INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF NEURAL ENGINEERING

J. Neural Eng. **3** (2006) 95–101 doi:10.1088/1741-2560/3/2/003

Compact self-wiring in cultured neural networks

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Received 9 January 2006
Accepted for publication 2 March 2006
Published 11 April 2006
Online at stacks.iop.org/JNE/3/95

Abstract

We present a novel approach for patterning cultured neural networks in which a particular geometry is achieved via anchoring of cell clusters (tens of cells/each) at specific positions. In addition, compact connections among pairs of clusters occur spontaneously through a single non-adherent straight bundle composed of axons and dendrites. The anchors that stabilize the cell clusters are either poly-D-lysine, a strong adhesive substrate, or carbon nanotubes. Square, triangular and circular structures of connectivity were successfully realized. Monitoring the dynamics of the forming networks in real time revealed that the self-assembly process is mainly driven by the ability of the neuronal cell clusters to move away from each other while continuously stretching a neurite bundle in between. Using the presented technique, we achieved networks with wiring regions which are made exclusively of neuronal processes unbound to the surface. The resulted network patterns are very stable and can be maintained for as long as 11 weeks. The approach can be used to build advanced neuro-chips for bio-sensing applications (e.g. drug and toxin detection) where the structure, stability and reproducibility of the networks are of great relevance.

1. Introduction

The link between structure and function in neuronal networks is fundamental for the precision and specificity of these networks to propagate, process and store information. It is widely accepted that cultured neuronal networks with engineered architecture provide powerful controllable model systems to test the issue of function–form relations, since it is possible to compare the activity of networks with different pre-designed structures [1–4]. Previous studies using lithography methods indeed revealed pattern-specific activities [2]. Owing to the growing interest in cultured neural networks for sensing applications and for basic investigation of a neural network activity, many attempts have been made, over the last several years, to construct neural networks with pre-defined connectivity. The novel method described here enables us to generate networks composed of controllable small-sized neuronal clusters (dozens of microns), linked by bundles of axons and dendrites with pre-designed geometry and topology.

Cell patterning at the micron scale is best achieved using modified micro-fabrication techniques. These techniques were originally developed for the production of semiconductor devices, but are routinely used today to construct heterogeneous ordered patterned surfaces with cell-attracting and cell-repelling chemistries or topographies which facilitate cultures with pre-defined arrangements [5, 6].

A large majority of the effort to pattern cultured neural networks has focused on chemical patterning with the specific aim of achieving cell positioning at pre-defined locations as well as network determination. A straightforward approach consists of patterning adhesion promoting proteins to desired

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locations by a direct lift-off process [2]. An equivalent process relies on a soft lithography process in which the adhesion proteins are transferred from a polydimethylsiloxane (PDMS) stamp to the substrate [7, 8]. Long-term stability of such patterns can be achieved by the addition of polyethyleneglycol (PEG) to the background. Several other related approaches have been successfully applied to achieve network patterning [9–11].

More recently, nano-topography was demonstrated as an alternative effective method to control the attachment and growth of cells [12–15]. Various studies have clearly demonstrated that surface texture at a scale of tens of nanometers to micrometers can influence the attachment of certain cells to surfaces and can be used as a mechanism to position cells at particular areas on a substrate [14, 16]. Cell type and surface roughness are important in determining the adhesiveness of the substrate.

Despite the apparent success of the techniques outlined above, a remaining challenge in neural network engineering is to achieve and ensure long-term stability, precise cell positioning and high fidelity of the formed neural network. The stability problem arises due to pulling forces exerted by axons and dendrites on the cell bodies, which often overcome the adhesion of the cells to the surface [17], leading to cell migration from designated locations [9, 11, 18]. A possible solution to the challenge stated above is the use of cell traps and physical barriers [4, 19]. However, these solutions are proved to be useful for only small and sparse cell populations.

Here, we describe a new approach in which by manufacturing isolated islands of cell clusters surrounded by a non-adhesive background, stable self-wired engineered networks of both cortical and hippocampal neurons are produced. This approach achieves precise cell positioning and controlled network geometry with wiring which consists of axons and dendrites. The method relies on a natural propensity of cells to form compact wiring between neighboring islands rather than on specific guidance. The clusters in our approach rapidly form in the first two days of culturing as the cells migrate on a mostly low affinity substrate toward high affinity, lithographically defined, adhesive templates on which they adhere and assemble.

We have previously demonstrated the formation of engineered neural networks using carbon nanotube (CNT) templates [20]. Here we expand our initial investigation. We begin by demonstrating that poly-D-lysine (PDL) islands are as useful as CNT islands in generating the engineered network. We then study the formation of different geometries and demonstrate the robustness of the presented approach. Finally, we study the details of the temporal dynamics of the forming networks in the first few days of their formation. Our data reveal that the self-assembly process is driven by neuronal aggregate mobility while stretching a connecting bundle.

2. Methods

2.1. Stencil fabrication

The realization of our new approach is based on a soft lithography process (using PDMS stencils) to define regions with the adhesive sites. An SU8-2075 (Micro Chem) mold on a silicon wafer is used to shape a PDMS stencil, which is later used to pattern the desired material on the surface. First, a layer of SU8-2075 photoresist is spin coated on the silicon wafer in two steps of 100 rpm s^{-1} to 500 rpm for 10 s and then 300 rpm s^{-1} to 2000 rpm for 30 s. Next, a pre-bake at 65 °C for 5 min and 95 °C for 20 min is performed followed by 4 min relaxation. The substrate is then exposed in a mask aligner (MA6, Karl Suss Inc.) for 50 s at 11 mW cm^{-2} followed by a post-bake at 65 °C for 1 min and 95 °C for 10 min, and a 2.5 min relaxation step. The substrate is then developed for 18 min in Shipley EC solvent, rinsed in isopropyl alcohol (IPA) and dried in air. This protocol results in a negative mold with a feature thickness of approximately 100 μm. The PDMS stencil is prepared by spin coating (2500 rpm for 1 min) a mixture of a 10:1 ratio base and curing agent on the silicon mold. After out-gassing for 10 min, the substrate is cured at 100 °C for 45 min. Finally, after detaching the substrate from the mold, the stencil is placed on the silicon or quartz substrate and acts as a chamber to hold the protein solution, or the iron nitrate catalyst (used as a catalyst for the CNT growth process) in IPA suspension.

2.2. Protein patterning

A PDL (Sigma p-7886) solution of 0.1 mg ml^{-1} in a sodium borate buffer of pH 8.5 (Sigma s-9640) was patterned on a glass slide using a PDMS stencil through the following procedure. Several drops of the PDL solution were dripped onto the PDMS stencil which was previously placed on a glass slide. After mild vacuum degassing for 15 min, the excess PDL solution was removed and the sample was dried at 37 °C for 30 min. The PDMS stencil was removed before cell plating.

2.3. Fabrication of patterned carbon nanotube (CNT) islands

Patterned CNT islands were fabricated as previously described in [20]. Briefly, a PDMS stencil was placed onto a quartz substrate (Electron Microscopy Sciences, cat 72250-03). A suspension of an iron nitrate catalyst in IPA (approximately 0.1 g in 20 cm^3) was sonicated, centrifuged, sprayed onto the PDMS stencil-covered substrate and air dried. After removing the PDMS stencil, the CNT islands were created on the patterned catalyst by a chemical vapor deposition (CVD) process. The substrates were introduced into a 1″ quartz tube furnace in an atmosphere of hydrogen gas (1000 sccm for 10 min). The temperature was ramped up to 860 °C. 20 sccm ethylene was used as the source gas for 8 min. Finally, the ethylene flow was terminated and the furnace turned off and cooled down to room temperature.

2.4. Cortical cell cultures

Cortical cells from 1 day old Charles River rats were prepared and maintained as described in [21]. Dissociated cells were plated onto the PDL patterned glass slides or onto CNT patterned quartz substrates at a density of 1×10^6 cells/dish (dish radius is 10 mm), suspended in 3 ml of a growth medium, containing 5% horse serum (Beith Hahemek 04-004-1A), 1 mg ml^{-1} gentamycin (Beith Hahemek 03-035-1c) and 0.02 mM glucose in MEM (Beith Hahemek 01-025-1A). The cultures were maintained under growth conditions at 37 °C with 5% CO_2 and 70% humidity.

2.5. Hippocampal cell cultures for immunofluoresence

For the immunofluoresence study, cells from hippocampal CA3 and dentate gyrus regions from the brains of 1 to 4 day old Sprague-Dawley rats were used. Cell cultures were prepared and maintained as described in [22, 23]. The dissociated cells were plated onto the PDL (20 $\mu g\ ml^{-1}$) patterned glass slides at a density of 0.6×10^6 cells/dish, suspended in 3 ml of the growth medium in MEM (Sigma) containing 10% heat inactivated normal goat serum, 1% L-glutamine and 0.8% D-glucose. One day after plating, the cells were transferred to a serum-free medium containing 45% MEM, 40% DMEM, 10% F12, 0.25% (w/v) BSA, 0.75% of 45% D-glucose, 0.5% B27 supplement, 0.25% L-glutamine, 0.01% kinurenic acid, 0.01% of mixed uridine and fluoro-deoxyuridine.

2.6. Immunocytochemistry

An immunocytochemical study was performed in order to validate the ability of the patterning method to restrict the cell bodies exclusively to the original PDL islands, without migration to the wiring section. The details of the immunocytochemical labeling are described in [23]. Briefly, cells were fixed for 10 min at room temperature with 4% paraformaldehyde, permeabilized with 0.25% triton and blocked with 3% normal goat serum. The cells were then incubated overnight at 4 °C with anti-microtubule associated protein 2 (MAP2) (1 $\mu g\ ml^{-1}$) (monoclonal, Sigma, Oakville, Ontario, Canada) for identification of dendrites, and anti-neurofilament M (NFM) (0.5 $\mu g\ ml^{-1}$) (polyclonal, Chemicon, Temecula, CA, USA) to identify axons. Then, immunolabeling was visualized with secondary antibodies conjugated to Alexa-488 for the MAP2 and Cy3 for the NFM, (2 $\mu g\ ml^{-1}$) (Molecular Probes, Eugene, OR, USA). Nuclear staining was done by incubating fixed cells with 4′,6-diamidino-2-phenyindole (10 $\mu g\ ml^{-1}$) (DAPI, Sigma-Aldrich, St Louis, MO, USA) for 15 min.

2.7. Light microscopy

Imaging of the fluorescent labeling was performed on a Zeiss Axiovert 200 M inverted microscope. The objective used was (from Zeiss) Plan-Neofluar 20X/0.5. The images were captured with a 12 MHz CCD camera (SensiCam, PCO, Kolheim, Germany) and an SK3 motorized stage (Marzhauser, Germany). Acquisition and analysis were performed with commercial software (Metamorph, Universal Imaging, USA). The figures were processed using PhotoShop 7.0 (Adobe Systems Inc.).

The images of cells in culture and time lapse recordings were taken using an optical microscope (IX-70, Olympus) equipped with a CCD camera (SC35, Olympus, Japan). Magnifications of ×40 or ×100 were used. Dry sample imaging was done using a metalorgical light microscope (MEIJI Techno, Japan), equipped with a CCD camera.

2.8. Scanning electron microscopy

For scanning electron microscopy observation, samples were fixated, dried and thereafter gold coated (Polaron Equipment Limited, SEM coating unit E5100). 1–2 weeks old neuronal networks were fixated for 30 min (37 °C) in 2.5% glutaraldehyde (49629, Fluka) in a PBS (79382, Fluka) solution. The fixed samples were then rinsed for 5 min in increasing concentrations of ethanol (25%, 50% and 75%), keeping the sample covered with each of the ethanol solutions, followed by 10 min rinses with 96% and 100% ethanol solutions. Finally, the dehydrated samples were critical-point dried using a Balzers Union critical-point drier. The samples were examined using a JEOL JSM-5610LV.

3. Results

The engineered networks, presented here, are formed by patterning adhesive regions of PDL or high density CNTs on glass or quartz substrates, respectively. The patterning is performed using reusable PDMS stencils [24] with 100 μm openings arranged at different geometries.

The cells were deposited on patterned substrates (with PDL or CNT islands) and after several days they aggregated and accumulated on the PDL, or CNT, coated regions while reducing cell density on the adhesive-free regions (figure 1(A)). Straight connections between the islands were clearly apparent, and interconnected networks were formed following the pattern of the adhesive templates. This process typically occurs by bridging gaps almost exclusively between nearest neighboring islands. Each cluster was composed of hundreds of cells, and extended between 1 and 8 bundles (figure 1(B)). These bundles were made of dendrites and axons, but not cell bodies (figures 1(C)–(F)). Interestingly, these bridges form very efficiently over quartz and glass surfaces, which are apparently very poor surfaces for cell attachment. In addition, few glial-like cells remained in between the clusters and were adherent to the surface.

The process described above relies on the cells' natural propensity to cluster and self-wire rather than on direct chemical or topographical guidance. This feature eliminates the need for high resolution lithography typically needed to pattern the links between cells [9–11, 18] and also allows the spatial separation between cells and processes. This advantage of the presented technique over conventional patterning is apparent in figure 1(A), which clearly reveals the fact that the cell bodies are exclusively restricted to the original PDL islands and do not migrate to the wiring section. This

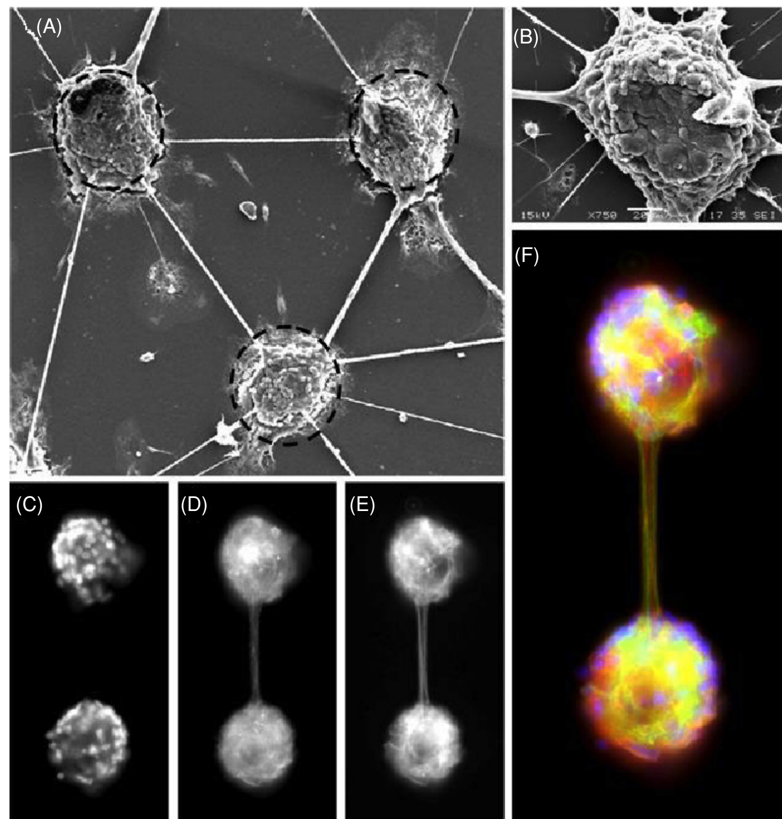

Figure 1. (A) A SEM micrograph of an engineered neural network which is self-assembled with 100 μm PDL islands as a template. The dashed circles mark the PDL islands used to define the network. No PDL was deposited in regions between these islands. Each PDL island contains a cell cluster, and the clusters are connected through bundles that are formed spontaneously. (B) Each of the cell clusters contains hundreds of cells and extends between 1 and 8 bundles. (C–F) Multiple staining of two interconnected clusters. The cells of these clusters are restricted to the PDL island (C, DAPI = cell bodies), and the bundle connecting them is composed of axons (D, NFM) and dendrites (E, MAP2). There are no cell bodies in the bundle. The axons and dendrites interact with the bundle (F, red—axons, green—dendrites, blue—cell body). The yellow color arises from coincidence of green and red fluorescence.

feature is best manifested in the immunofluoresence results (figures 1(C)–(F)), which reveal the efficient separation between the wiring region, made of axons and dendrites, and the cell clusters. This result stems from the fact that in our method the wiring occurs by process bridging over non-adhesive regions while in conventional methods the wiring path and the positioning of the cells are defined by a single chemical treatment. Therefore, in conventional patterning approaches, the tension exerted by axons and dendrites is likely to pull cells from their originally intended positioning to the surrounding surface.

We have used several different geometries for investigating the robustness of this method and found that the compact networks are successfully formed with different geometries, and almost always with connections between nearest neighbors. The networks formed are established with a well pre-defined grid (graph) connectivity which is determined by the position of the adhesive islands. Figure 2 illustrates the success of the presented approach in forming triangular, square and circular networks.

To investigate the origin of the compact wiring behavior presented here, we have examined the dynamics of the networks by placing cultures in an environmental chamber under a microscope, and performed time lapse recording by taking images every 4 min. The most conspicuous process revealed by investigating the temporal dynamics of these cultures takes place in the first 2 days after cells are plated on the surface of the substrate. Interestingly, network connectivity is not the result of the dynamics of single cells. Rather, the cells first aggregate into clusters at random locations on the substrate, after which they separate into individual clusters, which migrate and cling to the CNT sites (figure 3). As these clusters migrate, an isolated process bundle remains suspended between the islands and it stretches with the increased distance between the cell clusters. This process is depicted in figure 3, where a 10 to 15 h time lapse shows a clear separation between two clusters and the formation and extension of a single connecting bundle between them.

As we have shown above, the wiring process is driven by cluster formation and separation. All clusters form roughly at the same time and are simultaneously pulled to nearby anchoring islands. Consequently, eventual links form between mostly neighboring islands, and almost no cross-linking is observed (figures 2(A)–(E)).

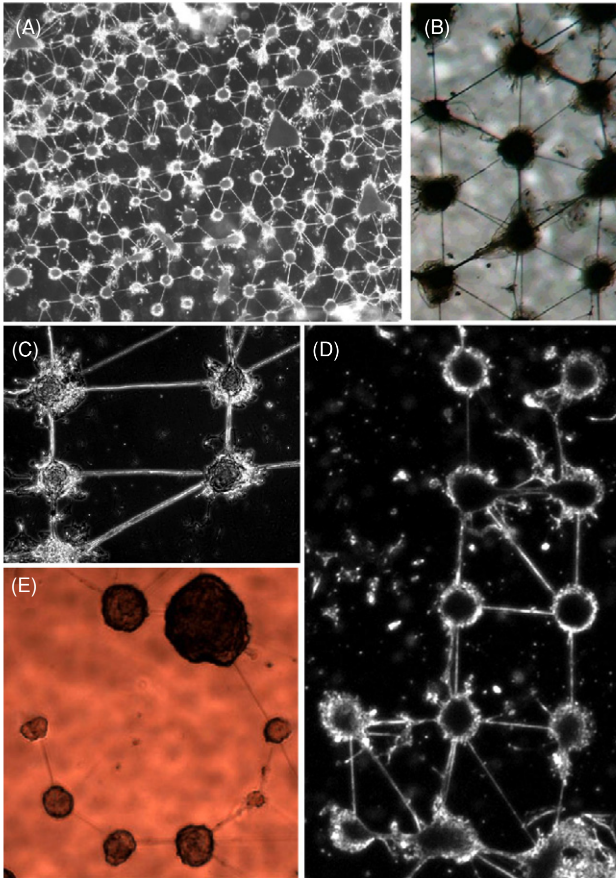

Figure 2. Engineered networks with PDL templates and different network geometries. (A) Low magnification showing a massive amount of interconnected ordered cell clusters, (B) triangular lattice, (C, D) a single (C) and a row (D) of square lattices and (E) circular arrangement. The wiring between the islands is achieved almost exclusively between neighboring islands. Adhesive sites (100 μm in diameter) were realized using PDL on glass.

The propensity of rat cortical neurons in high density cultures to cluster and form separated interconnected islands has already been described [25]. A similar behavior occurs in neurons from distinct biological systems such as insects [26], chicks [27] and vertebrates [25]. On adhesive surfaces (i.e. PDL or CNT coated), this phenomenon is marked by an initial uniform network formation, followed by spontaneous organization in aggregates after 2 to 3 days. Neuronal aggregation increases with time in culture, resulting in well-organized aggregates of a very high cell density, interconnected by fasciculating neurites [28]. The collapse into interconnected cell islands probably occurs due to strong tension in the dendrite and axonal connections between the neurons in the system, which overpowers the cell's relatively poor adhesion to the substrates.

Due to the PDL and CNT patterning we performed here, the new system we present is distinct and advantageous over the studies mentioned above. The cell aggregation results from the incapability of the cells to adhere to the non-adhesive surface, while the adhesive islands trap the clusters, anchoring them to specific pre-designed sites. The cluster connectivity that comes afterward may utilize the same mechanisms as in the works mentioned above, although it may differ by taking place on a non-adhesive surface.

At least two additional mechanisms are possibly contributing to the final result of the well-ordered networks. The first, which has already been described by us [20], occurs by outgrowth of processes from the cluster out. These processes ultimately reach other clusters or merge with processes originating in other clusters to form tightly connecting bundles. This process typically takes place several days after the cells are plated on the substrate. Another way through which large connecting bundles are formed is by association of single connecting processes with thick bundles, probably through dendritic bundling [29, 30] and axonal fasciculation [31]. This process is accompanied by cell motion along the bundles.

4. Discussion and conclusions

The results presented here demonstrate a simple and reliable method to form engineered networks consisting of well-ordered interconnected neuronal clusters. The cluster-to-cluster connectivity is made of neuronal processes alone. The resulting patterning is very stable and can be maintained for many weeks. The method is consistent with the formation of networks made of large cell populations.

There are several advantages for using large cell populations in ordered networks in culture. First, the viability of neuronal cells is positively correlated to the cell density. Although hippocampal cells can be grown in very low densities, such cultures require support of a glial feeder layer, whereas here, the cell clusters are self-maintained. Second, cell clusters, as opposed to single neurons, can extend few axons originating from different cells, highly increasing the chance and complexity of connectivity and activity in the system. Third, cell clusters have a higher total activity than single cells, increasing the sensitivity of the system if used as a biosensor. Fourth, the cell clusters can be considered as sub-networks which are connected to each other through the axonal fibers. The properties of a population activity within clusters and the pattern of signal propagation among the clusters as a network can be studied in response to specific wiring connectivity or during pharmacological manipulations. This last option is of great relevance both in basic structure–function relationship research in neuronal networks and in a bio-sensing application.

As the wiring process relies on a natural propensity of neurons in culture to form compactly wired networks, the properties of these structures heavily rely on the mechanisms that govern these assembly processes. For instance, an increase in cell density yields higher stress in the connecting bundles which eventually overpowers cell adhesion to the islands, resulting in pattern collapse [20]. Reduction in the cell density to yield a few cells per island is feasible, but is yet to be tested to verify the exact lower limit for which the self-wiring process still takes place. It is possible that a certain minimal number of cells are needed to maintain the activity needed for the formation of the wiring.

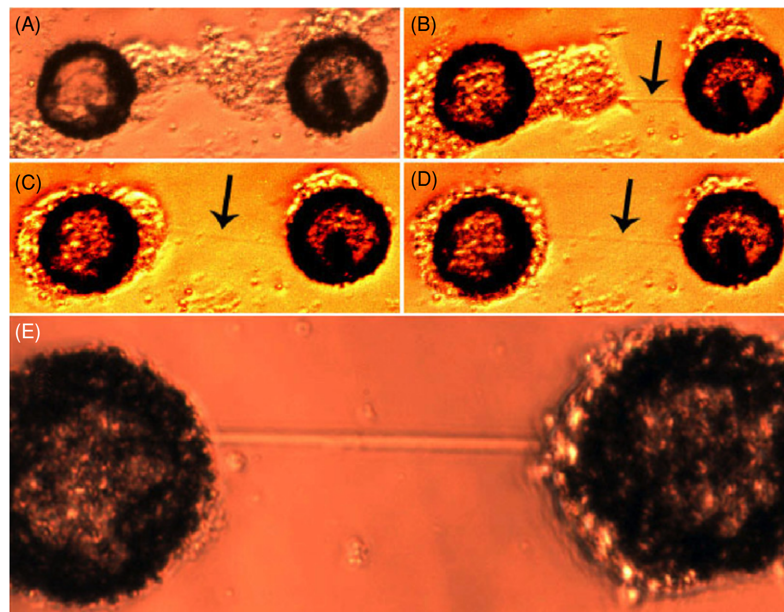

Figure 3. The dynamic of a network self-organization process on 100 μm patterned CNT islands, with 150 μm separation between the islands. The arrows indicate the process bundle interconnecting the two separating clusters. The sample was placed in the environmental chamber under the microscope immediately after plating. (A) 30 h after plating, (B) 40 h after plating, (C) 55 h after plating and (D) 69 h after plating. The final compact connectivity between the two isolated islands is depicted in (E).

A related issue is the maximal distance achievable between clusters. The limiting factor appears to be related to the flexibility of the neurite bundles to extend over long distances without exerting too much pulling forces. Preliminary data revealed links as long as 400 μm. A systematic investigation of this topic is currently underway.

With the mounting evidence supporting the link between a neural network function and form, we expect the various network geometries to have distinct patterns of activity propagation, information processing and sensitivity to external chemical stimuli. Accordingly, this approach may be used in bio-sensing applications where the structure, stability and reproducibility of the networks are believed to be of great importance. Indeed, the technology described above can be readily incorporated with a conventional multielectrode array setup using a simple alignment step: PDMS stencils can be mounted onto a support substrate and aligned (using a mask aligner) and anchored to electrode substrates [32]. Thus, electrical recording from compactly wired networks is conceivable and suitable for the construction of novel bio-sensors.

We believe that the advantages of the presented technique span beyond bio-sensing applications and may assist in studying fundamental questions in neural networks. Specifically, these networks can be used to investigate the link between a network's activity and its geometry, especially in light of the predilection of the networks to connect almost all nodes with the shortest wiring length possible. Furthermore, the system described here can be used to compare the processing of information in an ordered network, as opposed to a random one, as well as used for clarifying a link between efficient network structure and activity.

Acknowledgments

The authors thank Nadav Raichman and Itay Baruchi for helping with the protocols for the neural networks, Inna Brainis for technical assistance in extraction of the cell cultures and preparations of the cultured networks and Ze'ev Abrams for careful reading of the manuscript. The authors also thank the Electron Microscopy Center at the George Weiss Faculty of Life Sciences at Tel Aviv University. TG acknowledges the support of the Zandman–Slaner scholarship. This research was supported in part by an ISF grant and the Maguy-Glass Chair in Physics of Complex Systems.

References

[1] Chang J C, Brewer G J and Wheeler B C 2001 Modulation of neural network activity by patterning *Biosens. Bioelectron.* **16** 527

[2] Segev R, Benveniste M, Hulata E, Cohen N, Palevski A, Kapon E, Shapira Y and Ben-Jacob E 2002 Long term behavior of lithographically prepared in vitro neuronal networks *Phys. Rev. Lett.* **88** 118102

[3] Vogt A K, Wrobel G, Meyer W, Knoll W and Offenhäusser A 2005 Synaptic plasticity in micropatterned neuronal networks *Biomaterials* **26** 2549

[4] Merz M and Fromherz P 2005 Silicon chip interfaced with a geometrically defined net of snail neurons *Adv. Funct. Mater.* **15** 739

[5] Folch A and Toner M 2000 Microengineering of cellular interactions *Annu. Rev. Biomed. Eng.* **2** 227

[6] Bhadriraju K and Chen C S 2002 Engineering cellular microenvironments to improve cell-based drug testing *Drug Discovery Today* **7** 612

[7] Branch D W, Corey J M, Weyhenmeyer J A, Brewer G J and Wheeler B C 1998 Microstamp patterns of biomolecules for

high-resolution neuronal networks, *Med. Biol. Eng. Comput.* **36** 135

[8] Chang J C, Brewer G J and Wheeler B C 2003 A modified microstamping technique enhances polylysine transfer and neuronal cell patterning *Biomaterials* **24** 2863

[9] Scholl M, Sprossler C, Denyer M, Krause M, Nakajima K, Maelicke A, Knoll W and Offenhausser A 2000 Ordered networks of rat hippocampal neurons attached to silicon oxide surfaces, *J. Neurosci. Methods* **104** 65

[10] Kam L, Shain W, Turner J N and Bizios R 2001 Axonal outgrowth of hippocampal neurons on micro-scale networks of polylysine-conjugated laminin *Biomaterials* **22** 1049

[11] Sorribas H, Padeste C and Tiefenauer L 2002 Photolithographic generation of protein micropatterns for neuron culture applications *Biomaterials* **23** 893

[12] Mattson M P, Haddon R C and Rao A M 2000 Molecular functionalization of carbon nanotubes and use as substrates for neuronal growth *J. Mol. Neurosci.* **14** 175

[13] Fan Y W, Cui F Z, Hou S P, Xu Q Y, Chen L N and Lee I S 2002 Culture of neural cells on silicon wafers with nano-scale surface topograph *J. Neurosci. Methods* **120** 17

[14] Fan Y W, Cui F Z, Chen L N, Zhai Y, Xu Q Y and Lee I S 2002 Adhesion of neural cells on silicon wafer with nano-topographic surface *Appl. Surf. Sci.* **187** 313

[15] Hu H, Ni Y, Montana V, Haddon R C and Parpura V 2004 Chemically functionalized carbon nanotubes as substrates for neuronal growth *Nanoletters* **4** 507

[16] Zhang X, Prasad S, Niyogi S, Morgan A, Ozkan M and Ozkan C S 2005 Guided neurite growth on patterned carbon nanotubes *Sensors Actuators* B **106** 843

[17] Goldner J S, Bruder J M, Li G, Gazzola D and Hoffman-Kim D 2006 Neurite bridging across micropatterned grooves *Biomaterials* **27** 460

[18] Welle A, Horn S, Schimmelpfeng J and Kalka D 2005 Photo-chemically patterned polymer surfaces for controlled PC-12 adhesion and neurite guidance *J. Neurosci. Methods* **142** 243

[19] Zeck G and Fromherz P 2001 Noninvasive neuroelectronic interfacing with synaptically connected snail neurons immobilized on a semiconductor chip *Proc. Natl Acad. Sci.* **98** 10457

[20] Gabay T, Jakobs E, Ben-Jacob E and Hanein Y 2005 Engineered self-organization of neural networks using CNT clusters *Physica* A **350** 611–21

[21] Segev R, Shapira Y, Benveniste M and Ben-Jacob E 2001 Observations and modeling of synchronized bursting in two-dimensional neural networks *Phys. Rev.* E **64** 011920

[22] Baranes D, Lopez-Garcia J C, Chen M, Bailey C H and Kandel E R 1996 Reconstitution of the hippocampal mossy fiber and associational-commissural pathways in a novel dissociated cell culture system *Proc. Natl Acad. Sci.* **14** 4706

[23] Cove J, Blinder P, Abi-Jaoude E, Lafreniere-Roula M, Devroye L and Baranes D 2006 Growth of neurites toward neurite-neurite contact sites increases synaptic clustering and secretion and is regulated by synaptic activity *Cereb. Cortex* **16** 83

[24] Duffy D C, Jackman R J, Vaeth K M, Jensen K F and Whitesides G M 1999 Patterning electroluminescent materials with feature sizes as small as 5 μm using elastomeric membranes as masks for dry lift-off *Adv. Mater.* **11** 546

[25] Segev R, Benveniste M, Shapira Y and Ben-Jacob E 2003 Formation of electrically active clusterized neural networks *Phys. Rev. Lett.* **90** 1681011

[26] Shefi O, Ben-Jacob E and Ayali A 2002 *Neurocomputing* **44–46** 635

[27] Donowicz M S, Mangoura D and Schwartz N B 2003 Aggrecan regulates telencephalic neuronal aggregation in culture *Dev. Brain Res.* **143** 207

[28] Monnerie H, Boespflug-Tanguy O, Dastugue B and Meiniel A 1997 An in vitro cell culture system for the aggregation of embryonic chick central nervous system neurons *Brain Res. Protocols* **197** 344

[29] Hatton G I, Modney B K and Salm A K 1992 Increases in dendritic bundling and dye coupling of supraoptic neurons after the induction of maternal behavior *Ann. New York Acad. Sci.* **12** 142

[30] Zupanc G K 1991 Clustering of cell bodies, bundling of dendrites, and gap junctions: morphological substrate for electrical coupling in the prepacemaker nucleus *Neurosci. Lett.* **5** 29

[31] Van Vactor D 1998 Adhesion and signaling in axonal fasciculation *Curr. Opin. Neurobiol.* **8** 80

[32] Gabay T, Jakobs E, Ben-Jacob E and Hanein Y 2005 Carbon nanotube based neuro-chip for engineering, recording and stimulation of cultured networks *Proc. Transducers 2005, The 13th Int. Conf. on Solid-State Sensors, Actuators and Microsystems* vol 2 p 1226