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Towards a 'siliconeural computer': technological successes and challenges

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Electronic signals govern the function of both nervous systems and computers, albeit in different ways. As such, hybridizing both systems to create an iono-electric brain–computer interface is a realistic goal; and one that promises exciting advances in both heterotic computing and neuroprosthetics capable of circumventing devastating neuropathology. 'Neural networks' were, in the 1980s, viewed naively as a potential panacea for all computational problems that did not fit well with conventional computing. The field bifurcated during the 1990s into a highly successful and much more realistic machine learning community and an equally pragmatic, biologically oriented 'neuromorphic computing' community. Algorithms found in nature that use the non-synchronous, spiking nature of neuronal signals have been found to be (i) implementable efficiently in silicon and (ii) computationally useful. As a result, interest has grown in techniques that could create mixed 'siliconeural' computers. Here, we discuss potential approaches and focus on one particular platform using parylene-patterned silicon dioxide.

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2

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1. The brain–computer metaphor

Comparing the brain to a computer is the most recent in a long line of metaphors that reflect not only contemporaneous theoretical knowledge, but also popular technologies (and even prevailing social norms) of a given era. The water technology of antiquity (water clocks and the Archimedes screw, for example) inspired Greek pneumatic concepts of the soul [1]. The School of Hippocrates supported a hydraulic model of mind based upon the four humours. Centuries later, during the Enlightenment, clockwork mechanistic comparisons began to proliferate, reflecting new technologies of that era [2]. Even as recently as the mid-twentieth century, Sherrington compared the brain to '. . . an enchanted loom where millions of flashing shuttles weave a dissolving pattern' [3, p. 178].

In the 1820s, Charles Babbage pioneered early mechanical computing devices [4]. Man-made computers have since advanced remarkably and now have a predominantly microelectronic substrate. In the twenty-first century, many neuroscientists now try to understand the workings of the brain by comparing it to modern-day computers. This computational metaphor infers specific ideas about the data structure for representing and processing information. Contemporary reductionist ideas and neural network modelling argue that well-orchestrated populations of simple and 'stupid' elements (thresholded summators, linear combiners, Boolean gates) collectively achieve remarkable biological computational capacity. While this metaphor has many proponents, large gaps persist between our knowledge of neurobiology and the actual mechanisms and rules of neuronal computation. As such, in future we may well look back at current computing comparisons with astonishment. However, electronic signalling *is* central to the function of both nervous systems and contemporary computing. Electrons move in solid-state lattices of microelectronic semiconductors while ions in solution move through channels embedded in neuronal cell membranes. There is therefore a definite logic in attempting to hybridize the two systems. Heterotic computation is one motivation, while the development of neuroprosthetic devices is a clinically important extension of the same concept. This article discusses some of the theoretical challenges involved and some of the progress made to date.

2. Information processing in live nervous systems and the potential of hybridized systems

For both neuromorphic and heterotic computing paradigms, it is crucial to appreciate (as much as is currently possible) the machinery and mechanisms at work in real live nervous systems. In the brain, information processing is predominantly, though not entirely, the work of neurons. Neurons are specialized, electronically excitable, anatomically and functionally polarized, cells [5]. Ion channels maintain voltage gradients across their lipid bilayer membranes. This is achieved by maintaining different concentrations of ions inside and outside the cell. The key electrical components of neurons are voltage-gated ion channels. The open or closed state of these channels is connected to a displacement of electrical charge across the membrane. When the cross-membrane voltage is altered beyond a given threshold, voltage-gated ion channels change configuration, the cell membrane depolarizes and an action potential propagates along the cell's output wire—the axon.

Transfer of information between adjacent, connected neurons occurs at synapses [6,7]. The vast majority of synaptic transmission is chemically mediated. Pre- and post-synaptic membranes are separated by a synaptic cleft, into which a neurotransmitter is secreted. Neurotransmitter release from the pre-synaptic membrane is triggered by the arrival of an action potential. Membrane depolarization causes voltage-gated Ca^{2+} channels to open, consequent influx of Ca^{2+} ions, fusion of neurotransmitter-filled vesicles with the pre-synaptic cell membrane, and release of neurotransmitter into the synaptic cleft. Neurotransmitters bind to specific ligand-gated ion channels in the post-synaptic membrane. This results in a conformational change that opens
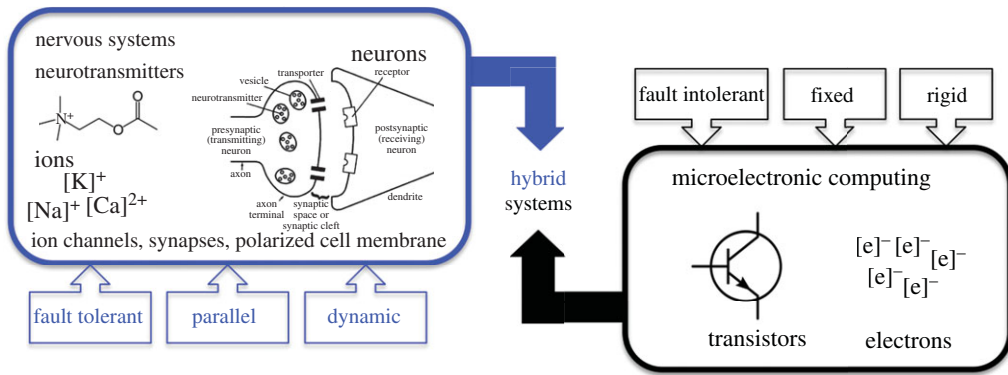
Figure 1. A comparison between the components and modus operandi of nervous systems and silicon-based computers.

the ion channel pore. The outcome is either a transient excitatory post-synaptic depolarization or inhibitory hyperpolarization (depending upon ion channel and neurotransmitter type). These inputs collectively determine whether the receiving cell reaches threshold for generation of an action potential. Most neurons in the central nervous system (CNS) receive thousands of synaptic inputs, each activating various combinations of transmitter-gated ion channels. The post-synaptic neuron transforms these complex chemical and ionic signals into a simple output. The process of converting many inputs into a single output constitutes a neural computation, of which a human brain performs billions per second in parallel. This computational process is fundamental to all neural information processing.

The human brain contains approximately 86 billion neurons equipped with an average of approximately 7000 synapses per neuron, collectively performing approximately 12×10^{15} calculations or computations per second.[1] This is further complicated by multiple different neurotransmitters, the variable influence of glial cells and a dynamic ultrastructure influenced by network activity. The 'connectome' and the 'synaptome' are terms relating to different scales of connectivity in nervous systems. The connectome describes macroscopic (major tracts, visible with human eye) and intermediate sized structures (visible with light microscopy). The synaptome refers to the smaller ultrastructural level (visible by electron microscopy). We remain largely ignorant of the highly complex microscopic anatomy of neuronal connections in the brain. Despite extensive efforts, we do not currently possess complete connectome data for the nervous system of any species, with the exception of the nematode worm *Caenorhabditis elegans* [8]. Work is underway to catalogue the human connectome with the overarching goal of building a realistic statistical model of the human brain (www.humanconnectome.org/consortia/). Although tempting to embrace a wiring diagram analogy, this is fundamentally inadequate. The connectome and synaptome evolve dynamically during development, life, ageing and in response to disease. The strength or weight of synaptic connections can be up- or downregulated, new synapses can be created and others eliminated, neurite architecture can be modified, and even neurons themselves can be created or destroyed.

Nervous systems thus operate in parallel, with dynamic interconnected neurons and an intrinsic degree of system fault tolerance. By comparison, most microelectronic computers work in a rigid, sequential, and fault intolerant mode (figure 1). Although a microelectronic computer's lack of parallelism is compensated in part by executing instructions at high speed, there is a significant time and energy tariff for shuttling data repeatedly through a central processing unit. For example, the Blue Gene supercomputer (capable of performing 10^{16} computations per second)

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[1]This approximation is based on assumptions of 86 billion neurons, connected via 7000 synapses per neuron, firing at an average frequency of 20 Hz, resulting in 1.204×10^{16} firing events per second. Moreover, this approximation fails to appreciate other 'calculations' attributable to glia–neuron interactions or neuropeptides, for example.

requires 1.5 MW to operate.[2] The human brain functions at at least 12×10^{15} bits s^{-1} and requires just 20–25 W.[3]

Interfacing silicon-based microelectronics with *in vivo* nervous systems (or simpler *in vitro* neuronal networks) has exciting potential. Theoretically, microscopic-level hybrids could offer a method of repairing or even enhancing nervous system functionality. Conversely, neuron-mediated computation could be incorporated into silicon-based computers to enhance their functionality, or used as a bridge to living biological transducers.

3. Interfacing neurons and silicon: challenges and potential utility

Interfacing on a meaningful multi-channel scale with living nervous systems (or *in vitro* neuronal networks) is a massive challenge. Theoretically, the ideal interface would record simultaneously from all component parts of a nervous system (quantitatively measuring electrical and chemical activity), and be equipped with complementary tools to stimulate specific targeted regions. However, the extremely small anatomical scale, proximity of non-neuronal cells, complex vasculature and a marked fragility all significantly restrict access. While long-established single-cell recording techniques have enhanced understanding of sensory responses in single neurons *in vivo*, they do not generate data regarding *broader* network activity, nor do they specify the spatial distribution and sub-threshold activity at synapses. Newer neuroscientific tools are improving resolution. For example, combining whole cell patch-clamp recordings[4] with two-photon microscopy allows *in vivo* measurement of calcium signals from dendritic spines of cortical neurons. Other techniques using optogenetics are also beginning to provide better *in vivo* system-level data [9], but have their own specific limitations, being highly invasive and requiring light to penetrate through tissues.

Hybridizing with simpler *in vitro* neuronal networks is arguably a sensible first step (and the primary focus of techniques described in this review). While such networks are far simpler, they offer a more practical environment in which to hone technologies that facilitate sympathetic interaction between neuron and silicon-based technologies. Such constructs have their own specific limitations. Most are two-dimensional and do not realistically reproduce certain facets of the *in vivo* nervous system environment (e.g. inter-relationship with glia or blood supply dynamics). However, the ability to control or rationalize (or even remove) these factors is also the core strength; simplifying the system into one from which dynamic activity may be recorded and emergent properties extrapolated. Unfortunately, traditional procedures for separating cells out for *in vitro* cell or organotypic cultures undermine normal neuronal network architecture and connectivity. Tools are therefore needed to engineer viable *in vitro* neuronal networks with defined topography and connectivity. Arguably, therefore, the core challenges are as follows:

 (i) to engineer a sympathetic connection between the key processing components of neurons (ion channels) and those of semiconductor computers (transistors and electrodes) and
 (ii) to do so in the context of an engineered neuronal network with defined topography (with respect to neuronal configuration and connectivity) and where long-term network maturation and survival is promoted.

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[2]According to IBM Research (see http://www-03.ibm.com/ibm/history/ibm100/us/en/icons/bluegene/).

[3]The average human male requirement of 2500 kilocalories per day equates to 0.0289 kilocalories per second. This translates to 121 joules per second or 121 W. The brain demands approximately 20% of cardiac output. As such, its power requirement can be grossly estimated as approximately 24.2 W.

[4]Patch clamp recording is an electrophysiological technique allowing the study of excitable cell types. An electrode, typically in the form of a fluid-filled glass micropipette with tip diameter of approximately 1 μm, is pressed against the cell membrane and suction applied. This forms a high resistance seal, enabling recording of currents measured across the membrane patch.

4. Establishing topographic control over *in vitro* neuronal networks

Imposing a given topography upon neurons demands the use of bio-patterning technologies. The resolution at which individual cells and subcellular components can be controlled is key. Importantly, this control may attenuate over time depending on patterning platform and cell type. This is particularly important for cells requiring time to mature into their final functional phenotype. Cell behaviour and phenotype are influenced significantly by interaction with an adjacent synthetic substrate. Undifferentiated cells are particularly susceptible to influence.

Also crucial is the issue of anatomic and functional polarity. During neuronal differentiation, symmetry is broken and morphology alters to create two functionally and structurally distinct compartments: dendrites and axons [10,11]. From an electrophysiological standpoint, neurons are polarized such that information flow is unidirectional (dendritic synapse → cell body → axon initial segment → axon → pre-synaptic terminal). This flow of information through an integrated network of neurons is fundamental to nervous system function. Achieving appropriate polarity in an *in vitro* engineered neuronal network is similarly critical. Functional polarity is dependent upon anatomic polarity, as manifest by the distinction between axonal and somato-dendritic domains. Polarity is also seen at a subcellular level, with protein complexes, organelles and ion channels found in distinct membrane regions or cellular compartments [12].

In vivo, genetically defined mechanisms (operating in concert with experience-based changes) enable neuronal cell bodies to locate correctly, form appropriate pathways and reach precise targets. Radial glial cells, for instance, provide a radiating fibre scaffold for cortex-bound daughter cells to migrate. Initially, neurites grow out from the cell body. One neurite becomes the dominant axon while others become dendrites. Fine structure depends upon environmental factors with neurons in different locations developing characteristic cyto-architecture and cell-to-cell connections. Axons reach appropriate targets through a combination of cell-to-cell communication and cell-to-extracellular matrix (ECM) communication. Integrins and other cell adhesion molecules in neuronal growth cones dictate migration, adhesion, guidance and outgrowth [13]. The growth cone probes the environment, seeking a suitable substrate upon which to take hold and advance. Growth and adhesion therefore occur in the context of appropriate ECM proteins, along haptotactic or chemotactic gradients.

(a) Neuronal patterning platforms

The concept of building topographically defined *in vitro* neuronal networks began to be explored over 40 years ago [14–16]. Several diverse approaches have been employed to date (figure 2), some being silicon-based, some piggybacking techniques used in the microelectronics industry (e.g. photolithography), and others using very different technologies (e.g. inkjet printing). Most are two-dimensional. The increasing availability of technologies such as three-dimensional printing and porous scaffolds is now enabling more three-dimensional approaches. However, while bringing a closer resemblance to aspects of *in vivo* histoarchitecture, such constructs are significantly more difficult to interact with and in some ways undermine the necessarily reductionist strategy.

(b) Potential technologies

Conventional inkjet printers pattern pigments by depositing droplets (of volume 10–$100\,\mu m$) under robotic control. The same technique can be used to pattern pro-adhesive cytophilic substances (e.g. collagen or poly-D-lysine) onto a cytophobic background [22]. In this way, inkjet printing has been used to pattern hippocampal rat neurons and glia [23]. Patterning resolution is significantly limited (to tens of micrometres at best). Moreover, uniting this method with silicon-based electronics raises the issue of alignment, component compatibility and the often-short functional lifespan of the printed pro-adhesive substance.
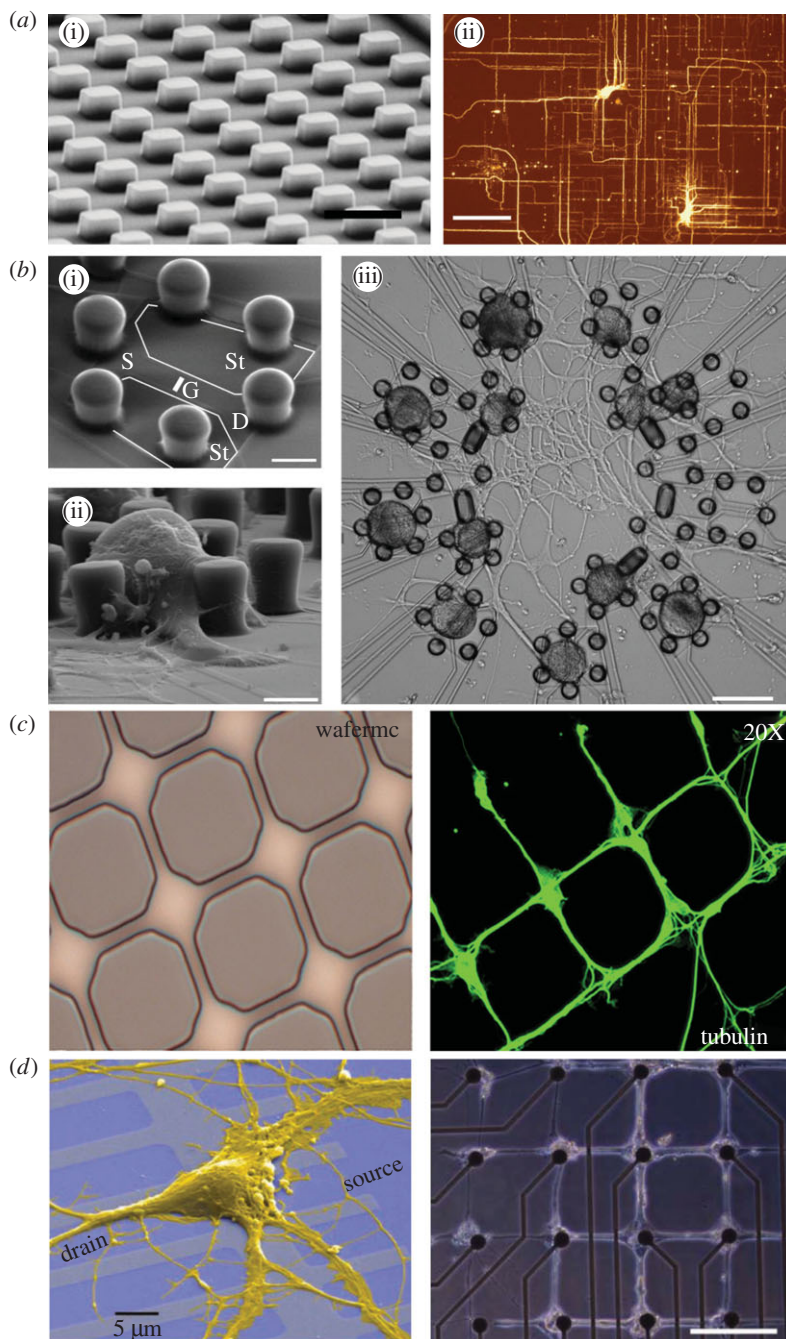
Figure 2. (*Caption opposite.*)

Microcontact printing involves the fabrication of an elastomeric[5] stamp. First, silicon is coated with a pre-designed pattern of photoresist termed the 'master'. A liquid phase pre-polymer (often polydimethylsiloxane, PDMS) is applied to the master to form the stamp. After curing, the stamp is 'inked' with cytophilic proteins (e.g. fibronectin) and applied to a given surface. This approach has been used with various 'inking' molecules and a variety of different cell types

[5] An elastomer is a polymer that has both viscosity and elasticity.

Figure 2. (*Opposite*.) Examples of techniques used for *in vitro* topographic control and/or interaction with neurons. (*a*) Using topographic variation to induce orthogonal neurite growth on silicon. (i) High magnification scanning electron microscopy image illustrating an array of pillars (created photolithographically on silicon) of height 1 μm. Scale bar, 4 μm. (ii) Automated tracing of neuron-specific β-III tubulin-labelled rat hippocampal neurons grown on the pillar array (after coating with poly-L-lysine). Note the orthogonal growth pattern of neurites. Scale bar, 100 μm. Adapted and reproduced with permission from Dowell-Mesfin *et al.* [17]. Copyright 2004 © IOP Publishing. All rights reserved. (*b*) Physical immobilization of individual neurons from snail (*Lymnaea stagnalis*) using a microscopic 'picket fence'. (i) Electron micrograph of a two-way contact with a 'picket fence' made of polyimide. Stimulator wings (St) and transistor (S, source; D, drain; G, gate) are marked (scale bar, 20 μm). (ii) Post fixation electron micrograph of neuron in the picket fence after 3 days *in vitro* (scale bar, 20 μm). (iii) Micrograph showing neuronal cell bodies in picket fences on a circle of two-way contacts connected by neurites after 2 days *in vitro* (scale bar, 100 μm). Adapted from Zeck & Fromherz [18]. (*c*) Microcontact printing in isolation and combined with multi-electrode arrays. (i) Silicon wafers with octagonal patterns designed to support a single neuronal cell body at each node. This wafer was used to create a polydimethylsiloxane stamp which was 'inked' with poly-L-lysine and then applied to a coverslip. (ii) Primary hippocampal neurons plated on micropatterned poly-L-lysine and immunostained for β-III tubulin. Adapted from Belkaid *et al.* [19]. (iii) Photomicrograph of a bio-patterned network (created using a similar microcontact stamping technique) but printed so as to align with an electrode array. This construct enabled recording of electrophysiological activity within the network. Adapted from Marconi *et al.* [20]. (*d*) Colourised electron micrograph of a murine hippocampal neuron on an electrolyte–oxide–silicon field effect transistor after 8 days of culture. Adapted Voelker & Fromherz [21].

including fibroblasts and keratocytes. Belkaid *et al.* used this approach to pattern neuronal cells, using PDMS stamps 'inked' with poly-L-lysine and printed onto untreated coverslips [19]. In this way, primary murine hippocampal and cortical cells were successfully patterned on linear and octagonal patterns. However, pattern degradation occurs fast (due to protein denaturation), demanding rapid use of the printed substrates. Considering downstream integration with microelectromechanical components, microcontact stamping is also problematic with respect to accurately aligning stamp with pre-fabricated components.

Microfluidics deals with behaviour, control and manipulation of fluids at sub-millimetre scale. For cell patterning, a substance such as PDMS is used to create a network of three-dimensional channels that are flooded with a cell suspension or irrigated with cell adhesive molecules. Morin *et al.* [24] combined a three-dimensional PDMS construct with a commercially available planar multi-electrode array (MEA). After flooding with either poly-L-lysine or laminin, neurons (derived from mouse or chick embryos) were successfully seeded into the microsystem. This approach suffers from the similar *post hoc* alignment issues as microcontact stamping. Moreover, the low flow in some microfluidic constructs can result in insufficient diffusion of nutrients in growth media. However, a clear benefit of such constructs is the ability to locally manage the fluid microenvironment, opening the way to targeted pharmacological therapy, for example.

Physical immobilization techniques range from fabrication of three-dimensional structures such as pillars or compounds to trap neuronal cell bodies, to the modification of surface topography or roughness to alter adhesion characteristics. Individual neurons from snail (*Lymnaea stagnalis*) have been immobilized using a microscopic 'picket fence' of polyimide on a semiconductor chip [18]. These physically restrained cells formed a network with post-synaptic excitation modulating the current of an on-chip transistor. A similar approach used 'nanopillar' arrays [25]. Here, in an effort to reduce neuronal migration on patterned substrates, arrays of vertical nanopillars (dimensions 150 nm × 1 μm) were created by ion beam platinum deposition. Movement of neurons in contact with nanopillars was significantly restricted. Such immobilization methods tend not to rely on biological agents or activation of potentially short-lived chemical cues. However, fabrication processes for these bespoke micrometre scale surfaces are complex and expensive. Moreover, while physical immobilization may serve to define the location of a cell body, there is limited control of *neurite* behaviour. With this in mind, topographic variation of substrate surface can be used to alter adhesive behaviour more subtly. Dowell-Mesfin *et al.* [17] explored the impact of specific topographical cues on murine hippocampal neurons.

Neurons were grown on poly-L-lysine-coated silicon surfaces containing regions of pillars created using photolithographic processes. One micrometre high pillars with different width and inter-pillar spacing were assessed. Neurite growth on smooth surfaces was random, while growth on regions with pillars of width of 2.0 μm and inter-pillar distance of 1.5 μm demonstrated more orthogonal growth.

Certain of these techniques can prove highly effective in defining aspects of network topography. Combined with MEAs or patch-clamping techniques, it has also been possible to record from different areas of patterned networks simultaneously. For example, Marconi *et al.* [20] combined the use of microcontact printing (using a silicon master) with accurate alignment upon a multi-channel MEA, to both inform topography of hippocampal neurons and also record electrophysiological characteristics. Similarly, Boehler *et al.* [26] have successfully developed a platform capable of recording from patterned hippocampal neurons for up to 21 days. This construct involves alignment of a PDMS stamp (inked with polylysine) with a separate MEA-incorporated substrate. Once refined, they achieved reliable patterning of neuronal networks with specified connectivity. Crucially, the underlying MEA electrodes also allowed recording of spike activity in the network. It was observed that spike rates were constant regardless of pattern complexity (4, 6 or 8 connections), suggesting that neurons may be optimized for a state of spike rate homeostasis. Identifying such emergent neuronal network computational properties exemplifies the potential of topographically defined neuronal networks and gives hints as to some of the computational mechanisms at work *in vivo*. However, while combining patterning with MEAs can prove effective there remain alignment and up scaling issues.

5. Neuronal patterning on parylene-C : SiO$_2$ constructs

Our approach has been informed by a desire to *fully* integrate the patterning platform into established silicon micro-fabrication processes. This would remove the alignment issues of two-stage (print-on-MEA type) processes and potentially open the door to silicon-integrated tools for neuronal recording and stimulation (such as embedded patch-clamps or other novel electrodes [21]). We also sought to generate a biologically stable patterning platform, amenable to long-term storage.

Photolithography involves transfer of geometric features from mask to substrate via illumination (usually ultraviolet light). A mask is designed using an appropriate computer-aided design platform and fabricated on a glass plate coated with a thin layer of non-transparent chromium that represents the desired geometric pattern. Standard feature resolution is 1 μm with some mask fabrication processes allowing sub-micrometre resolution. The substrate to be patterned is coated with a thin layer of photoresist (a UV-sensitive polymer). The coated polymer is aligned and brought into close contact with the mask. An UV source is applied such that unprotected areas are irradiated and therefore become soluble and removable in a subsequent development step. This process (and conceptually related spin-off technologies) has a long history of use in the fabrication of silicon semiconductor devices, where silicon dioxide wafers are frequently used as a substrate. As photolithographic techniques are at the core of microelectronic fabrication processes, our aim was to use similar processes to create a high fidelity neuronal patterning method on silicon. Cell patterning using photolithographic techniques is well described. For example, micro-patterns of different metal oxides have been created to alter the adhesive behaviour of human osteoblasts. These cells recognized the difference between aluminium, niobium, titanium or vanadium-patterned regions and showed differential surface adhesion and migration behaviours [27].

A balance between specific competitive binding, and non-specific repulsion, governs cell adhesion to an adjacent substrate. Specificity of cellular adhesion is controlled biologically by expression of cell surface receptors that bind with reciprocal ligands. In parallel, adhesion is also influenced more generically by surface energy, polarity and contact angle (or 'wettability'). If a material is too hydrophobic, pro-adhesive proteins tend to absorb in a rigid denatured state. This impairs access to specific binding sites and thereby impairs cell adhesion. Parylene-C is a notable

hydrophobic polymer with a baseline water contact angle of $87°$. This contrasts with a baseline water contact angle of $43°$ for SiO_2 [28]. Owing to low water and gas permeability, parylene-C has long been used to protect printed circuit boards. Being amenable to photolithographic etching processes, it is used ubiquitously in the semiconductor industry to create dielectric layers and as coatings for microelectromechanical systems. Low water absorption, the highly homogeneous nature of the parylene film, its thermal stability and high corrosion resistance have also promoted use in vascular stents, cardiac pacemakers and for insulating neural electrodes. Parylene is deposited by chemical vapour deposition, during which the monomer absorbs to a surface and then polymerizes spontaneously to form high molecular weight linear parylene films. Importantly, this process can take place at room temperature, making the coating process compatible with pre-fabricated heat-sensitive components [29].

On the basis of differences in contact angle, Delivopoulos *et al.* [30] hypothesized that photolithographically printed parylene-C might serve as a cell repellent domain, leaving cells to adhere specifically to a background of SiO_2. As such, oxidized silicon wafers were patterned with parylene features using standard clean room photolithographic process, then incubated with fetal calf serum (a standard component of cell culture media), and plated with murine hippocampal cells (a combination of neurons and glia). Although accurate cell patterning was achieved, cells in fact adhered to parylene domains and were repulsed by SiO_2 (figure 3). This unexpected finding led to questions about the impact that the photolithographic processes had upon the downstream adhesive behaviour of the two substrates, and also how the components of serum interact with the two substrates [31]. Subsequently, a number of other cell types (both neuronal and non-neuronal) have been trialled on this platform, revealing heterogeneous patterning behaviours [32,33]. Our recent work has focused on (i) trying to disentangle the cell adhesion mechanisms at work on parylene-C : SiO_2 constructs and (ii) refining neuronal patterning such that meaningful, functional neuronal networks might be generated.

The hippocampal cells initially tested were a combination of both neurons and glial cells. We first sought to pattern neurons alone, questioning whether isolated neurons can pattern or whether they rely on the presence of glial (or another) supporting cell type. Though the presence of glia among patterned neurons better reflects the *in vivo* environment, it complicates downstream efforts to interact with individual neurons. We used the Lund Human Mesencephalic (LUHMES) cell line which manifests well-described functional neuronal characteristics [34]. These are conditionally immortalized cells that can be induced to differentiate by shutting down the *myc* transgene. This promotes differentiation, resulting in a homogeneous source of functional post-mitotic neurons.

Attempting to pattern LUHMES neurons in isolation was unsuccessful. However, by pre-patterning the construct with a different template cell type, neurons were induced to adhere secondarily (figure 4). Interestingly, this finding was seen using both a glial cell type (derived from human glial tumours) and a non-glial cell type (human embryonal kidney cells, HEK 293). This is reminiscent of the glia–neuron patterning seen with the original primary rodent co-cultures [35]. HEK 293 cells performed a role analogous to glia. Moreover, LUHMES neurons that attached to HEK 293 cell clusters differentiated morphologically, evidenced by outgrowth of neurites onto surrounding bare silicon. Both cell types facilitated neuronal patterning by providing a physical point of attachment on-chip.

As discussed above, creating functional networks relies not only on dictating the location of the cell body, but also on controlling neurite growth direction, connectivity and polarity. More recent work using an array of circular nodes of parylene, with 'spokes' extending at $0°$, $90°$, $180°$ and $270°$ allowed the growth of orthogonally arranged neuronal networks. The size and spacing of parylene nodes influenced neurite directionality. Questioning whether the parylene node itself is capable of ushering the advancing neurite in a given direction, a different array of nodes (with varied morphology consisting of one or two spokes or one or two tapered extensions) was tested. This demonstrated that neurite directionality was not influenced by the underlying parylene. Neurites instead grew out onto the surrounding SiO_2 in a manner that suggested attraction to nearby cell clusters, possible mediated by an as yet unidentified chemotactic gradient.
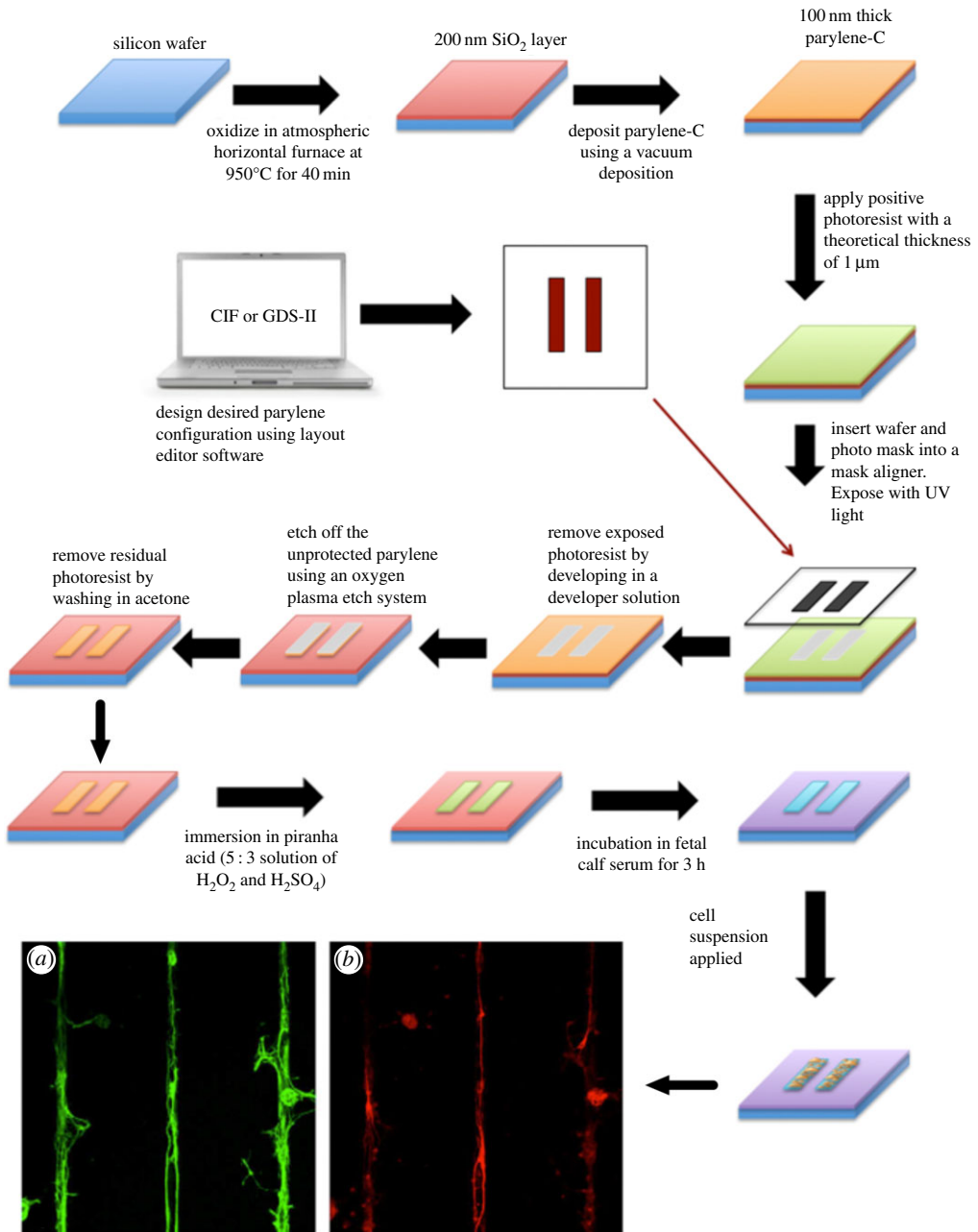
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Figure 3. Fabrication of parylene-C : SiO$_2$ substrates. Fabrication and activation processes for parylene-C-patterned SiO$_2$ wafers with photomicrographs of murine hippocampal cells cultured on-chip. Vertical parylene strips (20 μm thick) are separated from one another by 180 μm of SiO$_2$. (*a*) Stained with the glial fluorescence marker glial fibrillary acidic protein and (*b*) fluorescently stained with the neuron-specific marker β-III tubulin. Each image depicts an area of 500 × 500 μm.

This work has allowed neuronal cultures to be grown on silicon, with a degree of control over the architecture of the network. However, it currently has some significant limitations. The requirement for an underlying template cell, which continues to proliferate in contrast to the post-mitotic neurons, results in overgrowth from parylene domains. This, combined with tension in the interconnected neurites, results in cell lift-off and network obliteration after several days. Despite efforts to slow or arrest the growth of both template cells tested, overgrowth remains
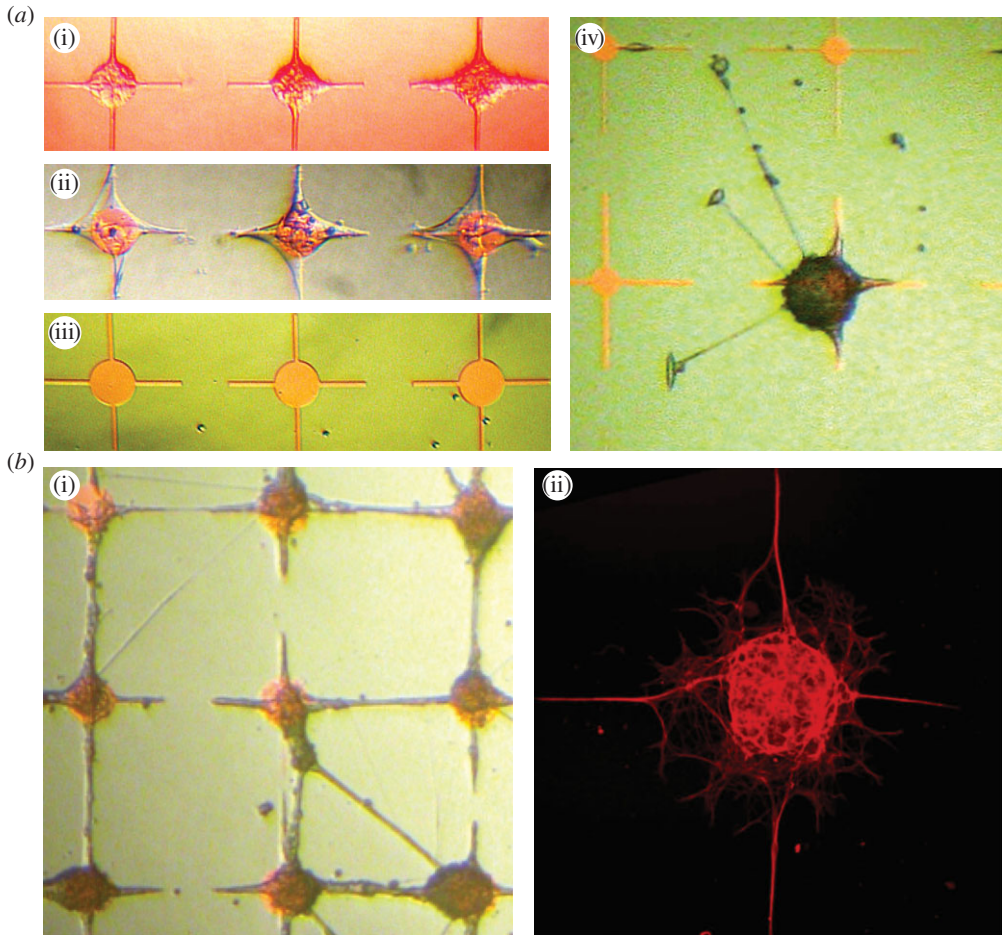
Figure 4. Neuronal networks on parylene-C : SiO$_2$. (*a*) (i) Human embryonic kidney (HEK 293) cells pattern accurately on parylene-C : SiO$_2$ chips. (ii) Glioblastoma-derived glial cells also pattern accurately on parylene-C : SiO$_2$ chips. (iii) Differentiated LUHMES neurons fail to pattern, nor adhere anywhere, on parylene-C : SiO$_2$ chips. Circular nodes are 100 μm in diameter. (iv) Co-culture of neurons with HEK 293 cells. HEK 293 cells were plated first, followed (48 h later) by the addition of pre-differentiated LUHMES neurons. In co-culture, neurons are seen to adhere to the HEK 293 cell template and show morphological differentiation with neurites extended out onto bare SiO$_2$. (*b*) (i) Glioblastoma-derived glial cells were plated first, followed (72 h later) by the addition of pre-differentiated LUHMES neurons. In co-culture, neurons are seen to connect and form an orthogonal network. Circular nodes are 100 μm in diameter. (ii) Immunofluorescence image of β-III tubulin-labelled co-culture, illustrating neurites extending outwards.

problematic [36]. Moreover, the presence of the template cell itself complicates creation of a suitable interface between neuron and silicon. That said, the bare SiO$_2$ domains (that contain only bridging neurites) represent a potential area of neuron-only interaction.

(a) Development of the parylene-C : SiO$_2$ platform

Future work will seek to better establish the rules governing neurite outgrowth and connectivity. Axon guidance molecules or other topographical features on-chip may allow another level of control. A suitable template cell that does not overgrow and overwhelm the network is needed. At present, little is known regarding the functional behaviour of patterned LUHMES neurons. Once longer term cultures are established, it will be important to confirm that neurons are electrophysiological active, firing action potentials and forming functional synapses.

12

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Fundamental to this platform, and other silicon-based patterning techniques, is the incorporation of a method for sympathetically uniting electrically active neurons with silicon-based components. Specifically, a strategy is needed to establish a reliable contact between the lipid bilayer of the polarized cell membrane and the oxide layer of silicon. Key factors are resistance and distance. One approach might involve incorporating novel electrodes. For example, carbon nanotubes (CNTs) are electrically conducting, allowing excellent interfacial electrical impedance [37]. Sorkin *et al.* [38] cultured neurons on 20 µm CNT islands on a background of quartz. Neurons anchored themselves to CNT islands, with processes curled and entangled among the nanotubes.

6. Future directions for optimized siliconeural devices

Once tools for patterning, interacting and recording from engineered neuronal networks reach appropriately high fidelity, a fundamental question arises regarding the type of network to engineer, and why. Equipped with a limited understanding of computation in nervous systems, it is currently difficult to connect design with intended functionality. However, should a silico-neuronal construct serve to augment standard electronic data processing, do we even need to understand how it operates? Perhaps not, though its potential use as a tool to help unravel the nature of *in vivo* neurodynamics would arguably be undermined by networks of totally undefined topography. Equally, one would not want to stifle an innate capacity of neuronal networks to self-organize into 'computational units' by dictating their organization too much. Some existing interfaces interact with completely random, non-patterned cultures. With this at one extreme, the question arises as to what degree of control is optimum.

Neuromorphic engineering is a means of synthesizing biologically inspired systems and elements. Neuromorphic hardware describes specially designed integrated circuits that incorporate such elements. If nervous systems can robustly be interfaced with silicon microelectronics, this form of biomimicry is arguably superseded. Why build components that mirror neurons when you can integrate neurons themselves? Rather than reaching this inflexion point suddenly, it is more likely that one approach will inform progress in the other. As better understanding of neurobiology prompts better informed neuromorphic constructs, the capacity to interact sympathetically with the 'real thing' will also advance.

Human brain–computer interfaces (BCIs) represent another heterotic computing construct. A BCI (or neuroprosthesis) is an artificial link between an organism's nervous system and the external world. Interaction with the tangible outside world normally occurs via our locomotor, sensory and special sensory systems. In the fledgling field of regenerative neurosurgery, BCIs offer a method of overcoming disability caused by neurological or musculoskeletal pathology. Pathology that interrupts a downstream component of behaviour (for example, a spinal cord injury) is theoretically amenable to therapy with a BCI. By recording from viable CNS domains, the region of pathology is bypassed and a meaningful interaction can be re-established with the outside world (for instance, via a robotic limb or a computer cursor). Clinically useful BCIs exemplify crude heterotic computing constructs: the patient provides biological computation (with their nervous system) and transduction (via sensory organs), while a connection to microelectronics lends the benefits of silicon-based functionality.

7. Conclusion

Evolved nervous systems and *in vitro* neuronal networks are biological computers with an efficiency, plasticity and fault tolerance that supersede that of current conventional computers. Therefore, to sympathetically hybridize and interact with them at a microscopic level represents an excellent opportunity to enhance the power of a silicon-based computational system.

Progress towards this goal is being made on several fronts. Neuronal patterning technologies are proliferating and some have potential for incorporation *in silico*. Many challenges, perhaps best faced *in vitro* at first, stand in the way. Arguably at the forefront is the need to combine a

degree of topographic control with suitable *in silico* components that allow long-term cellular interaction. Beyond heterotic computation, the prospect of *in vivo* human deployment offers great potential in the field of neuroprosthetics and regenerative neurosurgery and also represents a powerful tool for neurobiologists to help better define neuronal computational processes.

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