Neuronal Micro-Circuit on Chip: Design, Implementation and electrophysiological Results

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Abstract

The following in-depth critical literature review highlights the design and fabrication of neuronal microcircuits on chip-based architecture for both the stimulation and reception of biotransducer sensory information. To be able to fully study complex structures of the human brain under in-vitro conditions to which the scope of this report is confined, it is compulsory to be able to manipulate primary dissociated single cell neurons into different three-dimensional organizational structures, control their morphology and adapt them to sensory technologies such as the Microelectrode Array. A range of chemical patterning such as Parylene channelling and micro-molding physical confinement techniques such as polydiethyl-siloxane scaffolding are explored in addition to their relative strengths and limitations with respect to ease of fabrication, manipulability and accuracy and precision of resulting signal reception.

Word Count: 126

Introduction

Neuronal networks are extremely complex biological systems comprised of soma and their associated neurites (axons and dendrites) that relay electrical impulses to process, store and relay sensory information received by the brain in living organisms. These neuronal circuits can be bioengineered in a multitude of different methods with unique results. Microelectrode arrays (MEAs), growth substrate microfluidic channel enclosures, physical confinement and cell placement, NeuroArray patterning and other fabrication methods have been engineered to recreate the complex three-dimensional topology of neural networks seen in the human brain that may provide insight into connectivity changes that take place during aging or development of neural diseases for instance.

In-vitro bioengineering provides many advantages when synthesizing or analyzing neuronal networks. For instance, in-vitro microelectrode arrays (MEAs) enable long term storage of grown neural cell cultures for over a year. In-vitro methods do not require surgery or invasive operations to study living organisms, significantly reducing research study risks. Furthermore, they tend to be relatively inexpensive compared to in-vivo and metabolization of cells can be simulated effectively through the addition of chemical agents in solution for instance. They enable manipulation and control over the connectivity between dissociated

neurons or slices of the brain and facilitates investigation of cell morphologies and functionality via physiological monitoring ("In Vitro Studies", 2017). However, many parameters must be controlled to establish networks with significant functions, including but not limited to neuronal polarity, synaptic arrangement and the existence of dead cells in the matrix (Foroushani).

Of particular importance to pharmaceutical industries testing drugs prior to human exposure, bioengineering technologies for growing and analyzing neural networks synthetically will enable high throughput (e.g. droplet delivery) drug screening, particularly for those drugs designed to target neural diseases. Specifically, MEA can be used to screen selected compounds against ion channel targets (many diseases involve dysfunctional ion channel behaviour) and generate spatial maps of a drug's specificity of action by measuring the distribution of electrical response in tissue; as a biosensor it is invaluable for monitoring both chronic and acute effects of drugs and toxins on any electrogenic tissues (e.g. myocardial not just neuronal). As an example, quinidine applied as an antiarrhythmic drug is an HERG channel inhibitor that prolongs ventricular field potential and alters heart rhythm; MEA was able to be successfully utilized as a tool to generate dose dependent data and cardiac parameters and thereby record drug activity indirectly (Stett, Egert, Guenther, Hoffman, Meyer, Nisch & Haemmerle, 2003).

Critical Review and Evaluation of Literature

Background

The subsequent objective of this report is to detail contemporary design techniques for synthesis of neural circuits on microelectrode arrays, the engineering required to facilitate the

designs, and the resulting electrophysiological monitoring electrodes and importance of measured parameters to society.

Microelectrode arrays or MEAs (first produced in the 1970s) generally comprise of 8x8 or 6x10 grids of 10 to 30 micrometer titanium nitride based electrodes using Si_3N_4 insulation (Fejtl, Stett, Nisch, Boven & Moller, 2006). Plasma enhanced chemical vapour deposition of Ti under nitrogen generates a three-dimensional nano-columnar network of TiN with high surface area and electrical capacitance. In this way electrical signals are conducted from neurons to semiconductor circuitry layered below the nano-columnar network whereby they are transmitted to data acquisition systems and processed via CPU. In-vitro MEAs are non-implantable and require a medium chamber or cell culturing disc (Fejtl, Stett, Nisch, Boven & Moller, 2006).

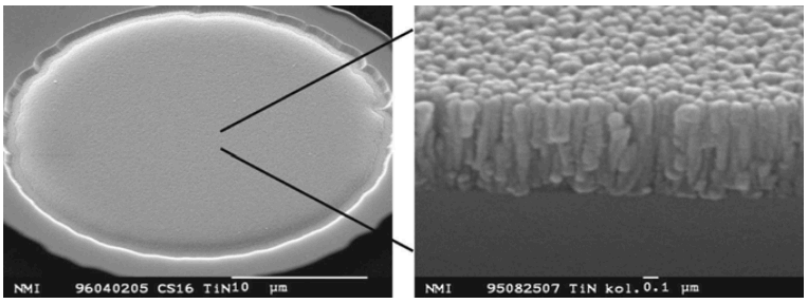

FIGURE 2.4. A single TiN MEA electrode is shown at µm and nm resolution. Note the nanocolumnar structure of the electrode, revealed by the REM image on the right.

It is critical to fabricate the electrodes to minimize impedance because doing so maximizes the signal to noise ratio (generally targeted at 5) and increases reading accuracy Obien, Deligkaris, Bullmann, Bakkum & Frey, 2014). The micrometer scale of the electrodes can often require augmenting effective surface area using Platinum black or conductive polymers (e.g. PEDOT or poly(3,4-ethylenedioxythiophene)) to achieve the necessary impedance. MEA is also utilized in neuronal stimulation by converting electron flow to ion flow in the encompassing electrolytic cell containing media (Obien, Deligkaris, Bullmann, Bakkum & Frey, 2014).

With respect to utility and biological answers provided, MEAs have resulted in numerous bioelectrochemical applications in cell analysis and engineering. Monitoring of cell release and consumption of compounds and enzymatic activity by MEAs is possible, for instance in oxygen concentration profiling to determine respiration rate using Pt electrodes for an embryo. Electro-catalysts and perm-selective films have been applied to electrode surfaces for the reduction and oxidation of secreted chemicals for electrochemical monitoring of neurotransmitters such as dopamine. Accumulation of enzymes such as SEAP (secreted alkaline phosphatase) in microwells can amplify redox reactions associated with substrate conversion, thereby monitoring enzymatic activity of single cells (Ino, Shiku & Matsue, 2017).

On the converse side, MEA's can be used for stimulating and fabricating activities rather than mere data parameter acquisition and analysis. Dielectrophoresis based microelectrode arrays using non-uniform electric fields can migrate cells with specific dielectric constants to fabricate cell culture arrays. Electrical stimulation between two electrodes can lyse cells via electroporation or even contract muscle cells by using hydrogel based electrically conductive polymers. Overall, between cell stimulation, fabrication and reception, MEAs function as a critical interface between hardware semiconductors and living biological systems through electric signalling pathways (Ino, Shiku & Matsue, 2017).

Single neuronal cells from dissociated cultures initially addressed electrophysiological activity via intracellular recording on a dynamic scale as opposed to static structural discoveries through fluorescence microscopy for instance. However, practical considerations such as time limitations (even for cultured brain slices) on lifespan of single cells inhibited data analysis of single neuronal cells. Neuroscientists depend on dissociation of single neurons from dissected brain tissue via mechanical/chemical methods grown into primary cultures to increase the accessibility of glia and other cells to techniques such as time-lapse

imaging. The single cell resolution enables in depth analysis of protein trafficking and morphology changes among other parameters even when maintained in appropriate media. The NeuroArray technique, to be detailed subsequently, addresses the patterning of neurons in a way that tracking, recording and manipulation of neural activity from single cells may be possible at a single cell resolution within large controlled networks.

The importance of maintaining the high single cell resolution information for a dense cluster of neurons is critical in the study of brain electrophysiology and neural diseases, as well as drug effects. Plating of single cell primary cultures on MEAs provides a host of technological benefits. The single neurons form complex synaptic networks in a monolayer form (the architecture output can be manipulated) resulting in ease of image monitoring and ensures all impacts of electrical stimulation can be observed (not possible with intact brain slices that are multi layer). Furthermore, lifespan of the microelectrode array neuronal network can be greater than a year, improving versatility for research studies. This bioengineering technology enables high resolution study including but not limited to synaptic plasticity, excitotoxicity and neurodegeneration induced by diseases such as Alzheimer's to improve probability of treating them in the future.

Microfluidic and Chemical based Patterning Techniques and their Strengths and Limitations

In this section, we will delve into the most commonly used microfluidic techniques utilized and their implementation, as well as challenges. Microfluidic patterning for growth of primary neuron cultures in microchannels will enable tailoring of drug concentrations for high resolution response testing of pharmaceuticals. Prior to microfluidic patterning on MEAs, electrical recording/stimulation were limited primarily to patch clamping techniques (Veitinger, 2011).

Patch clamping functions using a glass pipette containing electrolyte embedded into cell membrane such as that of a neuron, that induces a flux of ions through the membrane channel into the pipette and to an electrode connected to a highly sensitive operational amplifier. While improvements by the Ernst Sakmann in 1980 using a giga seal increased the signal to noise ratio to enable accurate recording of smaller currents such as single ion channels on a cell membrane, it cannot be practically deployed for a network of many neurons (Veitinger, 2011). Hence, MEAs in which neurons can be guided into predefined circuit destinations where the fixed electrodes are present address this issue effectively. Some of the issues associated with microfluidic patterning involve distribution of the cells in a way that nutrient uptake and cell concentration are viable for their long term survival, otherwise reversion to time constraints associated with single cell cultures occurs. While photolithography, in situ etching and microcontact printing technologies have been demonstrated to pattern cells on microelectrode arrays, fine resolution patterns are difficult to maintain which is problematic since growth of primary cultures into a network requires several weeks time.

One major method of patterning and guidance for primary neurons is performed through chemical attraction. Bovine serum albumin results in cytophobic regions, while poly-L-lysine results in cytophilic regions (that attract fatty neuron cells) when each are adsorbed on glass-perfluoropolymer, by which efficiency is tuned through adjusting the height of layering (Griscom, Degenaar, LePioufle, Tamiya & Fujita, 2002). Briefly, the microarray is built using a spin-coating, baking and layering sequence for Cytop CTL-809M type perfluoropolymer on glass followed by aluminum etching to the glass depth to generate the hydrophobic/cytophilic areas where poly-L-lysine will be selectively adsorbed through a membrane process to house the neurons in vertical channels (Griscom, Degenaar, LePioufle, Tamiya & Fujita, 2002). By contrast, the microfluidic methods feature a more physical based

"tunnel" system whereby neurons are guided through "total confinement" in three-dimensions (Griscom, Degenaar, LePioufle, Tamiya & Fujita, 2002).

The spatial structure of the microfluidic membrane comprises of 50 x 50 micrometer vertical wells spaced at 150 micrometers and interconnected with horizontal microfluidic channels of varying widths less than that of the vertical well width (Griscom, Degenaar, LePioufle, Tamiya & Fujita, 2002). Briefly, polydiethyl-siloxane (PDMS) are imprinted using multilevel SU-8 thick negative photoresist structures to generate the microfluidic channels. PDMS supports cell attachment and controls cell location and shape, as per the following fabrication methods. Microfluidic injection of PDMS followed by curing and extraction of the PDMS stencils (exposed to oxygen plasma to make them hydrophilic and reject neurons) onto glass covered with poly-L-lysine such that the bottoms of the PDMS molds attract neurons (cytophilic) as they fall in via gravity (Griscom, Degenaar, LePioufle, Tamiya & Fujita, 2002).

With respect to the chemical based patterning method, neurons only *grew* (resided even on cytophobic areas but in isolation) on the poly-L-lysine covered glass as reasonably expected. Variations in trench depth did not appear to have growth related effects on the primary culture, but variations in channel width did cause greater variation in neuron height (Griscom, Degenaar, LePioufle, Tamiya & Fujita, 2002).

For the microfluidic based patterning approach, while some neural connections were formed in the horizontal microfluidic channels, the primary cell cultures resulted in glial and other cellular debris scattered - controlling cell placement was more complex. The small chamber sizes can require can require significantly higher concentrations of neuronal cells (e.g. 10 000 per mm^2) since many of them do not reach the growth culture substrate, and is hence more resource intensive for use of primary cultures (Griscom, Degenaar, LePioufle, Tamiya & Fujita, 2002). The surface chemistry of the interior of PDMS microchannels is

regarded as having a hydrophobicity that causes trapping of bubbles impacting the distribution of the primary cell cultures, which may be addressed in the future with albumin treatment of the walls, yet another challenge for making this method more viable. However, cell lifespan was longer than the chemical patterning method and nutrient diffusion was strong. Both framework structures were situated on top of MED64 microelectrode arrays. One of the challenges in the fabrication procedure was to avoid etching of polyimide coating of the MED64 during plasma etching of the glass perfluoropolymer, and poorer selectivity of the polysilane growth substrate was observed in the microfluidic framework than the glass in the perfluoropolymer one (Griscom, Degenaar, LePioufle, Tamiya & Fujita, 2002).

With regards to circuit formation, interfacing the MED64 electrode probes to the microfluidic structures was achieved by aligning the PDMS membrane in three-dimensional space and pressing them together using a wet glass slide for adherence. The channeling architecture of the microfluidic structure causes optical diffraction that obscures dendritic growth in the instance of standard contrast microscopy, but did not affect fluorescence based imaging - for certain types of analysis this may be considered another drawback of this methodology (Griscom, Degenaar, LePioufle, Tamiya & Fujita, 2002).

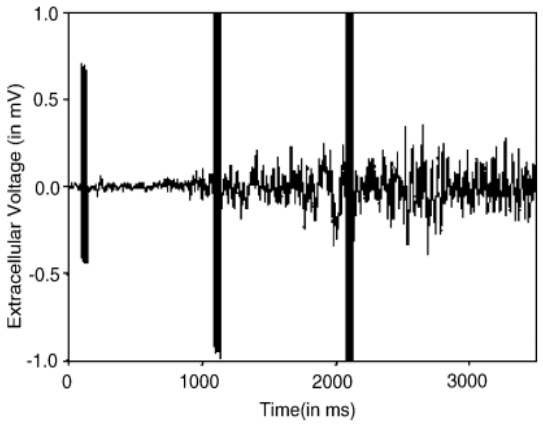

Fig. 5. Typical signals obtained in response to stimulation from the cells inside the PDMS microsystem shown in Fig. 3a). The three artifacts due to stimulation are clearly visible. The resulting composite signal is difficult to analyze due to the impossibility to extract single spikes.

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In studies involving plating of dissociated hippocampal neurons using PDMS stamps with laminin adhesion and growth substrate, researchers discovered interesting patterning dynamics for growth. Larger networks with more potential partners did not change the overall activity level of individual neurons (electrical impulse rate remained same overall), but the number of connections increased (each weaker). Furthermore, they found that the spatial arrangement of soma adhesion strips could be used to control the neuron polarity. Neurites growing on a single adhesion strip would preferentially form axons that transmit information while, punctuated strips favored dendrite formation to receive and could be further tuned based on the spatial dimensions of the strip and the micropatterning of adhesion gradients.

To mimic intact brain regional neuron structure, one must control the strength and directionality of pathways between individual neurons (e.g. sub-hippocampal neurons have uni-directional interaction only). Plating neurons at separate times in confinement to allow axon formation to fill microchannels and inhibit growth of axons from a subsequent plating, and manipulation of the geometry/form of channels (e.g. curved bypass channels or narrowing channels) to inhibit axon growth from certain cells have been used to accomplish patterning directionality to generate distinct emitting and receiving sides.

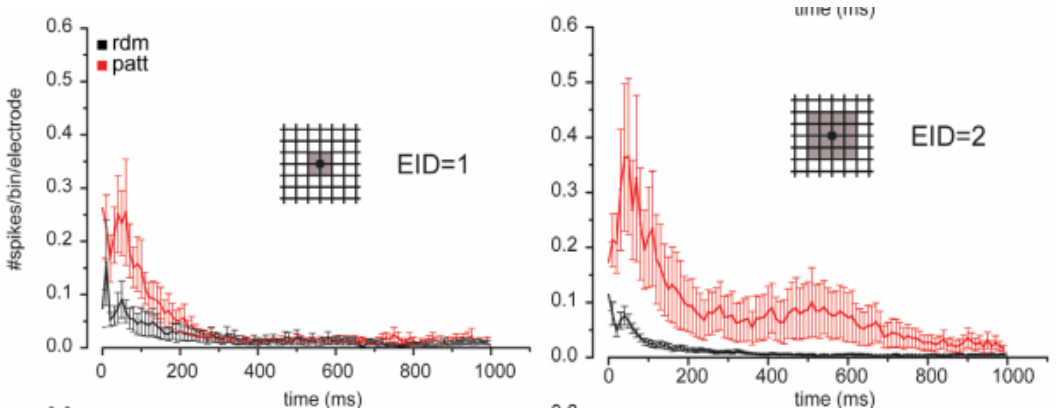
Because the monolayer neuronal structure in microelectrode arrays lacks three dimensional interaction necessary to simulate brain tissue, organic explants and three dimensional scaffolding using materials such as collagen have been developed to compensate this deficiency. Photothermal etching of the collagen gels can control direction of neural elongation in combination with alternating current electrokinetic forces in three dimensions. Exogenous silica beads used to bind neurons in two dimensional layers can also be mobilized to stack in wells generating (near brain level) high density three-dimensional neural circuits.

As discussed previously, microelectrode array structures have benefits over their patch clamp counterparts in their capability of detecting the activity of over 10000 neurons, but are expensive and cannot specifically stimulate individual soma for instance because of their reaction with extracellular fluids rather than direct contact approach.

The Characteristics and Advantages conferred by Patterned Topology versus Control

Control of the network topology, to reiterate, directly controls network synchrony in complex networks and does so through Graph theory based on clustering coefficients and mean path length among other parameters. Diseases such as Alzheimer's display topology changes that accompany those in functional connectivity. A study by Marconi et. Al used microcontact printing of a cell adhesion promoter and an agarose repulsive layer to achieve via topological confinement, patterning and growth of neuronal cultures for over three weeks successfully to allow for studies on mature networks (2012).

In comparing random to pattern growth for the neural structures, extracellular stimulation of the patterned cultures resulted in greater monosynaptic excitatory postsynaptic currents without multiple current peaks in the decay phase (Marconi et. Al., 2012).



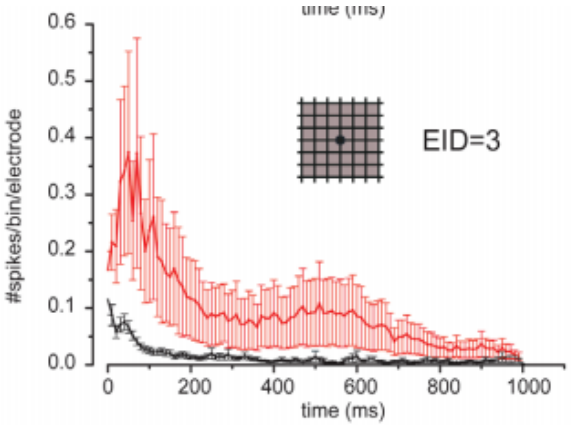


Figure 7. Post-Stimulus Time Histograms (PSTH) computed on random (*black*, n = 9) and patterned (*gray*, n = 6) cultures. Electrical stimulation of patterned MEAs elicited a higher number of spikes at various distances from the stimulation electrode. Increasing square areas around the stimulation electrode were analyzed. At electrode inter distance (*EID*) = 1, the differences were evident up to 200 ms. When patterned versus random cultures were compared at EID = 2 and EID = 3, the difference was systematic on the entire time interval, while PSTH shapes were quite similar. At different EIDs, the average number of spikes increased in patterned cultures, while it remained rather constant in random cultures.
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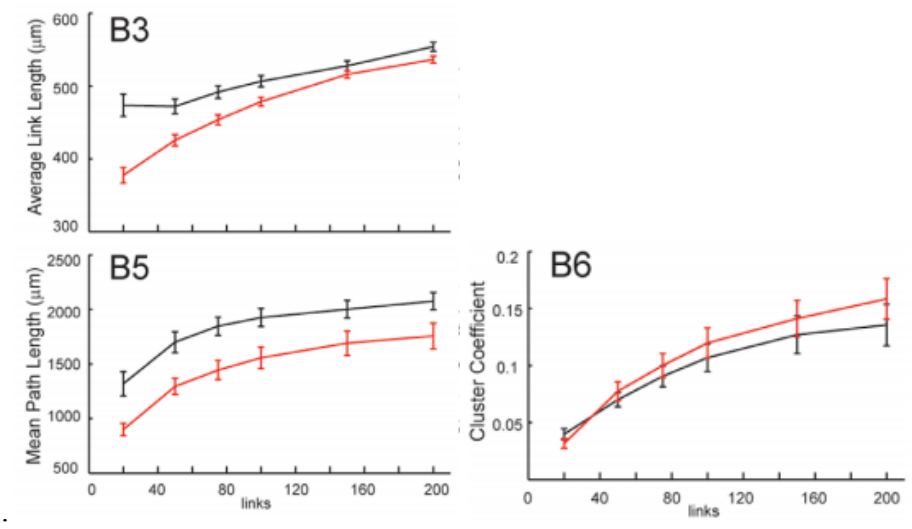
While patterning does not appear to modulate network activity such as quantity of synaptic connections and spontaneous firings, it does increase the incidence of synchronized bursting earlier on in the neural network maturation. Placing the neurons into a grid-like formation shortens the mean path length relative to randomized formations and increases the number of short range connections so that by aligning the network topology on MEAs electrodes, improved signal propagation and did not impede long range connections among multiple nodes (Marconi et. Al., 2012).

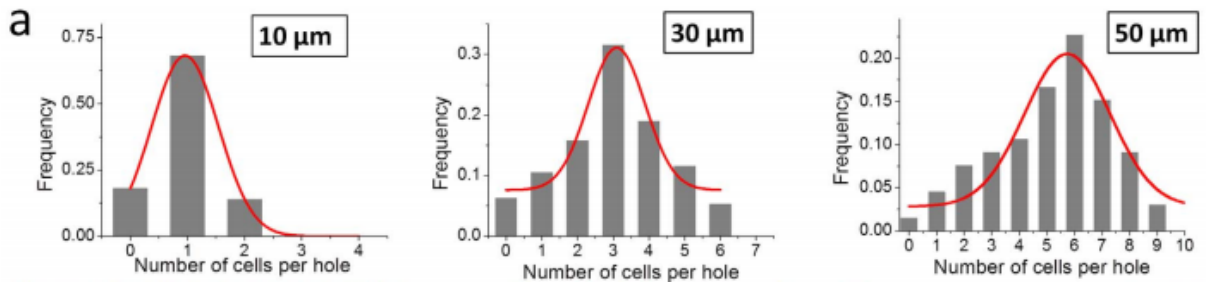


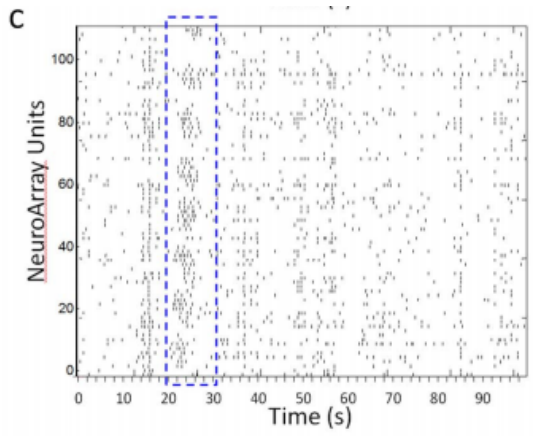
Despite the benefits of patterning discussed above, neuron cell growth, axonal branching and dendrite growth are constrained through their implementation. A three-dimensional solution known as the NeuroArray immobilizes neuron somata in an array of silicon membrane holes that allows neurites to grow and connect into complex spatial networks with minimal limitations that can closely simulate in-vivo neural hierarchical circuitry (Marconi et. Al., 2012).

Recent Evolution of Microfluidic and Confinement Based Approaches: NeuroArray

The NeuroArray uses microscale through holes in PDMS with supporting pillars lifting the stencil 3 micrometers above a glass surface to allow axon and dendrite projections to freely develop (Li, Xu, Huang, Lin,Luo, Chen & Shi, 2014). The fabrication of the NeuroArray uses a silane treated sacrificial layer to protect the SU-8 features so that after PDMS prepolymer is cured it can be removed without issues such as damage to the microscale features of the silicon wafer from etching or failure to produce through holes via high pressure assisted compression (Li et. Al., 2014).

The above array allows for 80% hole impregnation based on a seeding of 30-50k neurons per square centimeter with different sized holes of 10, 30 and 50 micrometers holding 1, 3, and 6 somata (on average normal distribution) respectively. By providing the capability to vary the number of cells in each through-hole, the neural network can be scaled up as desired (Li et. Al., 2014).





The stencil based manufacturing method of the NeuroArray device enables nearly 100% through structures in a thin layer of PDMS, specifiable down to approximately 3 micrometer approximately on par with expensive etching technologies described previously (Li et. Al., 2014). Furthermore, the array is compatible with optical microscopy methods such as calcium imaging using time lapse recording of Ca 2+ neuronal signals (Li et. Al., 2014).

Computational Modelling Control of Patterning Neuron Grid Networks using the Hodgkin-Huxley Model and associated parameters

Despite the patterning controls discussed to constrain soma and neurite channeling previously, there are still numerous parameters that impact network connectivity. Using computational models such as the Hodgkin-Huxley membrane model variations in in excitatory and inhibitory synapse arrangement, neuron polarity and the effect of dead cells can be assessed for influences on neuronal network connectivity.

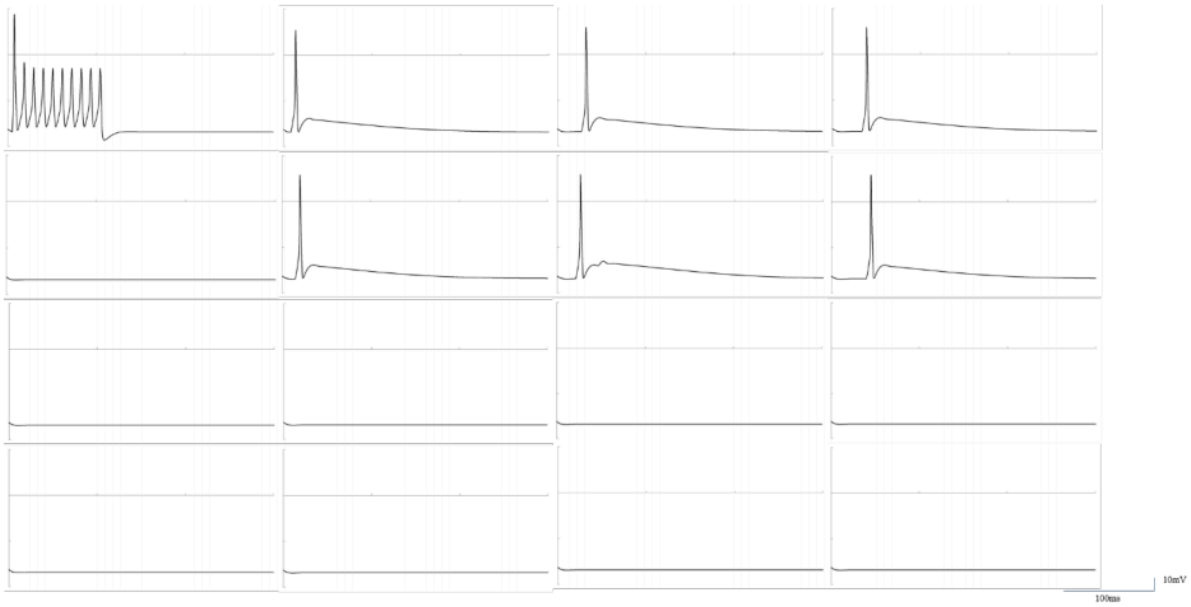
The lower synapse density in patterned growth procedures favours greater rates of cell death than randomized networks. Model calculations reveal 24 different network connectivity patterns between a system of just two neurons for instance, based on 6 different places for each cell's axon to grow and form synaptic connections with the other's dendrite in 4 states

(both alive, Neuron 1 dead, Neuron 2 dead, and both dead) (Foroushani). The conductance of voltage gated channels turns zero when neurons die and they gain a capacitor function in the form of storing electrical charge without transmission. Polarity and direction flow of synaptic information can be modulated through patterned line symmetry and width. The relative proportion of synapses that are excitatory versus inhibitory have been observed to vary vastly in studies, but for patterned networks the results lean slightly to a larger proportion being excitatory - one study revealed 27% of synaptic connections were GABAergic or inhibitory in nature (Foroushani).

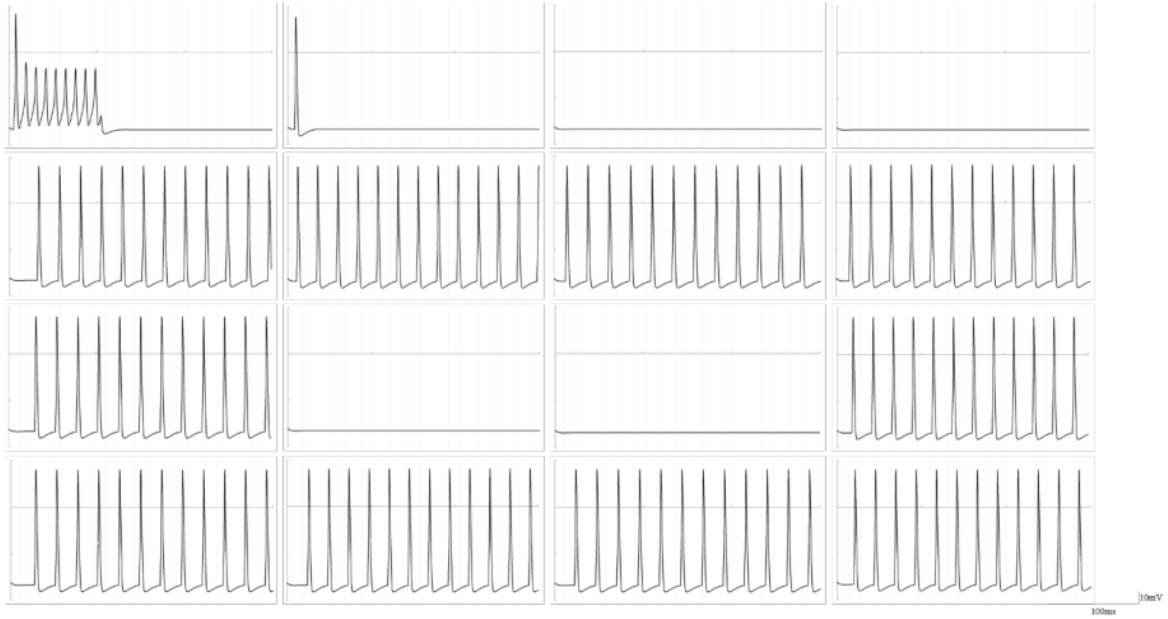
$$I = Cm\ dVm/dt + gK(Vm - VK) + gNa(Vm - VNa) + gl(Vm - Vl)$$

The above equation comprises the Hodgkin-Huxley model of membrane for the generation of action potential, relating the membrane potential to electrical current via channel reversal potentials, ion conductances and membrane capacitances. Based on assumed inputs such as 200pA electrical pulse stimulation to cells at 100 ms duration, patterns of elicited neural activity can be analyzed for different network topologies without requiring a physical microelectrode array construct (Foroushani).

To investigate neuron polarity, synaptic arrangement and effect of dead cells on the overall network activity, 2 models of anatomical connectivity, changing all synapses to either inhibitory or excitatory exclusively, and selection of dead cells under inhibitory synapse arrangement in different topologies are evaluated for both linear and 4 x 4 square grid networks. Since microelectrode arrays follow the square grid patterning for placement of electrodes we will only discuss the 4x4 grid network results of the model below (Foroushani).



The above graphs illustrate reveal the 4x4 grid response when synapses are exclusively inhibitory and only one of the 16 neural cells are stimulated in a state where the polarities are arranged for a maximum size 4 neuron loop. The reach of the inhibitory pulse was confined primarily to the small loop and adjacent cells and did not transmit to the other half of the grid despite being interconnected (Foroushani).



When the arranged synapses in the 4 x 4 grid were arranged to be excitatory in a polarity arrangement that promulgated a maximum size of 10 neurons in a loop, an oscillatory rather than single impulse with decay activity were observed rippling through the larger loop (Foroushani).

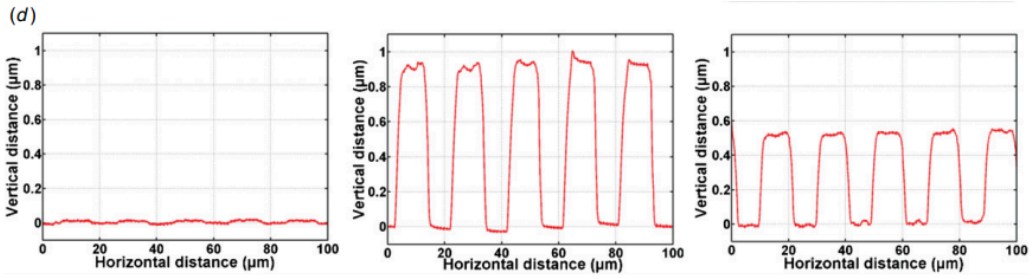
Overall, the signal time delay was minimized in the arrangement with the greater neuron loop size and excitatory synapses, and while an electrical response could be observed in dead cells to which signals were transmitted, they were unable to forward transmit the electrical impulse to neighboring cells which can cause bridge disruptions for instance between large neural loops and networks. The study suggests use of a series of parallel two-dimensional grid arrangements to facilitate neuron synapse formation that is critical to survival of the cells due to the impacts of microarray confinement on cell health in limiting connections (Foroushani).

Recent Evolution of Chemical Based Patterning Methods: Parylene C

Another chemical based scaffolding that utilizes hydrophilic/hydrophobic bonding to pattern biological molecules such as fibroblasts and myocytes, is that of Parylene C (Poly(chloro-para-xylylene)). Parylene C has a high Young's modulus, meaning it is very elastic and well-suited for cell patterning, has excellent hydrophobic encapsulation properties and easily integrates biosensors. The Parylene C method has advantages over the more traditional PDMS stamping approach discussed previously, most notably the difficulties in transporting cells and extracellular matrix reproducibly to the growth substrate locations and difficult precision alignment of the microelectrode arrays to the confinement cell. One of the

challenges in developing this patterning is associated with the fabrication removal of the parylene stencil where it is very thin (~1 micrometer) (Trantidou et. Al., 2014).

Briefly, the scaffold is constructed using a chemical vapour deposition on degreased/dehydrated glass, forming layers that can be manipulated for thickness. This is followed by spin coating with hexamethyldisilazane on top of which a 1.4 micrometer thick photoresist is layered (Trantidou et. Al., 2014). Oxygen plasma etching of the Parylene C film generates the hydrophilic patterning lines (separated by 10 micrometers of hydrophobic surface) 10 micrometers in width for optimal calcium imaging properties subsequently and vertical depth can be varied by plasma etching intensity and duration, as seen in the graphic profiles below (Trantidou et. Al., 2014). The hydrophilicity is believed to be generated via free carboxyl group formation on the Parylene polymer surface and the active sites have a longevity of at least four weeks (Trantidou et. Al., 2014).



Overall, the Parylene based chemical patterning method significantly enhanced alignment for both NRVM and fibroblast cell types compared to a non-engineered hydrophilic control surface group based on reductions in the modulus of angles of the cells from mean axes revealing alignment to the lines. Furthermore, the addition of grooves supported the physical confinement thesis of PDMS stamping method by producing better alignment through geometry to manipulate gravity effects. Furthermore, in terms of the biosensor analysis aspect, the cells in Parylene micro-engineered constructs elicited larger calcium ion signal magnitude and decline at all frequencies of stimulation, believed to be

caused by adjustment to the sarcoplasmic reticulum function from the organized structural geometry (Trantidou et. Al., 2014).

Proposed Research

Important protocols must be followed when preparing the microelectrode arrays, associated patterning regimes and microfluidic channels to ensure optimal functionality and sensitivity of sensor readings to neural networks. Frequently for standard MEAs, electrodes in their grid format are arranged in a glass ring, but care must be taken to avoid damaging the microscopic fragile electrodes when handling. Prior to use of MEAs, sterilization is frequently conducted through deionized water and ethanol application and/or autoclaving with near boiling temperature water. This includes autoclaving MEA lids primarily composed polytetrafluoroethylene Teflon that allows gas diffusion from cells but retains humidity control (Hales, Rolston & Potter, 2010).

While the primary neuronal are prepared from dissociation using papain solution, microstrainer and centrifugation with BSA solution to remove debris, microliter concentrations of polyethylenimine and then laminin solution are placed into the MEA center that will ensure the subsequent delocalization of and spreading of neuron cells across the electrode surfaces. During laminin binding to the dish, the neurons in BSA solution should have agglomerated into a pellet to be isolated, resuspended and analyzed for cell concentration. Repeat centrifugation or dilution may be necessary to bring the cell concentration into an optimal range, which for a standard 8 x 8 grid of 30 micrometer Titanium Nitride electrodes is approximately 1000 to 3000 cells per microliter of solution (Hales, Rolston & Potter, 2010).

Finally, after laminin incubation, careful application (so as to avoid introducing bubbles that may block electrode signals) via pipette of the cell suspension is used to cover all the electrodes. The Teflon lid should be resealed and the MEA placed into an incubator at 5% CO_2, 9% O_2 and 65% humidity at 35-37°C to prevent oxidative stress to the neural cells and avoid water damage to electronic components (Hales, Rolston & Potter, 2010). The associated patterning and microfluidic protocols have been discussed previously in detail in the Discussion section, for further information on the parameters that must be controlled and steps, please consult the references section of this report.

Acknowledgements

I would hereby like to acknowledge resource contributions from scholarly authors with regards to the novel approaches taken for the patterning and guidance of primary culture neurons on microelectrode arrays to generate complex organized neural networks that have the capacity to replicate elements of the enigmatic human brain. They have provided me with a solid foundational resource for proposed research based on the strengths and limitations inherent in various alternatives.

Most importantly, I would like to address the helpful and involved guidance provided by my course Professor, (Insert Professor Name Here) for (Insert Course Here), who provided me the resources and instruction necessary to see this in-depth critical literature review through to completion.

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