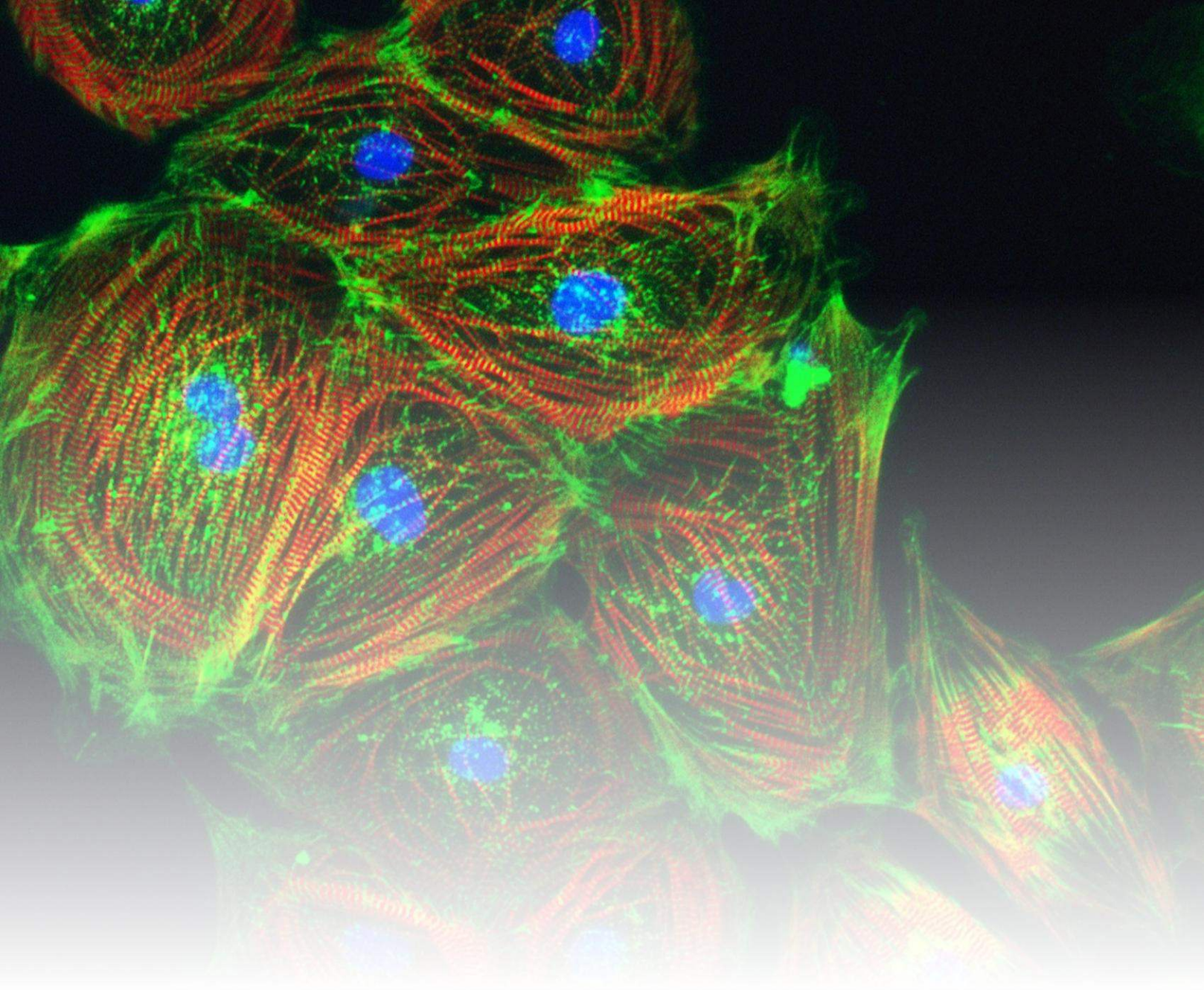

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Electrophysiological analysis of atrial and ventricular axoCells™ cardiomyocytes utilizing 3Brain's CorePlate ™ enabled HD-MEA

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3Brain IT SRL | Genova, Italy

Abstract

This study evaluates the electrophysiological properties of Axol's iPSC-derived atrial and ventricular cardiomyocytes using 3Brain's CorePlate™ enabled high-density microelectrode array (HD-MEA) technology. Over a 7-day protocol, spontaneous activity and β-adrenergic responsiveness were assessed via (HD-MEA) recordings, and analysis performed with BrainWave5 software. Atrial cells showed a higher spontaneous beating rate than ventricular cells, reflecting chamber-specific functional properties. and atrial cardiomyocytes responded predictably to isoproterenol. These findings highlight the effectiveness of combining CorePlate™ technology and iPSC-derived cardiomyocytes for cardiac modeling and drug screening.

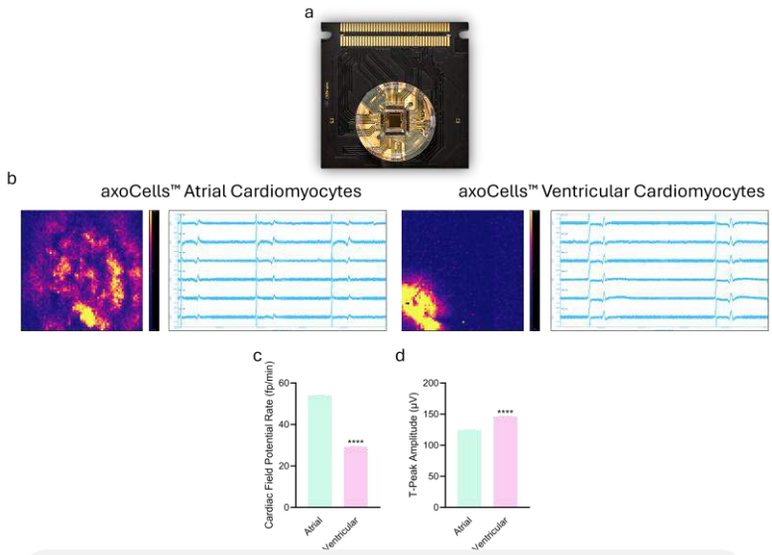


Figure 1. a) CorePlate™ 1W 38/60 b) Activity Map (left) and Raw Signal Map (right) of axoCells™ Atrial and Ventricular Cardiomyocytes. c) Beating Rate and d) T-Peak amplitude comparison between Atrial and Ventricular Cardiomyocytes.

Methods

Cell Preparation

Atrial (ax2518) and ventricular (ax2508) iPSC-derived cardiomyocytes were thawed and cultured in Axol's Cardiomyocyte Maintenance Medium (ax2530) on fibronectin-coated 3Brain CorePlate™ 1W 38/60 arrays. Cells were allowed to mature for seven days post-thaw.

Electrophysiological Recording

Electrophysiological activity was recorded using the 3Brain BioCAM DupleX system in conjunction with CorePlate™ 1W 38/60 featuring 4,096 electrodes within a 3.8 × 3.8 mm² active area (21 × 21 µm² electrodes, 60 µm pitch). Recordings were sampled at 20 kHz with a 5 Hz high-pass filter.

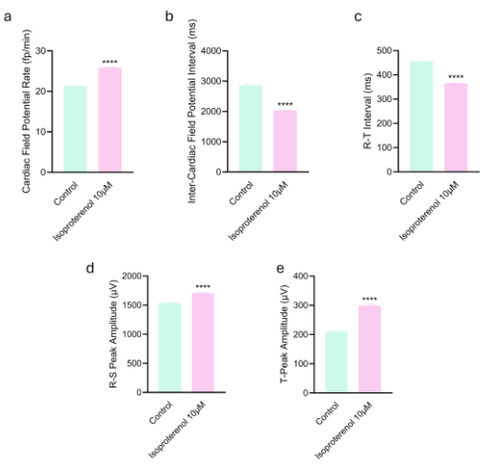


Figure 2. a) Cardiac Field Potential Rate, b) Inter-Cardiac Field Potential Interval, c) R–T Interval, d) R–S Peak Amplitude, and e) T-Peak Amplitude of axoCells™ Atrial Cardiomyocytes in the absence and presence of 10 µM isoproterenol.

Pharmacological Assay

At day 9 in vitro, 10 µM isoproterenol was applied to atrial cardiomyocytes to assess responsiveness to β-adrenergic stimulation. Electrophysiological responses were measured over a 3-minute period before and after treatment.

Analysis Software

Data were analyzed using BrainWave5 software to extract parameters such as beat frequency, field potential duration, R–T interval, and T-peak amplitude. The same software was also used to extract waveforms and propagation maps.

Results

At Day 7 post-thaw, both atrial and ventricular iPSC-derived cardiomyocytes showed robust spontaneous activity when cultured on 3Brain's HD-MEA platform. Atrial cardiomyocytes exhibited a higher beating rate compared to ventricular cells, aligning with their physiological roles *in vivo* (Fig. 1). The beating rate for atrial cells was notably faster, demonstrating their intrinsic pacemaker activity, which is characteristic of atrial tissue in the human heart.

Isoproterenol Stimulation

When treated with 10 µM isoproterenol at Day 9, atrial cardiomyocytes exhibited significant changes in electrophysiological activity. Among all changes, T-peak moved closer to the QRS complex, indicating faster repolarization, and both the R–S and T-peak amplitudes increased (Fig. 2 and 3).

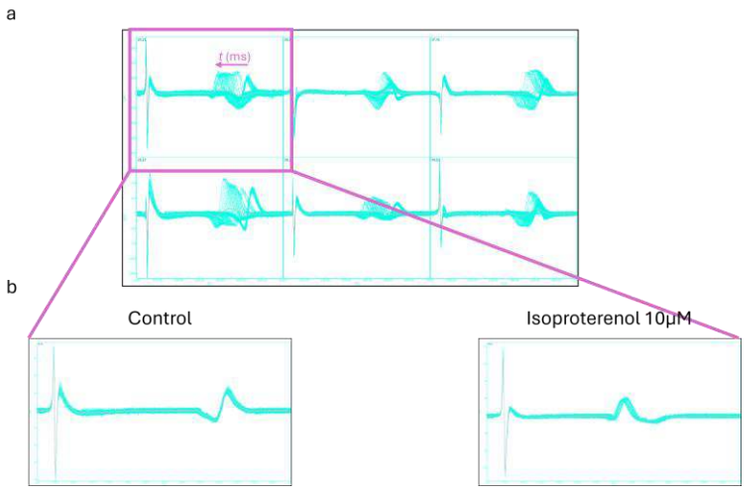


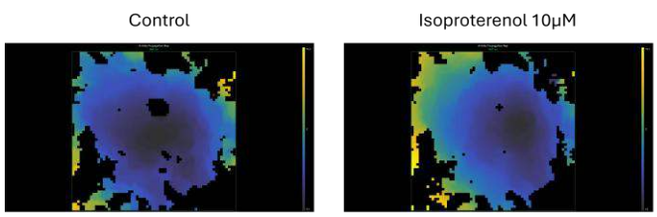
Fig. 3 a) Event waveforms recorded during a 3-minute exposure of atrial cardiomyocytes to 10 µM isoproterenol. The T-peak is visibly closer to the QRS complex following treatment. b) Zoomed-in view of selected channels showing the average waveform calculated over 30 seconds before treatment and the final 30 seconds after treatment.

Signal Propagation

Changes in activity propagation were observed, with propagation duration increasing from 40 ms (control) to 45 ms (post-isoproterenol), consistent with enhanced calcium influx and underdeveloped repolarization mechanisms in immature cardiomyocytes (Fig. 4). This increase in propagation duration was accompanied by a corresponding rise in network burst duration. These findings demonstrate the ability of HD-MEA to capture detailed electrophysiological signatures and differences between cell types, making it an effective tool for studying arrhythmias and electrophysiological properties.

Conclusions

The findings from this study demonstrate the power of 3Brain's CorePlate™ enabled HD-MEA system in characterizing the distinct electrophysiological properties of axoCells™ Atrial and Ventricular Cardiomyocytes. Significant differences in basal activity were observed, with atrial cardiomyocytes exhibiting a higher beating frequency compared to ventricular cells. After isoproterenol treatment, atrial cells showed a notable increase in cardiac field potential rate, a shift in the T-peak, and an increase in T-peak amplitude. These results underscore the chamber-specific electrophysiological behavior of atrial and ventricular cardiomyocytes, which closely reflect their physiological roles, thus showcasing the ability of CorePlate™ 1W 38/60 to capture real-time, high-resolution data on electrical activity propagation providing invaluable insights into arrhythmogenesis, particularly for studying re-entrant circuits.



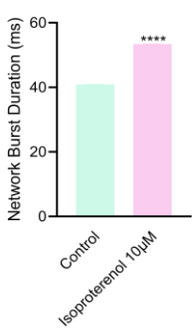


Figure 4. Propagation Map (left) and Network Burst Duration (right) of axoCells™ Atrial Cardiomyocytes before and after the treatment with isoproterenol.

This technology allows for the differentiation of electrophysiological properties at both the single-cell and network levels, offering a deeper understanding of the cellular mechanisms underlying common arrhythmias, such as atrial fibrillation. By leveraging this advanced platform, researchers can investigate the detailed dynamics of arrhythmogenesis and the response to therapeutic interventions, opening new avenues for cardiovascular research. Additionally, the ability to utilize iPSC-derived cardiomyocytes as a model for complex heart diseases provides an exciting approach for personalized medicine, offering great potential for the development of novel therapies targeting arrhythmic conditions.

BiomimX
THE BEATING ORGANS-ON-CHIP

Beating heart-on-chip to model atrial and ventricular cardiac chambers

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Abstract

Developing **chamber-specific *in vitro* cardiac models** using hiPSC-derived cardiomyocytes (CMs) remains challenging to accurately predict drug-induced cardiotoxicity. Here, we present atrial and ventricular beating heart-on-chip models (uHearts) developed within the uBeat® Platform, which applies physiological mechanical stimulation to microtissues, to enhance their functionality. The microtissues showed chamber-specific gene expression, synchronous contraction and distinct electrophysiological profiles. uHearts were effectively used to evaluate the cardiotoxic effect of compounds.

Figure 1. BiomimX experimental set-up used to generate atrial and ventricular beating heart-on-chip models (uHeart) exploiting iPSC-derived cardiomyocytes from Axol Bioscience.

Methods

The chamber-specific 3D microtissues were generated using 75% of atrial (or ventricular) human iPSC-CMs (ax2518, ax2508) and 25% of supportive cardiac fibroblasts. Cells were encapsulated in fibrin hydrogel and cultured in the uBeat® Stretch Platforms (BiomimX Srl). A finely tuned mechanical stimulation (i.e. 10% uniaxial strain, 1Hz) was applied for up to 11 days. Microtissues development was monitored daily to assess cell self-organization and to determine the onset of a spontaneous and synchronous beating. PCR analysis was performed to assess ion-channels and chamber-specific structural-related genes (e.g.

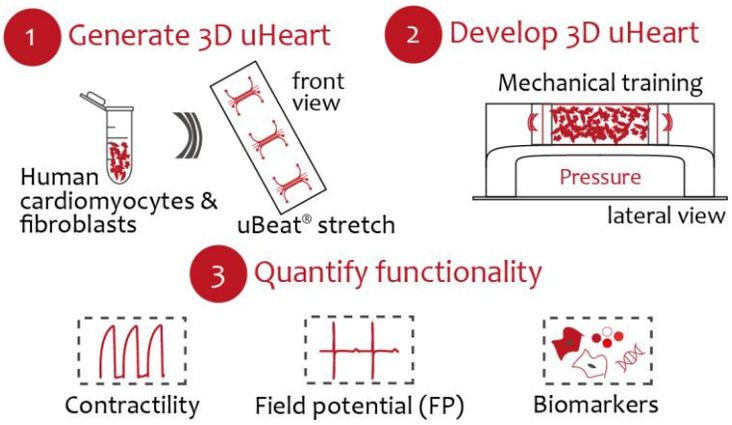

Figure 2. Experimental plan to develop chamber-specific uHeart models: 1) cells are inserted in the uBeat® stretch platforms, 2) mechanical stimulation is applied up to 11 days; 3) readouts are performed (also upon drug administration).

SCN5A, *KCNH2*, *CACNA1C*, *MYH6*, *MYH7*, *MYL2* and *MYL7*). Immunofluorescence staining was exploited to investigate cell distribution within the microtissue (e.g. Troponin I, typical of cardiomyocytes) and to determine atrial and ventricular phenotypes (i.e. Myosin Light Chain 2 -MLC2-V for ventricle and Sarcolipin-SLN for atrium). Contractile properties were derived by analyzing bright field videos through Muscle Motion software (Sala et al, 2018). Beating period-BP, contraction amplitude-CA, contraction duration-CD and relaxation time-CT were assessed. Electrophysiology was measured by means of a BiomimX proprietary technology, μECG, which allows the recording of microtissue field potentials. BP, spike amplitude-AMP and field potential duration-FPD were evaluated to assess the properties of atrial and ventricular microtissue. Drug-induced alteration of electrophysiology was finally evaluated.

Results

Mechanical stimulation appeared to promote the **atrial and ventricular uHearts** development, eliciting an earlier onset of synchronized beating already after 5 days in culture. Atrial microtissues showed a time-dependent increasing expression of sodium, potassium and calcium channel-related genes, while ventricular uHeart had a stable expression of them. Structural genes such as *MYH7* and *MYL2* showed higher expression level in both types of microtissues cultured for 11 days, respect to day 0. At protein level, as expected, uHearts were positive for cardiac troponin I, confirming the formation of highly interconnected microtissue.

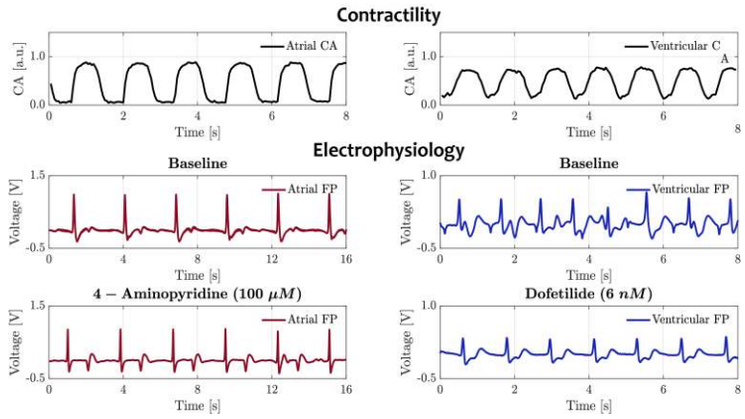

Figure 3. Contractility traces of atrial (top left) and ventricular (top right) uHearts after 11 days in culture. Electrophysiological signals measured before and after drug administration in atrial (left) and ventricular (right) uHearts.

Additionally, chamber specific SLP and MLC2-V was observed exclusively in atrial and ventricular models, respectively. Dynamically cultured atrial uHearts showed a progressive reduction in BP and a significant increase in CA over time, while ventricular uHeart exhibited a stable BP and a mild CA increase. While contraction and relaxation times were variable across both models, ventricular uHearts generally exhibited shorter RT. Clear electrophysiological signals were successfully acquired from both atrial and ventricular uHearts, allowing for the identification of the typical depolarization spikes and the smoother repolarization peak. Pharmacological tests highlighted the capability of uHearts to respond to reference compounds: 4-Aminopyridine mainly affected atrial microtissues by prolonging the FPD_{cF} and dofetilide elicited a significant prolongation of the FPD_{cF} in ventricular microtissues.

Conclusions

Chamber-specific cardiac models developed using human iPSC-derived cardiomyocytes represent a significant advancement in *in vitro* systems for cardiovascular research and cardiotoxicity screening. Advanced OoC platforms have proven invaluable for applying physiologically relevant mechanical stimulation, enabling the generation of cardiac tissues that closely mimic the structure and function of their *in vivo* counterparts. In particular, the application of controlled uniaxial stretching via the uBeat® technology enhanced microtissue functionality, accelerated the onset of synchronous contraction, and promoted more physiologically accurate behavior.

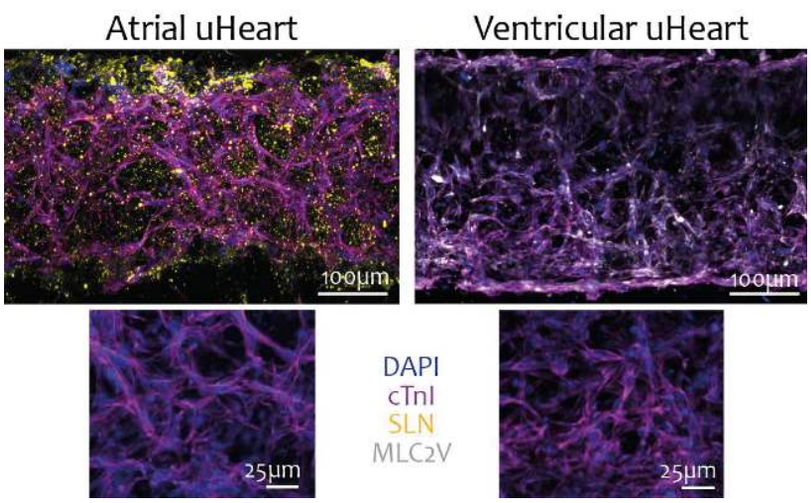

Figure 4. Immunofluorescence staining of atrial (left) and ventricular (right) uHeart, stained with Cardiac Troponin I (magenta), Sarcolipin (yellow), Myosin Light Chain 2 (grey) and DAPI (blue).

The resulting atrial and ventricular uHearts exhibited distinct structural, functional, and electrophysiological features, faithfully reflecting chamber-specific phenotypes. Furthermore, the uHearts responded predictably to reference pharmacological agents, validating their applicability for high-fidelity drug screening. Beyond cardiotoxicity assessment, these models offer a reliable and scalable platform for disease modeling and personalized medicine. Their tunability—through adjustment of cardiomyocyte-to-fibroblast ratios and the potential incorporation of immune components—enables the simulation of pathological conditions such as fibrosis, inflammation, and arrhythmias. Continued optimization using broader compound libraries and patient-specific iPSC lines will further strengthen the translational relevance of the uBeat® Platform, supporting its role as a next-generation tool for cardiac research and drug development.

Advancing ALS Modeling: High-Dimensional Insights with CytoTronics' Pixel

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Abstract

In this application note, we demonstrate the use of CytoTronics' Pixel to simultaneously capture morphological and electrophysiological data from iPSC-derived motor neurons from Axol Bioscience. Using impedance-based electrical imaging and extracellular voltage recordings, we characterized the structural and functional properties of motor neurons from healthy and ALS-associated genotypes (TDP43, C9ORF72, and SOD1) over 28 days. Pixel revealed genotype-specific differences in morphology, network development, and neuronal firing dynamics, enabling high-resolution, longitudinal monitoring of disease-relevant phenotypes. This integrated approach supports applications in cell line validation, disease modeling, and phenotypic drug screening.

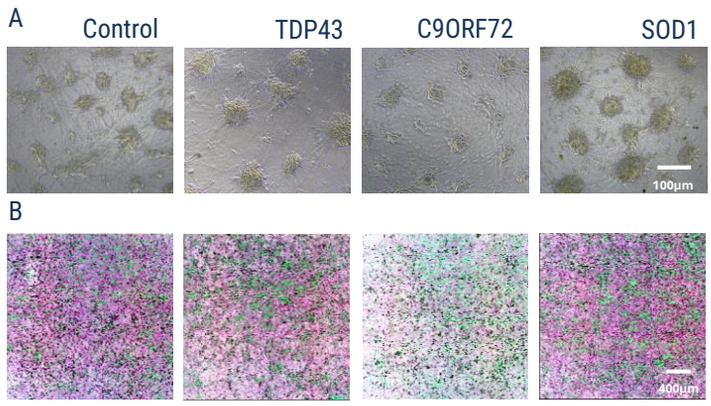

Figure 1. Morphological Phenotypes on DIV20. (A) Brightfield images of motor neurons on sister glass-bottomed dish on DIV20 showing expected phenotypic clustering patterns. (B) Multi-parametric electrical images of motor neurons showing distinct phenotypic signatures.

Methods

Human iPSC-derived motor neurons representing healthy control (ax0076), TDP43 (ax0079), C9ORF72 (ax0074), and SOD1 (ax0735) genotypes were plated onto CytoTronics' Pixel microplates pre-coated with poly-D-lysine and vitronectin. Each well was seeded with either 25,000 or 50,000 cells, corresponding to 190,000 and 380,000 cells/cm^2, respectively. Cultures were maintained under standard conditions for 28 days, with medium changes performed according to recommended protocols. To monitor morphological phenotypes and overall cell health, a sister culture was maintained in parallel on a glass-bottomed dish.

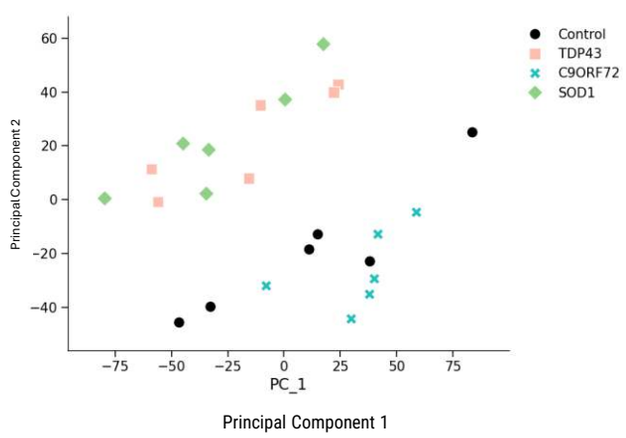

Figure 2. Principal component analysis (PCA) of control and disease motor neurons show distinct clustering and separation of cell lines based on electrical imaging measurements.

Pixel and Measurement Overview

The Pixel enables simultaneous acquisition of two complementary data types from live cells: impedance-based electrical imaging and extracellular neural measurements. All measurements were performed using the integrated Pixel hardware and software suite.

Electrical Imaging

Electrical imaging was conducted every 2 hours throughout the 28-day culture period. This non-invasive, label-free technique captures cell-induced perturbations in electric fields within each well. More than 20 orthogonal impedance measurements were collected per well at each timepoint, providing detailed structural and morphological characterization. Variations in impedance reflect key biological properties such as cell attachment, barrier integrity, morphology, and motility. Different geometries and frequencies of the electric field allow selective probing of these cellular traits. High-dimensional analyses, such as principal component analysis, can then integrate these multiple measurements into a single comprehensive output.

Neural Recordings

Extracellular neural recordings were acquired every 12-24 hours. Each well on the Pixel microplate contains dedicated recording channels that detect changes in local extracellular voltage associated with neuronal firing. These signals reflect ion channel activity and action potential propagation near the electrode surface. Voltage fluctuations were recorded and processed in real time by the Pixel software, which automatically extracted neural activity features including spike rate, burst frequency, and network activity.

Results

The integration of Pixel's impedance-based electrical imaging and neural recording capabilities enabled dynamic, multi-parametric monitoring of iPSC-derived motor neurons across all four genotypes. Electrical imaging measurements captured clear differences in morphology, including cell attachment, clumping, and network formation (Figure 1). Principal component analysis (PCA) of impedance parameters revealed distinct phenotypic patterns between healthy and disease-associated lines (Figure 2). These structural signatures were consistent across replicate wells, highlighting Pixel's utility in detecting subtle morphological phenotypes in live cultures without the need for staining or fixation.

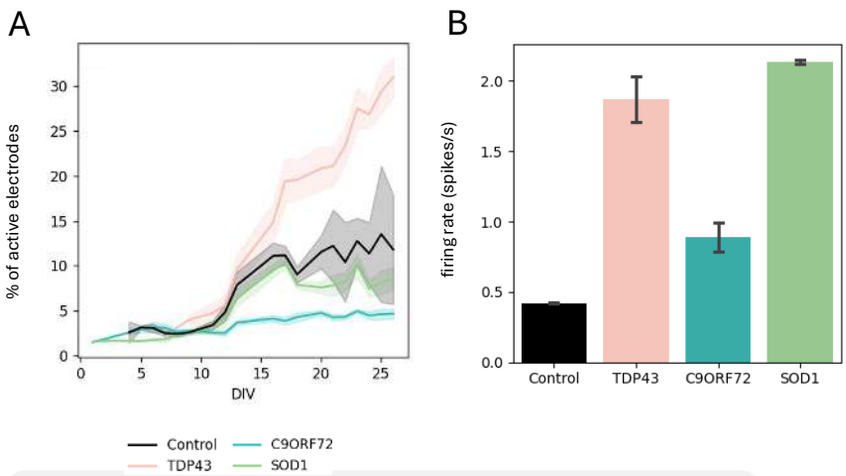

Figure 3. (A) Longitudinal traces showing percent active electrodes per well over 28 days in culture. (B) Firing rate (spikes/s) on DIV28.

In parallel, neural recordings captured the progressive maturation of the neuronal activity over the culture period, revealing distinct differences in firing patterns across genotypes (Figures 3, 4). Compared to healthy controls, disease-associated neurons exhibited increased firing rates and altered burst durations, highlighting functional impairments characteristic of each genotype.

Conclusions

This study highlights the power of CytoTronics' Pixel combined with Axol Bioscience's iPSC-derived motor neurons to enable comprehensive, label-free analysis of both structural and functional neural phenotypes. Using simultaneous electrical imaging and neural recordings, Pixel revealed clear, genotype-specific differences in morphology, network development, and neuronal activity. The ability to track longitudinal changes in the same wells without perturbing the culture offers a distinct advantage for disease modeling, cell line validation, and phenotypic drug screening.

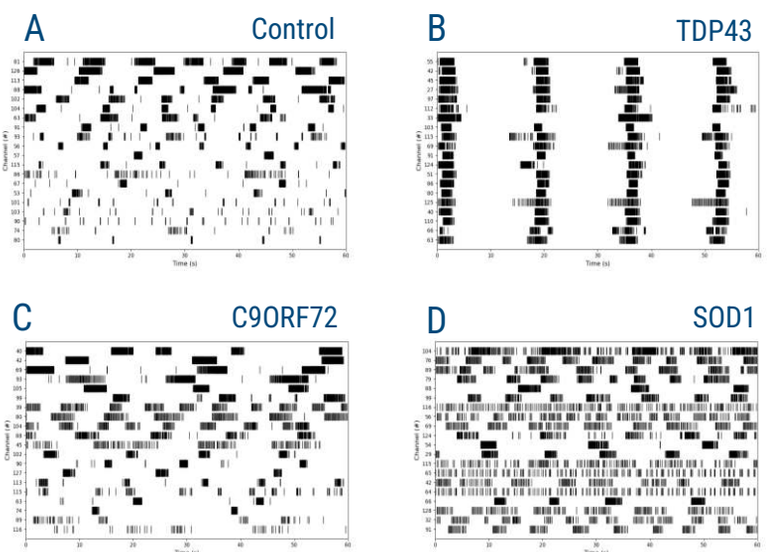

Figure 4. Neuronal firing raster plots across genotypes showing distinct functional phenotypes for 20 channels on DIV28. (A) Control, (B) TDP43, (C) C9ORF72, and (D) SOD1.

Together, CytoTronics' Pixel and Axol's high-quality neuronal models provide a robust, scalable solution for researchers seeking deeper insight into neurodegenerative disease mechanisms and therapeutic discovery.

systasy

Pathway Profiling of axoCells™ Sensory Neurons

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Abstract

Human iPSC-derived sensory neurons are an important model system to study the biology of this cell type in health and disease. Systasy Bioscience GmbH, Axol's partner for NGS assay technologies, profiled axoCells™ sensory neurons using Systasy's *pathwayProfiler* technology. Two batches of axoCells sensory neurons were assessed by the multiplexed *pathwayProfiler* assay. Cultures that were stimulated by nerve growth factor (NGF) or phorbol myristate acetate (PMA) showed a selective and highly similar response for both batches. This supports the notion that sensory neurons derived from different batches of Axol's human iPSC-derived sensory neuron progenitors (ax0055) provide cultures with reproducible properties in terms of cellular signaling.

Methods

pathwayProfiler assay. Cellular signaling is complex, with different stimuli triggering specific cellular responses through intracellular pathways (**Fig. 1A**). These complex cellular signaling activities can be profiled in living cells using Systasy's lentiviral *pathwayProfiler* assay (**Fig. 1B, C**), which contains 30 biosensors designed to monitor multiple signaling pathways simultaneously. The *pathwayProfiler* assay allows multiplexing on multiple levels, namely for pathways, samples, and groups of samples, e.g. plates. This allows a highly parallel measurement of multiple experimental conditions such as in compound profiling campaigns.

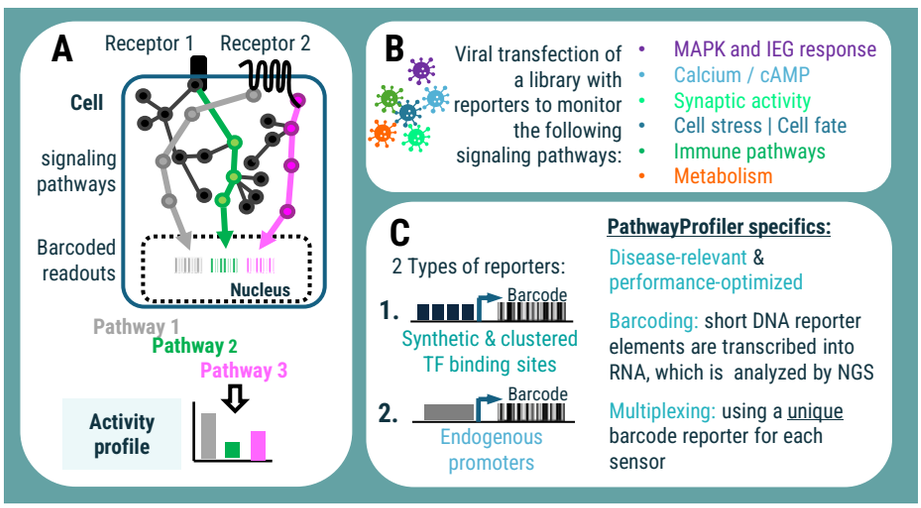
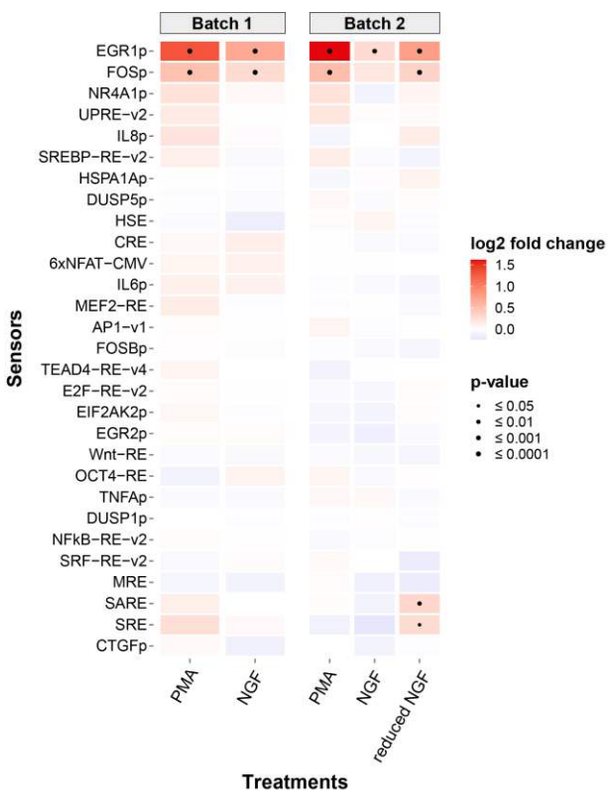


Figure 1. Principles of Systasy's *pathwayProfiler* assay. (**A**) Synthetic barcoded sensors measure pathway activities in living cells. (**B**) The sensors are delivered into cells using lentivirus-mediated infection. (**C**) Synthetic sensors consist of two types of reporters. The first type consists of synthetic & clustered response elements (RE) formed of transcription factor (TF) binding sites. They capture defined cellular cues such as calcium signaling or serum response. The second reporter type contains endogenous promoters, reflecting the transcriptional activity of target genes.

Cell culture. axoCells Sensory Neuron Progenitors (ax0055) were cultured following Axol's user guide. On day 22, mature sensory neuron cultures were stimulated with either 200 ng/ml NGF or 10 ng/ml PMA for 4 hours and then analyzed by the *pathwayProfiler* assay. Two batches were differentiated and analyzed in parallel. With more wells available for batch 2, an additional, "reduced" condition was tested. For this, the medium levels of NGF and BDNF were lowered from 25 ng/ml and 10 ng/ml to 2 ng/ml on day 20, to reduce background activity of NTRK1 and NTRK2-mediated activation of MAP kinase (MAPK) signaling. The "reduced" condition was stimulated with 100 ng/ml NGF on day22.

Results

axoCells Sensory neurons were analysed applying Systasy's proprietary *pathwayProfiler* assay. Pathway responses to the stimuli NGF or PMA were analysed in two different axoCells batches in parallel. The PMA stimulus resulted in a selective activation of MAPK sensors, i.e., EGR1p and FOSp, comparable in both axoCells batches (**Fig. 2**). The NGF stimulus resulted in activation of the EGR1p sensor in both axoCells batches. In batch 1, the FOSp sensor was activated under regular, non-reduced conditions with regular levels of NGF and BDNF in the medium during stimulation. In batch 2, the FOSp sensor was only significantly activated under NGF- and BDNF-reduced conditions. Notably, this reduction resulted in the additional activation of two sensors, namely the SARE (synaptic activity response element) and SRE (serum response element) sensors. Thus, reducing the activation of MAP kinase signaling through reduced NGF and BDNF, mediated by NTRK1 and NTRK2, respectively, enabled the detection of NGF signaling profiles using the *pathwayProfiler* assay. This suggests that reducing selective baseline signaling activity increases the capacity for overall response to a given pathway in these cultures. This feature may be important when implementing screening conditions in sensory neurons. Importantly, comparing the PMA- and NGF-induced sensor response of all 30 sensors between batch1 and batch2 revealed a high correlation among sensor responses for the two different axoCells batches (**Fig. 3**).



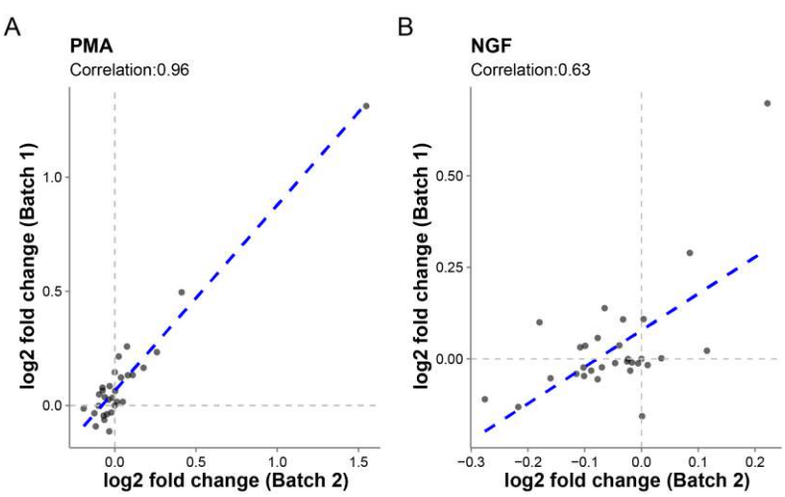


Figure 3. axoCells sensory neuron-batches 1 and 2 correlate when stimulated with PMA and NGF. Correlation plot of 30 individual sensor responses of batch 1 vs. batch 2 for PMA (A) and NGF (B).

Figure 2. Sensory neuron cultures responded to PMA and NGF stimuli. Sensor responses were normalized to unstimulated controls. A condition with reduced levels of NGF and BDNF before stimulation (labelled as reduced.NGF) was normalized to a similarly treated control.

Conclusions

Two sensory neuron progenitor batches obtained from Axol were cultured in parallel for 22 days following Axol's user guide. Systasy's *pathwayProfiler* assay was used to assess the cellular signatures in these cells, and found that signatures were highly reproducible between the two batches. Sensor activation for the 30 sensors of the *pathwayProfiler* assay highly correlated between the two batches. PMA treatment led to the activation of the MAPK sensors EGR1p and FOSp. Similarly, NGF stimulation led to the activation of these two MAPK sensors as well as the SRE and SARE sensors, both of which also respond to MAPK stimuli. These data support the notion that different batches of axoCells Sensory Neuron progenitors have a cellular physiology with reproducible properties.

Neurotoxicity Evaluation via Nerve MPS and Machine learning

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Abstract

Peripheral neuropathy is a frequent adverse effect of chemotherapy, yet its preclinical prediction remains limited by the lack of reliable human-relevant models. This study presents a microphysiological system (MPS) using human iPSC-derived sensory neurons to evaluate neurotoxicity. By integrating microfluidic engineering with deep learning-based morphological analysis, the platform enables reproducible, high-throughput detection of drug-induced neuronal damage. It supports quantitative biomarker integration and offers a practical *in vitro* evaluation strategy for improving drug safety profiles.

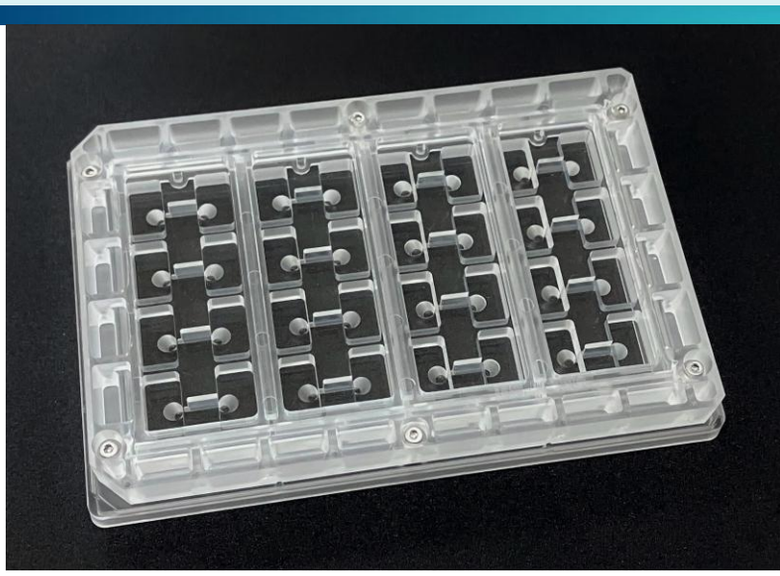

Figure 1. Nerve Plate (USHIO Inc.)

Methods

To develop an *in vitro* model for peripheral neurotoxicity, Nerve Plate was created that includes a chamber for neuron somas, a gate to separate neurites from somas, and a channel guiding unidirectional axon growth. The chip is made with cyclo-olefin polymer.

The human iPSC-derived sensory neurons progenitor (ax0055, Axol Bioscience) were seeded into the soma chamber and cultured for 21 days to establish robust polarized neurite networks.

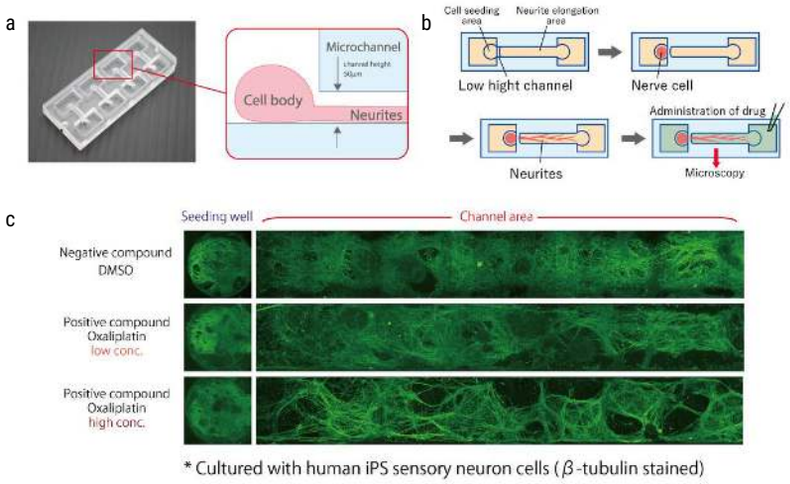

Figure 2. (a) Schematic of side view of the device (b) Schematic of a series of test processes from culture to drug stimulation. (c) Immunofluorescence image of neurite elongation in the MPS.

After differentiation, neurons were exposed to known neurotoxicants including paclitaxel, vincristine, oxaliplatin, bortezomib, colchicine, and acrylamide. Immunocytochemistry with βIII-tubulin and nuclear staining was performed to visualize structural integrity post-treatment. Confocal images of soma and neurite regions were acquired and preprocessed for input into a convolutional neural network (CNN). The CNN was trained to classify compounds based on morphological features indicative of neuronal damage. To assess robustness, dimensionality reduction and clustering techniques were used to evaluate inter-compound feature distinctions. Additionally, culture media were collected post-exposure for measurement of NF-L levels using ELISA, serving as a secondary validation of neuronal damage.

Results

The Nerve chip supported clear compartmentalization and unidirectional growth of iPSC-derived sensory neurons. Upon compound exposure, neurons exhibited distinct morphological phenotypes depending on the agent. Paclitaxel and vincristine caused pronounced neurite beading and fragmentation, whereas bortezomib induced soma condensation and reduced axon outgrowth.

The trained CNN successfully identified compound-specific toxicity patterns, achieving high classification accuracy across all tested drugs. Principal feature extraction using t-SNE revealed clear separation between compound groups, suggesting that the deep learning model effectively captured drug-specific morphological signatures. This morphology-based approach enabled detection of subtle neurotoxic effects even in cases where visual differences were less apparent.

In addition, NF-L quantification revealed increased levels following exposure to certain agents, consistent with observed neurite degeneration. These biochemical results supported the morphological findings and reinforced the platform's biological relevance.

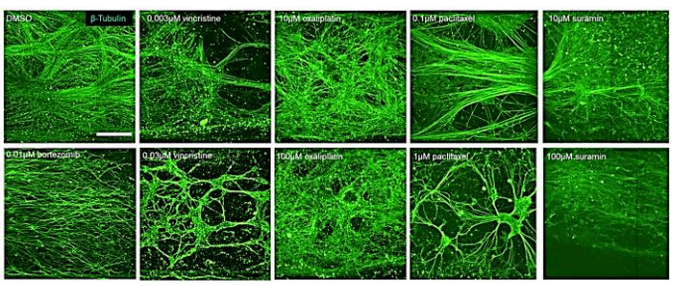

Figure 3. Representative β-tubulin immunofluorescence images in the MPS device after drug administration.

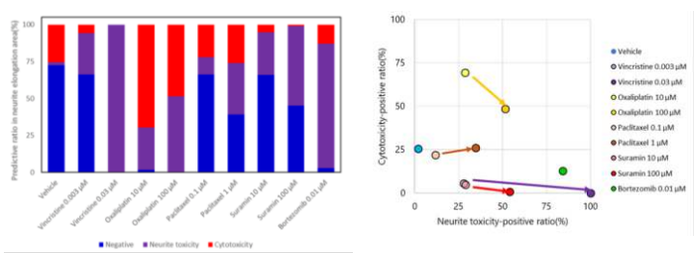

Figure 4. Prediction results of representative compounds (a) Distribution of the predicted ratio as negative, positive for neurite toxicity, or positive for cytotoxicity. (b) Classification of compounds based on the prediction results.

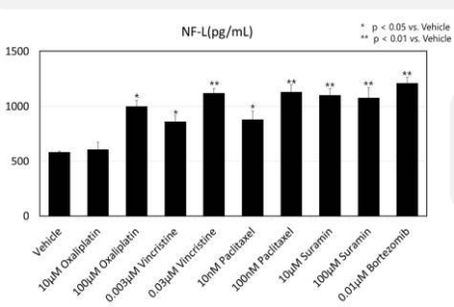

Figure 5. Expression value of human neurofilament light chain (NF-L) from medium supernatant obtained from channels after drug administration.

Conclusions

This study introduces a novel microphysiological system (MPS), Nerve Plate for peripheral neurotoxicity evaluation that combines iPSC-derived sensory neurons with image-based deep learning. The platform provides a human-relevant, reproducible, and scalable approach for detecting drug-induced morphological alterations, offering enhanced resolution over conventional visual scoring methods.

By guiding unidirectional neurite outgrowth, the MPS design enabled compartmentalized imaging and targeted analysis. Integration of deep learning allowed automated detection and classification of morphological damage patterns, distinguishing subtle variations induced by different neurotoxic agents. The ability to separate compounds based on their impact on neuronal substructures offers valuable mechanistic insights.

The exclusion of non-human cell types and the reduced reliance on animal models address current translational gaps in neurotoxicity testing. This system is particularly suited for early-phase drug development and compound prioritization, providing a functional and interpretable platform for neurotoxicity prediction with strong applicability in both academic and industrial contexts.

Toxics 2024, 12(11), 809; https://doi.org/10.3390/toxics12110809
https://www.ushio.co.jp/en/feature/organs-on-chip/

A 3D neurite outgrowth model for studying motor axon biology

Mimetas | Oegstgeest, The Netherlands

Abstract

MIMETAS developed a 3D *in vitro* neurite outgrowth model using human iPSC-derived motor neurons cultured in the OrganoPlate® platform. The system enables directed axonal growth by embedding neurons in an extracellular matrix and guiding axons into an adjacent gel layer, creating physiologically relevant compartmentalization.
The model is well-suited for studying axonal biology, neuropathies, and chemotherapy-induced peripheral neuropathy (CIPN). Axons show predictable responses to both neurotoxic agents like vincristine and axonal guidance cues, making it an ideal tool for mechanistic research and drug screening.

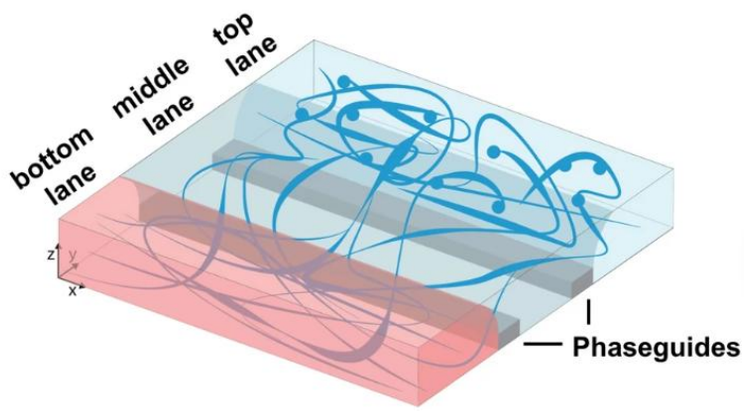

Figure 1. A schematic overview of the segregated neurite outgrowth model containing motor neuron somate in the top lane and axons in the middle and bottom lanes of the OrganoPlate platform.

Methods

Human iPSC-derived motor neurons (Axol Bioscience) were embedded in an extracellular matrix and cultured in the OrganoPlate® 3-lane platform, which supports parallel processing of up to 40 microfluidic chips for medium-throughput experimentation. This setup enables clear separation of somas and axons by directing axonal outgrowth into a neighboring gel channel. The model supports detailed analysis of axonal structure and behavior.

It was applied to evaluate neuronal responses to neurotoxic compounds, excitotoxic stress, and guidance molecules. Axonal outgrowth was visualized and quantified over time, leveraging the platform's compatibility with imaging and automation for streamlined analysis.

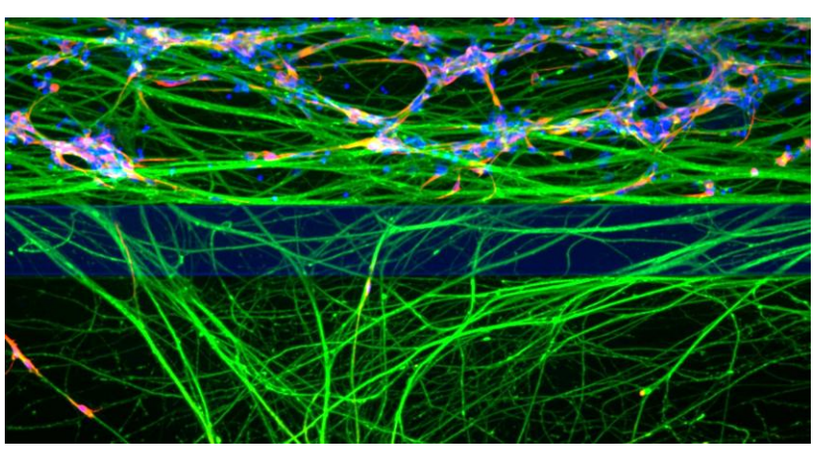

Figure 2. Motor neurons (day 17) were immunostained for dendritic marker MAP2 (red) and axonal marker TAU (green). Neurites protruding into the middle lane are mostly TAU-positive and MAP2-negative, indicating a predominantly axonal nature. Maximum projection images, the scale bar is 200 µm.

Results

The model supported consistent and functional maturation of iPSC-derived motor neurons, with directional axonal outgrowth that increased over time. Axons responded predictably to vincristine in a dose-dependent manner, revealing selective axonal toxicity without compromising cell viability—ideal for neurotoxicity screening.

Pathological responses relevant to disease, including axon degeneration and stress granule formation, were induced by glutamate and sodium arsenite exposure. The platform also enabled manipulation of axonal behavior: axons were attracted by growth factor-rich media and repelled by semaphorin gradients, demonstrating its utility for modeling axon guidance and regeneration.

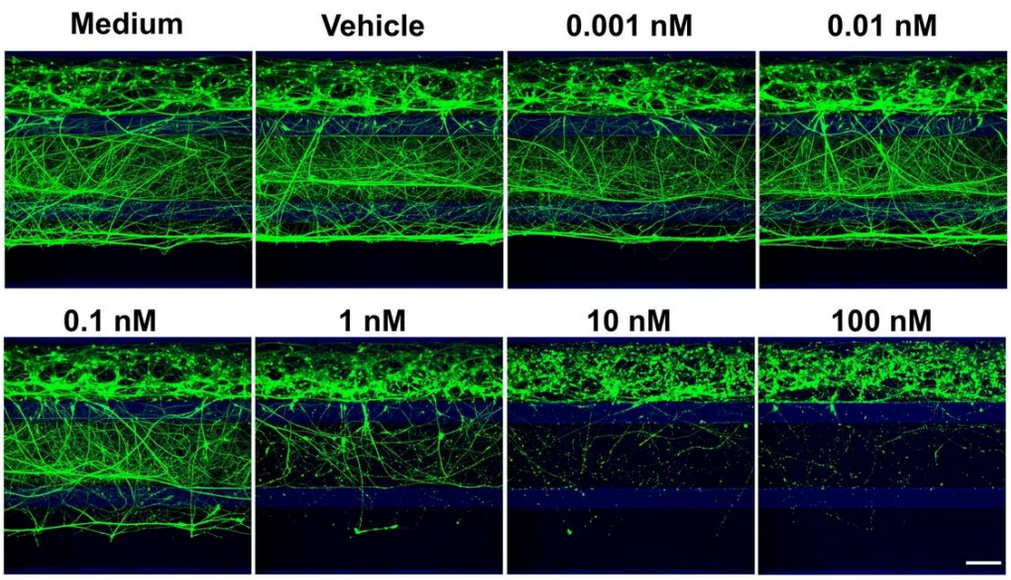

Figure 3. Neurite outgrowth was quantified following 96h exposures to different chemotherapeutics. Compounds could be ranked in terms of toxicity. Motor neurons (day 17) were exposed to various concentrations of vincristine for 4 days. Cultures were visualized with calcein-AM dye. Maximum projection images, the scale bar is 200 µm.

Conclusions

This 3D neurite outgrowth model offers a robust and physiologically relevant platform for studying motor neuron biology, axon pathology, and regeneration. Its ability to isolate axons and measure specific responses to compounds and signaling cues makes it well-suited for disease modeling and neurotoxicity testing.

With built-in scalability, automation readiness, and high-throughput compatibility, this platform empowers efficient and predictive research in neurobiology and therapeutic development. We envision use of the model studying axonal biology and disease, drug discovery and regenerative medicine.

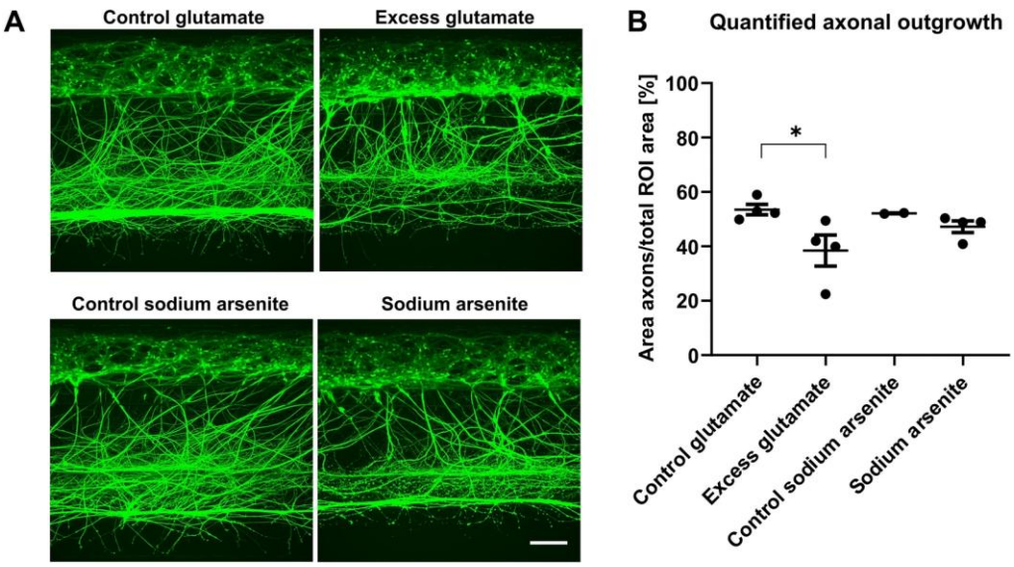

Figure 4. Excitotoxic conditions induce reduced axon outgrowth and formation of stress granules. (B) Motor neurons (day 17) were exposed to 5 mM glutamate for 7 days or 0.05 mM sodium arsenite for 3 h and labeled with calcein-AM dye. The scale bar is 200 µm. (B) Quantification of the axon outgrowth depicted in (A).

A high content imaging screening assay to identify novel neuroinflammation modulators using human iPSC-derived microglia

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Abstract

Neuroinflammation is an area of growing interest for the treatment of central nervous system (CNS) disorders. Although novel biological targets have been identified that modulate neuroinflammation, robust and validated *in vitro* cell models of disease are missing to support effective early drug discovery (DD) programmes.

Neuroinflammation is instigated by microglia, which are the resident immune cells of the CNS. Microglia play a role in the clearance of dying cells due to the accumulation of misfolded proteins such as amyloid β (Aβ), cellular debris, and maintain homeostasis.

Here we describe the optimization and validation of a miniaturized human iPSC-derived microglia *in vitro* model to study phagocytosis of a pathological protein by using complimentary imaging platforms.

Methods

We cultured terminally differentiated axoCells™ WT human iPSC-derived microglia (iMGL), obtained from Axol Bioscience, in 384-well plates, as per the manufacture's protocol. Phenotypic characterization was determined at DIV7 by examining the expression of key microglial markers (TMEM119, P2RY12) by immunofluorescence (IF) staining using selective antibodies.

Human iMGL from healthy donors (DIV7) were incubated with pH sensitive pHrodo® deepred labelled Aβ1-42 peptide. Assay plates were read on an IncuCyte® S3 Kinetic Live Cell Analysis System (Sartorius) for between 0 -24 hours.

To complement the kinetic, live cell assay, we also developed a 384-well plate-based end-point high-content phagocytosis phenotypic assay. At the end of the IncuCyte assay, cells were fixed with 4% (v:v) paraformaldehye and read on a Molecular Devices ImageXpress confocal instrument. A custom-built image analysis algorithm was generated using MetaXpress® 6 software (Molecular Devices) to recognize cell nuclei and phagocytosed Aβ1-42 peptide.

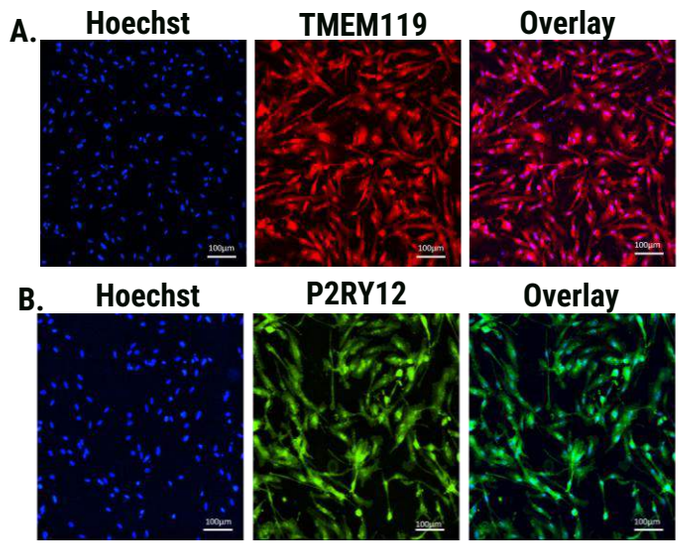


Figure 1. Representative images from immunofluorescence (IF) staining of iMGL expressing the microglia markers A. TMEM119 (red) and B. P2RY12 (green) and nuclei (Hoechst: blue

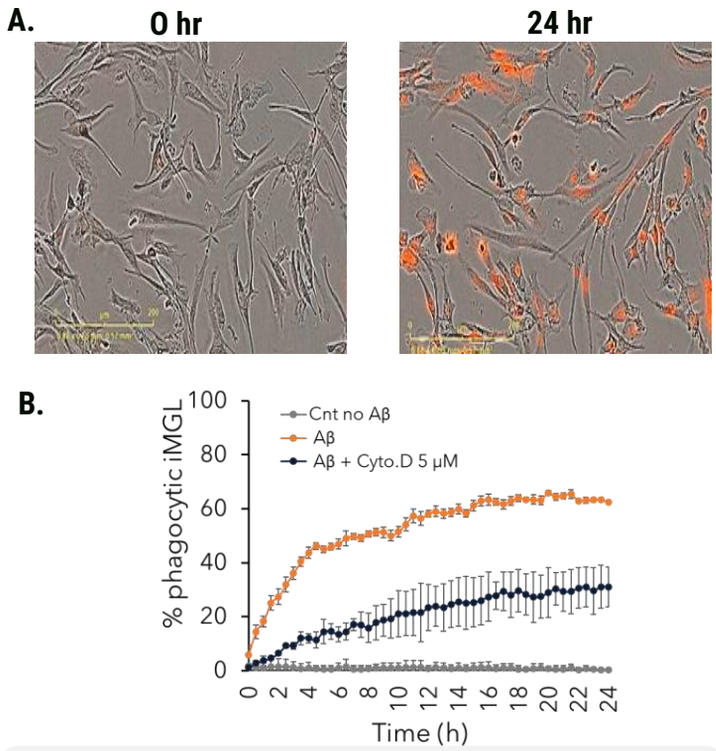


Figure 2. A. Representative images showing WT iMGL uptake of Aβ 1-42 peptide at 24 h. B. Percentage phagocytic iMGL plotted over 24 h

Results

Maintaining microglia for 7 days in culture (DIV7) resulted in a mature phenotype as measured by expression of key microglial markers, to include TMEM119 and P2RY12 (Figure 1.)

Phagocytosis of Aβ1-42 peptide by acidic lysosomes (pH 4.5–5.5), causes an increase in pHrodo fluorescence intensity that increases with time. The phagocytosis can be inhibited by agents that affect cytoskeleton proteins such as cytochalasin D (Figure 2.).

The end-point phenotypic imaging assay provides excellent resolution and allows the sub-cellular distribution pattern of the phagocytosed Aβ1-42 peptide to be examined. In addition, information on the population of microglial cells that are actively involved in clearing the Aβ1-42 cargo can also be studied (Figure 3).

We have multiplexed the immunofluorescence imaging assay to provide information on cell health, phagocytosis, TREM2 expression and sub-cellular localisation (Figure 4.)

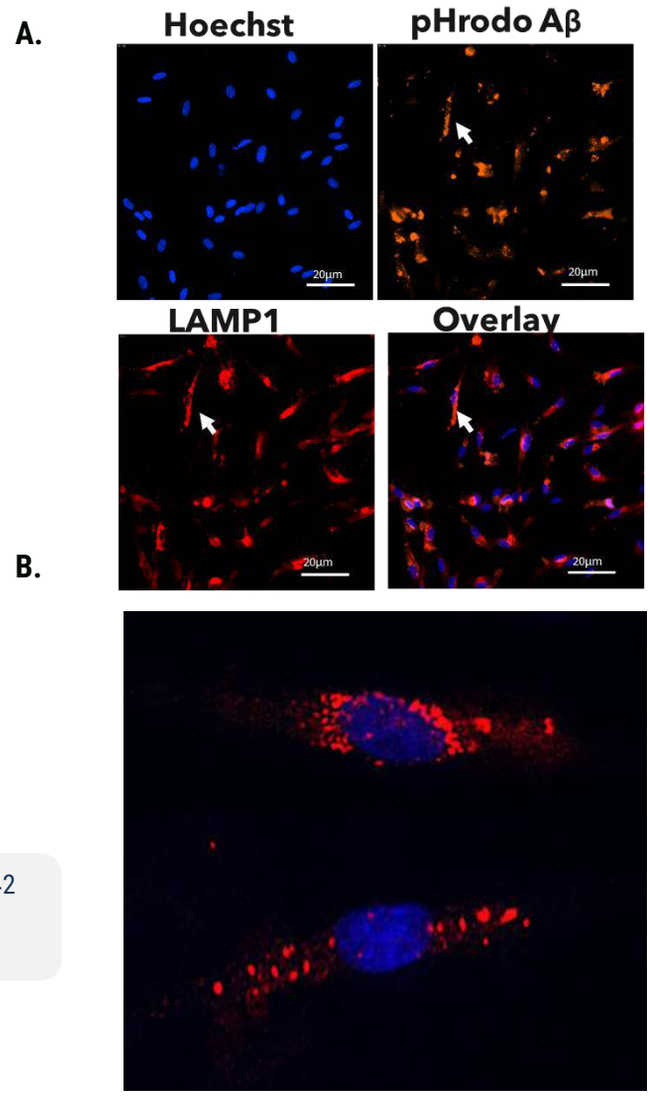


Figure 3. End-point phagocytosis assay showing engulfment of Aβ 1-42 peptide. A. Co-localisation (white arrow) of Aβ1-42 (orange) and LAMP1(red), images taken at 40X. B. Digitally magnified images

Conclusion

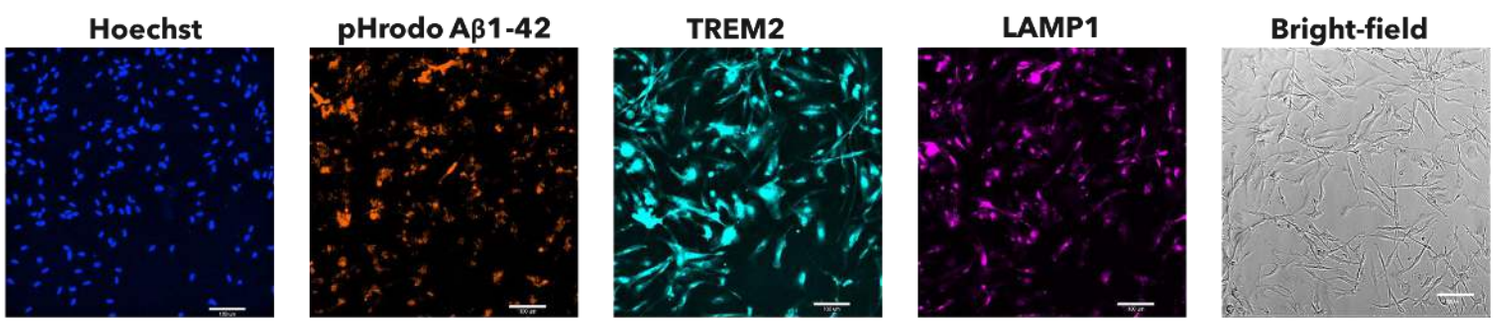


Figure 4. A Multiplexing high-content imaging assay. Representative image of wild-type (WT) iMGL shows nuclei (blue), Aβ1-42 (orange), TREM2 expression (cyan), LAMP1 (magenta) and bright-field.

We have successfully used human iPSC-derived microglia to develop and optimize two different 384-well based imaging assays. Both assay platforms provide a deep insight into how microglia phagocytose a pathological protein found in numerous neurodegenerative conditions. In particular, the high-content assay is able to multiplex up to 5 different parameters, including (i) total nuclei count, (ii) phagocytosis of Aβ1-42 peptide, (iii) TREM2 expression and distribution, (iv) LAMP-1 lysosome co-localisation, and (v) cell morphology.

These assays will be used to profile pharmacological compounds that may help to identify novel therapeutics that can modulate neuroinflammation. Our goal has been to incorporate more predictive humanized screening assays into our drug discovery programmes, deliver more successful projects, and identify neuroinflammation modulators to treat CNS diseases.

Multi-electrode array characterization of a human iPSC-derived motor neuron disease model for ALS drug discovery

Axion BioSystems | Atlanta, USA

Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects spinal motor neurons. Although the pathology of ALS is still poorly understood, several disease phenotypes are recognized to be associate with motor neurons from ALS patients[1]. A lack of standardized, easy-to-use, and readily accessible human cell models is a significant hurdle in ALS drug discovery. Hyperexcitability is a key trait relating to ALS pathology. This altered electrical activity is believed to contribute to the degeneration of motor neurons and the progression of ALS and, therefore, should be an essential attribute in an *in vitro* model of ALS.

1.

Figure 1. Axion BioSystems' Maestro Pro MEA (multielectrode array) platform

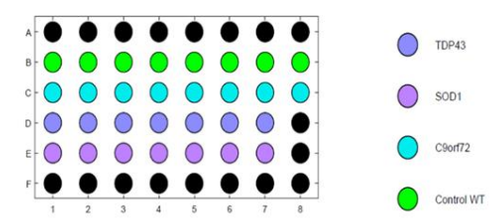
The Axion BioSystems' Maestro Pro MEA (multielectrode array) platform provides label-free assessment of neural activity and connectivity over time within a multi-well format, allowing for the simple analysis of complex biology. The Maestro Pro detects key aspects of neural networks, including activity, synchrony, and oscillation, to provide a comprehensive functional profile for disease modelling. In this study, we characterized motor neurons (Axol Bioscience) derived from iPSCs from 3 donors with ALS and one unaffected donor, to investigate functional phenotype differences between cell lines, including the key attribute of hyperexcitability.

Methods

MEA measurements are based on the detection of extracellular field potentials, which are the electrical signals generated by the activity of neurons or other excitable cells. These potentials arise due to the flow of ions across the cell membrane during action potentials or other electrical events.

To verify the cells as suitable disease models for functional screening, it is critical to identify whether the disease models demonstrate a disease relevant phenotype when compared to controls. axoCells™ Motor Neurons derived from unaffected and ALS patients were plated at 60K cells per well in a CytoView MEA 48-well plate and cultured for up to 20 days before their morphology and functional neuronal electrical activity were assessed. Firing activity of cells was assessed by 5-minute MEA recordings from DIV 10. Data was recorded using the "Neural Spontaneous" configuration in AxIS Navigator software (Axion BioSystems). Action potentials were detected via adaptive thresholding to assess a selection of both non-networked and networked parameters. Samples were from a minimum of 3 electrodes in the same well, demonstrating the differences or uniformity of firing patterns within each cell type.

2.a.



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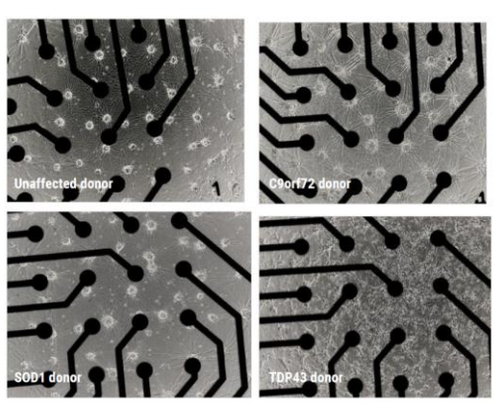

Figure 2. a. CytoView MEA 48 plate layout. b. Motor neuron coverage of the CytoView MEA plates. iPSC-derived motor neurons from four lines

1. (Neural Regen Res. 2024 May; 19(5): 1036–1044. doi: 10.4103/1673-5374.382985).

Results

Characterization by MEA

Quantification of activity profiles and raster plots shows that the ALS motor neurons have distinct firing profiles compared to unaffected donor motor neurons. Differences are observed in firing rate, spike amplitude, bursting profiles and networking profiles. All neurons produced clear burst and network burst activity and differing ALS disease lines displayed clear network firing differences in keeping with their phenotype and disease complexity (Figure 3).

3

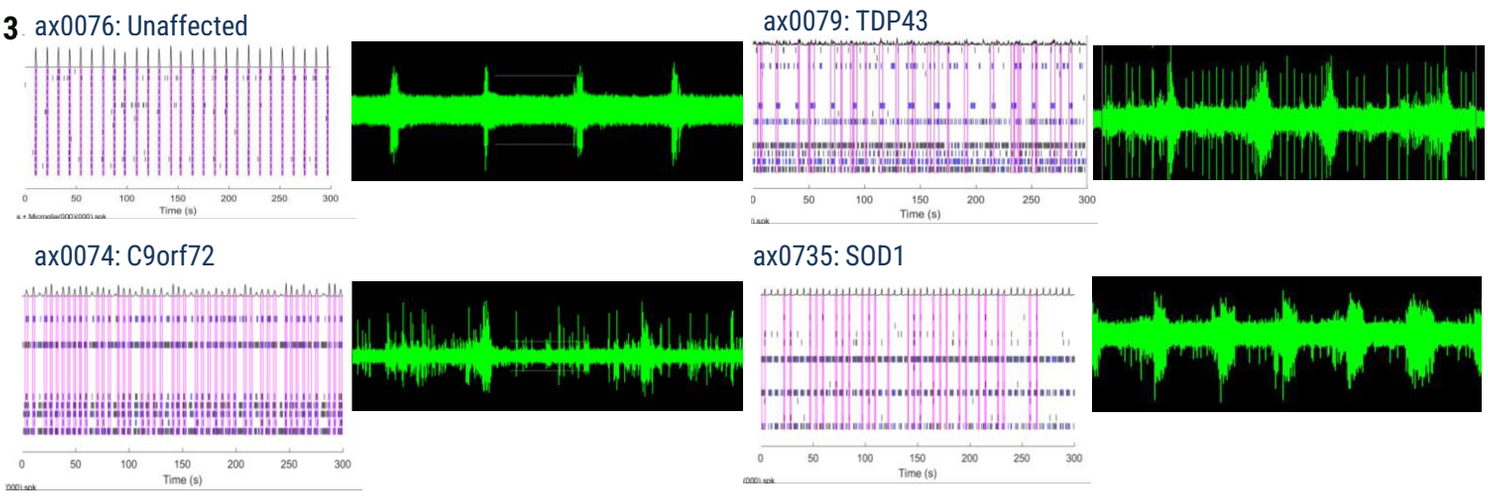

Figure 3: MEA Recordings of axoCells Motor Neurons (DIV 20). Raster plots (left) and voltage traces (right) show firing patterns of unaffected, TDP43, C9orf72, and SOD1 motor neurons over 300 seconds. Raster plot ticks are spikes; blue ticks are single-electrode bursts; pink boxes are network bursts. Different cell lines exhibit distinct electrophysiological activity.

Neural activity recording and analysis with the Maestro MEA platform

Action potentials were detected via adaptive thresholding to be processed and assessed quantitatively as both individual and network parameters, including measurements of firing, bursting, and synchrony. Data analysis was performed using the AxIS Metric Plotting Tool and wells were excluded if below 0.1. Hz Mean Firing Rate to generate the following raster plots: (Figure 4). Error bars indicate SEM, n=3–10 independent technical repeats against 10 wells per condition.

4.

Firing parameter	Wildtype	C9orf72	SOD1	TDP43
No. of spikes	→	→	→	↑
Mean firing rate	→	→	→	→
No. of spikes per burst	→	→	↘	↓
Inter-burst interval	→	↑	↑	↘
ISI-COV	→	↘	↘	↓
No. of bursts	→	↘	→	↑
Burst duration	→	↘	↘	↓
No. of network bursts	→	→	→	↑
Network burst duration	→	↘	→	↑
No. of spikes per NB	→	→	↘	↓
Network burst %	→	↑	↓	↘
Network normalised duration IQR	→	→	→	↑
Synchrony index	→	↘	→	↓
Spike amplitude	→	↑	→	↑

Table Key	
Increased parameter value	⇨
Decreased parameter value	⇨
Comparable to wildtype	⇨
Small Decrease or increase – extent of change reflected in arrow angle for parameter	↘
Large Decrease or increase	↓ ↑

Figure 4. Summary of MEA graphical data, changes in the parameter indicated by color and arrow direction, as described in the table key above. We were able to visualize key parameters to distinguish between each of the axoCells motor neurons, ALS phenotypes.

Conclusions

Motor neurons were assessed for ALS related morphology and neuronal firing activity using the Maestro MEA platform to compare network activity and functional network formation of individual cell types. In an 'unaffected control' line we saw synchronization, short burst events and constant signal amplitude. In comparison, motor neurons from the ALS lines demonstrated a reproducible loss or gain of function for certain parameters in each phenotype.

The reproducible functional characterization of these cells allows for functional QC to be incorporated into the standard QC testing of iPSC-derived motor neurons improving the QC process and to demonstrate batch-to-batch consistency.

All ALS phenotype lines displayed a reproducible loss of synchronous firing and different degrees of a hyperexcitability phenotype. This is in accordance with the understood ALS clinical pathology and supports the case for the use of these cells in an experimental *in vitro* model to study ALS pathology and disease.

SmartHeart: An innovative high throughput assay to generate and assess cardiac micro tissues from hiPSCs answering to the current challenges of cardiac toxicity and efficacy

Ahmed Khedher, Patricia Davidson, Stijn Robben, Magali Seguret, Cyril Cerveau, Maël Le Berre, Rita S. Rodrigues Ribeiro
4Dcell | 14 rue de la Beaune 93100 Montreuil

Abstract

Cardiac adverse events are a major cause of drug development failure, with cardiotoxicity accounting for one-third of regulatory setbacks, which highlights the need for predictive human-relevant cardiac models. This app note describes the validation of 3D cardiac tissues using Axol Bioscience's iPSC-derived cardiomyocytes and cardiac fibroblasts on the SmartHeart® platform, developed by 4Dcell. The tissues exhibited reproducible contractile behavior and responded to pharmacological agents in a physiologically relevant manner. Isoprenaline increased heart rate and contractility stress, while dofetilide reduced contractile parameters, confirming the platform's ability to detect diverse drug effects. Combining physiological relevance with high-throughput capabilities, this system represents a robust alternative to traditional *in vitro* models.

Methods

The SmartHeart® platform integrates Axol Bioscience's high-quality iPSC-derived cardiomyocytes and cardiac fibroblasts to create physiologically relevant 3D cardiac tissues. The workflow begins with seeding Axol's cardiomyocytes and fibroblasts in an engineered 96 - well plate, facilitating their self-organization into contractile ring-shaped tissues. The 3D cardiac tissues develop spontaneous contractility with consistent beating patterns, enabling detailed functional assessment.

The platform enables direct drug exposure and imaging, supporting video recording for contractility (brightfield), and electrophysiology/calcium handling (fluorescence) assessment. This all-in-one approach eliminates the need for tissue transfer between platforms, maintaining tissue integrity throughout the studies while generating rich, multi-parametric datasets that better predict human cardiac responses compared to traditional 2D cultures or animal models.

1.

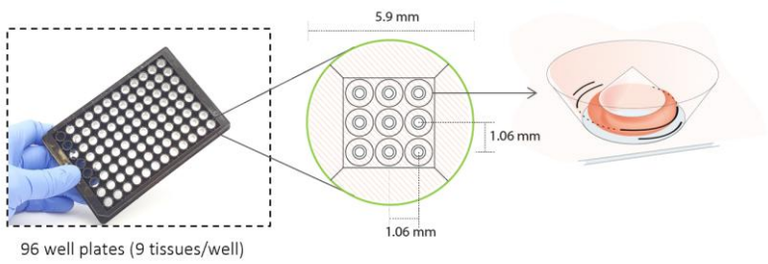

Figure 1. Overview of the SmartHeart platform. The technology is based on standard 96-well plates coated with a structured hydrogel (PEG based), which features an array of conical-shaped microwells, each surrounding a central pillar, enabling the formation of ring-shaped cardiac tissues.

2.

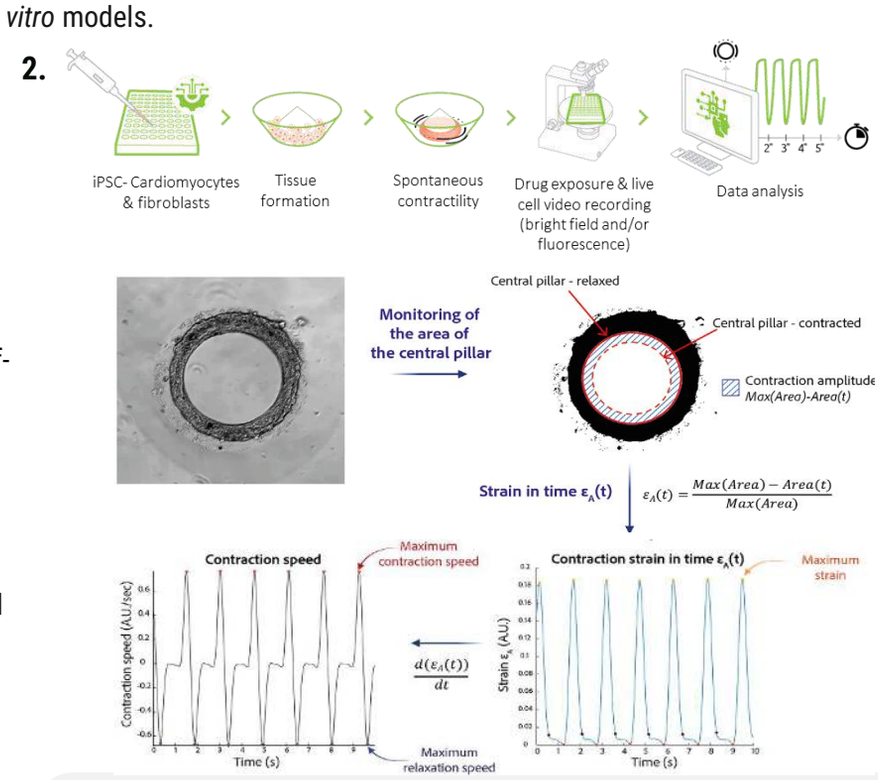

Figure 2. Workflow for cell seeding and contractility analysis. Beating tissues are assessed using 4Dcell's SmartX software, which employs machine learning to detect the central pillar and track area changes over time, calculating strain (εA) and its first derivative (contraction and relaxation speeds) at day 14. Stress is derived as the product of strain and the Young's modulus of the PEG-based hydrogel.

Results

The functional response of the ring- shaped tissues was validated using well-established pharmacological agents. Treatment with the β-adrenergic agonist isoprenaline demonstrated dose-dependent positive chronotropic and inotropic effects, characteristic of mature cardiac tissue.

The organoids exhibited a rapid response beginning at low nanomolar concentrations, with beating frequency increasing approximately 1.7-fold and reaching a plateau at ~1000 nM. Simultaneously, contractile parameters showed significant enhancement, with contraction stress increasing by ~20%, maximum contraction speed by ~55%, and maximum relaxation speed by ~95% at 1000 nM, maintaining these elevated levels through the highest tested concentration (10,000nM). Conversely, exposure to the hERG channel blocker dofetilide produced pronounced negative inotropic effects, dramatically reducing maximum contraction speed, relaxation speed, and contraction stress by approximately 60-80% at concentrations as low as 10 nM, with continued modest decreases at higher concentrations. This bidirectional pharmacological response profile confirms the presence of functional adrenergic signaling pathways and ion channels within the tissues, demonstrating its relevance for detecting both stimulatory and inhibitory drug effects on human derived cardiac tissues with high sensitivity.

Furthermore, these tests can be combined with electrophysiology and/or calcium assessments, leveraging the platform's flexibility to acquire multiple readouts on a single platform.

Conclusions

The SmartHeart® platform combining Axol Bioscience's hiPSC-derived cardiac cells demonstrates remarkable utility for evaluating drug-induced effects on human cardiac function. These results confirm that self-assembled 3D cardiac tissues recapitulate key aspects of native cardiac physiology, including appropriate responses to both positive inotropic stimulation and ion channel modulation. The multi-parameter analysis capabilities—simultaneously measuring beating frequency, contraction stress, and contraction/relaxation kinetics—provide comprehensive insights into cardiac contractile function. Moving forward, it is planned to expand the pharmacological validation by evaluating additional compounds targeting various cardiac ion channels and exploring the effects of other classes of inotropic drugs. This continued development will further establish the predictive value of the SmartHeart® platform for cardiac safety assessment and efficacy testing, offering pharmaceutical researchers a powerful tool for evaluating compound effects on human derived cardiac tissues with high physiological relevance and translational potential.

3.

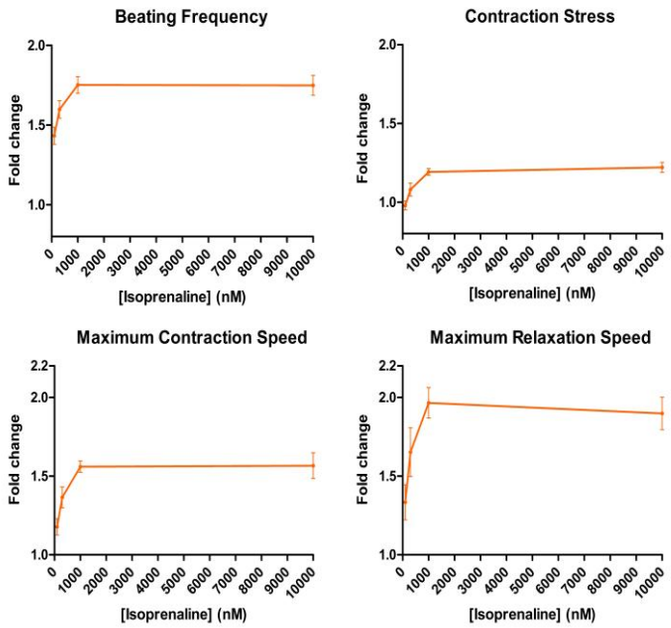

Figure 3. Drug testing on the cardiac tissues. Effect of isoprenaline on tissue contractility: beating frequency, contraction stress, and maximum contraction and relaxation speeds. These parameters are expressed as a ratio between their value for each concentration and the value with DMSO control (N = 2 ind. exp).

4.

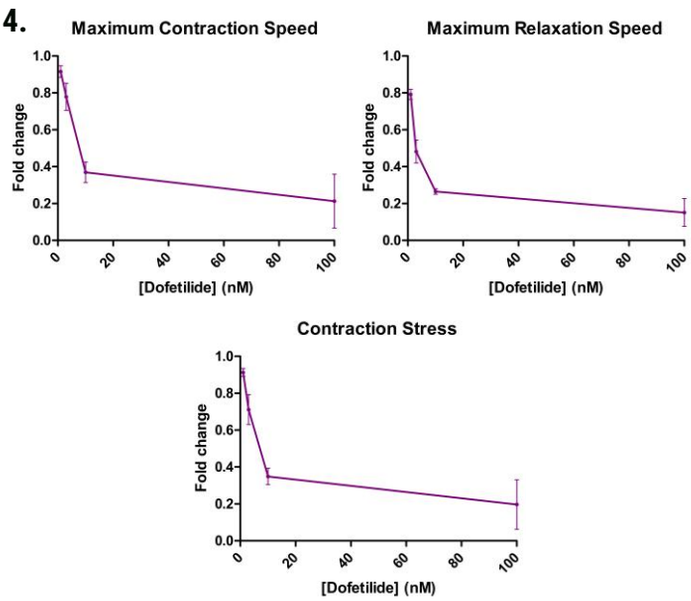

Figure 4. Drug testing on the cardiac tissues. Effect of dofetilide on tissue contractility: contraction stress, and maximum contraction and relaxation speeds. These parameters are expressed as a ratio between their value for each concentration and the value with DMSO control (N = 2 ind. exp).

AF on Demand : A Human iPSC-Derived Screening Assay Targeting Atrial Fibrillation

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innoVitro GmbH | Juelich, Germany

Abstract

Atrial fibrillation (AF) is the most common form of cardiac arrhythmia, significantly impacting morbidity and mortality worldwide.

Using commercially available human induced pluripotent stem cell derived atrial cardiomyocytes (hiPSC- atrial CMs) from Axol Bioscience (axoCells™), we developed an AF-like human phenotype *in vitro* disease model.

The drug screening assay is designed to assess AF-related functional changes in a human-relevant context, with extracellular field potential duration (EFP Duration, CardioExcyte 96) as readout parameter.

Methods

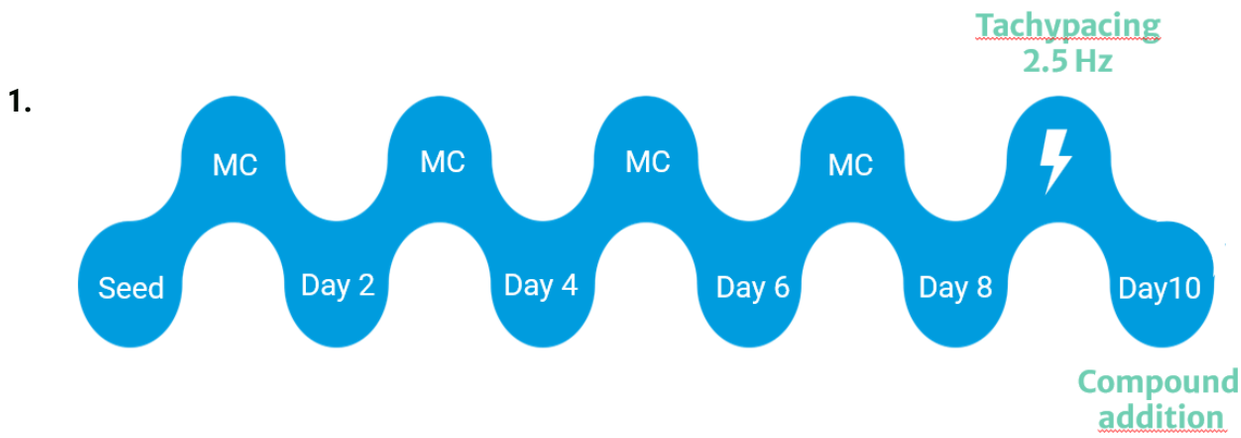


Figure 1. hiPSC-derived atrial CMs tachypacing protocol.

- Preculture of atrial hiPSC-CMs on CardioExcyte plates for 9 days including medium change (MC) every other day
- Begin tachypacing on day 9 for 24h using the CardioExcyte 96 system -> induction of AF phenotype with reduced EFP duration
- Compound addition 24h after beginning of tachypacing and chronic EFP duration assessment

Results

Tachypacing of hiPS-derived atrial cardiomyocytes for 24 h resulted in a significant EFP duration shortening down to 60 % in comparison to pre-tachypacing EFP duration (100%). Class III antiarrhythmic drugs ibutilide, dofetilide and sotalol are known therapeutics for restoring normal heart rhythm in AF. All three compounds show respective EFP duration prolonging effects in the here presented in vitro disease model using hiPSC-atrial CMs.

2.

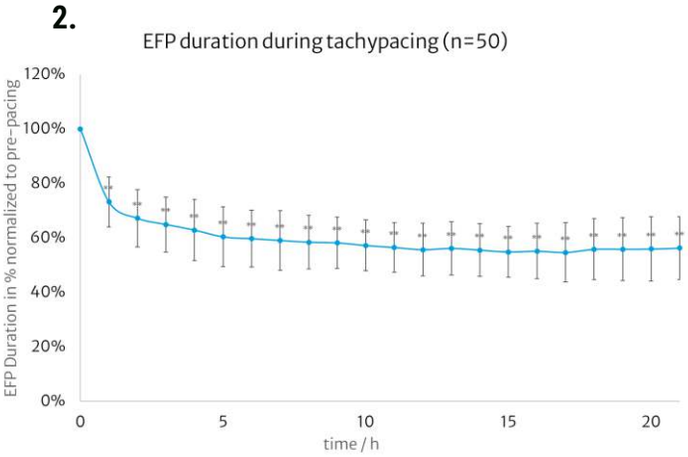


Figure 2. Effect of tachypacing (2.5Hz) on human iPSC-atrial CMs. Tachypacing for 24h shows a 40% decrease in EFP duration after approx. 5 h (n=50). Statistical analysis performed with Wilcoxon-Mann-Whitney (WMW) test.

3.

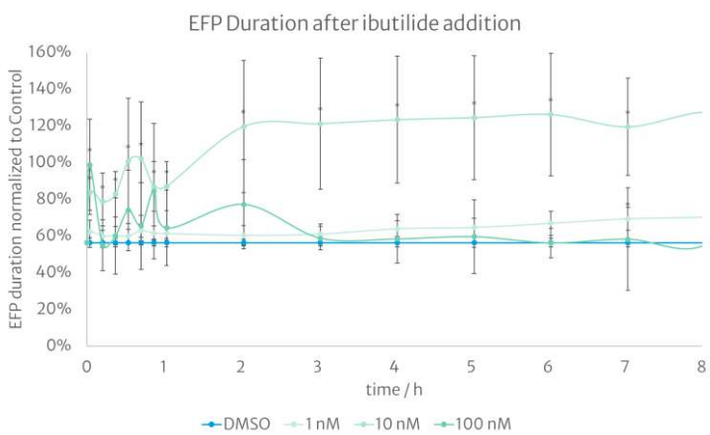


Figure 3. Effect of ibutilide on human iPSC-atrial CMs under 2.5Hz tachypacing condition. 10 nM of ibutilide exhibit a significant increase in EFP duration from 60% - due to tachypacing - up to 120%. Statistical analysis: WMW test.

4.

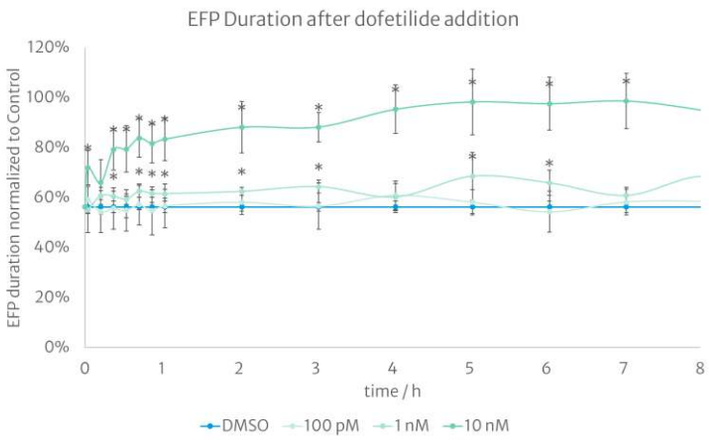


Figure 4. Effect of dofetilide on human iPSC-derived atrial CMs under 2.5 Hz tachypacing shows a concentration-dependent increase in EFP duration from 60 % - due to tachypacing - to almost 100 % after 10 nM dofetilide addition. Statistical analysis: WMW test.

5.

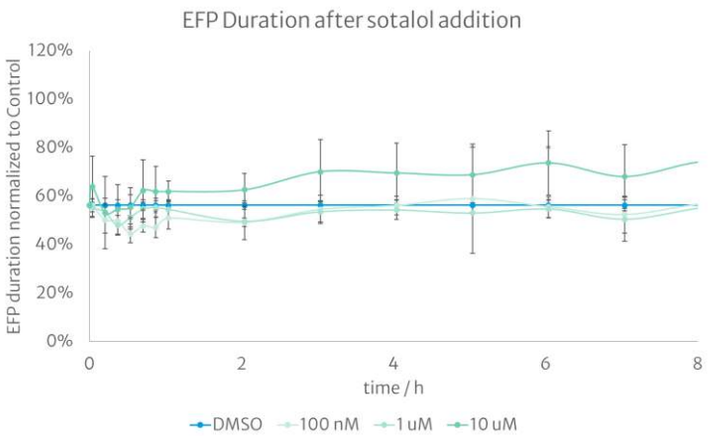


Figure 5. Effect of sotalol on human iPSC-derived atrial CMs under 2.5 Hz tachypacing shows an increase in EFP duration from 60 % - due to tachypacing - up to 75% after 10 µM sotalol addition. Statistical analysis: WMW test.

Conclusions

This human-based model represents a meaningful advancement in atrial fibrillation research by delivering data that's more predictive of clinical outcomes. It enables earlier, smarter decisions by providing a platform that better reflects human cardiac biology. For drug developers, that means lower attrition, faster timelines, and greater confidence in moving the right therapies forward.

Characterizing sensory neurons as universal bio-digital sensor to explore PNS applications

NETRI | 76 rue Georges Gouy, 69007, Lyon, France

Abstract

By combining NETRI's NeuroFluidics™ platform with Axion's Maestro MEA system, we developed a method to transform hiPSC-derived sensory neurons into reliable bio-digital sensors.
Fluidic isolation of somas and stimulation of endings enables reproducible electrophysiological responses in a setup mimicking human physiology.
This supports compound screening in PNS-related fields, from dermato-cosmetics to pain. Multi-metric analysis enables digital fingerprinting and advanced functional screening.

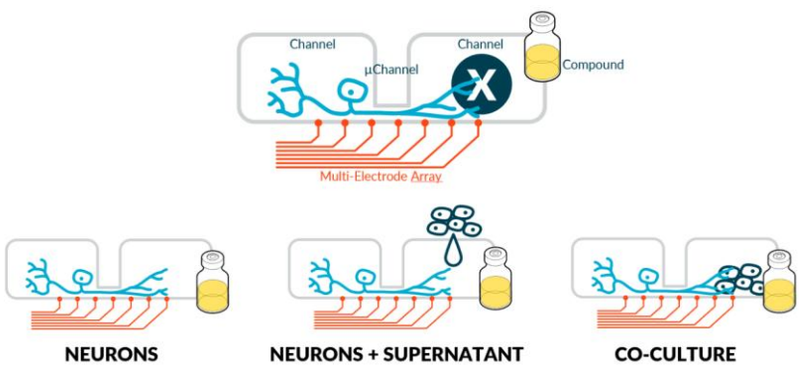
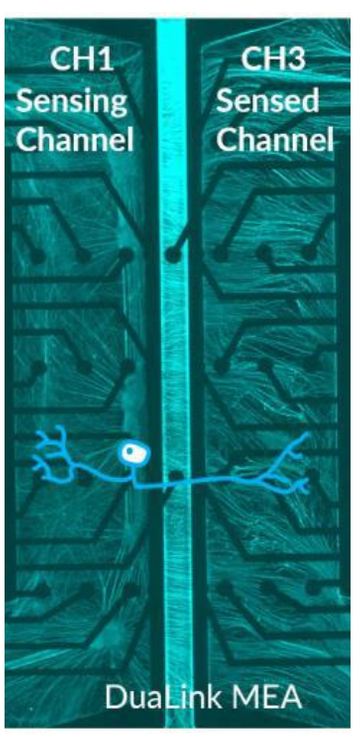


Figure 1. Schematic representation of neurons as a sensor approach.

Methods

We used the NeoBento™ standard SBS format (Hardware, HW) outfitted with up to 16 DuaLink™ MEA chips, that combine NETRI's microfluidic architectures with a bespoke MultiElectrode Array (MEA) surface from Axion BioSystems. Metrics are extracted using NETRI's UpLink™ (Software, SW) piloting Axion BioSystems' AxIS Navigator. Extracted data was processed in NETRI's database DataLink.

This HW/SW platform allows users to fluidically isolate somas from their endings (and eventually target organs) through microchannels. It can also perform compartmentalized functional activity recording, specifically in the ultra-sensitive µchannels that host the sensory neuron axons that ferry data from the sensed compartment to the sensing compartment.



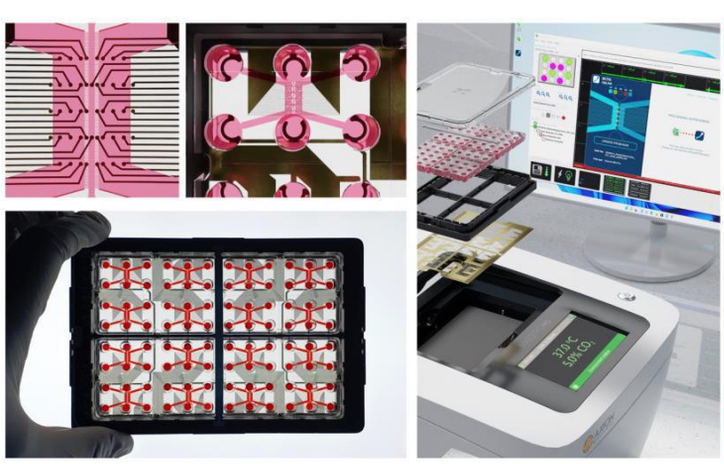


Figure 2. Hardware and Software setup. DuaLink™ MEA Chip in it's NeoBento™ SBS format, Axion BioSystems' Maestro Pro™ MEA system, Axion BioSystems' AxIS Navigator, and NETRI's UpLink software. A sensory culture in DuaLink™MEA with somas in Channel 1 and Ending in Channel 3.

Results

Sensor Repeatability

To ensure repeatability, we partnered with Axol Bioscience for axoCells™ batch selection, standardized the culture protocol on DuaLink™ MEA chips, and seeded neurons only in Channel 1.
Cultures ran for 20 days. A QC method based on basal MEA activity excluded outliers.
Non-destructive recordings allowed self-referencing: comparing each chip's baseline to its response after compound addition. This mitigates variability and enables robust statistical analysis.

Sensor Sensitivity

Sensor sensitivity was evaluated using reference compounds via two protocols: Time Response, for prolonged effects, and Fast Response, for transient responses. Time Response compares sequential baseline and compound-exposed periods; Fast Response captures real-time differences between vehicle, compound, and control. In both, stimuli applied to Channel 3 elicited statistically significant responses based on DuaLink™ MEA microchannel metrics, demonstrating robust, application-relevant sensitivity in sensory neurons.

Sensor Sensitivity

MEA metrics enable sensor specificity beyond sensitivity limits. We tested veratridine, lidocaine, and their vehicles (DMSO, H_2O) applied to Ch3 neurites. Metrics with Pearson scores <0.8 and high information gain or PCA relevance were selected. Discriminative metrics, primarily from microchannels, revealed clear distinctions between all states, including vehicle types. These findings show that beyond mean firing rates, specific MEA patterns enable altered state fingerprinting, supporting digital signature development.

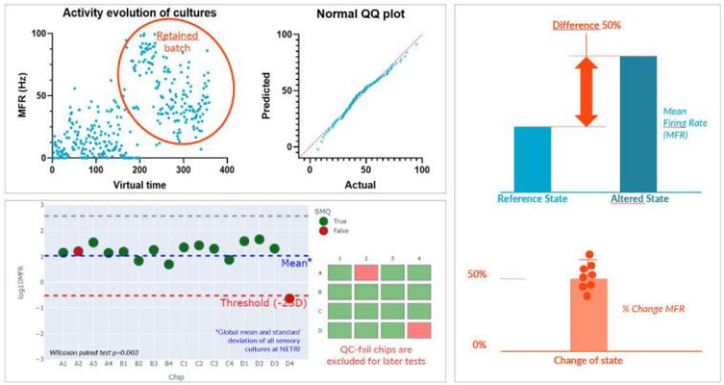


Figure 3. Supplier batch monitoring and selection. QC test and chip selection hiPSC sensory neurons DIV20. Focusing on proportional change of state.

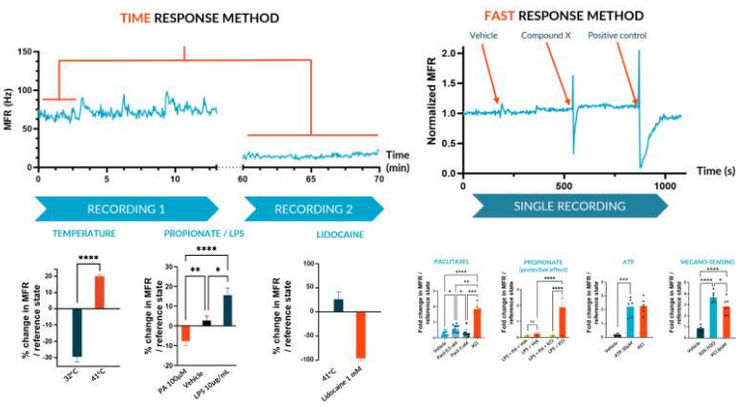


Figure 4. Sensor response (TIME and FAST)

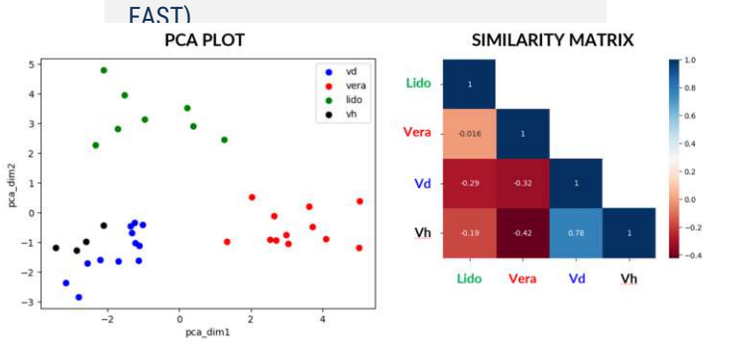


Figure 5. Principal component analysis over standardized Axion metrics. Compound similarity matrix based on Axion metrics with highest information gain (top5). All based on microchannel metrics.

Conclusions

Neurons-as-sensors enable standardized PNS functional analysis via fluidically isolated cultures.

This allows compound, nutrient, or drug effects to be assessed across diverse fields. By defining digital signatures as MEA-derived n-dimensional ratio matrices between healthy and altered states, we reduce culture variability and quantify drug effects reliably. This non-destructive method works on acute or chronic timescales, without imaging or chemical assays.

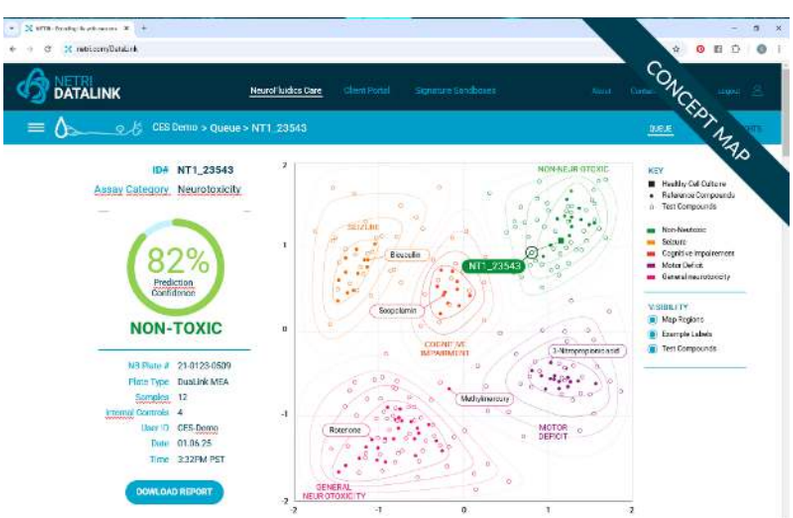


Figure 6. Concept map of Digital Library for PNS Applications.

Thank you to our collaborators who are working on breaking new ground in new approach methodologies

3Brain - Electrophysiological analysis of atrial and ventricular axoCells™ cardiomyocytes utilizing 3Brain's CorePlate ™ enabled HD-MEA

BiomimiX - Beating heart-on-chip to model atrial and ventricular cardiac chambers

Axion Biosystems (CytoTronics) - Advancing ALS modeling: high-dimensional insights with CytoTronics' Pixel

Systasy Bioscience - Pathway profiling of axoCells™ sensory neurons

Ushio - Neurotoxicity evaluation via nerve MPS and machine learning

Mimetas - A 3D neurite outgrowth model for studying motor axon biology

Sygnature Discovery - A high content imaging screening assay to identify novel neuroinflammation modulators using human iPSC-derived microglia

Axion BioSystems - Multi-electrode array characterization of a human iPSC-derived motor neuron disease model for ALS drug discovery

4DCell - SmartHeart: An innovative high throughput assay to generate and assess cardiac micro tissues from hiPSCs answering to the current challenges of cardiac toxicity and efficacy

innoVitro - AF on demand: a human iPSC-derived screening assay targeting Atrial Fibrillation

NETRI - Characterizing sensory neurons as universal bio-digital sensor to explore PNS applications

Quality manufacturing
We manufacture iPSC-derived cell types from our ISO9001:2015 production facility.

Global logistics
We supply our products worldwide to pharma, biotech, research and technology providers.

Dedicated team
With a highly skilled, science-rich team, we work collaboratively to realize better human disease models.

If you would like to discuss the adoption of iPSC-derived complex models into your workflow, or our specialist services provision, please contact us at operations@axolbio.com

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