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# THE FUTURE OF MICROELECTRODE ARRAY IN EARLY TOXICOLOGY ASSESSMENT

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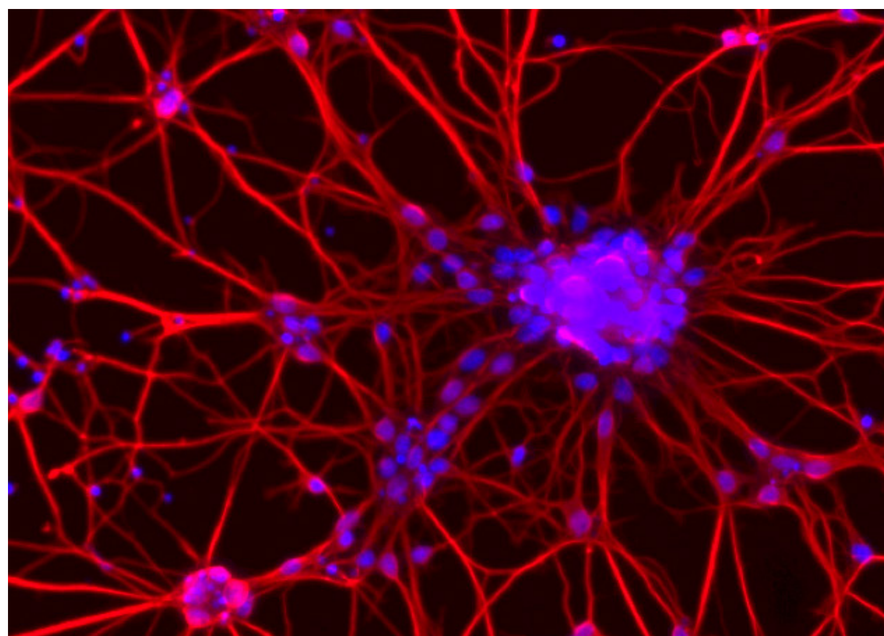
## BACKGROUND INFORMATION

Late phase failures are all too common with 90% of compounds failing during clinical development[1]. Much of this late stage attrition can be attributed to safety issues. Over the last 15 years, there has been an emphasis on developing *in vitro* techniques which can more accurately reflect and predict toxicity issues much earlier in the drug discovery and development process to reduce the cost and time burden associated with late stage attrition.

In this article, we review the current challenges within the pharmaceutical industry and how companies are addressing these challenges using human relevant cell-based models. The article will delve deeper into microelectrode array (MEA) and its key role in *in vitro* toxicology testing.



## THE COST OF FAILURE

Recent estimates suggest that the cost of getting a new drug to market is in the region of $2.6 billion[2] and can take up to 15 years. When considering these statistics, it is easy to understand why drug attrition is a major source of concern for the pharmaceutical industry especially when it occurs in late stage clinical development where it is reported approximately 90% potential drugs fail[1].

Although the reasons for attrition are varied, the majority of the failures can be attributed to the key factors of toxicology/safety, efficacy, pharmacokinetics, formulation or commercial issues[3]. An analysis by Waring *et al.,* (2015)[4] in *Nature Reviews* reviewed the attrition of drug candidates from four major pharmaceutical companies (AstraZeneca, Eli Lilly, GlaxoSmithKline and Pfizer). Interestingly, the review identified that safety and toxicology were the largest sources of drug failure during preclinical and clinical development for the data set analysed. Identifying these safety issues earlier is key to improving this statistic. In fact, this has been successfully demonstrated in the case of pharmacokinetics where the late stage attrition has reduced dramatically[3] as a consequence of standardised early stage ADME testing and relatively stringent regulatory requirements for *in vitro* drug-drug interaction evaluation. To replicate this approach in the field of toxicology, reliable and predictive human relevant models need to be available to complement or even replace traditional pre-clinical animal testing, and, over the past 10–15 years, there has been a drive within the industry to develop these models. This first gathered momentum with the publication of the report 'Toxicity Testing in the Twenty-First Century'[5] in 2007, which recognised the inefficiency in expensive and lengthy *in vivo* animal testing and proposed a shift towards high throughput human relevant cell-based models with mechanistic quantitative endpoints for the prediction of toxicity. Several initiatives now exist to investigate, research and/or promote alternatives to animal testing within the field of toxicology for pharmaceuticals and chemicals. These include ECVAM, NC3Rs, Safer Medicines Trust, CiPA, REACH, EPA Toxcast, HESI and FDA Tox working group to name a few. Through these initiatives, more robust and predictive human relevant models are now becoming available which will assist in standardising early stage toxicology testing and radically changing the way in which the industry conducts this testing.

Developing cell-based models is only one part of the puzzle and these new models need to be coupled with sophisticated analytical tools which provide relevant and highly sensitive and selective readouts. One such tool which is showing immense value in cardiotoxicity and neurotoxicity testing is MEA. In this article, we evaluate this technology and demonstrate how it has been instrumental in transforming our understanding of organ-specific toxicity.

## BACKGROUND TO MEA

It is almost 50 years since the first publication on the use of MEA on cultured cells[6]. However, the power of this technology in toxicology testing has only just been realised due to the advent of the multi-well MEA allowing for its utility in high throughput analysis.

MEA uses plates composed of up to 96 wells, each of which contains multiple individually embedded microelectrodes which continuously monitor the electrical behaviour of cells. Electrically active cells, such as neurons or cardiomyocytes, are then cultured over these microelectrodes. Once the cultures become established, they form cohesive networks and present an electrophysiological profile. The resulting electrical activity (either firing of neurons, or the uniform beating of cardiomyocytes) is captured from each electrode in each well on a microsecond timescale providing both temporally and spatially precise data across an entire population of cells. These MEA plates can then be used to assess the behaviour of the cultured cells in the presence of new chemical entities and, therefore, predict drug-induced toxicity. Due to the technology being label-free and non-invasive, continuous monitoring over long periods can occur, which allows the system to mimic extended dosing regimens in clinical trials.

**Figure 1:** *An example of the design of a typical MEA plate. Each well contains multiple individually embedded microelectrodes which continuously monitor the electrical behaviour of cells. Image provided by Axion Biosystems.*

MEA is one of the most sophisticated and sensitive technologies for measuring changes in such spontaneously-active cells. This technology is rapidly becoming the cross-industry standard for assessing cardio- and neurotoxicity. Let us explore the technology in more detail to understand how it is being used in toxicology testing.

## ROLE OF MEA IN CARDIOTOXICITY TESTING

### Predicting Cardiotoxicity – Is hERG Enough?

Cardiotoxicity is one of the leading causes of toxicity-related attrition leading to discontinuation of clinical trials and withdrawal of drugs from the market[7]. The current preclinical (ICH S7B[8]) and clinical (ICH E14[9]) safety guidelines require a preclinical electrophysiology test using the human-ether-à-go-go Related Gene (hERG) assay followed by an *in vivo* QT measurement. However, these measures are surrogates for, rather than automatic predicators of, Torsades de Pointes (TdP) and proarrhythmia. It has become clear that these guidelines may have resulted in the premature discontinuation of potentially valuable new therapies by focusing solely on hERG block and QT prolongation.

### Changing Regulatory Landscape

The Comprehensive *in vitro* Proarrhythmia Assay (CiPA)[10] initiative was established following a workshop at the United States Food and Drug Administration (US FDA) in 2013. The objective of the initiative is to develop new methods for assessing proarrhythmic risk with improved specificity over existing approaches. The CiPA Steering Team is composed of members from the US FDA, Health and Environment Sciences Institute (HESI), Cardiac Safety Research Consortium (CSRC), Safety Pharmacology Society (SPS), European Medicines Agency (EMA), Health Canada, Japan National Institute of Health Sciences (NIHS) and the Pharmaceuticals and Medical Devices Agency (PMDA). The key goal of the initiative is to inform future regulatory guidance for ICH S7B[8] and ICH E14[9] with more relevant methods for predicting nonclinical cardiotoxicity.

One of the methods which has been validated by CiPA for proarrhythmic potential assessment is MEA using spontaneously beating human-induced pluripotent stem cell (iPSC)-derived cardiomyocytes[10]. Human iPSC-derived cardiomyocytes are

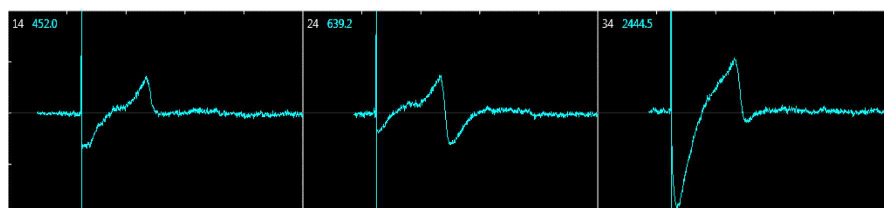



**Figure 2:** *MEA trace illustrating a typical ECG-like response.*

very similar to human primary cardiomyocytes both in their physiology and in their electrical 'ECG-like' response to drugs. Although the CiPA validation is not yet fully complete, it is expected that data generated by the MEA platform will be an important component of the new regulatory guidance.

**Monitoring Cardiotoxicity in Real Time using MEA**

Many disorders of the heart are the result of subtle changes to cardiomyocyte excitability, contractility, or both. MEA can measure the four key aspects of functional cardiac performance, label-free and in real-time in every well of the multi-well plate. These include propagation, field potential contractility and action potential. From these, the MEA system detects key parameters of cardiomyocyte activity, including depolarisation, propagation of excitability, repolarisation, beat timing and irregular beating (arrhythmia). These non-invasive electrical measurements mean that there is no need to use dyes or other reporters which can limit the sensitivity and specificity of the analysis.

Typically, a highly purified population of cardiomyocytes differentiated from human iPSCs are used. The cells are a mixture of spontaneously electrically-active atrial, nodal, and ventricular-like myocytes. They possess typical human heart cell characteristics forming electrically connected layers that beat in synchrony and exhibit expected electrophysiological and biochemical responses upon reference drug exposure.

MEA has the advantage that the cells are directly probed in the same plate in which they are cultured. Other high throughput platforms, such as the automated patch clamp or flow cytometry, require cell samples to be transferred into a single-cell suspension before testing. This process does not mimic the network of connected cells in the heart, and the multiple cell harvesting steps add time and cost to the analysis. MEA captures cardiomyocyte functionality while preserving the morphology of a cardiac cell model.

**Acute Cardiotoxic Effects – Defining Mechanism using MEA**

MEA can be used to define characteristic responses to specific ion channel or receptor interaction as it measures the contribution of all channels involved in the electrophysiological response rather than just a single ion channel. In this section, we explore this concept further by analysing the traces observed following cell treatment with fast sodium channel blockers, hERG inhibitors and ß-adrenergic receptor agonists[11].

## CASE STUDY 1:
## FAST SODIUM CHANNEL BLOCKERS AFFECT THE SODIUM AMPLITUDE

The MEA trace in Figure 3 shows that the amplitude of the signal is reduced in the presence of the 2 µM tetrodotoxin, a potent neural sodium channel blocker. It has an $IC_{50}$ of between 1 and 2 µM in the cardiac fast Na channel, Nav1.5.

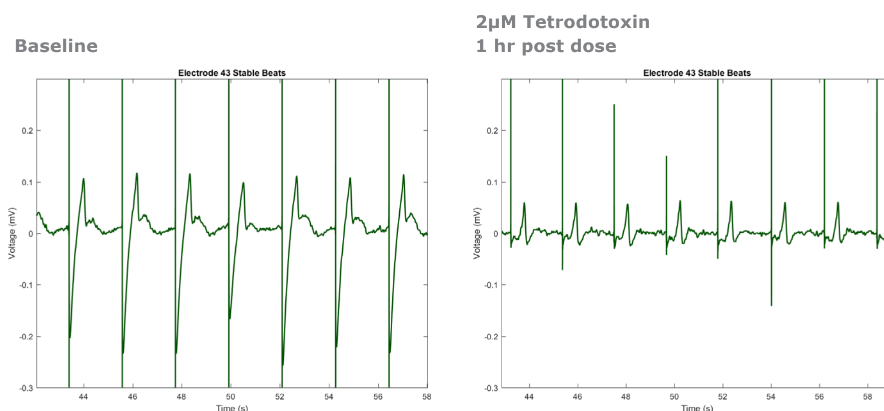



**Figure 3:** *MEA traces illustrating the effect of treatment of human iPSC-derived cardiomyocytes with the fast sodium channel inhibitor, tetrodotoxin, compared to untreated cells[11].*

## CASE STUDY 2:
## hERG INHIBITORS AFFECT BEAT RATE, T-WAVE AND PROVIDE EVIDENCE OF EARLY AFTER DEPOLARISATION

hERG inhibition is one of the key mechanisms involved in QT prolongation which potentially can lead to Torsades de Pointes. From the traces in Figure 4, it can be seen that both quinidine and E-4031 inhibit the T-wave response. There is also evidence for the formation of Early After Depolarisation (EAD) events which have been linked to Torsades de Pointes and arrhythmias. The beat rate is also decreased in both instances.

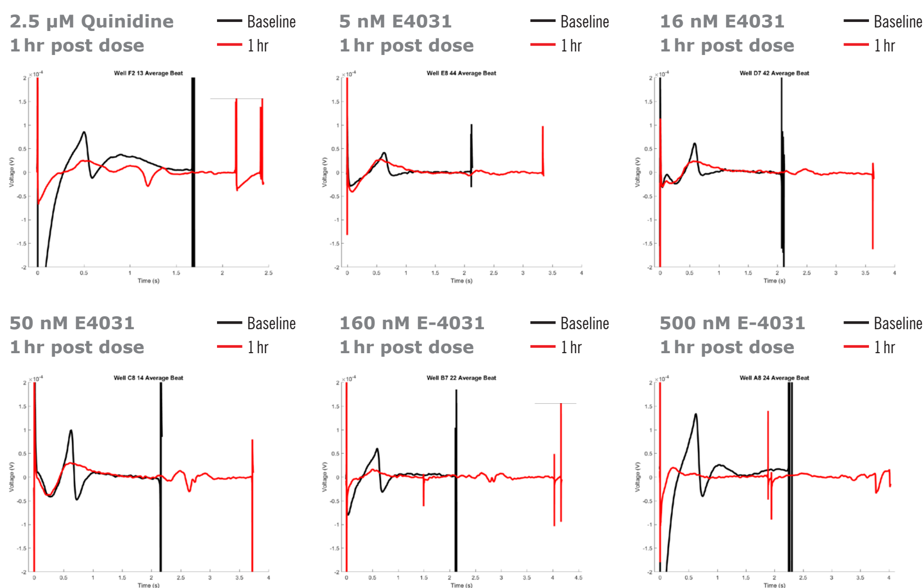



**Figure 4:** *MEA traces showing effect of hERG inhibitors (A) quinidine and (B) E-4031 on human iPSC-derived cardiomyocytes*[11].

## CASE STUDY 3:
## ß-ADRENERGIC RECEPTOR AGONISTS REDUCE FIELD POTENTIAL DURATION AND INCREASE BEAT RATE

The ß-adrenergic agonist, isoproterenol, has no effect on the T-wave amplitude but decreases the field potential duration which correlates to beat rate (Figure 5). This effect demonstrates effective expression of ß-adrenergic GPCR in the cells. Case Studies 1–3 illustrate how MEA in combination with human iPSC-derived cardiomyocytes is a powerful screening tool for acute cardiac effects – highlighting mechanism of the effect through specific perturbations of the electrophysiological profile.

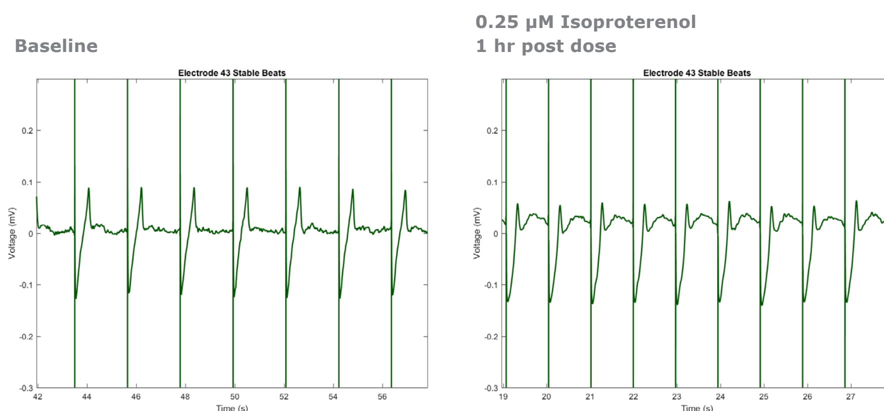



**Figure 5:** *MEA trace following treatment of human iPSC-derived cardiomyocytes with the ß-adrenergic agonist, isoproterenol*[11].

**Picking Up Chronic Cardiotoxic Effects**
Not all cardiac adverse effects are apparent during acute dosing regimens. The real power of iPSC-derived cardiomyocytes on an MEA platform is predicting unexpected liabilities and long term chronic effects *in vitro.* Due to the ability to measure and maintain cells over long periods of time using a label-free platform, this technology is ideal for evaluating short and long term exposures early in drug development. Presently, these type of long term studies are performed in telemerised dogs at great expense. The majority of effects on the hERG channel occur via direct block of the channel. However, an alternative mechanism of drug-induced hERG blockade has been discovered which involves inhibition of hERG trafficking to the cell surface[12]. This effect may go undetected in most conventional safety assays.

In the examples below, we highlight a couple of case studies where MEA has been used to detect hERG trafficking and unexplained chronic cardiotoxic effects[13].

## CASE STUDY 4: PENTAMIDINE EFFECT ON HERG TRAFFICKING

The antimicrobial, pentamidine, is one example of a drug that affects hERG trafficking. Over an extended incubation period of 24 and 48 hr in the presence of pentamidine, it was possible to detect the hERG trafficking effects as demonstrated in Figure 6.

Figure 6 shows the time course of the MEA following incubation with pentamidine. The baseline (black) and the 2 hr (red) MEA trace showed no acute effects. At the 24 hr time point (green) there was a significant delay in repolarisation as well as in the beat length. In addition, there was also a slight decrease in the amplitude of the repolarisation. At 48 hr (blue), the effects were increased with a greater delay in the repolarisation and a further decrease in the amplitude.

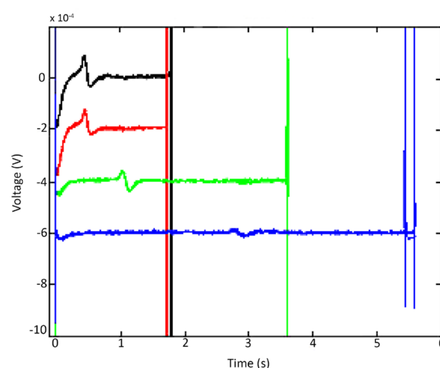



**Figure 6:** *Human iPSC-derived cardiomyocytes treated with pentamidine over 2 hr (red), 24 hr (green) and 48 hr (blue)[13]. Baseline is black.*

## CASE STUDY 5: CHRONIC CARDIOTOXIC EFFECTS WITH BMS-986094

BMS-986094 was developed by Bristol-Myers Squibb (BMS) as a prodrug of a guanosine nucleotide analogue to treat the hepatitis C virus. During Phase II clinical trials, one patient died and a further eight patients were hospitalised due to cardiotoxicity. The patients were found to have multiple cardiac adverse effects including ST depressions, T-wave inversions and loss of T-wave amplitude. The development of BMS-986094 was subsequently stopped in 2012. Notably, these cardiotoxic effects were only observed after extended use following administration of the 200 mg dose. At that time, *in vitro* cardiac toxicity tests were unable to assess the impact of extended dosing – one of the key reasons for the failure to detect this liability prior to Phase II trials.

Human iPSC-derived cardiomyocytes in conjunction with MEA were subsequently used to investigate the mechanisms behind BMS-986094 cardiotoxicity. Over the course of 14 days the impact of BMS-986094 was assessed at a range of dose levels[14].

A

**% OF VEHICLE**

| Test Article | Time (hr) | Test Concentration (μM) | Average Beat Period | Average Na+ Slope | Average Na+ Amplitude | Average Field Potential Duration |
|---|---|---|---|---|---|---|
| BMS-986094 | 1 | 10 | 106 ± 5.4% | 78 ± 10.8% | 80 ± 11.7% | 105 ± 6.2% |
| | | 2 | 106 ± 2.7% | 112 ± 9.6% | 111 ± 7.3% | 107 ± 5.5% |
| | | 0.4 | 105 ± 1.8% | 91 ± 23.1% | 93 ± 26.2% | 99 ± 8.5% |
| | | 0.08 | 106 ± 3.4% | 122 ± 18.9% | 129 ± 16.1% | 105 ± 5.3% |
| | | 0.016 | 106 ± 4.6% | 115 ± 16.7% | 115 ± 16.3% | 107 ± 5.9% |
| | | Minimum effective concentration (MEC) | Not applicable | 10 | 10 | Not applicable |
| | 120 | 10 | Not determined | Not determined | Not determined | Not determined |
| | | 2 | 100 ± 4.8% | 57 ± 26.0% | 41 ± 24.3% | 102 ± 8.0% |
| | | 0.4 | 85 ± 2.0% | 93 ± 35.1% | 96 ± 36.0% | 82 ± 10.6% |
| | | 0.08 | 88 ± 3.2% | 185 ± 47.5% | 203 ± 60.5% | 86 ± 8.3% |
| | | 0.016 | 96 ± 3.3% | 121 ± 50.7% | 125 ± 53.1% | 92 ± 4.7% |
| | | Minimum effective concentration (MEC) | 10 | 0.08 | 0.08 | 10 |
| | 288 | 10 | Not determined | Not determined | Not determined | Not determined |
| | | 2 | Not determined | Not determined | Not determined | Not determined |
| | | 0.4 | 70 ± 5.1% | 33 ± 13.1% | 7 ± 4.6% | Not determined |
| | | 0.08 | 66 ± 3.0% | 130 ± 19.5% | 138 ± 24.2% | 72 ± 6.5% |
| | | 0.016 | 91 ± 6.4% | 107 ± 9.9% | 107 ± 12.2% | 89 ± 6.9% |
| | | Minimum effective concentration (MEC) | 0.08 | 0.08 | 0.08 | 0.08 |
| | 336 | 10 | Not determined | Not determined | Not determined | Not determined |
| | | 2 | Not determined | Not determined | Not determined | Not determined |
| | | 0.4 | Not determined | Not determined | Not determined | Not determined |
| | | 0.08 | 62 ± 5.5% | 128 ± 11.7% | 136 ± 19.1% | 68 ± 7.0% |
| | | 0.016 | 93 ± 6.9% | 108 ± 2.9% | 110 ± 3.1% | 90 ± 5.5% |
| | | Minimum effective concentration (MEC) | 0.08 | 0.08 | 0.08 | 0.08 |

B

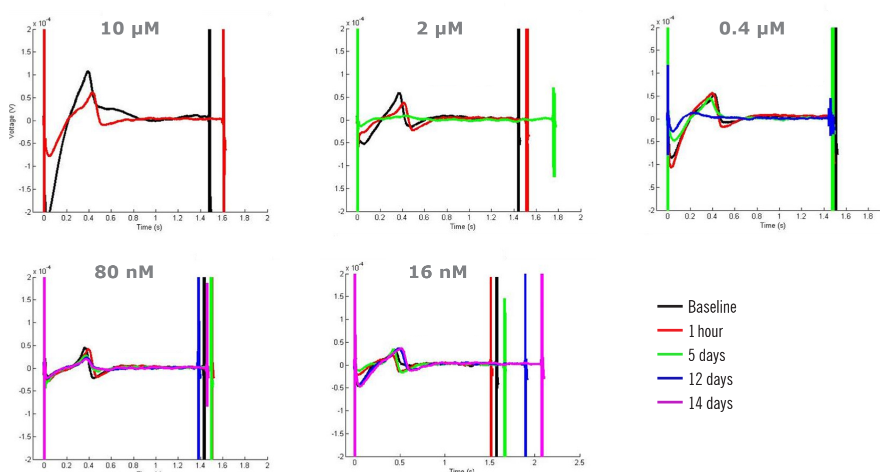



**Figure 7:** *MEA data for BMS-986094 (A) Measured cell response (as a % of the baseline corrected to the vehicle control) following increasing doses of the drug over the 14 day (336 hr) time period and (B) MEA traces following incubation of increasing concentrations of BMS-986094 over time[14].*

The table in Figure 7A shows the changes that occur across the dose levels over the 14 day (336 hr) time course of the experiment. As the experiment progresses to later time points, the effects become more pronounced and the cells eventually stop beating. Even at the 80 nM concentration, a significant change in the beat rate as well as a reproducible and elevated increase in the sodium amplitude is observed at the 14 day time point.

Figure 7B shows MEA traces of BMS-986094 concentration over time demonstrating the corresponding effect on beat length and the amplitude of the T-wave at the different doses and the later time points.

Traditionally, detecting chronic cardiac effects using *in vitro* methods has been challenging and has led to compounds progressing through the drug development process even though they pose a significant cardiac risk. It is clear that MEA is a powerful technique which can be applied in drug discovery, to effectively detect these risks earlier.

**Detecting Cardiotoxic Pharmacodynamic DDI**
Due to our aging population, prescription of multiple drug therapies including cardiovascular drugs is becoming more widespread, increasing the potential incidence for drug-drug interactions (DDI). DDI pose significant drug safety issues and, although investigations into pharmacokinetic DDI are now routine *in vitro* and in clinical trials, the same cannot be said for pharmacodynamic effects. Pharmacodynamic DDIs are generally undetectable or not assessed at all in high throughput *in vitro* screening assays. All of these aspects mean that potentially dangerous pharmacodynamic DDIs often do not come to light until the drug has progressed to market.

A long history of DDIs exists for cardiovascular drugs. The use of MEA with human iPSC-derived cardiomyocytes allows DDIs to be flagged early in development. The comprehensive nature of human iPSC-derived cardiomyocytes in MEA also enables the mechanism of cardiac adverse effect as well as the impact to be assessed.

## CASE STUDY 6:
## PHARMACODYNAMIC DDI BETWEEN SOFOSBUVIR AND AMIODARONE

The pharmacodynamic DDI observed between sofosbuvir and amiodarone serves as a recent example of an unanticipated interaction with cardiac effects. Sofosbuvir-based drugs have significantly advanced care for hepatitis C virus-infected patients. Although sofosbuvir did not exhibit adverse cardiac effects in clinical trials, post-marketing reports indicate that severe symptomatic bradycardia can occur through the co-administration of sofosbuvir and amiodarone. The underlying mechanistic basis of this DDI was unknown, but hypotheses suggested an interaction with the P-glycoprotein (P-gp) drug transporter. MEA and human iPSC-derived cardiomyocytes were used to recapitulate the interaction between sofosbuvir and amiodarone *in vitro,* and more generally assessed the feasibility of human iPSC-derived cardiomyocytes as a model system for DDI (Figure 8)[15].

MEA recordings demonstrated that sofosbuvir and amiodarone interact to affect human iPSC-derived cardiomyocyte electrophysiology. The study illustrated that the effects elicited by the DDI were not caused by the more commonly observed mechanism of direct block of sodium, potassium, or calcium currents, inhibition of the P-gp drug transporter, or metabolite production. Instead, they were associated with a disruption of intracellular calcium handling at clinically relevant concentrations, and cessation of contractile beating at the highest supra-physiological concentrations, indicating a pharmacodynamic DDI with a cardiac mechanism of action. The results provide new mechanistic insight into the sofosbuvir-amiodarone interaction, and more generally suggest that human iPSC-derived cardiomyocytes could serve as a comprehensive model system for evaluating cardiac pharmacodynamic DDI.

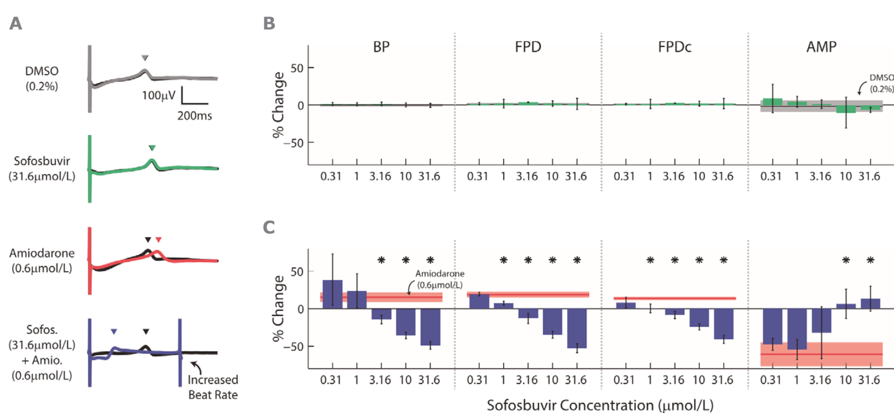



**Figure 8:** *Electrophysiological effect of sofosbuvir when co-administered with amiodarone in vitro. A: Example field potential waveforms for the vehicle control, sofosbuvir alone, amiodarone alone, and the combination of sofosbuvir and amiodarone. B: Percent change from baseline for BP (beat period), FPD (field potential duration), FPDc (corrected field potential duration), and AMP (spike amplitude) in response to the addition of sofosbuvir alone. C: Percent change from baseline for BP, FPD, FPDc, and AMP in response to the addition of sofosbuvir in combination with amiodarone*[15]*. Reprinted with permission from Oxford University Press.*

In addition to cardiotoxicity testing, MEA is showing considerable promise in CNS-related screening. In the second part of this review, we focus on this rapidly evolving field and how MEA is driving new research and providing a valuable tool for *in vitro* neurotoxicity screening.

## ROLE OF MEA IN NEUROTOXICITY TESTING

### Neurotoxicity – A Concern for Both the Pharmaceutical and Chemical Industry

Neurotoxicity is a considerable concern for not only the pharmaceutical industry where an attrition rate of 21% has been reported in Phase I to Phase III clinical trials[16] but also for the chemical and agrichemical industry where unwanted side effects can affect the general population through pesticide use and industrial contamination of the air or water supply. The latter is of specific concern for developmental toxic effects in embryos or children where the CNS is not fully developed and where susceptibility to these types of toxins is high. Robust early stage models for predicting neurotoxicity are essential to reduce the number of animals used during toxicology studies and to decrease the potential for late stage failure.

### Lack of *In Vitro* Models for Predicting Neurotoxicity

The brain and nervous system is by far the most complex organ in the body but is still relatively poorly understood. Animal studies still play a major role in neurotoxicity testing. For example, in pharmaceutical testing, the modified Irwin's test assesses behavioural changes, sensory/motor responses, coordination and body temperature, and is a regulatory requirement prior to first in human studies. Seizurogenic responses are often assessed in repeat dose toxicity studies, the pentylenetetrazol (PTZ) pro-convulsant model in rodents or gold standard EEG recordings in rats. Despite these models being available, it is important to note that the percentage of human neurological toxicities detected in animal studies still only reaches approximately 60%, so better models are required which are more predictive of human neurological adverse events[17]. Early identification of these risks using *in vitro* techniques would have benefits in decreasing late stage failure and in the reduction of animal usage.

Neurotoxicity and seizurogenic liabilities are difficult to detect using standard *in vitro* cytotoxicity assays. This is mainly due to the inherent limitations of these assays to predict adverse neural network disruptions and chemically-induced perturbations[18]. Traditionally, the benchmark assay for *in vitro* detection of seizure liability has been the rat hippocampal brain slice assay. This technique is limited by its low throughput and requirement for sophisticated instrumentation and a high level of expertise. Electrophysiologically based *in vitro* assays such as ion channel assays can detect specific agonists and antagonists, but cannot predict how drugs interact with a neural network as a whole. Neurotoxicity can also be detected using a neurite outgrowth assay with a high content imaging platform, but this does not detect disturbances in the overall electrophysiological function. Patch-clamp assays, although high-throughput, often require ion channel subunits to be expressed in non-neuronal cell models. This means that the impact on native neurons cannot be inferred and multiple ion channels must be assessed individually, making the process lengthy and inefficient. None of these assays allow for the impact of a drug to be assessed on functionally connected neural networks.

MEA overcomes these issues, and is now showing promise as a valuable tool for predicting neurological effects. The platform can test multiple compounds on one plate with electrophysiologically active cell types. It is a high throughput, functional, and reproducible method for the detection of neurotoxic and seizurogenic drug-induced effects[18].

### MEA – A Game Changer for Assessing Seizurogenic and Neurotoxic Response

Seizures and neurotoxic responses pose a serious risk in the drug discovery and development process. These adverse central nervous system effects are one of the most common reasons for the attrition of drug discovery pipelines[19]. Yet efficient and accurate methods to detect the effects are lacking. MEA offers a robust, reliable, high throughput solution that tracks functional cell excitability in real-time using label-free technology. Using this approach, neural networks are grown on the multi-well plates

enabling the morphological complexity of the neural cell model to be preserved. Using neuronal cells, MEA recapitulates many features of this cell type *in vivo,* including spontaneous activity (spiking and bursting), plasticity, organisation and responsiveness to a wide range of neurotransmitters and pharmacological agonists/ antagonists. This technology provides a unique *in vitro* system for preclinical drug discovery, neurotoxicity assessment and disease modelling.

**MEA Spike Train Analysis – A Wealth of Valuable Data**
Spike train data produced by the MEA is presented in raster plots which provide a qualitative analysis of the data. In order to perform quantitative statistical analysis of the data, multivariate spike train descriptors are extracted from the raster data[20]. Although numerous descriptors are extracted, there are three main categories in which the parameters fall, namely, activity (i.e. firing rates), spike train organisation (i.e., interspike interval (ISI) and burst statistics) and cross-channel synchronisation (correlation of spike trains across wells). Figures 9 and 10 demonstrate how the raster plots are interpreted and the spike train descriptors which can be generated from these plots.

In the presence of picrotoxin, the raster plots illustrate (i) increased burst organisation, (ii) changes to spike activity (most spikes occur in bursts) and (iii) increased synchrony between electrodes. The raster plots can then be analysed further using spike train multivariate descriptors as shown in Figure 10 for picrotoxin.

**Figure 9:** *MEA raster plots of spontaneous spike activity from two active electrodes in a single well for (A) baseline activity and (B) 1hr post dose treatment with the seizurogenic compound, picrotoxin (10μM)[20].*

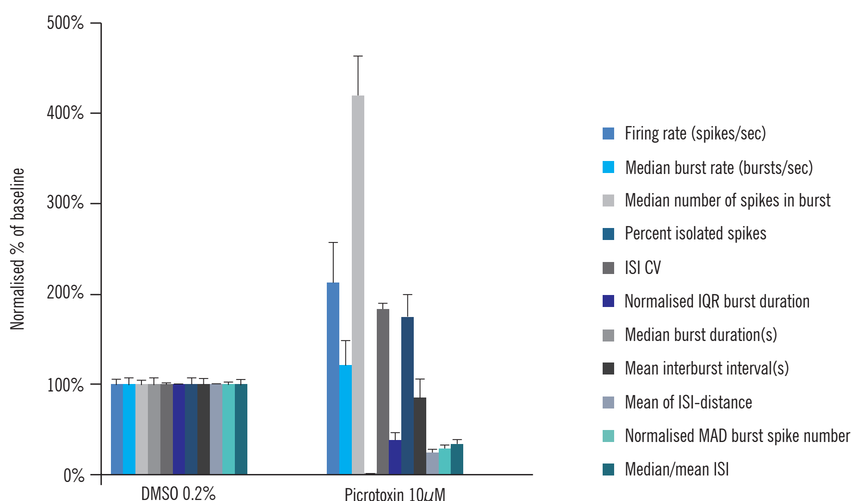



**Figure 10:** *A subset of endpoints quantifying activity (e.g. firing rate), burst characteristics (e.g. burst rate, spikes in burst, isolated spikes, burst duration, ISI CV, normalised IQR burst duration, normalised MAD burst spike number and median/mean ISI) and synchrony (ISI distance) for picrotoxin compared to the vehicle control[20].*

**Predicting Seizurogenic Response using MEA**
Using the spike train descriptors generated, specific patterns of response (or finger-prints) emerge which act as a classification method for binning neuroactive compounds into specific classes or target related clusters.

Although technological developments are now being made, primary rat neurons have traditionally shown advantages over human iPSC-derived cells in terms of their robust electrophysiological response to a broad range of chemicals using MEA, and have proved to be a reliable surrogate for human neurological behaviour. Using rat cortical neurons, distinct patterns of electrophysiological response have been observed for seizurogenic compounds using the MEA, which is predominantly dependent on their mechanism of action[21]. This is illustrated in Case Study 7 below.

## CASE STUDY 7:
## DETERMINE MECHANISM OF ACTION OF NEUROTOXINS THROUGH SEIZUROGENIC PATTERNS

A specific seizurogenic prediction pattern in the MEA is observed for the $GABA_A$ antagonists, picrotoxin, gabazine, bicuculline, pentylenetetrazol, tutin, tranexamic acid and endosulfan (Table 1). The pattern is characterised by an overall increase in activity (firing rate and burst rate) as well as changes in burst organisation and synchrony patterns with the burst characteristics having a greater significance over the activity rates. Interestingly, in addition to the $GABA_A$ antagonists, 4-aminopyridine, a potassium channel blocker produces a similar pattern of response. The second pattern of phenotypic response observed is quite different to the $GABA_A$ antagonists, namely a reduction in spike and burst rates, a deconstruction in burst formation and a complete abolition of network synchrony. These compounds (NMDA, SNC80, linopirdine, strychnine, amoxapine and thioridazine) are reported to have a seizurogenic response *in vivo* but are not $GABA_A$ antagonists. Two of the neurotoxins (domoic acid and tetrodoxin) were so potent that the neurons had complete loss of firing and so quantitative analysis of the different parameters was not possible. As expected, the negative control compounds (acetaminophen and ibuprofen) showed no effect on neural activity.

Although research in this field is at a fairly early stage, it is clear that seizurogenic response can, in general, be classified into 2 distinct and opposing fingerprints dependent on mechanism of action. In this respect, MEA provides a wealth of valuable specific endpoints for probing the electrophysiological response in neuronal cells.

| Compounds | Firing Rate | Median Burst Rate | Median Num. of Spikes in Burst | Percent Isolated Spikes | ISI CV | Norm. IQR Burst Duration | Median Burst Duration (s) | Mean IBI (s) | Mean of ISI-distance | Norm. MAD Burst Spike Number | Median/ Mean ISI | Median ISI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Picrotoxin | ↑ | ↑ | ↑ | ↓ | ↑ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ |
| Gabazine | ↑ | ↑ | ↑ | ↓ | ↑ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ |
| Bicuculline | n/c | ↑ | ↑ | ↓ | ↑ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ |
| Pentylenetetrazol | ↑ | ↑ | ↑ | ↓ | ↑ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ |
| Tutin | ↑ | ↑ | ↑ | ↓ | ↑ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ |
| Tranexamic Acid | ↑ | ↑ | ↑ | ↓ | ↑ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ |
| Endosulfan | ↑ | ↑ | ↑ | ↓ | ↑ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ |
| 4-Aminopyridine | ↑ | ↑ | ↑ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ |
| NMDA | ↓ | ↓ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↑ | ↑ |
| SNC80 | ↓ | ↓ | ↓ | ↑ | ↓ | ↓ | ↓ | ↑ | ↓ | ↓ | ↑ | ↑ |
| Linopirdine | ↓ | ↓ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↑ | ↑ |
| Strychnine | ↓ | ↓ | ↓ | ↑ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↑ | ↑ |
| Amoxapine | ↓ | ↓ | ↓ | ↑ | ↓ | ↓ | ↓ | ↑ | n/c | ↓ | ↑ | ↑ |
| Thioridazine | ↓ | ↓ | ↓ | ↑ | ↓ | ↓ | n/c | ↑ | n/c | ↓ | ↑ | ↑ |
| Domoic Acid | LF | LF | LF | LF | LF | LF | LF | LF | LF | LF | LF | LF |
| Tetrodotoxin | LF | LF | LF | LF | LF | LF | LF | LF | LF | LF | LF | LF |
| DMSO (0.2%) | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c |
| Acetaminophen | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c |
| Ibuprofen | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c | n/c |

n/c, no change; LF, complete loss of firing; ↓, lowering of endpoint activity to 0%; ↓, decrease; ↑, increase.

**Table 1:** *Heatmap of the endpoint results highlighting patterns of responses for proconvulsive compounds, neurotoxins and negative control compounds[21]. Reprinted with permission from Oxford University Press.*

### Maturation Matters in Forming Neuronal Networks

Pilocarpine is a muscarinic receptor agonist. The pilocarpine-induced seizure model is one of the earliest models for inducing epileptic response in rodents. It was therefore a surprise when pilocarpine, which is a muscarinic receptor agonist, failed to generate a significant change in activity over control levels using the MEA in combination with primary rat neurons[21].

### CASE STUDY 8:
### EFFECT OF NETWORK MATURATION ON NEURONAL RESPONSE

Follow-up studies were performed to evaluate pilocarpine further in order to explain this result[22]. Three different cell models (rat cortical neurons, rat hippocampal neurons and human iPSC-derived cortical neurons co-cultured with human iPSC-derived astrocytes) were assessed over different maturation times (14 days *in vitro* compared to 21 days *in vitro*). Only the human co-culture model was sensitive enough to measure significant neuronal activity at 14 days *in vitro* (DIV). However, following 21 DIV, both rat neuronal models responded well to pilocarpine both exhibiting significant effects on bursting and synchrony. Using high content screening, it was determined that this effect was likely to be caused by a time dependent expression of muscarinic acetylcholine receptor subtypes with M3 and M4 only expressed after 21 DIV[22].

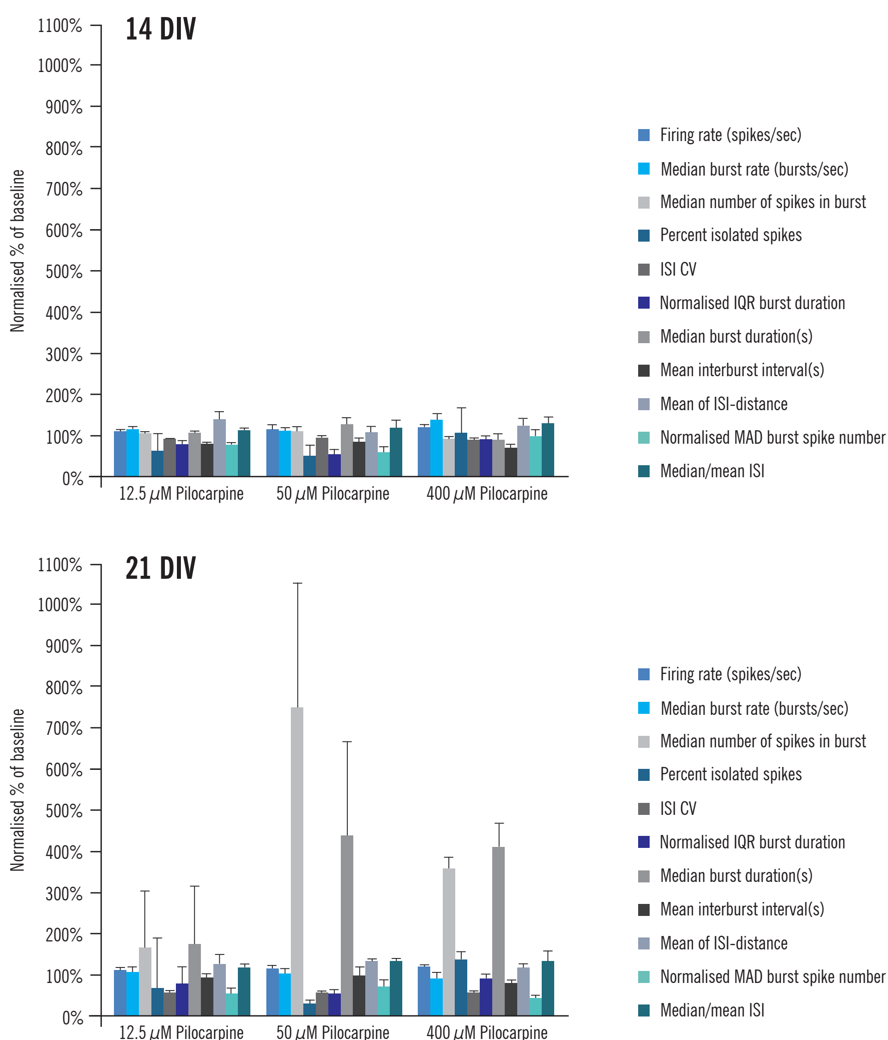



**Figure 11:** *Comparison of concentration dependent neuronal response to pilocarpine in cryopreserved rat hippocampal neurons at 14 and 21 DIV[22].*

The data presented in Figure 11 clearly highlights the importance of neuronal cell maturation during these studies and the importance of characterising these models for specific ion channels/receptors prior to use in routine screening.

## ABOUT CYPROTEX

**Cyprotex** was founded in 1999 and specialises in *in vitro* and *in silico* ADME-Tox. This includes *in vitro* ADME screening to support discovery projects, regulatory *in vitro* ADME and DDI studies during preclinical and clinical development, specialist mechanistic *in vitro* human and animal toxicity models (e.g. 3D models and MEA electrophysiology) and modelling and simulation expertise. The company has sites at Alderley Park near Manchester in the UK and in Watertown near Boston in the US.

In 2016, Cyprotex was acquired by Evotec AG. As a whole, the Group offer integrated and stand-alone drug discovery capabilities as well as full CMC and IND-enabling services, allowing the company to provide expert support across the value chain from early discovery through to preclinical development and beyond.

## SUMMARY

**Standardised reliable *in vitro* models are still lacking in the field of toxicology. Many decisions are still based on regulatory preclinical animal testing. Too often these tests are left too late and liabilities are only detected when considerable time and money has been consumed. Furthermore, animal studies may not provide reliable predictions of human toxicity. Consequently, there has been a drive to develop new robust *in vitro* methods which are predictive of human toxicity.**

**The recent introduction of human iPSC-derived cells has improved the relevance of these *in vitro* models and when combined with sensitive electrophysiological technologies such as MEA are causing considerable interest within the industry. As a consequence of CiPA and other initiatives, and as our understanding of the data continues to improve, it is expected that this technology will become a standardised mainstream assay for acute and chronic cardiotoxicity and neurotoxicity assessment.**

**The future of MEA in *in vitro* toxicology testing is looking strong – a prospect which is really something to get excited about!**

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