitor cell growth in three dimensions, including bacteria. This study aims to improve the detection of *R. erythropolis*, a readily available nonpathogenic organism, using a flexible microelectrode array (Flex-MEA) fabricated on a flexible printed circuit board (PCB). The Flex-MEA enables improved electrode arrangement compared with previously published planar Pt electrodes made on a glass substrate [5]. First, the capability of the Flex-MEA to monitor cell growth will be demonstrated by imaging *R. erythropolis,* cultured in a 3D cell structure. EIT will then be employed to study the behaviour and responses of *R. erythropolis* to environmental changes. Successful detection of *R. erythropolis* has excellent potential, not only in the biomedical field but also in the marine field for the study of biofouling.

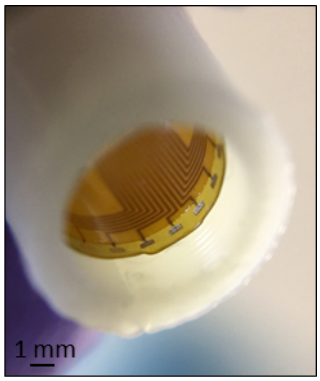

Figure 1: The Flex-MEA fitted in a 6.4 mm fluid chamber

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Invited Talk: Impedance-based analysis as a dedicated technique to characterize efficacy of novel antitumour compounds

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Evaluation of candidate molecules as dedicated antitumour compounds is a complex, multistep process, which prefers high throughput and real-time cell physiological assays. Application of impedimetry-based systems (ECIS, xCELLigence etc.) in combination with other cell physiology/molecular biology high content screening platforms (e.g. CellDiscoverer7) provides the possibility to perform large number of accurate measurements required by pharmaceutical research.
In the last decade, hundreds of drug candidates (free active drugs, targeting moiety and their conjugates) have been screened in our laboratory.
(i) In these investigations the most frequently tested drug-carrier constructs were belonging to peptide- or protein-based conjugates: daunomycin- (Dau), doxorubicin- (Dox) or methotrexate- (Mtx) peptid hormone (e.g. GnRH, neurotensin) conjugates; while there were other targeted constructs selected by phage display technique as well as another list of novel chemotherapeutic agents (e.g. cinchona alkaloids, tamoxifens, TIC-10 and their ferrocene containing hybrids).
(ii) The most often used target cells were: human pancreas ductal carcinoma (PANC-1), human acute monocytic leukemia (Mono Mac-6); human melanoma (A2058 and HT168-M1); human colorectal carcinoma (COLO-205) and choriocarcinoma (BeWo) cell lines.
(iii) While the monitored cell physiological characteristics (proliferation, cell adhesion, migration) as well as apoptosis were analysed by impedimetry and colorimetric assays (e.g. AlamarBlue). The registered antitumour effects were confirmed by computer assisted analysis of morphometry and migratory behaviour in holographic microscopy.
In impedimetry-based cytotoxicity/viability assays (e.g. in PANC-1) showed that in the group of peptide-based compounds (i) some conjugates containing Dau (e.g. KK031), selected by phage display technique, expressed the highest cytotoxic effect (viability: 8.6 % at 10^{-5} M); (ii) the neurotensin-based conjugates were also effective (viability 4.6 %) in tumour cells, nevertheless in these type of constructs the dual targeting or formation of bifunctional conjugates could also enhance the cytotoxicity of the monofunctional compound. In the group of non-peptide-type ingredients (iii) cinchona alkaloids and TIC-10 derivatives proved to be the most effective ones (IC50 = 3E-6 - 6E-6 and IC50 = 7E-7 - 3E-6 respectively); (iv) formation of ferrocene hybrids of cinchona alkaloids resulted also enhanced cytotoxic effects both in PANC-1 and COLO-205 cells; while (v) the increased cytotoxicity of the halogenated TIC-10 (IC50 = 3.4E-7) vs. the parent molecule was also detected (IC50 = 1,7E-6).
Results presented above as well as series of data gained by other probes of cell physiology (to be presented in the oral presentation) support our opinion that impedimetry is a strong arm of experimental screening and complex evaluation of a wide range of antitumour compounds.

The project was supported by the National Research, Development and Innovation Office (NVKP_16-1-2016-0036)

Electric Cell-substrate Impedance Sensing (ECIS) as a method to test marine bioactive compounds in wound healing

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Wound healing is a physiological response to tissue injury [1]. Epithelial layers serve as a protective barrier against toxic compounds, infectious agents and other external stressors, and thus raid repair after damage is vital. In this highly complex process cell migration has a pivotal role.

Scratch wound assays are the classical *in vitro* method to analyse wound healing as they are simple and low cost. However, the demand for more sensitive assays has led to the development of new techniques such as, **E**lectric **C**ell-substrate **I**mpedance **S**ensing (ECIS). The major advantages of ECIS assays are that they are highly reproducible and permit wound healing to be observed in real-time. ECIS assays monitor living cells in a non-invasive manner and have been shown to successfully detect cell growth, cell proliferation, cell migration and invasion and cell cytotoxicity [2].

The aim of the present study was to assess if the ECIS system could be used to screen the bioactivity of Marine bioactive compounds such as fish proteins, and microalgae extracts on wound healing and more specifically the migration/proliferation of dermal fibroblasts. To evaluate the approach, primary human dermal fibroblasts (NHDF) were used and the effects of piscine Cartilage acidic protein 1 (CRTAC1) on wound healing was determined. Human CRTAC1 is an extracellular matrix protein which has already been associated with important cell-cell or cell-matrix interactions [3], but its potential use to aid wound healing remains to be fully characterized. Two different methodologies were used: the ECIS assays using IBIDI 8W1E PET arrays and introducing in the procedure a preliminary damage step in confluent dermal cells and the classical scratch assay. Our results indicate that CRTAC1 promotes dermal fibroblasts migration and confirms the proposed role for this protein in cell-cell interactions. Based on this study, the ECIS system will be a useful high-throughput method to identify bioactive compounds with a positive effect on dermal fibroblasts and wound healing but also as a method to identify novel protein functions.

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Connectivity and reorganization of cardiomyocytes and fibroblasts in co-cultures

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The electromechanical function of cardiac muscle of healthy as well as remodeled tissue depends critically on the communication of myocytes with fibroblasts. Upon cardiac fibrosis, fibroblasts translocate into infarcted necrotic tissue, transform into myofibroblasts and alter their communication capabilities. In our recent Electric Cell-substrate Impedance Sensing (ECIS) based studies, we determined a multiple parameter space relevant for fibrotic cardiac tissue development comprising the following essential processes: (i) adhesion to substrates with varying elasticity, (ii) dynamics of contractile motion, and (iii) electromechanical connectivity. We quantified the accompanying parameters *in vitro* using monolayers of co-cultures formed from rat fibroblasts and cardiomyocytes.
By combining Electric Cell-substrate Impedance Sensing (ECIS) with conventional optical microscopy we could measure the impact of fibroblast to cardiomyocytes ratio on the aforementioned parameters in a non-invasive fashion. Cells were grown on gelatin-fibronectin coated gold electrodes with variant elasticity quantified with Atomic Force Microscopy. We monitored adhesion and formation of co-cultures of cardiomyocytes and fibroblasts and determined spreading rates derived from impedances changes. Period analysis of impedance time series and propagation induced phase contrast imaging allowed us to measure contraction dynamics, thus the change in beat-to-beat intervals as a function of increasing fibroblast concentrations. Finally, we followed modulations of the barrier resistance as a measure of connectivity as a function of fibroblast fraction both optically and via impedance spectroscopy.
Combining results from adhesion assays, contraction and connectivity analysis, we can claim that: (i) a preferred window for substrate elasticity around 7 kPa for low fibroblast content exists, which is shifted to stiffer substrates with increasing fibroblast fractions. (ii) Beat frequency decreases nonlinearly with increasing fraction of fibroblasts, while (iii) the intercellular resistance increases. Our results show a maximal - yet still functional - connectivity for a fibroblast content of 75 percent [1].
More recently, we have focused on dynamics of shear stress stimulated cardiac cultures using flow based ECIS approaches and quantified reorganization on the cytoskeletal level [2]. Furthermore, ECIS based wounding or barrier assays were empolyed as a first step towards an *in vitro* model for scar remodelling [3].

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Impedance-based Characterization of pH-dependent Cell Behavior

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Changes in cellular behavior, caused by alterations of the extracellular pH, are of fundamental interest. Cells exhibit a characteristic pH gradient across their plasma membrane, which is different for distinct cell types and reveals differences in cell metabolism. It is known that certain cell behaviors are strongly dependent on a physiological extracellular microenvironment. Therefore acidification can lead to crucial changes regarding membrane-associated enzyme activities, ion transport activity, protein and DNA synthesis, and intracellular calcium levels [1]. Several phenotypic key features like proliferation, migration, respiration or wound healing efficiency have also been found to be pH-sensitive. It is noteworthy that differences in migration are closely linked to enhanced invasive and metastatic properties. Monitoring the speed of migration serves as an important experimental parameter to describe metastatic potential as well as to screen drugs and toxins with respect to their impact on cell dissemination.

In our studies we used ECIS® to search for potential impacts of extracellular pH on (i) cell migration, (ii) proliferation and (iii) adhesion of normal rat kidney epithelial (NRK) cells as a model cell line.

(i + ii) NRK cells were grown to confluence, before they were incubated with buffers of varying pH value. The cells on the electrode were then killed by invasive voltage pulses. As the cells in the electrode periphery are not compromised, the electrode is repopulated in time by migration and proliferation of neighboring cells.

To study the pH-dependency of cell spreading, cells were suspended in buffers with the respective pH value, seeded on the electrodes and the time course of cell spreading was monitored.

We observed that healing as well as proliferation rates were strongly pH-dependent: with increasing pH value of the applied buffer, wound closure was observed to be faster. Lower pH values, in contrast, resulted in a drastically decreased healing and proliferation velocity, ultimately resulting in cell death. In contrast, cell spreading is only affected under severe extracellular acidification.

In addition to these impedimetric studies, we investigated the respiratory activity under varying extracellular pH values by monitoring the oxygen concentration beneath the cells using oxygen sensitive sensor foils and an imaging system provided by PreSens®. Cellular respiration is reduced by lowering the extracellular pH value in accordance with the functional ECIS assays. The combination of various ECIS modes to characterize cell phenotypes with studies on their metabolism may help to analyze specific aspects of tumorigenesis.

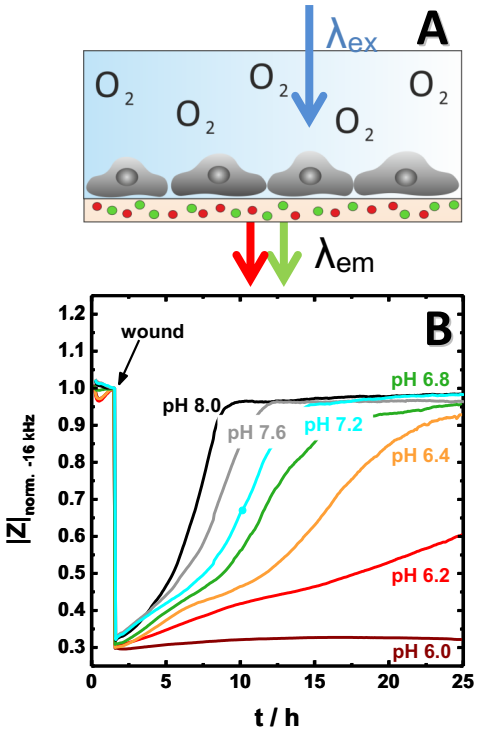

Fig. 1**A** Scheme of the ratiometric oxygen measurement setup, **B** Normalized impedance signal of wound healing process of NRK cells as a function of time.

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Invited Talk: The push and pull of endothelial integrity is balanced by Cdc42 and RhoB GTPases

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Endothelial integrity is essential for vascular health, and its loss will cause edema and tissue damage through plasma leakage and the exit of leukocytes into the tissues. Vascular-Endothelial (VE)-cadherin is the major cell-cell adhesion molecule connecting neighboring endothelial cells, thus preserving endothelial barrier function. VE-cadherin is regulated by tyrosine phosphorylation and by actin dynamics. Cortical actin polymerization will drive membrane protrusion, stabilize VE-cadherin and improve barrier function, while actomyosin-based contraction will 'pull' on VE-cadherin inducing intercellular gaps and loss of barrier function.

We found that in unstimulated, primary human endothelial cells (EC) barrier function is dynamic and is the net outcome of balanced pulling and pushing forces, regulated by the RhoGTPases RhoB and Cdc42, respectively. We showed that in resting EC RhoB is continuously degraded following its ubiquitylation by the Culln3-Rbx1-KCTD10 E3 ligase. Inhibition of RhoB ubiquitylation rapidly increases its protein levels 10-20-fold, driving actomyosin based contraction ('pull') and loss of barrier function. Thus, RhoB ubiquitylation protects endothelial barrier function.

Simultaneously, a basal level of active Cdc42 in resting EC maintains actin polymerization and membrane protrusion, preserving EC contact and barrier function. We found that the ouput of Cdc42 signaling in EC is controlled by the Cdc42GEF FARP1 and the Cdc42GAP SYDE1.

Finally, we showed that the balance between Cdc42-RhoB signaling which controls integrity in resting endothelium is distinct from agonist (e.g. thrombin)-induced barrier dynamics, in which RhoA drives the loss of integrity and Rac1 is required for barrier restoration.

In summary, the vascular endothelium employs differential, RhoGTPase governed signaling pathways driving the gain and loss of barrier function in resting (Cdc42-RhoB) and agonist-stimulated (RhoA-Rac1) cells.

Investigating blue light illumination on human retinal pigment epithelial cell lines and its potential to model AMD in vitro

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Age-Related Macular Degeneration (AMD) accounts for 50% of the blindness cases in Western countries. With age, cumulative oxidative damage leads to Retinal Pıgment Epıthelial (RPE) and photoreceptor cell death. Due to its high energy blue-light disrupts the electron transfer mechanism of mitochondria [1] (Godley et al., 2005; Rozanowska, 2012) and chromophores to induce reactive oxygen species (ROS), as well as disrupting lysosomal activity [2] (Rozanowska, 2012) ROS interacts with the surrounding molecules and triggers a lipid peroxidation chain reaction [3] (Rozanowska, 2004) Oxidated molecules cannot be digested properly in the lysozyme and they form lipofuscin granules, an hallmark of ageing. Lipofuscin granules are highly reactive to blue-light and the cumulative damage leads to RPE cell death.

Our aim is to generate blue-light induced in-vitro models of AMD. To this end we exposed ARPE-19 cell lines cultured in 96-well plate to a gradient of intensities of blue-light for various durations with two different ad hoc LED array illumination systems, to investigate the contribution of cumulative energy on cell viability and accumulation of ROS. Loss of cell viability following exposure was assessed with presto blue assay while CellRox Green oxidative stress assay confirmed that the cause of the cell death is related to oxidative stress. Our results showed that the cell viability decreases due to oxidative stress cell death as the blue-light intensity and exposure time increase, while a two-way ANOVA underlined an interaction between exposure and intensity. In a novel approach, we investigated, with Electrical Cell-substrate Impedance Sensing, ECIS, and fluorescent microscopy of ZO-I staining, whether or not blue-light induced damage in the tight junctions between the cells, and decreased the barrier function of ARPE-19. This study showed a potential to generate with blue light exposure in vitro models that recapitulate some of the aspects of AMD. These models will be improved in the future with lipofuscin-like autofluorecence expression in ARPE-19 cells.

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Chronic high glucose exposure induces cellular dysfunction in Endothelial Colony Forming Cells

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Endothelial Progenitor Cells (EPCs) play important roles in vascular repair. However, it has recently been shown that EPCs may be impaired in diabetes. The purpose of this study was to examine the effect of a diabetic milieu on a subset of EPCs called Endothelial Colony Forming Cells (ECFCs).

Short term (3-5 days) *in vitro* exposure to high D-Glucose (DG) (25mM) resulted in a significant increase in ECFC tubule formation. On the contrary, long-term (4 week) exposure to high DG resulted in a significant decrease in ECFC tubulogenic capacity ($p<0.001$). Long term high DG also negatively affected ECFC function, shown by a significant reduction in ECFC migratory capacity using an *in vitro* scratch wound assay ($p<0.001$) and a decreased ability of high DG exposed ECFCs to form a tight barrier using the XCELLigence system when compared to controls. Interestingly, long-term exposure to high DG resulted in premature senescence of ECFCs, characterised by a significant increase in senescence-associated β-galactosidase activity ($p<0.001$) and a significant increase in 53BP1 foci ($p<0.05$). The Seahorse Bioscience XF^e96 analyser was used to compare the Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) of short and long term high DG treatment. The glycolytic reserve was exclusively diminished by long term exposure to high DG as cells failed to increase ECAR after ATP synthase inhibition by Oligomycin.

Our results demonstrate the deleterious effect of long-term high DG on ECFC functionality and indicate that this may be due to the induction of premature cellular senescence.

Invited Talk: Impedance Sensing with Thin-Film-Transistors (TFT) Array Bio-sensors

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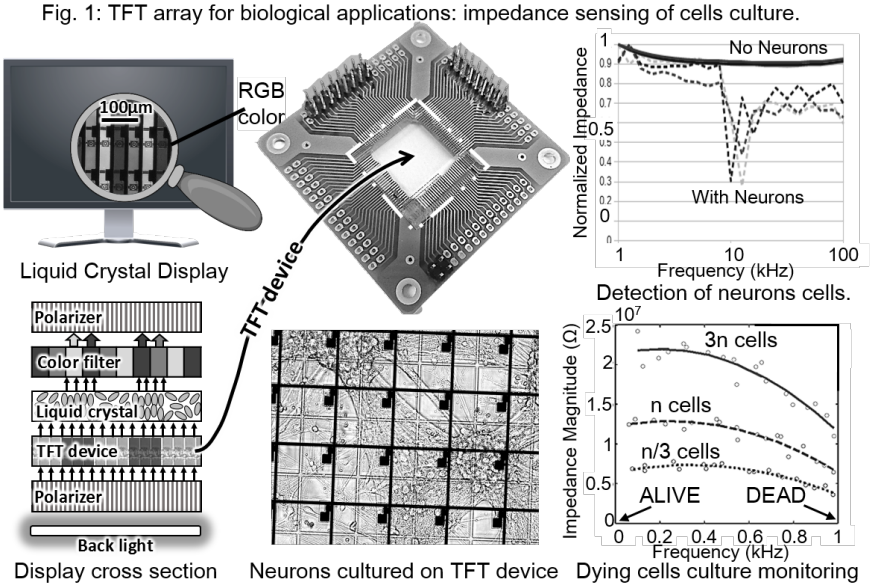
The study of biological cells is usually performed by means of optical observation, with a microscope, and often requires fluorescence staining. On another side, devices with integrated electronics propose electrical approaches in cells studies. The advantages of such approach are that they allow in situ, fluorescent dye-free and long term study, which can be directly quantified by measuring electrical parameters.
Multi-Electrodes-Arrays (MEAs) are the most standard category of devices used, in particular for neurons and brains in vitro studies. MEAs are fabricated using standard microfabrication: an array of eventually transparent microelectrodes can be obtained on transparent substrates. However, density and number of microelectrodes are limited, due to the wiring congestion. CMOS technology can propose MEAs with much higher density of smaller and more numerous microelectrodes. But the substrate is quite small and not transparent [1].
Besides these two technologies, our group has been working with Thin-Film-Transistor (TFT) technology, originally used for the fabrication of LCD, to develop advanced MEAs with high density and large number of microelectrodes, fabricated on transparent substrates, for biological applications. With these devices, cells manipulation using dielectrophoresis, cells monitoring by impedance sensing, neurons cells excitation and sensing, displacement of droplets by electrowetting-on-dielectric (EWOD), and biomolecule sensing are under investigation [2,3]. Cells detection, monitoring of a cell culture as well as biomolecule sensing were performed by impedance sensing and will be presented in detail during the conference.

Fig. 1: TFT array for biological applications: impedance sensing of cells culture.



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Expanding the information depth of impedance based assays by using piezoelectric growth substrates

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Impedance measurements have been established as a versatile technique to monitor cell-based assays under label-free, non-invasive and time-resolved conditions. These approaches are capable of studying a huge variety of in vitro scenarios by observation and quantifying the behavior of adherent cell monolayers grown upon planar gold-film electrodes while they are exposed to different stimuli.
By using electric cell-substrate impedance sensing (ECIS) several fundamental processes in cell physiology such as cell adhesion, cell proliferation, cell migration, intracellular signal transduction or cell death have been successfully examined (Fig. 1A, [1]). In all cases the sensitivity of the measurement is either based on changes in cell coverage of the electrode or on changes in cell morphology. The technique does not provide information on the molecular level and is thus referred to as a *wholistic* readout. One strategy to get more specific information about the cells requires combining ECIS with other techniques. We have been exploring the quartz crystal microbalance (QCM) as an additional label-free and non-invasive transducer to monitor adherent cells (Fig. 1B, [2]). The core component of this technique is a piezoelectric quartz resonator with two evaporated gold-electrodes on each side. By applying an AC potential difference between the surface electrodes the shear-wave resonator is excited to perform mechanical oscillations close to its fundamental resonance frequency of about 5 MHz. This mechanical oscillation goes hand in hand with an electrical oscillation that can be probed by impedance analysis. If mass is deposited on the quartz surface, e.g. during cell adhesion, the resonant oscillation is affected by the mass loading and all mechanical changes within the cell bodies. So even though the readout parameter is the electrical impedance, the information content is different from ECIS and reports on changes in *cytomechanics* rather than *cell shape*. Both techniques are *wholistic* in nature but provide complementary information of the cells under study.
This abstract reports on experiments in which cells were monitored during attachment, spreading and polarization or treatment with Concanavalin A with both techniques. The individual parameters together provide a more comprehensive picture on what happens on a cellular level than any of the two techniques for itself.

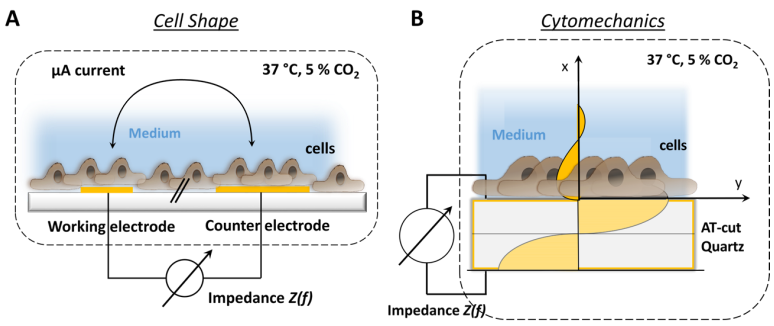

Fig. 1: The basic principles of two impedance-based cell monitoring approaches: electric cell-substrate impedance sensing (ECIS, A) and quartz crystal microbalance (QCM, B).

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Optical Imaging of Electrical Impedance using Surface Plasmon Resonance Sensors

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We present a new technique that is capable of performing optical measurements of electrical impedance with sub-micrometer resolution using surface plasmon resonance sensors. Surface plasmons (SPs) are longitudinal collective oscillations of free electrons at metal-dielectric interface. They are excited with light under specific conditions, commonly using an attenuated total internal reflection setup. The excitation is observed as a drop in the intensity of the reflected light occurring at the resonant angle. This resonance position is sensitive to both optical and electrical properties at the metal-dielectric interface.
SPR sensing structure consists of a thin film of gold (~50nm) deposited on a glass substrate. SPR sensors are commonly used to study molecular interactions within a confined axial scale (i.e. a penetration depth in the order of 100nm), but they are also sensitive to voltage perturbations at the gold-electrolyte interface [1]. Their demonstrated ability to spatially resolve electrical current provides a new class of two-dimensional electrodes with optical readout. Additionally, measurements are performed with a total internal reflection microscopy setup (Fig.1) that uses an oil immersion objective lens (NA: 1.49) and the transparent SPR "electrodes" allow standard microscopy to be performed simultaneously with optical imaging of impedance. The sensors are also used as substrates to adhere cells under investigation.
The principles of impedance imaging has been demonstrated previously [2]. Electrical impedance is computed from: (i) a small alternating voltage applied between the gold surface and a reference electrode and (ii) the resulting current flow obtained from the experimental SPR response. However, the accuracy of the present approach is affected by the sensitivity of the sensor to the optical heterogeneity of the adherent cells. Here, we present a scan-less measurements of localized electrical impedance of microscopic protein patterns adhered to the electrode surface as well as a resistive "phantom" outside the penetration depth of the sensor. We also discuss the factors that affect the sensitivity and dynamic range of the instrument as well as the accuracy of impedance measurements.

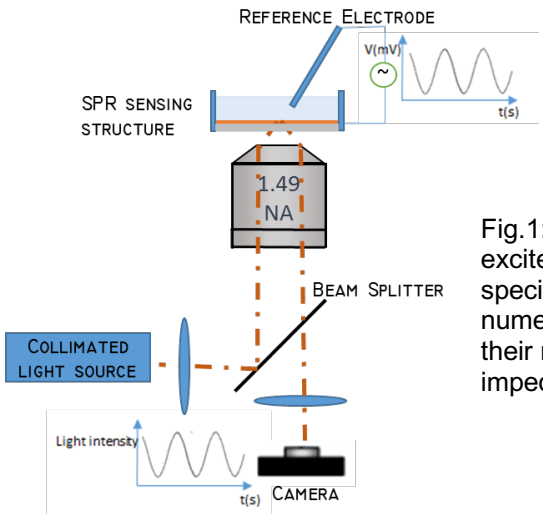

Fig.1: Impedance microscopy setup: surface plasmons are excited at gold-electrolyte interface with p-polarized light at specific angle of incidence using an objective lens with high numerical aperture. They are sensitive to applied voltage and their response to voltage modulation depends on the electrical impedance at the interface.

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Invited Talk: Light-addressable potentiometric sensors for cell imaging applications

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Light addressable potentiometric sensors (LAPS) can record surface potentials and impedance with spatial resolution using photocurrent measurements at electrolyte-insulator-semiconductor field-effect structures. Good lateral resolution was achieved using silicon-on-sapphire (SOS) substrates. To improve the sensitivity of LAPS, the traditional insulator was replaced with an ultra-thin organic monolayer [1]. An SU-8 photoresist pattern on a LAPS substrate was used to assess the resolution. An intensity-modulated laser was focused through the sapphire substrate into the 0.5 µm thick silicon layer and scanned from the uncoated to the coated part of the sample (Fig. 1). The results show that the resolution obtained using a single photon effect can be improved by reducing the laser wavelength. Two-photon imaging resulted in a resolution of 800 nm at a wavelength of 1250 nm.

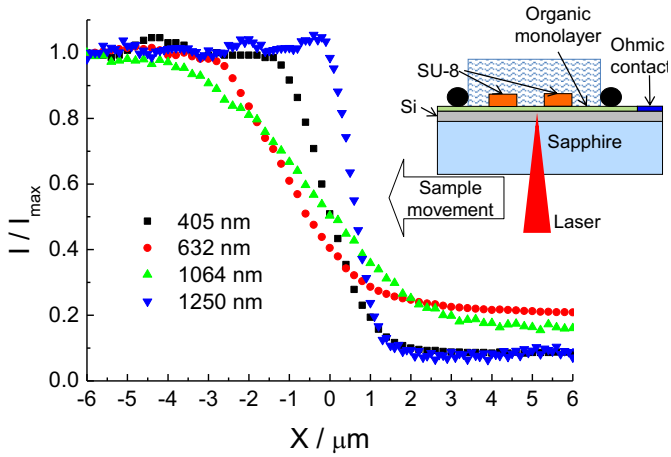

Figure 1 Photocurrent line scans across SU-8 edge at different laser wavelengths

LAPS is a promising tool for cell imaging. The technique has been used for the measurement of cell metabolism detecting the release of different types of ions, and for the measurement of extracellular potentials. The sensitivity of LAPS to charge and impedance has been exploited for visualising cell surface charges and impedance, but only when there is a direct contact between insulator and cell [2]. In normal viable cell culture, the gap between the insulator surface and the cell and the short Debye length in physiological salt solutions prevents any response of LAPS to the cell surface charge.

Recently, electrolyte-semiconductor structures without an insulator have been shown to be suitable for ac-photocurrent imaging [3]. The ac-photocurrents at these structures feature redox currents in addition to the depletion layer charging currents observed in LAPS. Preliminary experiments on indium tin oxide (ITO) have shown that this technique is suitable for the electrochemical imaging of the substrate-facing side of single cells.

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‘Instant ECIS’: A concept for storing frozen cells on electrode surfaces for instant use

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Impedance-based cell observation belongs to the most widespread non-invasive, label-free research tools for monitoring mammalian cells and their holistic response to a given stimulus in real time [1]. In the past, ECIS (electric cell-substrate impedance sensing)-based cell monitoring was applied to study a multitude of cell physiological processes, such as cell adhesion, proliferation, migration, intracellular signal transduction or cell death [2]. The common first step when performing impedimetric cell-based assays is seeding of initially suspended cells in a defined density upon the electrode surface. After establishment of a confluent cell layer, the attached cells are subjected to various assays in presence of a compound of interest. This requires a preceding cell culture in flaks or petri dishes as well as their preparation and seeding just before the intended assay is planned.
‘Instant ECIS’ describes a novel approach based on cryopreserved cells. Initially suspended living mammalian cells are seeded on the electrode surface in cryopreservation medium and are frozen to − 80 °C. Whenever needed, the cells are thawed and revitalized by adding culture medium. Subsequently, the cells attach and spread and the ready-to-use electrode array can be applied in impedimetric cell studies. The rationale behind this approach is to separate the unavoidable but time-consuming cell culture work (i.e. cultivation, seeding etc.) from conduct of the assay. Thus, impedimetric monitoring of cell-based assays becomes possible at any time and independent of the presence of a cell culture lab.

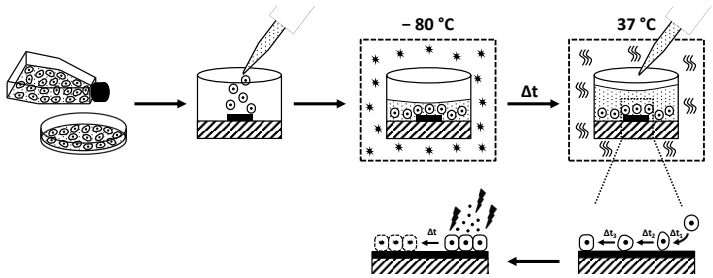

Fig. 1: Concept of the ready-to-use impedimetric biosensor based on cryopreserved cells on the electrode surface. The cells can be thawed at a selected time and subjected to various impedimetric assays without preceding cell culture work (patent pending).

With optimized freeze/thaw protocols the cells show high post-thaw viability and function. Compared to non-cryopreserved, freshly seeded controls of the same cell density, frozen and revitalized cells show a similar spreading rate onto the electrode surface, as indicated by the time course of the impedance. Cryopreserved cells also retain their typical physiological phenotype and sensitively reflect the bioactivity of test compounds. ECIS-readings revealed a similar response profile upon activation of cell-surface receptors or exposure to toxins for cryopreserved cells compared to monolayers of freshly seeded, non-frozen cells. This new approach vastly improves scheduling as well as reproducibility of cell-based assays and may pave the way for further in-field applications.

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Optimized Multilevel Discrete Wavelet Transform Analysis for Mitochondrial Respiration Regulation of Human Mesenchymal Stem Cells

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Mitochondria plays an important role in human mesenchymal stem cells (hMSCs) proliferation and differentiation.[1][2] Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP), which uncouples mitochondrial oxidative phosphorylation from the electron transport chain, is known to increase oxygen consumption rates. In this study, hMSCs derived from umbilical cord were chronically exposed to different concentrations of FCCP. Cell proliferation and mitochondrial activities were measure by CyQUANT® NF cell proliferation assay and Seahorse XF-24 extracellular flux analyzer respectively. Compared with control group, chronic exposure of hMSCs to 1 μM and 3 μM FCCP for two days led to approximately 10% and 60% reduction of proliferation. Furthermore, hMSCs exposed to 3 μM FCCP for 20 hours significantly reduced their mitochondrial function.

Electric cell-substrate impedance sensing (ECIS) was also used to detect the alternation of hMSCs micromotion and wound healing migration in response to different concentrations of FCCP treatment for 20 hours. Under rapid time collect (RTC) mode, the resistance time series at 4 kHz and capacitance time series at 40 kHz were both recorded in two different experiment sets. Multilevel discrete wavelet transform (DWT) detail coefficients were used to quantify time series described above. And the optimized procedure assisted to choose the best mother wavelet to retrieve detail coefficients. We named this optimized procedure “Wavelet Scan” in honor of the greatest “Frequency Scan” in the ECIS system.

The resistance results demonstrated a dose-dependent decrease from 1μM to 3μM FCCP, while the capacitance results has better distinguishment from 0.1μM to 3μM implying the dose-dependent reduction of cell micromotion. Furthermore, multilevel simulation results suggested that these tests could all be implemented under 16wells single frequency time course (SFT) mode with 1Hz sampling rate. Our results demonstrate that the consequent of DWT detail coefficients quantification was an appropriate method to analyze FCCP effect on hMSCs.

For wound healing migration, the recovery curves were fitted by sigmoid function and the hill slope showed a dose-dependent decline from 0.3μM to 3μM FCCP, indicating the decrease in migration rate. Also, dose dependent incline of the inflection points from 0.3μM to 3μM FCCP implied the increase of the half time for wound recovery migration. Moreover, the Capacitance time series has showed a significant dose-dependent results from 0.1μM to 3μM FCCP. Together, these results demonstrate that partial uncoupling of mitochondrial oxidative phosphorylation reduces proliferation, micromotion, and wound healing migration of hMSCs.

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Invited Talk: Working toward the delivery of automated and real time reporting assays from pluripotent stem cells

Prof. David Hay
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Invited Talk: Development of Real-Time Potency Assays for Cellular Therapies and Regenerative Medicine Using Impedance Technology

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Cellular therapies hold tremendous promise in prevention, treatment, cure and mitigation of diseases and injuries. Whether derived from allogenic or autologous sources, one of the biggest hurdles in manufacturing of cellular therapies is understanding and managing donor to donor variations and maintaining consistent and stable functional activity of the cells. Therefore, developing tools and assays which can monitor critical quality attributes of the cells throughout the manufacturing process is of utmost importance. We will discuss and present data on the uses of cellular impedance technology as a critical quality assay for different types of cellular therapies including chimeric antigen receptor T cells (CART), mesenchymal stem cells (MSC) and human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM). For CART cells, we will discuss the development of real-time potency assay that can monitor the specificity and efficacy of CART cells towards target cancer cells expressing different types of antigens. For MSCs, we will discuss measurement of key quality attributes such as adhesion and proliferation and importantly we will discuss how such parameters can guide and predict the extent of functional activity of the cell or its propensity to differentiate into a particular lineage. For hiPSC-CM, we will discuss how cellular impedance can be used to assess critical quality attributes of the cells such as extent and rate of contractility. In summary, key features of cellular impedance technology such as non-invasive, real-time and quantitative readout can provide incisive information about key aspects of cells and cellular therapies which can guide manufacturing and potentially clinical decision making process.

A novel highly parallelized multimodal bioelectronic real time High Content Screening platform for hiPSC derived 2D and 3D cardiomyocyte cultures

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Negative side effects on the human heart are still the major reason for withdrawal of approved drugs from the market. Although over the last decades there were distinct improvements to eliminate the bottleneck of preclinical studies, today's preclinical in vitro cardiac safety assays are insufficiently predictive for humans and highlight the need for improved in vitro assays to detect cardiotoxic effects. In this context, most drug tests for side effects are focused on acute effects, there is a big gap especially for in vitro testing of chronic and repeated dose toxicity.
To overcome these limitations, we developed an optoelectronic real-time monitoring platform. In combination with mature human stem cell derived cardiomyocytes, a novel High Content Screening for active pharmaceutical ingredients (API) for cardiotoxicity testing was realized. The platform combines a hybrid bioelectronic system that allows a sensitive non-invasive label-free monitoring of drug-induced cellular and electrophysiological changes quantitatively measured by impedance spectroscopy (EIS) and field potential monitoring (FPM) [1]. More strikingly, the integration of novel high-speed impedance analyzers allows sampling frequencies of at least 500 Hz and therefore, the quantitative analysis of cardiac contraction mechanics. In combination with FPM, API effects on the electromechanical coupling (EMC) can be analyzed. Beside micro- and multielectrode arrays based on classical highly conductive electrode materials like gold and platinum, we developed arrays made of transparent semiconductive electrode materials. Thus, the hybrid bioelectronic monitoring can be combined with optical analysis. Furthermore, we developed multi-well microcavity arrays, a special feature for 3D cardiomyocyte real-time monitoring. To demonstrate the performance of our platform, we analyzed several reference compounds like chronotropic as well EMC disturbing compounds. More strikingly, using our long-term stable 3D hiPSC derived cardiomyocyte clusters, we demonstrated detection of acute and chronic cardiotoxic effects for the reference compound doxorubicin. In conclusion, our novel hiPSC derived cardiomyocyte based optoelectronic high content screening platform represents a step forward to a predictive in vitro cardiotoxicity analysis.

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Invited Talk: Use of cross-linked polypeptide multilayer-coated electrodes to monitor osteogenic differentiation of human dental pulp stem cells

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The biomaterials on which stem cells attached play a key role in stem cell proliferation and differentiation. Among various biomaterials, polypeptide multilayer films are promising for the development of artificial extracellular matrix for the regulation of stem cell differentiation since they are entirely synthetic and can mimic crucial features of extracellular matrix proteins [1]. Polypeptide multilayer films made of synthetic peptides are non-cytotoxic and biocompatible. In addition, this material allows for modulation of thickness, stiffness, and adhesiveness [2, 3]. In this study, human dental pulp stem cells (hDPSCs) derived from teeth were cultured on polypeptide multilayer films fabricated by the alternate deposition of cationic poly-L-lysine and anionic poly-L-glutamic acid, cross-linked with EDC/sulfo-NHS, and coated with type-I collagen. Osteogenic differentiation of hDPSCs was evaluated by qPCR of osteogenic specific genes such as ALP (Alkaline Phosphatase), OCN (osteocalcin), and OPN (osteopontin), and monitored by cross-linked polypeptide multilayer-coated electrodes using electric cell substrate impedance sensing (ECIS). The results showed that hDPSCs demonstrated better cell attachment and spreading on even number layer of films, where the top layers are poly-L-glutamic acid films. Furthermore, the osteogenic differentiation of hDPSCs showed a significantly higher differentiation rate on cross-linked films than on culture dishes or cover glasses.

In the ECIS monitoring of osteogenic differentiation, hDPSCs were seeded into electrode wells and allowed to form confluent cell layers, and the impedance time courses of the cell-covered electrodes throughout the osteogenic induction were measured up to 21 days at 11 frequencies ranging from 62.5 Hz to 64 kHz [4]. Compared with the data of undifferentiated hDPSCs, significantly lower impedance time courses of hDPSCs treated with osteogenic differentiation medium were observed. In addition, these two distinct time course profiles were detected as early as 24 hours after induction. To detect subtle changes in cell morphology in the early stage of cardiac differentiation, the frequency-dependent impedance data were analyzed with a theoretical cell-electrode model [5]. The results showed that the decrease of measured impedance of differentiated cells was mainly due to a decrease of the junctional resistance between cells (R_b) and an increase of the cell-substrate separation (h). We also analyzed the impedance changes between the differentiated groups with the cross-linked polypeptide multilayer film and without it. The ECIS results also validated that the osteogenic differentiation of hDPSCs was enhanced when cross-linked polypeptide multilayer films were used.

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Characterisation of *in-vitro* Cardiac Cell Models for Preclinical Assessment of Oncology Drug-Induced Cardiotoxicity

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Detrimental effects upon the cardiac system are a major cause of drug attrition. In-vitro methodologies currently used for cardiac safety screening offer sub-optimal assessments of non-cardiac cell lines engineered to express cardiac ion channels, or primary tissue with limited utility for clinical translation. The emergence of innovative technologies combined with the use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), with the ability to synchronously beat (contract) in-vitro, opened up opportunities for improved identification of cardiotoxicity. However, the costly and complex maintenance techniques pose limitations in terms of widespread use.
One resolution is the use of cardiac cell lines, with capability for continuous growth and clinical translation. However, the limitations of these models for detecting both structural and functional cardiotoxicity is unknown.
In this study we explore the predictive value of a ventricular cardiac cell line (AC10), an atrial cardiac cell line (HL-1) and functionally-responsive hiPSC-CM to determine toxicity of the histone deacetylase inhibitor (HDACi) class of drug, thus highlighting the advantages of each type of cell model in relation to structural and functional cardiotoxicity. This not only supports the value of comprehensive cellular screening models, but offers a predictive tool to assess cardiotoxicity that would allow development of efficacious and safer drugs in this class.

Posters Only		
P1	Anna-Maria Pappa	Impedance monitoring of native cell membranes: A biomimetic system to study membrane- compound interactions
P2	E. Brotin	ImpedanCELL: a core facility to monitor in real-time the cellular activity: focus on two high-throughput screening studies in ovarian cancer
P3	Francesca Cavallini	ECIS based wounding and reorganization of cardiomyocyte-fibroblast co-cultures
P4	Katie Morgan	Using an impedance based assay in the investigation of drug induced liver injury comprising the gut/liver axis
P5	Krisztina Juhasz	Assessment Of Drug Effects On Cardiomyocyte Function: Comprehensive In Vitro Proarrhythmia Assay (CIPA) Result
P6	Orsolya Lang	Impedance-based screening of novel heterocyclic molecules designed for apoptosis induction
P7	Miroslav Karabaliev	Constant Phase Element Behaviour of Electrode-Supported Lipid Films in the Study of Drug-Membrane Interactions
P8	Miroslav Karabaliev	Effect of Thioridazine on the Dielectric Relaxations of Spectrin-based Under-Membrane Skeleton of Human Red Blood Cells
P9	Florian Urban	Transepithelial permeation of bioactive molecules determined online by impedance-based monitoring in a co-culture setup
P10	Patricia Ruas Madiedo	Validation of impedance monitoring for the formation and removal of biofilms from clinical related pathogens
P11	Eszter Lajkó	Investigations of the effects of neuroprotective molecules on endothelial functions by impedimetry and holographic microscopy
P12	Jurgen Wuyts	Multisine impedance spectroscopy as a tool to determine antifungal mode-of-action
P13	Tom Van Loy	Unraveling G protein-coupled receptor mediated chemokine signaling with cellular electric impedance.
P14	Jordi Doijen	Investigating CXCR4 internalization and heterodimerization with CXCR7 using cellular electric impedance
P15	Adam Obr	Impedance analysis of hematopoietic cells: the role of Src family kinases in leukemia cell adhesion
Posters from Talks		
T7-5	Stefanie Michaelis	'Instant ECIS': A concept for storing frozen cells on electrode surfaces for instant use
T4-3	Nadira Jamil	Flexible Microelectrode Array (Flex-MEA) Design for Micro-Bioimpedance Tomography of *Rhodococcus erythropolis*

List of Posters

T7-2	Simone Ruckdäschel	Expanding the information depth of impedance based assays by using piezoelectric growth substrates
T1-4	Christian Kade	Time-resolved Response Profiles of GPCR Activation: Combining Two Independent Impedance-Based Approaches
T2-4	Patricia Ruas Madiedo	Selection of probiotics and prebiotics against *Clostridium difficile* using an *in vitro* intestinal model based on impedance real time monitoring
T4-1	Hancong Wu	Towards 3D impedance-based cellular assays with Electrical impedance tomography
T2-3	Fabian Bonetto	Discrimination between Normal and Cancerous Cells using ECIS and Artificial Intelligence Techniques.
T6-3	Christina O'Neill	Chronic high glucose exposure induces cellular dysfunction in Endothelial Colony Forming Cells
T1-3	Katie Morgan	Chlorpromazine disrupts structural integrity of cell membranes in HepaRG cells and initiates a proinflammatory response
T3-2	Yi-Ting Lai	Impedance Analysis of Adherent Cells Cultured on Various Sizes of Electrodes
T2-9	Maria Zinkl	Monitoring the Toxicity of Bisphenol A using Multiple Impedance-Based Cellular Assays
T2-8	Krisztina Juhasz	Cell Monitoring using impedance and impedance spectra for high content toxicity and cell proliferation screening
T8-5	Carol de Santis	Characterisation of *in-vitro* Cardiac Cell Models for Preclinical Assessment of Oncology Drug-Induced Cardiotoxicity
T3-3	Michael Skiba	Impedance Analysis of Heterogeneous Cell Populations: Impact on Data Analysis and Modeling
T5-4	Lisa Sauer	Impedance-based Characterization of pH-dependent Cell Behavior
T2-2	Come Thieulent	Real-Time monitoring of Equid alphaherpesviruses infectivity in equine dermal cell based on impedance measurements: effects of aciclovir and ganciclovir treatments

Impedance monitoring of native cell membranes: A biomimetic system to study membrane- compound interactions

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Cellular membranes represent an obvious, yet relatively neglected target for developing new drugs. Using membrane models (*e.g.*, supported lipid bilayers, SLBs) for sensing and drug screening is limited by incompatibility at the biotic/abiotic interface, lack of molecular complexity, and lack of integrated functionality to transduce at the molecular level. We have recently demonstrated that SLBs are compatible with electroactive materials able to transduce molecular interactions of toxins with a model membrane (1). In parallel our teams have developed methods to assemble *complex* (native) membrane structures into planar supported bilayers on solid supports, using materials directly harvested from live cells. (2) By using hydrated polymer- based transducers, instead of solid supports one can achieve a fluid environment- close to the physiologically relevant environment- i.e., facilitating mobility of the transmembrane proteins, which is essential for such native plasma membrane systems. These features enhance the native structural and functional properties of the membrane and ensure the credibility of our platform to be physiologically relevant and predictive of membrane molecular interactions. In this work, we demonstrate the formation of native SLBs on top of the conducting polymer *poly*(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) using both optical and electrochemical impredance spectroscopy (EIS) analysis. EIS dynamic monitoring reveals useful information regarding plasma membrane formation and allows for sensitive detection of minute changes. This work is a first step towards the use of native plasma membranes with highly-sensitive, label-free and fast electrical readout to transduce minute changes in biological ion flux into electrical signals for early diagnosis/sensing of disease as well as drug testing.

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ImpedanCELL: a core facility to monitor in real-time the cellular activity: focus on two high-throughput screening studies in ovarian cancer

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ImpedanCELL is an innovative and original core facility for studying real-time high-throughput cellular activity using impedance measurement (xCELLigence technology) and real-time cellular imaging. It is localised on two different geographical sites in Caen (Normandy, France): Comprehensive Cancer Center F. Baclesse for all non-microbiology applications and LABÉO (Equine Hippolia Health research platform and BIOTARGEN research team) for all applications in virology and in bacteriology (biosafety level 2). The core facility is equipped with cutting-edge technologies (3 xCELLigence MP, 1 xCELLigence DP, 1 iCELLigence, 1 Cellavista,…) allowing the study of adhesion, proliferation, cell death, migration and invasion in various domains including oncology, neurosciences, toxicology, immunology, marine biology, virology and bacteriology. In line with one ImpedanCELL's mission, the core facility is open to any scientists with high-throughput study needs for dynamic tracking of real-time cellular behaviour. ImpedanCELL is open to collaborations and services for both academic and industrial partners and offers theoretical and practical courses.

Firstly, based on xCELLigence technology, a high-throughput functional screening of a library of 1200 microRNAs (miRNAs) was performed on two several chemoresistant ovarian cancer cell lines and allowed to identify several cytotoxic candidate miRNAs on their own ("apoptomiR") or in association with chemotherapy (chemosensitizer miRNA). This work allow us to identify a first potent "apoptomiR" called miR-491-5p. An individual functional associated study showed that miR-491-5p induces apoptosis in IGROV1-R10 ovarian cancer cell lines by targeting directly EGFR and Bcl-xL. Interestingly, the apoptotic effect of miR-491-5p could be mimicked by a combining an EGFR inhibitor with a BH3-mimetic molecule (two drugs already used in clinical practice). This work highlights the potential of phenotype-based miRNA screening approaches to decipher, in the absence of a preconceived idea, new relevant therapeutic targets harboring synthetic lethal interactions to propose new rationale drug combinations [1].

Secondly, the development of direct Mcl-1 inhibiting molecules constitutes a major challenge for the success of Bcl-xL targeting strategies in ovarian carcinoma. After computational modelling to identify molecules able to target the Mcl-1 hydrophobic pocket, a high-throughput functional screening assay based on xCELLigence technology was implemented to evaluate the ability of designed and synthesized molecules called oligopyridines to sensitize ovarian carcinoma cells (in addiction to both Bcl-xL and Mcl-1) to Bcl-xL-targeting strategies. After establishing structure-activity relationships and identifying a potent lead (hit) called Pyridoclax, surface plasmon resonance assay demonstrated that pyridoclax directly binds to Mcl-1. Without cytotoxic activity when administered as a single agent, pyridoclax induced apoptosis in combination with Bcl-xL-targeting siRNA or with ABT-737 in ovarian cancer cells. These results open up interesting perspectives for the clinical use of Mcl-1 inhibitors to improve the clinical management of ovarian cancer [2].

Overall, these two high-throughput studies demonstrated the interest to use impedance-based technologies to identify some pertinent targets and molecules for therapeutic intervention in the context of the precision medicine in ovarian cancer.

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ECIS based wounding and reorganization of cardiomyocyte-fibroblast co-cultures

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Cardiovascular ischemic heart diseases are the leading causes of natural death worldwide. Cell death during a myocardial infarction leads to a multiphase reparative response in which the damaged tissue is replaced with a fibrotic scar produced by fibroblasts and myofibroblasts. The fibrotic response, although it is crucial for preventing rupture of the ventricular wall, leads to progressive impairment of cardiac function and eventually to heart failure. In both experimental and clinical evidence it has been suggested that fibrotic changes in the heart are reversible. Hence, it is important to understand the mechanism involved and to have a simple model system to test potential treatments.

In vitro co-cultures of cardiac cells can be used to model the fibrotic condition and to gain knowledge on the electromechanical crosstalk between myocytes and non-myocytes [1] under different physiological states.

In our work [2] we applied two different Electric Cell Substrate Impedance Sensing (ECIS) mediated wound healing assays, to assess the dynamic of the reorganization process on co-culture and pure cultures of cardiomyocytes and fibroblasts from neonatal rats. Comparing the electrical wound healing with a barrier assay (i.e. electric fence) we found that especially the cell-cell connectivity, and thus cell layer integrity, dominates the healing dynamics for the two intrinsically different assays.

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Using an impedance based assay in the investigation of drug induced liver injury comprising the gut/liver axis

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Background/Aims: Drug induced liver injury (DILI) accounts for approximately one half of all cases of acute liver failure [1]. Before entering the liver, drugs pass through the gut. Determining the dynamics of the gut/liver axis would contribute to a better understanding of how drugs are transported prior to metabolism by the liver. Using model hepatotoxins we highlight the use of impedance based assays in identifying possible mechanisms of damage in both hepatocyte and intestinal cell lines. Paracetamol (APAP) overdose is a leading cause of DILI [2]. Previously, using ECIS technology and the hepatic HepaRG cell line, we showed a dose dependent loss of impedance with specific disruption to tight junctions (TJs) at sub-therapeutic, therapeutic and toxic concentrations of APAP [3]. Here we investigate the effect of APAP on the Caco2 intestinal cell line to determine if any dose dependent changes in impedance can be detected. Understanding the mechanisms whereby APAP and its metabolites contribute to DILI could improve therapy thus reducing the number of fatalities per year.
Method: Caco2 cells were seeded on 8W10+E ibidi arrays coated in collagen and monitored using the ECIS Z theta platform. Previously established concentrations of APAP [2] were administered at day 14, when the cells were confluent, and impedance was monitored for 24 hours.
Results: Measurements of total impedance after application of APAP showed a different profile of impedance on Caco2 cells compared to that of hepatocytes. Hepatocytes showed dose dependent damage at a sub therapeutic, therapeutic and toxic concentrations while, Caco2 intestinal cells showed no loss of impedance at any concentration (Figure 1b) which indicated the enteric cells are an effective barrier with regards to APAP toxicity.
Conclusion: Impedance based assays can provide useful insight into the effect of APAP on the gut/liver axis. Here we show no loss of impedance in Caco2 intestinal cells challenged with sub therapeutic, therapeutic and toxic doses of APAP comprising overall membrane integrity and no loss of adhesion or tight junctions. This differs from a dose dependant toxic response seen in hepatocytes using the same concentrations. These results suggest that the enteric cells form a barrier in regards to APAP toxicity and may inform further investigation into the effects of APAP on the gut/liver axis.

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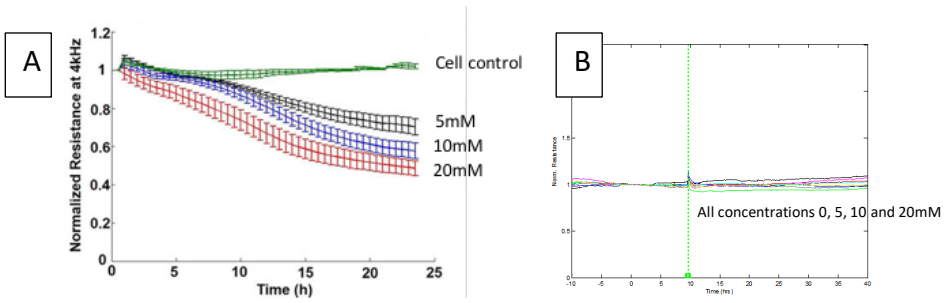

Figure 1: Impedance measurement of serial concentrations of paracetamol on human hepatic HepaRG cells and human intestinal Caco2 cells

Assessment Of Drug Effects On Cardiomyocyte Function: Comprehensive In Vitro Proarrhythmia Assay (CIPA) Results

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The on-going comprehensive in vitro pro-arrhythmia assay (CiPA) initiative aims to improve drug safety testing and help more beneficial chemical entities reach the market. The project consists of three main parts, which include:

1) electrophysiological investigation of drug effects on human ventricular ion channels,
2) characterization of the in-silico cardiosafety model predictions,
3) assessment of discrepancies and gaps in fully integrated biological systems (human induced pluripotent stem cell- derived cardiac myocytes (hiPSC-CMs) and the human ECG).

To assess and validate drug effects on hiPSC-CMs in a combined measurement of cardiac excitation and contraction, a subset of compounds which are part of the Phase II CiPA study were tested. A prospective comparison study using combined extracellular field potential (EFP) and impedance technology was conducted across 4 independent laboratories testing 23 reference compounds in 4 independent commercially available hiPSC-CMs.

Our data demonstrates that hERG channel blockers, such as Sotalol and Dofetilide prolonged field potential duration (FPD) at low concentration and induced arrhythmia as measured by field potential (FP) recording and impedance (IMP) recordings at higher concentrations. The cross-cell comparison displayed different minimal effective concentrations regarding FPD prolongation and cessation of beating. On the contrary, Diltiazem, a calcium channel inhibitor, weakened cell contractile activity and shortened FPD. Multichannel inhibitors, such Quinidine increased FPD, weakened the contraction force and induced arrhythmia. Furthermore, validation on low risk proarrhythmia compounds such as Tamoxifen or Verapamil and intermediate risk compounds such as Cisapride and Chlorpromazine will be presented. Comparison of the compound effects across the 4 different sites showed the consistent trend of the effect.

Taken together, commercially available hiPSC-CMs in conjunction with combined measurements of ion channel activity and contractility is a reliable approach for risk assessment of proarrhythmic compounds.

Impedance-based screening of novel heterocyclic molecules designed for apoptosis induction

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Pancreatic adenocarcinoma is one of the most aggressive tumor with poor prognosis, the five-year survival rate is 5% [1]. The resistance of pancreatic carcinoma to treatment regimens represents a major challenge, whereas pancreatic carcinoma cell resistance to apoptosis is well known and may contribute to treatment failure [2]. A potential drug selectively restoring the regulation of apoptosis or inducing apoptosis of the tumor cells may reduce the therapeutic failure.
Aims of our study were: (i) to design a new library of heterocyclic molecules for apoptosis induction; (ii) to screen the novel molecules on different tumor cell lines by HTS impedimetry; (iii) to identify the molecular target of the hit molecules; (iii) to check how the hit molecules act on the apoptotic pathway.
Methods: the drug-like library were developed upon non-flat 3-dimensional templates and libraries (Smart Diversity Approach™). Compounds with small molecular weight, more favorable physicochemical properties, higher sp3/sp2 atom ratio, and novel 3-dimensional shapes with various functionalities were selected and synthetized. Biological tests were performed on different tumor cell lines, PANC-1(pancreatic adenocarcinoma), COLO 205 (colon cancer), A2058 (melanoma), EBC-1 (lung cancer). Cytotoxicity was measured by impedance based technique in xCELLigence SP (ACEA) system. Flow cytometry were applied to detect the apoptotic effect of the molecules, caspase 3 or 7 activity were measured by BD FACSCalibur™.
Results: In total 193 novel heterocyclic molecules containing linker connection functional groups with a relatively low molecular weight, below 600 Daltons were synthetized in high purity (> 95 %). 18 of the tested compounds were significantly cytotoxic on pancreatic tumor cells (PANC-1). These molecules were able to reduce the cell viability of other tumor cell lines (COLO 205, A2058 and EBC-1) as well. Screening in databases based on structural similarities revealed that the potential target of several components is the XIAP (X-linked inhibitor of apoptosis). However, the apoptotic pathway analysis proved, that some of the hit molecules were able to induce caspase 3 and 7 activation in the model cells, further examination is required to test the interaction of the XIAP and the hit molecules.
Although XIAP is not expressed in normal pancreatic ductal cells, several clinical study recently proved that it is overexpressed in pancreatic carcinoma cells, associate with invasiveness and shortens the survival of pancreatic cancer patients [2]. Selective inhibition of XIAP may have a favorable therapeutic effect.
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Constant Phase Element Behaviour of Electrode-Supported Lipid Films in the Study of Drug-Membrane Interactions

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Electrode-supported lipid films have been often used to study drug-membrane interactions [1]. In this work, the lipid films were obtained through thinning of a film-forming lipid solution deposited between an electrolyte phase and a glassy carbon electrode (GCE) [2].

The effects of three different drugs on the lipid film structure were investigated by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). Two antipsychotic drugs, chlorpomazine (CPZ) and thioridazine (TDZ), and the spin-labeled chloroethylnitrosourea containing glycine SLCNUgly were used in the study. SLCNUgly is an analogue of the clinically used drug lomustine (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, CCNU). CPZ, TDZ and SLCNUgly are electroactive and their penetration in the lipid phase could be demonstrated by using CV. The occurrence of defects in the lipid structure was additionally assessed with CV by the aid of the hydrophilic electroactive ions ferri/ferrocyanide $Fe(CN)_6^{3-/4-}$. However, when the lipid phase of the film remains continuous and without water-filled defects, the hydrophilic electroactive probes $Fe(CN)_6^{3-/4-}$ cannot penetrate the film and the method of CV is not enough informative concerning other structural changes in the lipid arrangement.

To shed additional insight on the drugs effects and interactions, the impedance of the films was analysed. The impedance of the GCE-supported lipid films is modeled with an equivalent circuit consisting of parallel capacitance C_p and resistance R_p [3]. These capacitance and resistance are not frequency independent but could be calculated as equivalent C_p and R_p for each measured frequency of the impedance spectrum and presented as functions of the frequency f, $Cp = C_p(f)$ and $R_p = R_p(f)$. For the lipid films used in this work, it is demonstrated that both $C_p(f)$ and $R_p(f)$ are well approximated with power-law functions. This behaviour implies that the impedance Z of the films could be analysed in terms of the well-known constant-phase angle element (CPE), which is often used to describe the interfacial impedance of solid working electrodes:

$$Z_{CPE} = Q^{-1}(i\ 2\pi f)^{-\alpha}$$

The analysis of the changes in the CPE-coefficient Q and the CPE-exponent $\boldsymbol{\alpha}$ after the interaction of the lipid films with different concentrations of CPZ, TDZ and SLCNUgly suggests that the CPE-exponent $\boldsymbol{\alpha}$ could be used as convenient parameter to describe the effects of the drugs on the lipid arrangement and the lipid structure of the films.

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Effect of Thioridazine on the Dielectric Relaxations of Spectrin-based Under-Membrane Skeleton of Human Red Blood Cells

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Deformability and elasticity of human red blood cell (RBC) membrane largely depend on the segmental flexibility of spectrin-based under-membrane skeleton (MS) and the attachment of MS to lipid membrane. The segmental flexibility and attachment of MS can be elucidated studying its intrinsic dielectric polarization. The latter can be expressed by the frequency dependence of the difference in dielectric properties of RBCs prior to and after the thermal denaturation of spectrin assuming the dielectric activity of denatured spectrin nil.

Isolated RBCs were suspended in isotonic low salt mannitol media and heated across the denaturation temperature of spectrin (49.5 °C). At this temperature, the complex impedance of suspension sustained change, $\Delta Z^* = \Delta Z_{re} + j\Delta Z_{im}$, which was a measure for the dielectric polarization of native MS. The ΔZ_{re} and ΔZ_{im} were determined at 16 frequencies between 50 kHz and 10 MHz and corrected for the temperature dependence of conductivity. The complex plane (Nyquist) plot of $-\Delta Z_{im}$ against ΔZ_{re} depicts two semicircle arcs revealing two dielectric relaxations on MS, called beta and gamma relaxations. While beta relaxation reflects the piezo effect on spectrin, induced by low frequency electrostriction of lipid membrane, the gamma relaxation is a response to the direct interaction of electric field with MS [1].

We now evaluated the effects produced by the phenothiazine derivative thioridazine, a lipophilic cation and effective drug in psychiatry, on the beta and gamma relaxations of MS. At concentrations of 100-200 μM Thioridazine strongly subdued, and at 300 μM fully eliminated the gamma relaxation with slight effect on beta relaxation. This outcome was related to the known ability of cationic amphiphiles to intercalate in the inner negatively charged leaflet of lipid bilayer severing the attachment of anion exchanger to the MS.

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Transepithelial permeation of bioactive molecules determined online by impedance-based monitoring in a co-culture setup

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Standardized assays to determine the permeability of epithelial and endothelial cell layers are commonly based on labeled tracer molecules [1,2]. Fluorescent-labeling of tracer compounds is preferred over radio-labeling to reduce laboratory safety requirements and more complicated waste management. However, covalent attachment of a fluorophore to a compound of interest might alter the physicochemical properties of the tracer significantly. Hence, a label-free permeation assay would overcome all the limitation described above.
This study describes a new assay strategy to measure the permeability of unlabeled, bioactive molecules that is based on impedance monitoring in a co-culture setup. Similar to the existing assays, the barrier-forming cell layer is grown to confluence on a porous polymer support so that the cell layer separates an apical and a basal fluid compartment. The unlabeled, bioactive tracer compound is added to the apical compartment at the beginning of the assay. Its time-dependent permeation into the basal compartment is followed with the help of a second cell population (sensor cells) that is grown on indium-tin-oxide (ITO) electrodes deposited on the bottom of the lower compartment like in regular impedance-based monitoring setups (cf. figure 1). The sensor cells are selected for their ability to respond to the presence of the bioactive tracer compound by cell shape changes that are sensitively reported by impedance readings of the ITO-electrodes. Thus, the arrival of the tracer in the basal compartment is reported by changes in impedance of the cell-covered electrodes in the basal compartment. In *proof-of-concept* experiments as presented here we have made use of sensor cells that endogenously express G-protein coupled receptors (GPCRs) and we used agonists to these receptors as tracer molecules to probe their transepithelial permeability. After the agonist (e.g. histamine), added to the apical compartment at the beginning, has permeated across the barrier forming cell layer into the basal compartment, it is detected by the response of the sensor cells to the activation of the corresponding GPCR. Dependent on the type of sensor cells and their individual signal transduction that is triggered by receptor activation, a characteristic time course of impedance is observed and analyzed with respect to the permeation rate of the tracer.
The new impedance-based readout detects the permeation of unlabeled receptor ligands and offers semi-quantitative information about the permeability of the probed ligands. The described label-free permeation assay combines GPCR target screening assays with ligand permeability studies.

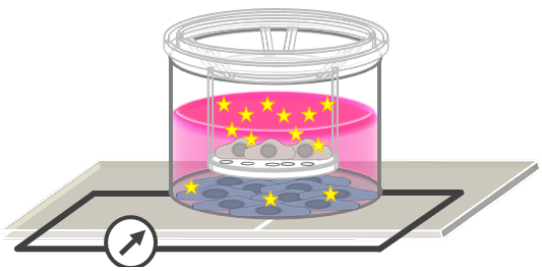

Figure 1: Experimental setup to probe the permeation of bioactive tracers across endothelial or epithelial cell layers by impedance readings of sensor cells in the basal compartment.

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Validation of impedance monitoring for the formation and removal of biofilms from clinical related pathogens

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Microbial biofilms are complex communities composed of one or multiple species adhered to a solid surface and surrounded by a polymeric extracellular matrix secreted by the microbes. This matrix, conferring protection to the microorganism, can be composed of different molecules, such as polysaccharides, proteins, teichoic acids, nucleic acids and lipids. Bacterial biofilms are often found in nature, being especially relevant those involved in clinical infections or formed on food industry settings. *Staphylococcus aureus* and *Staphylococcus epidermidis* are cause of nosocomial infections linked to the colonization of medical devices; on the other hand *Streptococus mutans* is the key player in the formation of dental plaque, being involved in the initial steps of cariogenic biofilm formation. The search for novel anti-biofilm molecules is a very active research area and the use of fast, reliable and accurate techniques allowing the screening of such molecules, is of great interest. We have assessed the suitability of the impedance-based xCELLigence RTCA-DP equipment (ACEA Bioscience Inc.) as an alternative to standard methods, for monitoring in real-time the biofilm formation of nine strains from the previously described species [1]. Furthermore, the suitability of this method to address the effect of bacteriophage-derived anti-biofilm compounds on the development and removal of staphylococcal biofilms was evaluated [1, 2].
Our results demonstrated that this approach allowed the distinction between biofilm-producers and non-producers of *S. aureus* and *S. epidermidis*, as well as the formation of *St. mutans* biofilms only when sucrose was present in the culture medium as it allows the synthesis of the polysaccharide matrix. Besides, the impedance-based results showed high correlation with those obtained with standard approaches, such as crystal violet staining and bacteria enumeration, as well as with those obtained upon other abiotic surfaces (polystyrene and stainless steel). The inhibition of staphylococcal biofilm formation by the bacteriophage phi-IPLA7 and the bacteriophage-encoded endolysin LysH5, as well as the removal of a preformed biofilm by this last antimicrobial treatment, were also continuously monitored [1].
Finally, the antibiofilm ability of phage-derived compounds [three lytic proteins (LysH5, CHAP-SH3b, and HydH5-SH3b) and one exopolysaccharide depolymerase (Dpo7)] against biofilms formed by four *S. aureus* strains (two clinical isolates and two from food origin) was assessed. The RTCA data generated after biofilm treatment allowed the calculation of different antibiofilm parameters: i) the minimum biofilm eradicating concentration that removes 50% of the biofilm, $MBEC_{50}$ ii) the lowest concentration needed to observe an antibiofilm effect, LOABE, and iii) the specific antibiofilm activity and the percentage of biofilm removal, that revealed LysH5 as the best antibiofilm compound [2].
In conclusion, the impedance-based technology can be used to quickly assess and compare, by standardized parameters, the disaggregating activity of antibiofilm proteins; therefore, it is a suitable method to search for novel bio-active molecules with effectiveness against bacterial biofilms.

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Investigations of the effects of neuroprotective molecules on endothelial functions by impedimetry and holographic microscopy

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Stimulation of the re-endothelization process is considered to be a promising approach towards the treatment or prevention of diseases attributed to the endothelial injury (e.g. neurodegeneration). In case of some endogenous (e.g. kynurenic acid – KYNA [2]) and synthetic (e.g. *R*-deprenyl – RD [1]) neuro/cytoprotective molecules, it was indicated that influential endothelial cell-ECM adhesion and locomotion may be involved in their effects.

The present work aimed to investigate the effects of KYNA derivatives and deprenyls on cellular behavior (e.g. adhesion and locomotion) and on morphology of GP8 rat cerebral and HMEC-1 human dermal endothelial cell lines.

The tested molecules were KYNA and its analogues (KYNA-A1, -A2, -A4, -A5 [3]) as well as *R*- and *S*-deprenyl (SD). To detect their adhesion modulator and cytoprotective effects the impedance-based xCELLigence SP System was used. The holographic microscope, HoloMonitor M4, was applied for the morphometry and migration studies. The endothelial cell viability was measured by flow (FACSCalibur) and image (NucleoCounter) cytometers.

Both KYNA and RD had significant adhesion inducer effect at physiological concentrations (nanomolar range). The KYNA derivatives containing an amide side chain at the C2 position (KYNA-A1 and A2) had lower adhesion inducer effects compared to KYNA. All synthetic analogues (except KYNA-A5) had a time-dependent inhibitory effect on GP8 cell adhesion at a supraphysiological concentration (10^{-3} mol/L) [3]. The observed changes in the morphometry parameter (area and optical thickness) corresponded well with the adhesion modulator effects of KYNA and deprenyls. Based on the impedance curves and morphological indices (e.g. irregularity) as viability markers KYNA and the deprenyls did not have toxic effect. KYNA caused a slow and random movement of the endotehlial cells [3], while RD (10^{-9} M) provoked the straight movement of HMEC-1 cells by increasing the migration (distance) and decreasing the motility (actual path). The impedimetric measurements showed that RD (10^{-12} - 10^{-9} M) could moderate the cytotoxic effect of L-homocysteine on HMEC-1 cells known as an inductor of endothelial damage.

Our results indicated that the endothelial cell adhesion and locomotion could be a new therapeutic target of KYNA derivatives and deprenyls. KYNA analogues could contribute to maintain the integrity of brain endothelium, while RD could have a potential role in rescuing endothelium and facilitating endothelial repair. The applicability of impedimetry and holographic microscopy for characterizing endothelial repair was also clearly suggested.

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Multisine impedance spectroscopy as a tool to determine antifungal mode-of-action

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So far, high-throughput target-based drug screening of synthetic molecules has not resulted in new antifungal drug classes. One of the proposed reasons for this failure is the lack of penetration of synthetic molecules in the fungal cell, the high false-positive rates and the lack of selectivity [1]. Three major classes of antifungal drugs are currently in use including the polyenes, the echinocandins and the azoles. Two of them, the polyenes and the echinocandins, are natural products or based on natural products produced by micro-organisms. Small molecules produced by micro-organisms have several desirable characteristics. First, they are highly specific due to their high degree of chirality. Second, they have evolved to be active and finally, they occupy a more diverse chemical space. However, natural product-based antifungal drug discovery is associated with a number of technical challenges. Fermentation extracts from microorganisms are composed of complex mixtures containing hundreds or thousands of unknown compounds and concomitantly natural product drug discovery is plagued by a high background of known and/or nuisance compounds. Recent efforts have revealed that the vast majority of microorganisms is still unable to grow under laboratory conditions, indicating a huge unexplored source of new natural compounds. Mining this so-called 'microbial dark matter' has recently proven successful in the search for novel antibiotics [2], but has not been yet used in the search for novel antifungals. Even though previously uncultured micro-organisms can be a rich source of novel antimicrobials, they still produce known antimicrobials. Therefore, a high-throughput platform to filter out known or undesirable compounds would decrease costs and time consumption in antifungal drug discovery. In this work, we have used *in situ* cultivation to isolate and screen novel micro-organisms for the production of antifungals. Next, we have developed a multisine impedance based platform to characterize unfractionated microbial extracts and interestingly, to determine the mode-of-action of the responsible compounds. Here, *Candida albicans* biofilms were grown in modified microtiter plates with gold electrodes coated at the bottom of the plates. Next, the cells were subjected to a small alternating potential inside the wells of the 96 well plates during biofilm development or destruction for 24h. The measurement of the resulting micro-current through the developing biofilm is made up of multiple frequency components (1 - 60 kHz). Therefore, we have developed a proprietary data reduction algorithm to model our data and obtain a relevant impedance signature that we have named the 'antifungal index'. When we challenged growing *C. albicans* biofilms with different antifungals we were able to obtain specific signatures for each class. By comparing the signature profile of unknown compounds from unfractionated microbial extracts with a library of signature profiles of known compounds we were able to identify the antifungal mode-of-action of the unknown compounds in the microbial extracts. The results in this study show that impedance based mode-of-action determination can become a valuable tool in the next-generation natural product-based drug discovery platforms.

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Unraveling G protein-coupled receptor mediated chemokine signaling with cellular electric impedance.

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CXC chemokine receptor 4 (CXCR4) and CXC chemokine receptor 7 (CXCR7) are G protein-coupled receptors (GPCRs) that are associated with various human diseases including cancer, its metastasis and HIV infection [1,2]. Both receptors are considered to be promising therapeutic targets. As such, robust and specific cell-based assays are instrumental for the identification and characterization of molecules that act upon this important class of therapeutic targets. CXCR4 can be stimulated by a single natural ligand, the chemokine CXCL12. While CXCR7 binds CXCL12 with high affinity as well, this receptor can also be activated by a second ligand, the chemokine CXCL11. Unlike CXCR4, CXCR7 is an atypical GPCR devoid of intracellular activation of heterotrimeric G proteins that are composed of $G\alpha$ and $G\beta\gamma$ subunits. Nevertheless, CXCR7 can still recruit β-arrestin molecules upon ligand binding, which leads to receptor internalization and the initiation of other signaling events, including the activation of MAP kinases [3,4].

We used cellular electric impedance as an alternative approach to study canonical G protein-mediated signaling pathways downstream of CXCR4. Dose-dependent and receptor-specific stimulation of CXCR4 was recorded upon addition of its unique chemokine ligand CXCL12. The magnitude of the response correlated well with the level of CXCR4 expression at the cell surface [5]. The technology turned out to be sensitive enough to generate responses with cells that endogenously express CXCR4 at lower levels than normally achieved with recombinant cell lines. $G_{\alpha i}$ signaling largely contributed to the CXCR4-mediated response as demonstrated by strong abolishment of the impedance response upon pretreatment with pertussis toxin (PTX). $G_{\alpha q}$- and $G_{\beta\gamma}$-related events only had minor impact on the response [5]. Overall, small molecule compounds inhibiting intracellular signaling events further downstream of G protein activation had minor impact on the response profile whereas small molecule receptor antagonists (*i.e.* compounds that prevent receptor activation) could entirely block the induced impedance response. Addition of the natural chemokine ligands (CXCL11 and CXCL12) to CXCR7-expressing cells did not result into detectable cellular electric impedance responses, at none of the tested frequencies [5]. This suggests that non-G protein-mediated receptor signaling events induced upon stimulation of atypical chemokine GPCRs are not easily detectable by cellular electric impedance measurements.

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Investigating CXCR4 internalization and heterodimerization with CXCR7 using cellular electric impedance

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The CXC chemokine receptor 4 (CXCR4) and its ligand CXCL12 have defined roles in immune cell trafficking, HIV infection, and cancer progression [1]. *In vivo*, dysregulated CXCR4 signaling is known to support disease progression. In addition, defined CXCR4 mutants with impaired receptor internalization have been associated with pathological conditions [2]. The *in vivo* signaling function of CXCR4 can be modulated by CXCR7, a second CXCL12-interacting chemokine receptor devoid of G-protein activation. Two mechanisms for this modulation have been proposed: 1) scavenging of CXCL12 by CXCR7 and 2) dimer formation between CXCR7 and CXCR4 that reduces the signaling capacity of CXCR4. We studied these phenomena, relevant for *in vivo* CXCR4 activity, using cellular electric impedance [3].

We previously demonstrated that $G_{\alpha i}$-dependent CXCR4 activation can be measured using cellular impedance [4]. Here we assessed, with the same technology, mutant forms of CXCR4 (R334X and E343X) that are associated with WHIM syndrome, a congenital immunodeficiency disorder. Both mutants lack part of the C-terminus, leading to impaired receptor internalization [2]. CXCL12 evoked dose-dependent responses on cells expressing mutant or wildtype (WT) CXCR4 with similar magnitude, but the response of mutant CXCR4-expressing cells was more prolonged and remained elevated. Together with data from internalization assays, this suggests that impaired receptor internalization can result into a more prolonged activation profile that can discriminate mutants from WT CXCR4.

CXCR4 and CXCR7 form heterodimers when co-expressed *in vitro*, whereby CXCR7 can negatively modulate the signaling capacity of CXCR4 [3]. Here, CXCR4-expressing cells were co-transfected with increasing amounts of CXCR7. When challenged with CXCL12, CXCR4-mediated responses decreased with increasing levels of CXCR7 cell surface expression. Stimulation of these cells with CXCL11, a chemokine that only activates CXCR7, did not result into detectable impedance changes. This demonstrates that CXCR7 can alter CXCR4 activity, by CXCL12 scavenging and/or by directly interfering with CXCR4 signaling. Co-cultures of cells exclusively expressing CXCR4 or CXCR7 were used to explore the possibility that the observed decrease in CXCR4 activity was solely due to CXCL12 scavenging by CXCR7. The strong negative correlation between the CXCR4 response and CXCR7 surface expression obtained with the co-transfection experiments could, however, not be replicated by increasing the amount of CXCR7 positive cells in co-cultures. Hence, the decreased CXCR4 response in cells co-expressing CXCR4 and CXCR7 cannot be entirely explained by scavenging, but is likely to be established by a direct interaction between CXCR4 and CXCR7 [4].

References:

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Impedance analysis of hematopoietic cells: the role of Src family kinases in leukemia cell adhesion

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Adhesion signaling is a field of biology studied mostly on adherent cell types. However, hematopoietic cells grow in suspension, and use adhesion to the extracellular matrix (ECM) only in their early development, or – in the case of differentiated cells – to perform the tasks they are specialized for. Peripheral leukemic cells are derived from more or less immature hematopoietic precursors that have, among other alterations, defects in adhesion to the bone marrow microenvironment. On the other hand, leukemic stem cells (LSC) use adhesion to the bone marrow ECM as a mean to evade chemotherapy, and are a source of the minimal residual disease, and of the disease relapses. Kinases of the Src family (SFK) are known regulators of adhesion signaling in adherent cell types, and their overexpression and/or hyperactivation is often seen in malignant diseases. They are also involved in hematooncologic disease progression and resistance to therapy, particularly in several types of leukemias. In the present work, we used a variety of methods including microimpedance measurement, fluorimetric measurement of adhered cell fraction, immunoblotting, confocal microscopy, and interference reflection microscopy. Our results indicate that active Lyn kinase, a hematopoietic SFK, is present in adhesion structures of leukemic cells as well as in those of adherent cells. In leukemic cells, elevated Lyn activity correlates with loosening the cell-fibronectin interactions. However, Lyn is not necessary for the structure formation, and unlike in adherent cells, the effect of its kinase activity on the structure stability is limited. Given the established role of Lyn in many cellular processes of hematopoietic cells, we assume that its function in adhesion structures is to regulate cell proliferation and survival. Lyn may also affect the adhesion structures in kinase-independent manner.

Logistic

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