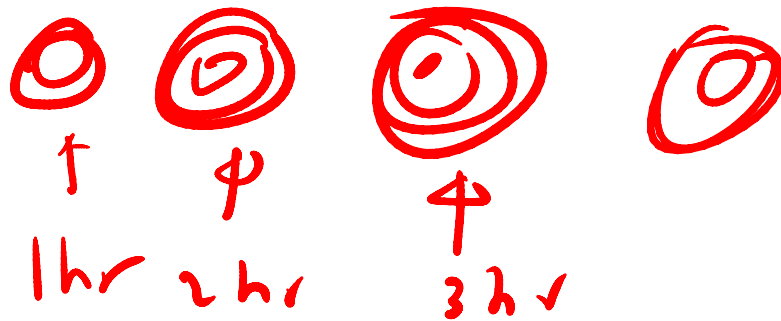


# Chapter 12

## Polymer Nanocontainers



# Nanocontainers

Liposomes

Dendrimers

Layer by Layer Deposition

Block Copolymer

Shell Cross-Link

## Stability

# Liposomes

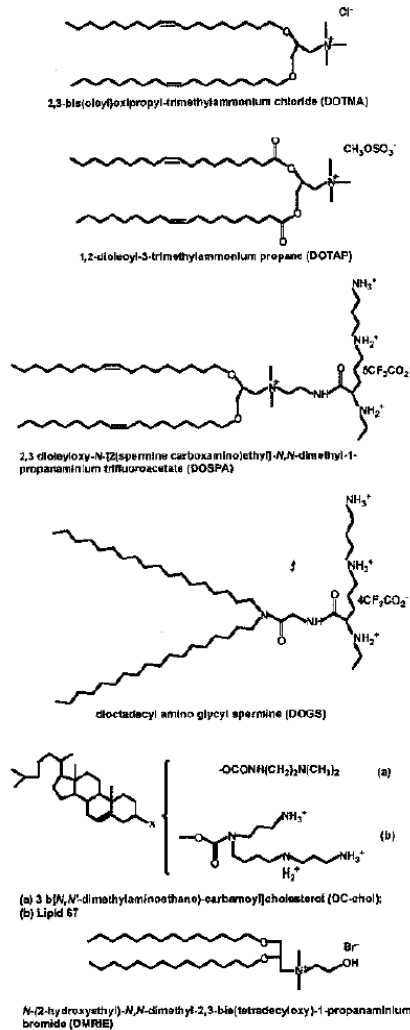


Figure 21.7 Structures of some cationic lipids commonly used in gene therapy.

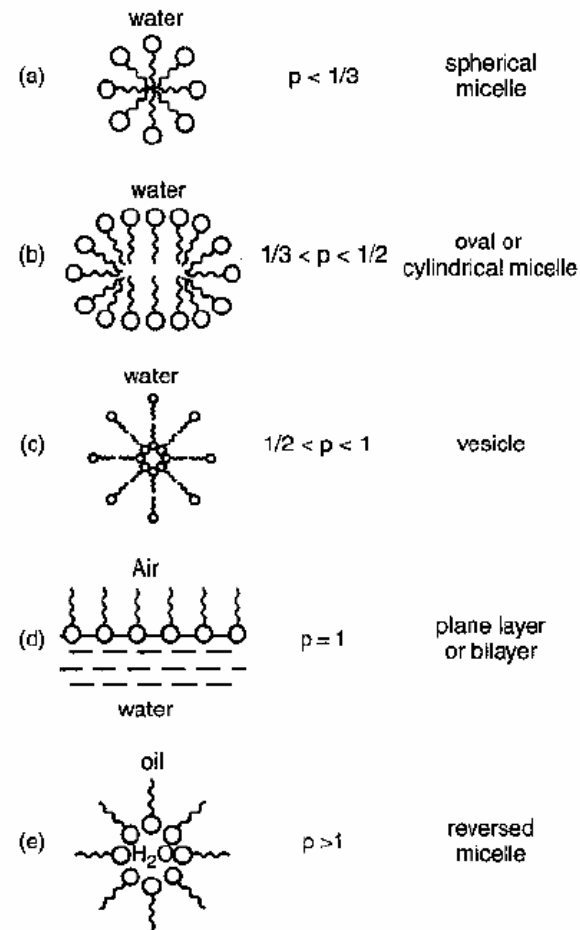
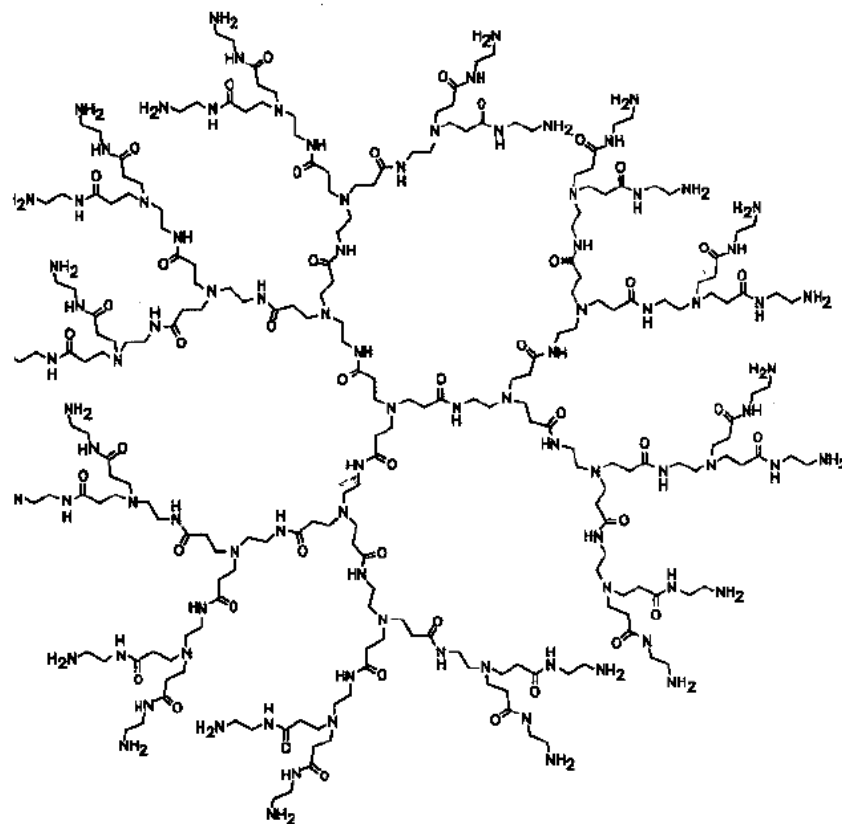


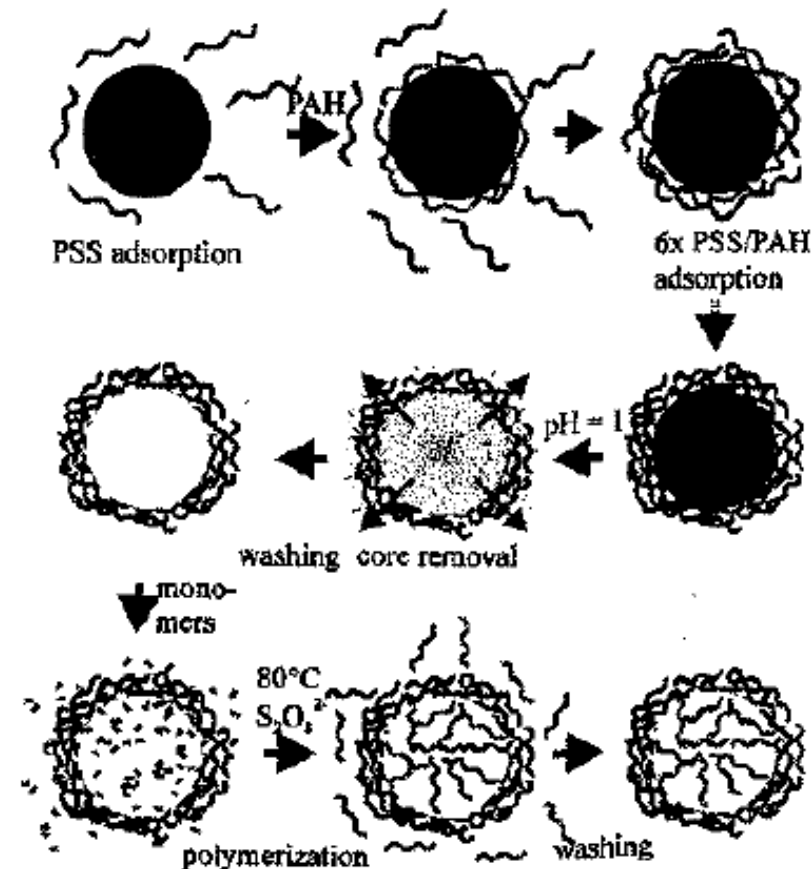
Figure 12.14. Sketch of structures formed by amphiphilic molecules at water-oil or water-air interfaces for various values of the packing parameter  $p$  of Eq. (12.6). [Adapted from E. Nakache et al., in Nalwa (2000), Vol. 5, Chapter 11, p. 580.]

# Dendrimers



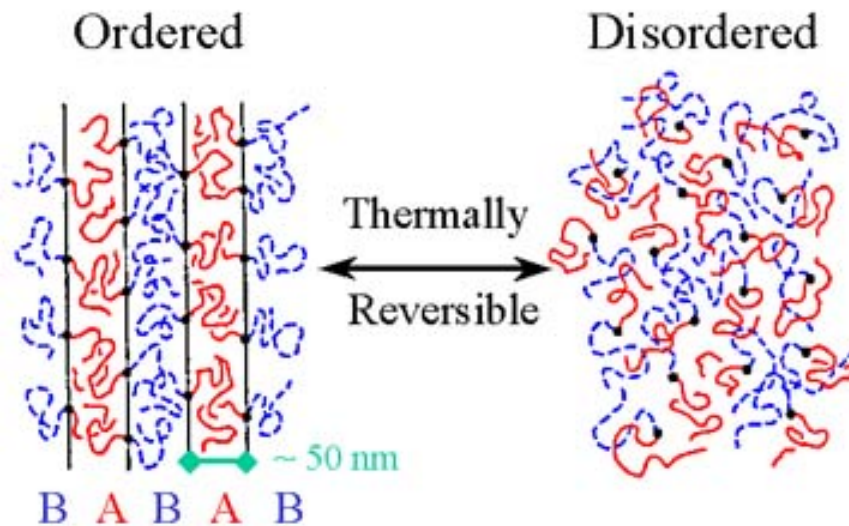
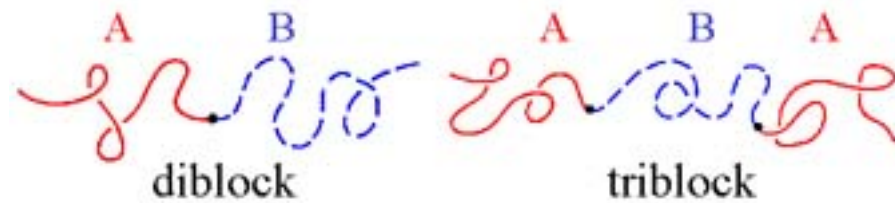
**Figure 11.16.** Fifth-generation polyaminoamine (PAMAM) dendrimer. [Prepared by D. A. Tomalia, H. Baker, J. R. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, and P. Smith, *Polym. J.* **17**, 117 (1985).]

# Layer by Layer

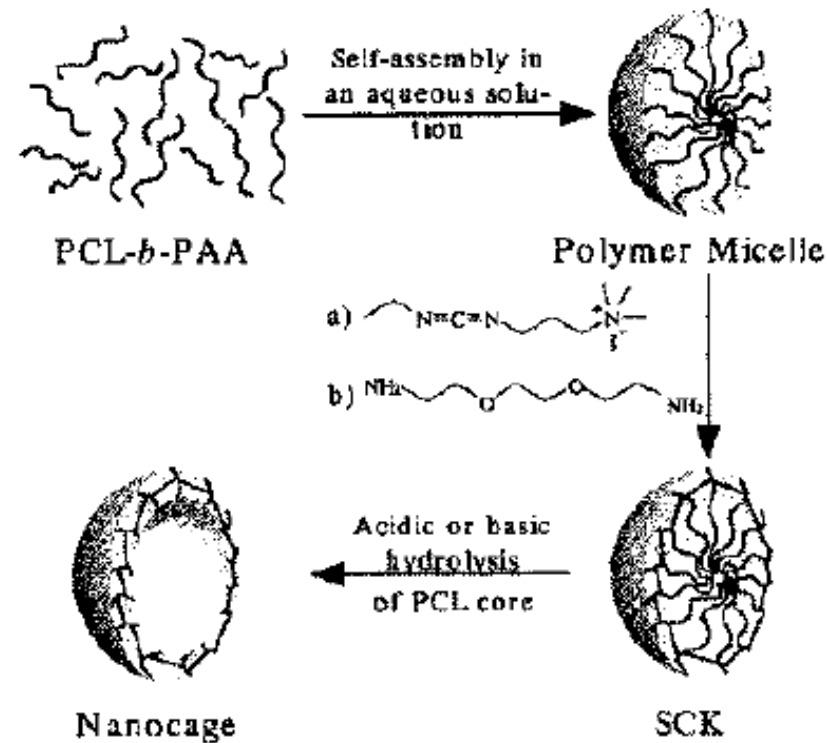


**Figure 12.1** Schematic representation of the procedure for preparing hollow spheres using layer-by-layer deposition of oppositely charged polyelectrolytes on colloidal particles and subsequent encapsulation of polymers in a "ship in a bottle" fashion. PSS: sodium polystyrene sulfonate; PAH: poly(allylamine)hydrochloride. (Reproduced from Ref. [81], with permission.)

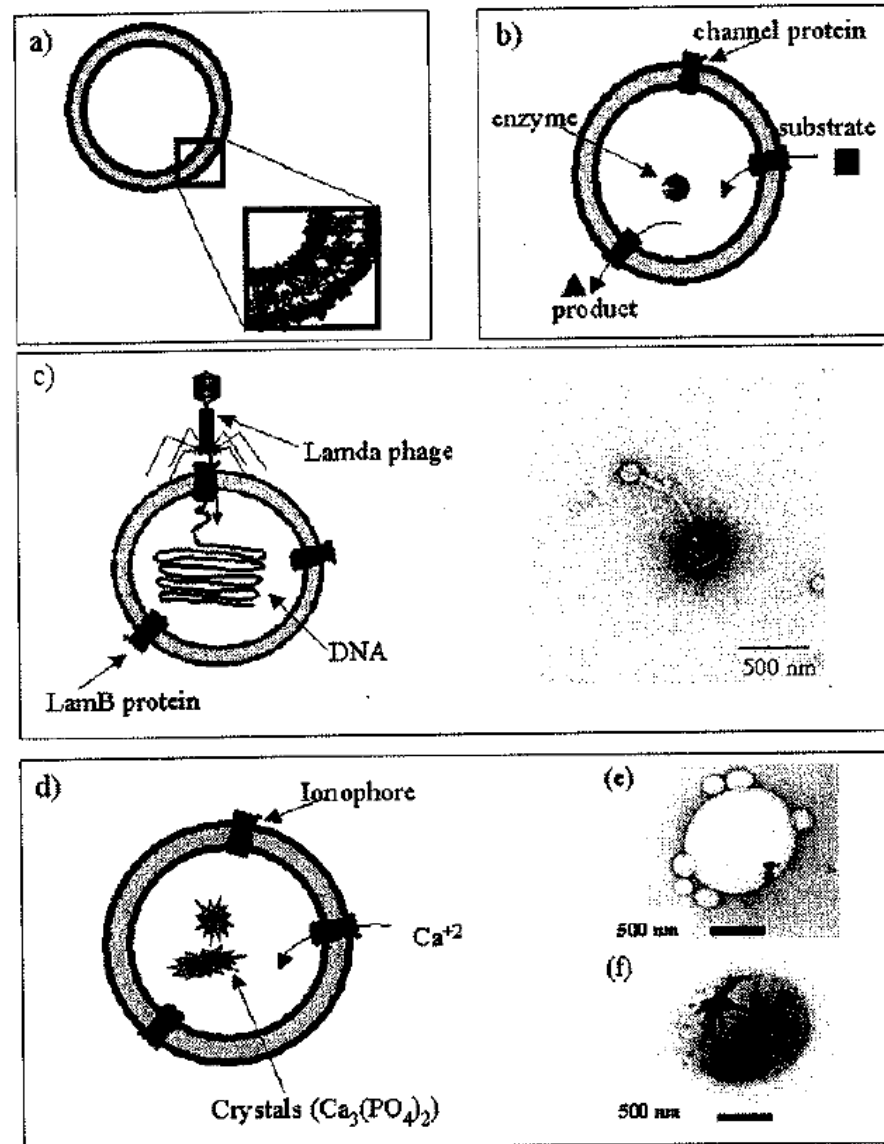
# Block Copolymers



# Shell Cross-Link



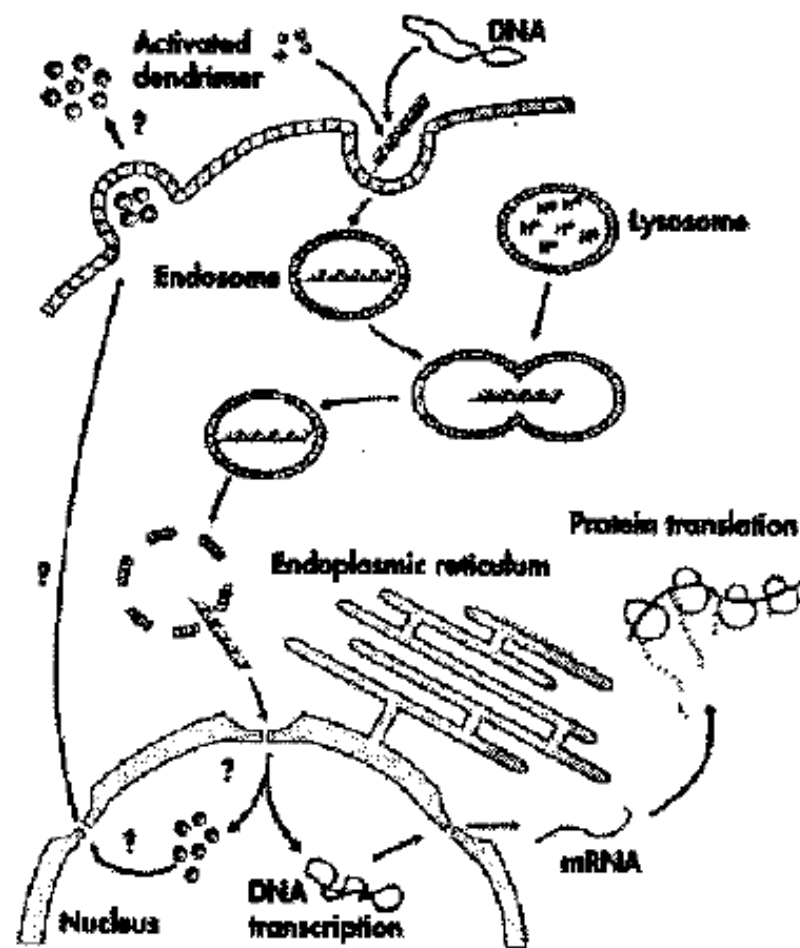
**Figure 12.2** General procedure for the preparation of hollow shell cross-linked nanocages (SCKs) from amphiphilic diblock copolymers. (Reproduced from Ref. [50], with permission.)



**Figure 12.3** (a) Schematic view of a ABA triblock copolymer vesicle and magnification of the structure of its membrane, showing the constituting polymer chains. (b) Representation of a BioNano-reactor with encapsulated  $\beta$ -lactamase and inserted membrane channel proteins to facilitate diffusion of substrates and products in and out of the nano-reactor. (c) Model of viral DNA encapsulation via

phage binding and injection into nanocontainers, and transmission electron micrograph (TEM) showing the binding of a phage onto an ABA triblock copolymer vesicle. (d) Schematic representation of an ABA nanocontainer with incorporated ionophores in its membrane used as biomineralization device. TEMs showing (e) calcium phosphate crystals after 1 h and (f) after 24 h.

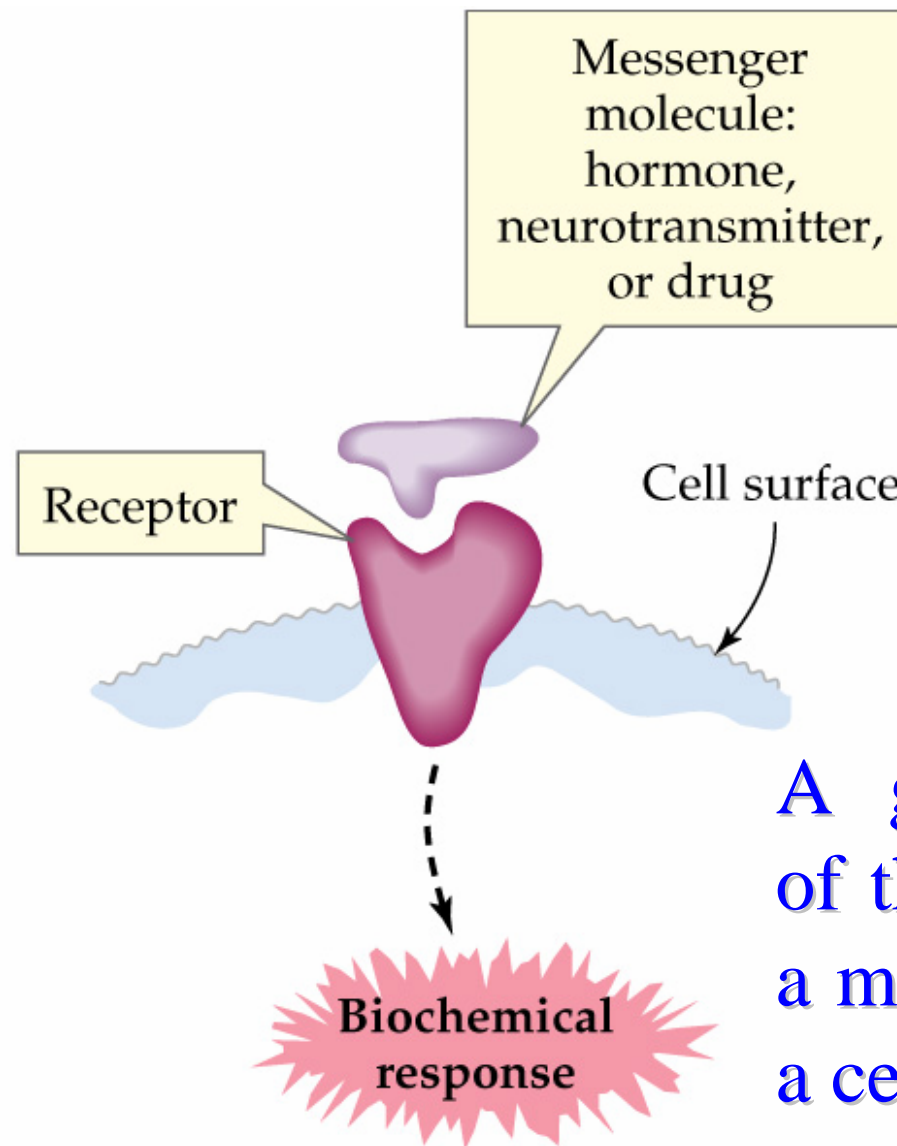




**Figure 12.5** Model mechanism of DNA transfection using active dendrimer-mediated uptake. (Reproduced from Ref. [88], with permission.)

# **Chapter 5**

## **Defined Networks of Neuronal Cell**



A general representation of the interaction between a messenger molecule and a cellular receptor.

- ◆ Since hormones travel through blood stream, the responses they produce require minutes to hour to begin.
- ◆ The chemical messages of the nervous system travel along nerve fibers, taking only a fraction of a second to reach their destination.
- ◆ Most nerve cells do not make direct contact with their target, neurotransmitters carry the messages across a small gap between the nerve cell and the target.
- ◆ Neurotransmitters are released in very short bursts and are quickly broken down or reabsorbed by the nerve cells.

# Neurotransmitters

- ◆ Neurotransmitters are the chemical messengers of the nervous system. Most neurotransmitters are amines.
- ◆ They are released by nerve cells (neurons) and transmit signals to neighboring target cells.
- ◆ Synapses: The place where the tip of a neuron and its target cell lie adjacent to each other.  
Signal transmission through neurotransmitters is shown in Fig 20.5

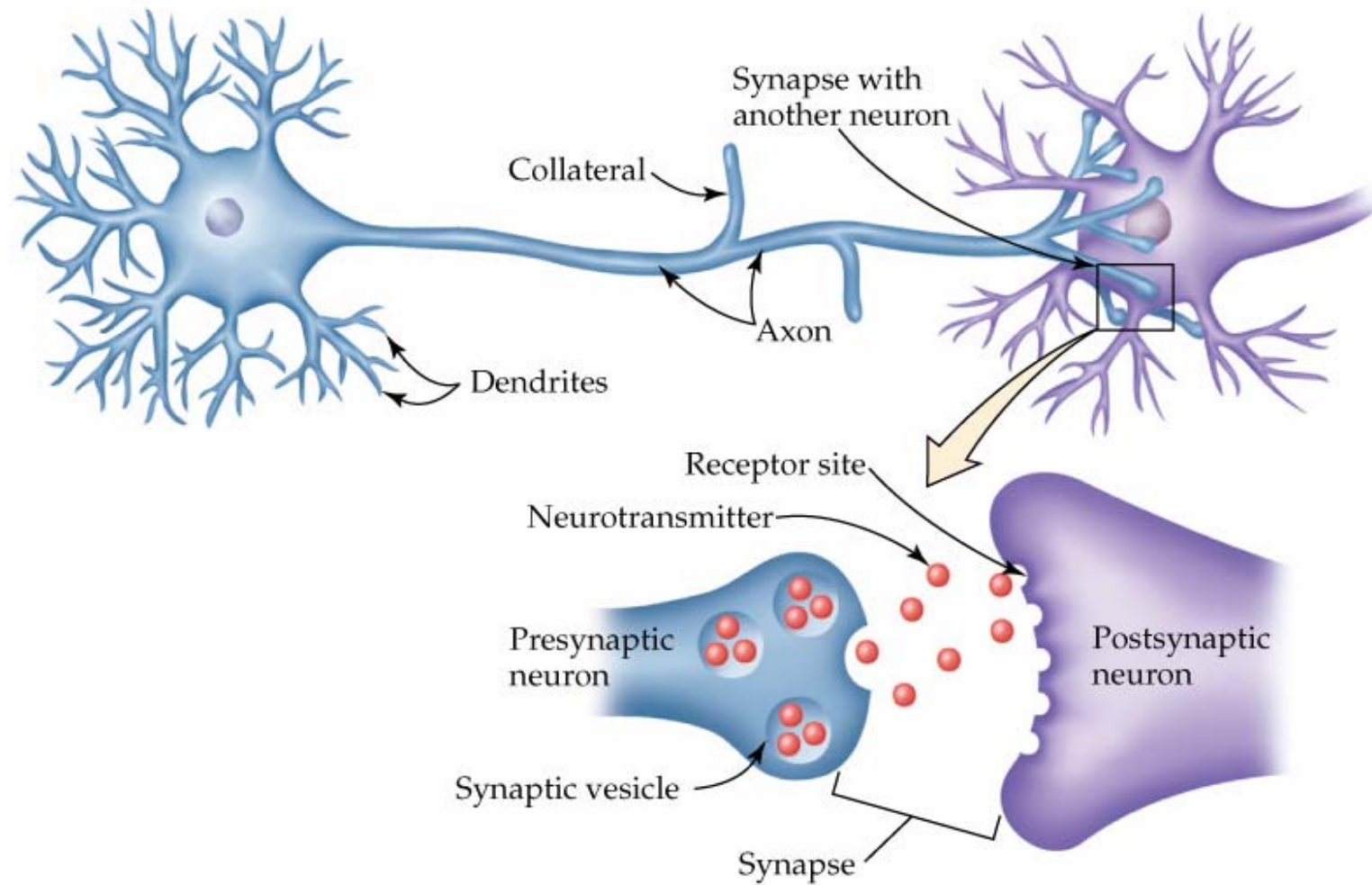


Fig 20.5 Transmission of a nerve signal by neurotransmitter

# **How Neurotransmitters Work: Acetylcholine, Its Agonists and Antagonists**

Acetylcholine (ACh) is a neurotransmitter, produced in presynaptic neuron and stored in their vesicles is responsible for the control of skeletal muscle. It may play role in the sleep-wake cycle, learning and memory, and mood. The rapid sequence of events in the action of acetyl choline in communicating between nerve cells is illustrated in the following Fig 20.7.

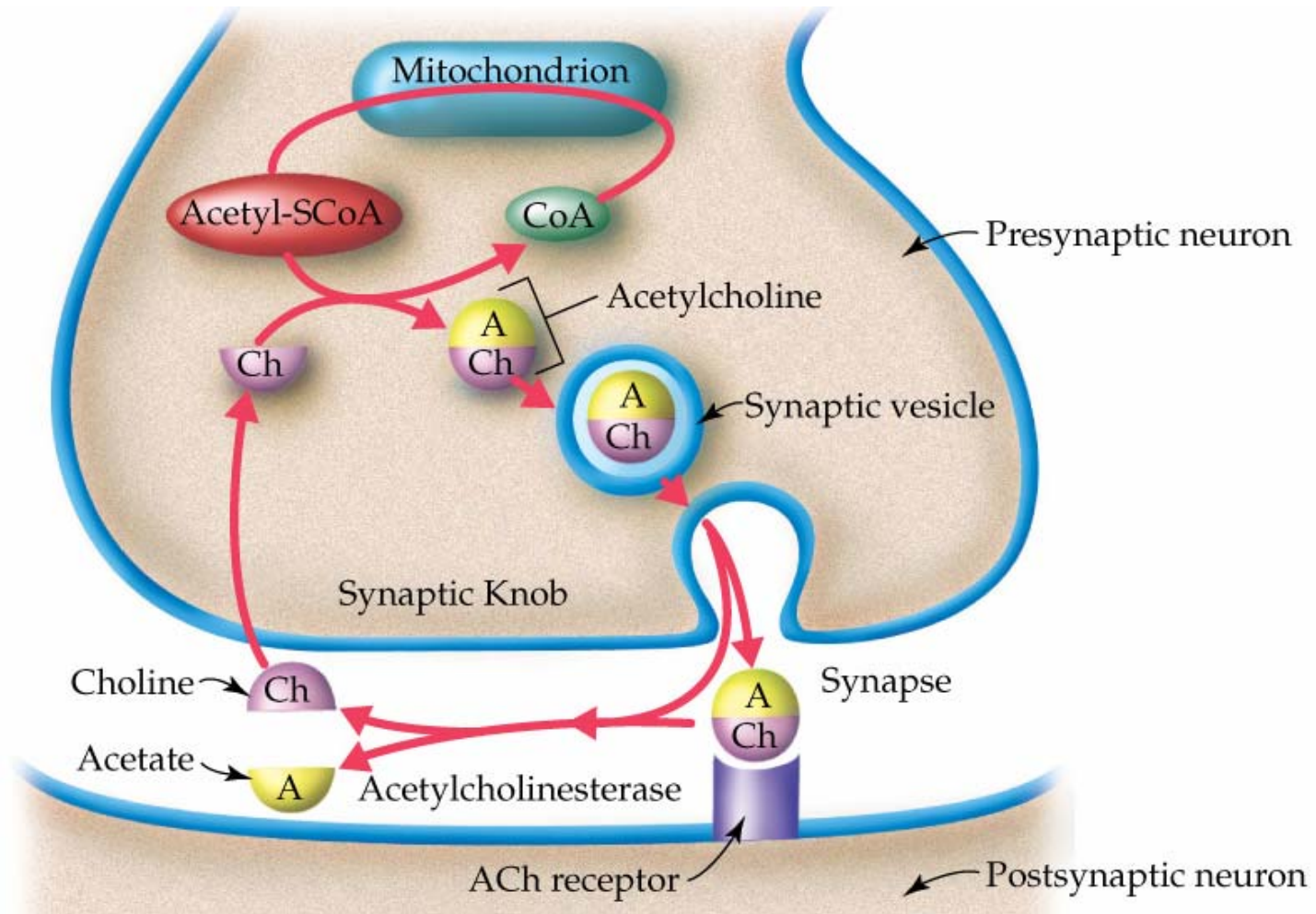


Fig 20.7



- ◆ A nerve impulse arrives at the presynaptic neuron.
- ◆ The vesicle move to the cell membrane, fuse with it, and release their ACh molecules.
- ◆ Ach crosses the synapse and binds to receptor on the postsynaptic neuron.
- ◆ The resulting change initiates the nerve impulse in that neuron.
- ◆ With the message delivered, Acetyl cholinesterase present in the synaptic cleft catalyzes the decomposition of acetylcholine.
- ◆ Choline is absorbed back into the presynaptic neuron where new ACh is synthesized.

# Patterning

Topographic Patterning

Chemical Patterning

# Development of nanostructured biomedical micro-drug testing device based on in situ cellular activity monitoring

Shalini Prasad\*, Jorge Quijano

Biosensors and Bioelectronics 21 (2006) 1219–1229

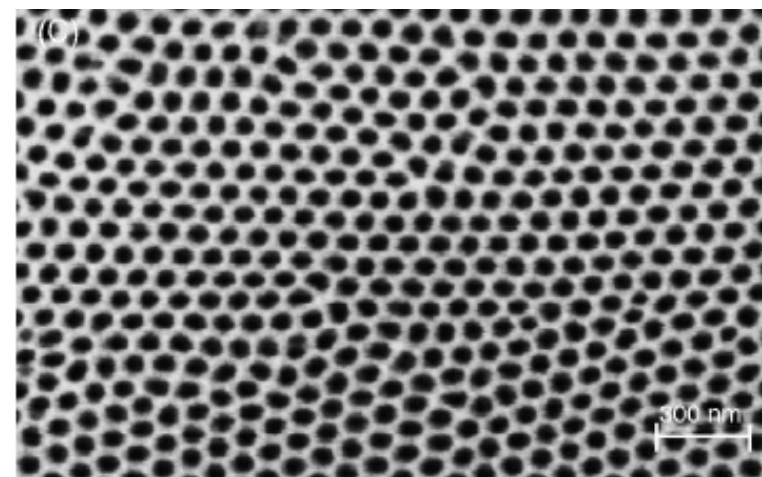
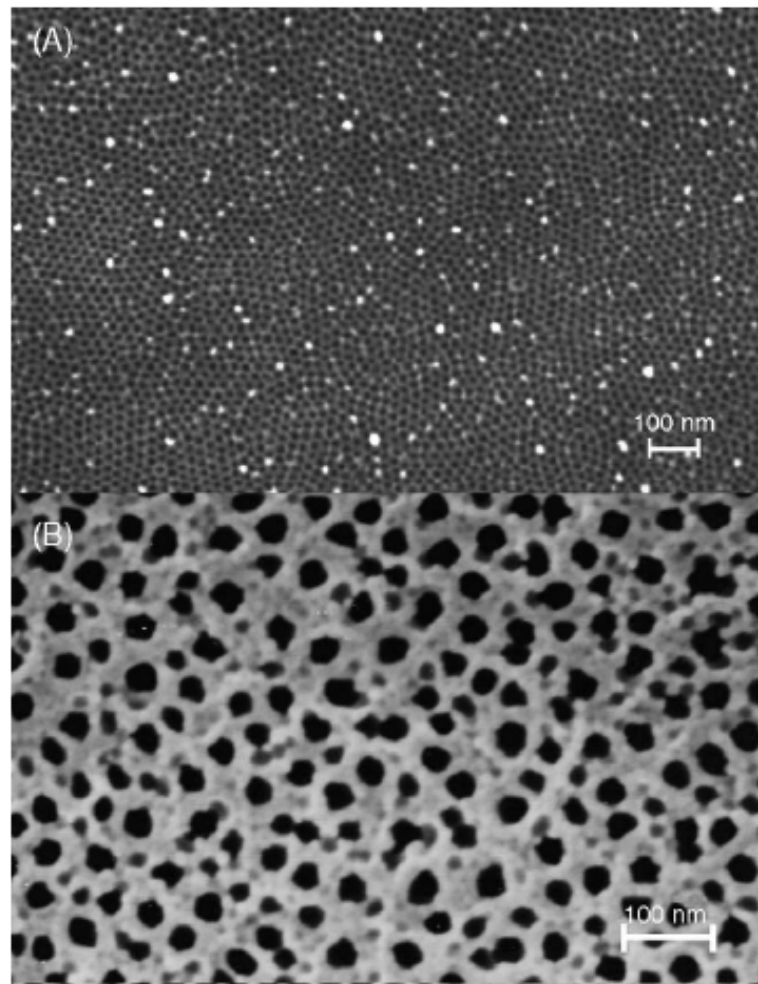


Fig. 1. (A–C) Scanning electron micrographs of porous alumina membranes fabricated using the two step anodization process. (A) Membranes with pore size of 25 nm. This is suitable for small molecule transport through the membrane. (B) Porous membranes with pore diameter of 50 nm. (C) Pore size of the membrane is 100 nm. The pores are more even and ordered as the pore diameter increases.

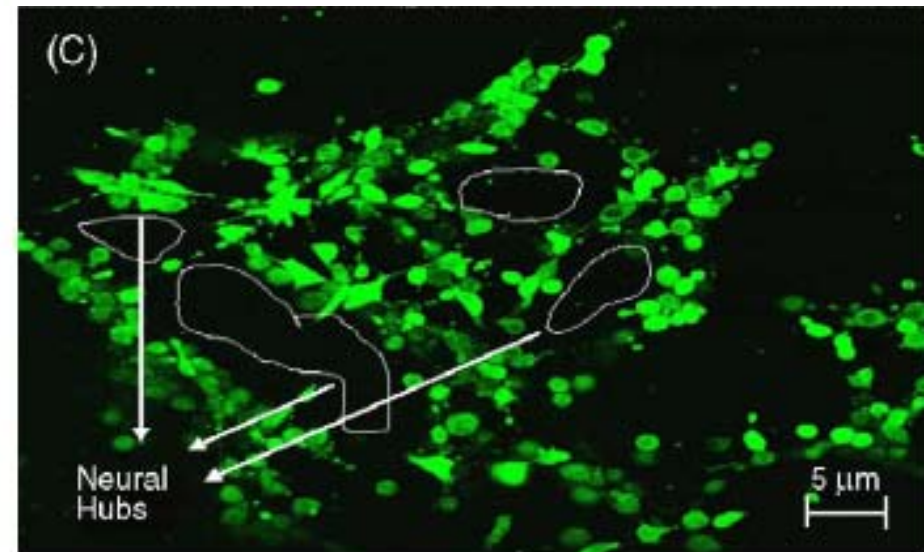
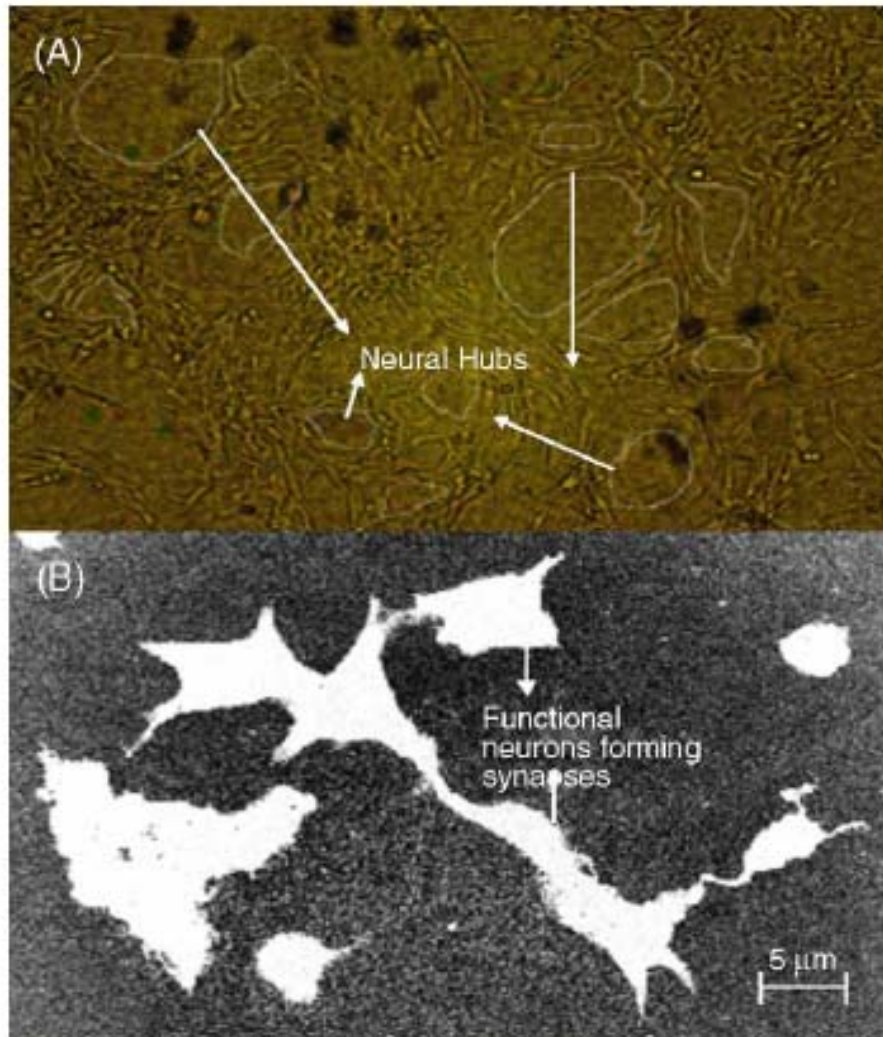


Fig. 2. (A) Optical micrograph demonstrating the formation of neural hubs on porous alumina membrane 24 h after seeding and incubation on the nanoporous substrate. (B) Scanning electron micrograph of functionally synaptic neurons forming communication hubs on the nanoporous alumina membrane. (C) Fluorescent optical micrograph of differentiating neurons on porous alumina substrate. The neurons were preloaded with fluo-3 calcium stains to establish their viability (molecular probes, Eugene, OR) and visualize using Leica Confocal SP-100.

Poly-L-lysine

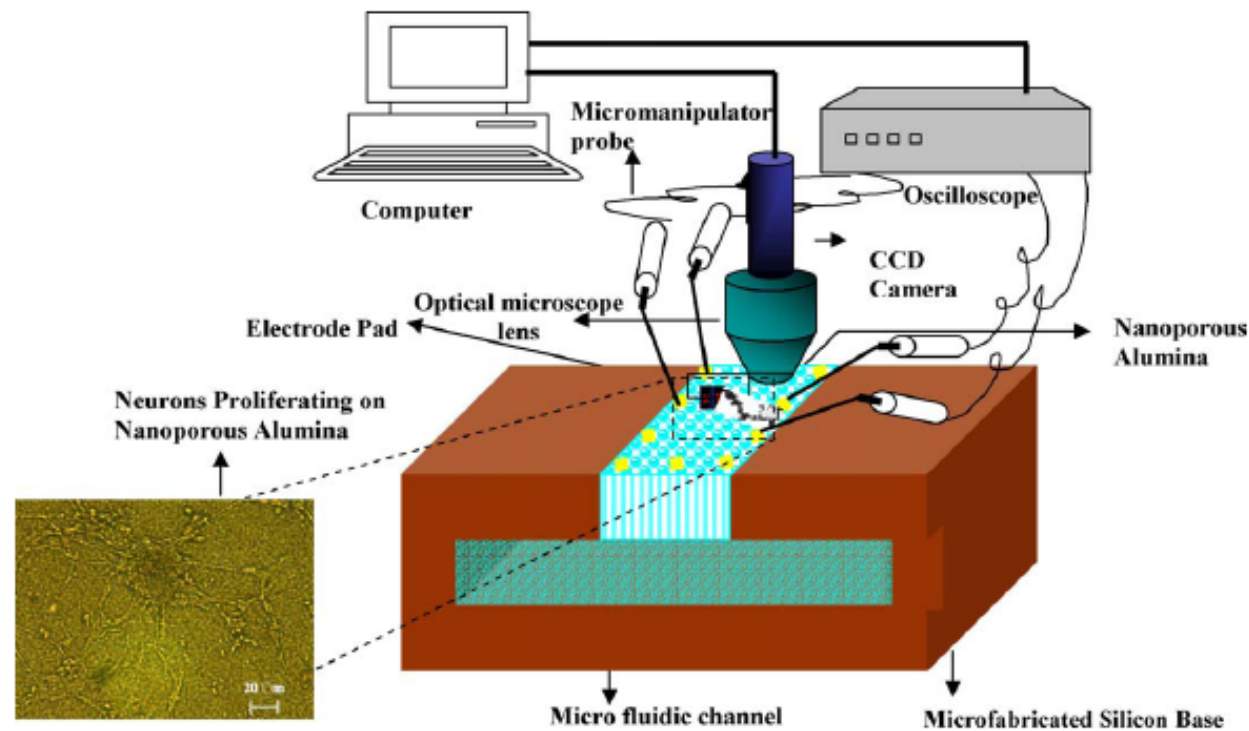


Fig. 3. Schematic representation of the experimental set up for simultaneous electrical as well as optical characterization of variations to cellular behavior due to the effect of drug molecules. The cells (neurons) are patterned on the nanoporous alumina substrate. Metallic pads are selectively deposited on the porous alumina substrate. Solution containing specific drug molecules is flowed into the silicon micro fluidic channel. Due to capillary and diffusion effects the drug molecules in solution come in contact with the cell membranes and cause variations to the cellular electrical activity which is probed and recorded in situ using the micro manipulators and the oscilloscope. The entire process is remotely controlled using Lab VIEW.



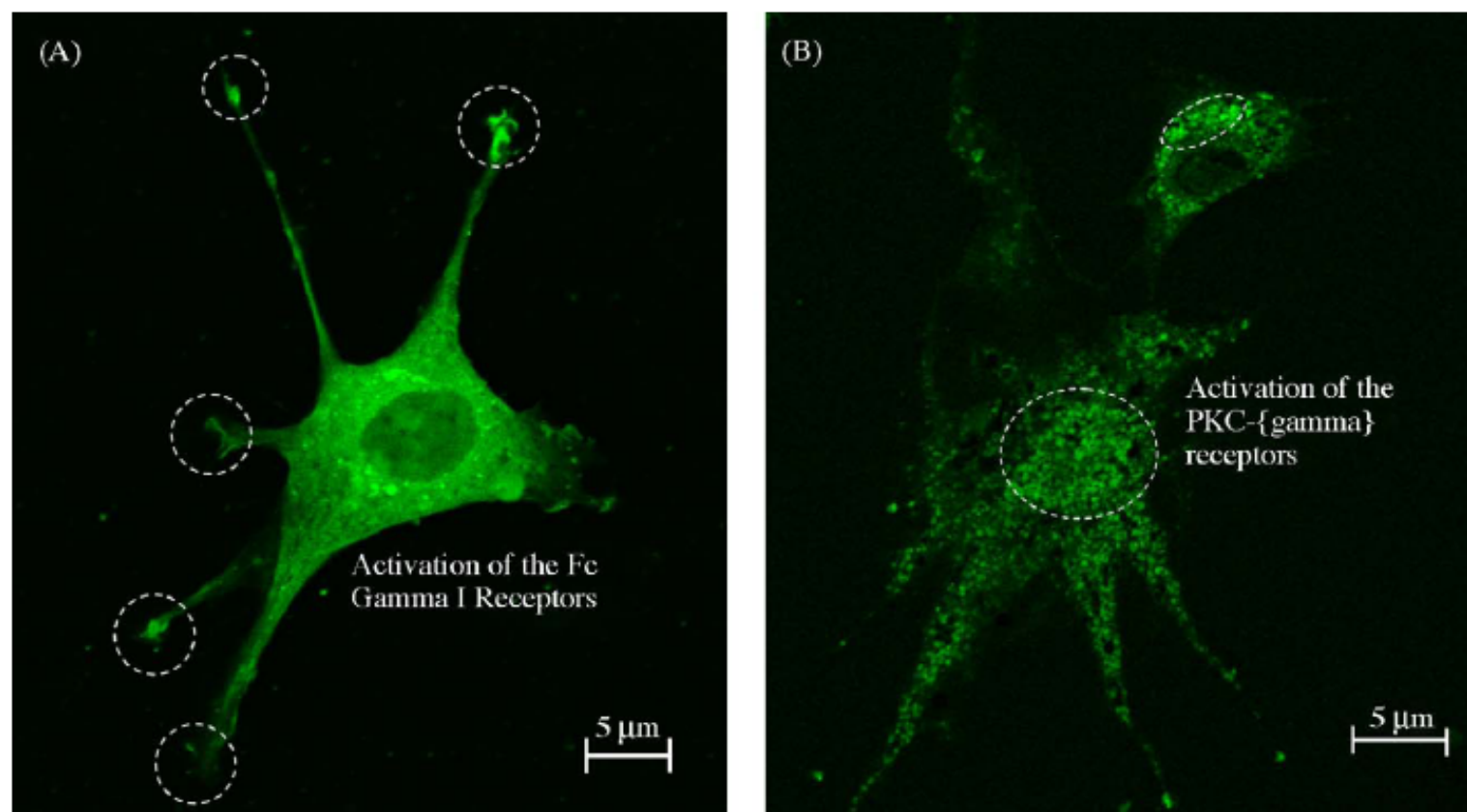


Fig. 5. (A) Fluorescent confocal optical micrograph demonstrating the activation of the PKC- $\gamma$  receptors at the neurite terminals (as shown by the circled area), resulting in potassium transients due to the effect of glucose on the neuronal cell membrane. The cells were pre-loaded with GFP (Molecular Probes, Eugene, OR) and the fluorescent transients were visualized during the interaction of the glucose molecules with the cell membrane. (B) Fluorescent confocal optical micrograph demonstrating the activation of the Fc- $\gamma$  I receptors on the neuron cell membrane at the soma (as shown by the circled area), resulting in potassium transients due to the effect of IgG on the neuronal cell membrane. The cells were pre-loaded with GFP (molecular probes, Eugene, OR) and the fluorescent transients were visualized during the interaction of the IgG molecules with the cell membrane.

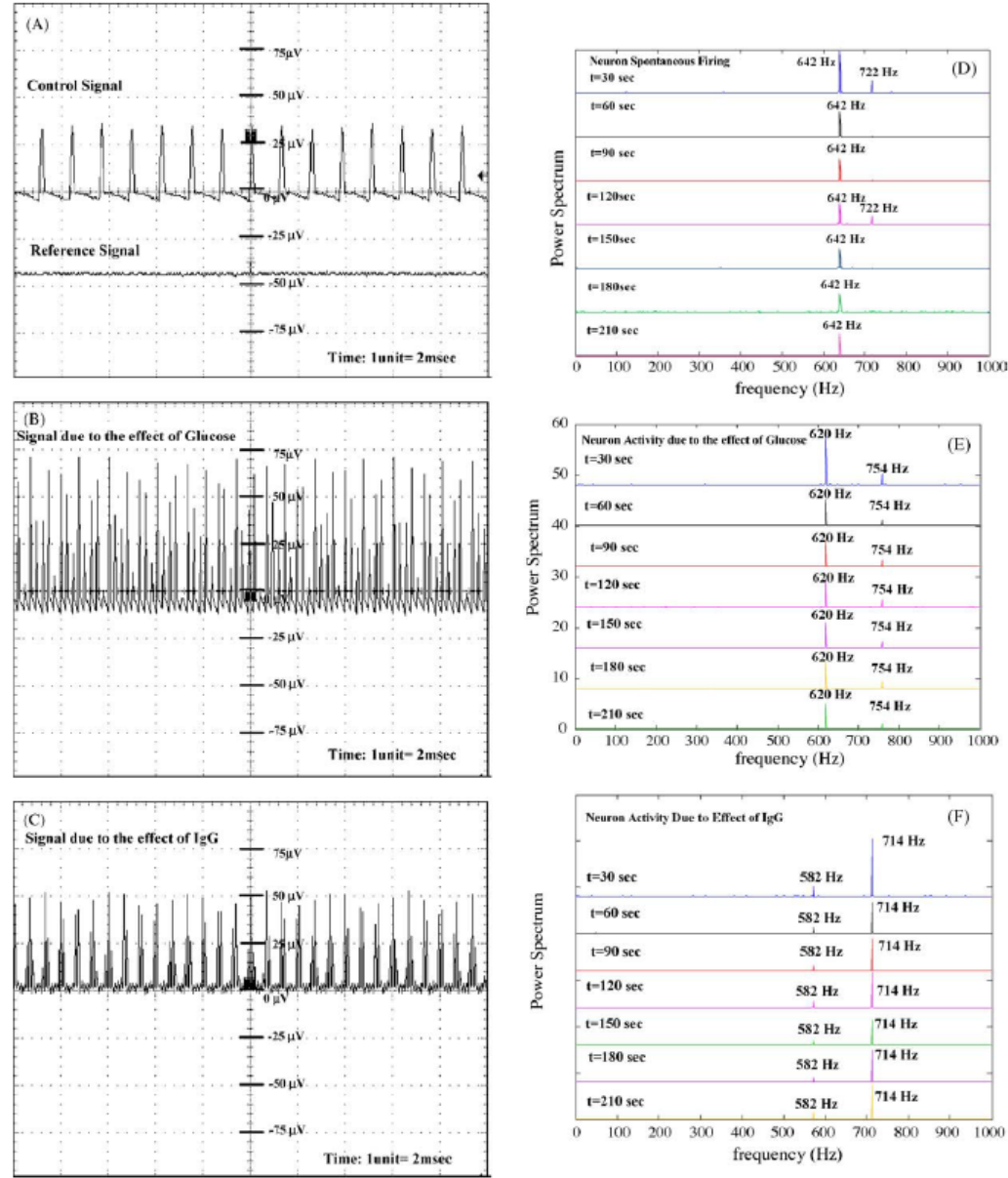
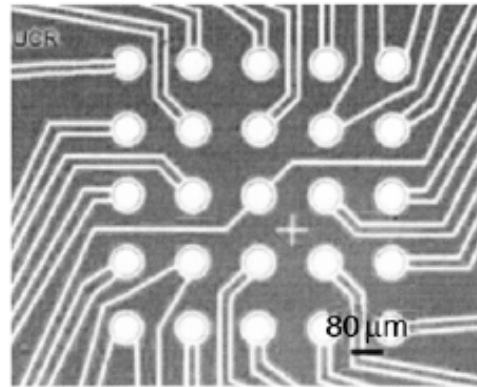
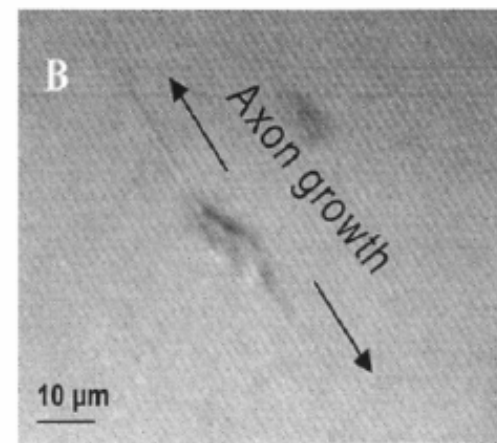
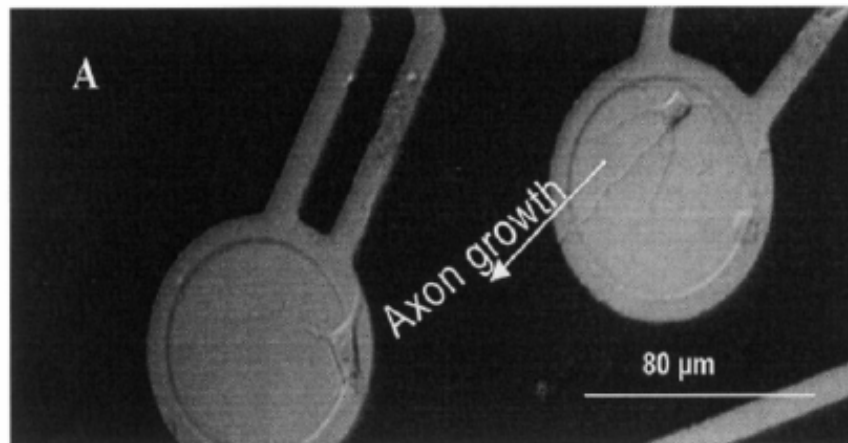


Fig. 6. (A) Snapshot of the time domain signal obtained from the patterned electrodes that show the spontaneous extra cellular electrical activity from neurons developing functional synapses and demonstrating viability on the nanoporous substrate. The reference signal is that obtained from the electrode pads in the presence of the cell buffer but in the absence of the cells. (B) Extra cellular electrical activity snapshot obtained from the microelectrode pads from neurons due to the effect of glucose at 250 mM. (C) Extra cellular electrical activity snapshot obtained from neurons due to the effect of IgG at 250 mM.



*Fig. 2. Optical micrograph of the electrode array. There are 25 platinum electrodes of 80  $\mu\text{m}$  diameter and 200  $\mu\text{m}$  center to center spacing arranged in a  $5 \times 5$  array.*



*Fig. 10. (A) Directed growth of neurites on applying DC potential of 0.8 V. Growth direction is along decreasing field strength. (B) Control experiment, neurites growing along micro tenches in direction of decreasing field strength.*



## Techniques for patterning and guidance of primary culture neurons on micro-electrode arrays

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Eiichi Tamiya<sup>b</sup>, Hiroyuki Fujita<sup>a</sup>

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<sup>b</sup>*Japan Advanced Institute for Science and Technology, 1-1 Asahidai, Tatsunokuchi, Ishikawa 923-1292, Japan*

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In this work, two different micro-patterning methods for use in the growth guidance of dissociated primary culture neuron cells are compared: open chemically patterned growth substrates and enclosed micro-fluidic channels. Open, chemically patterned growth substrates are prepared by photolithographically patterning perfluoropolymer barrier structures on glass substrates. Neural pathways are created when poly-L-lysine is selectively adsorbed on the glass to form a cytophilic growth matrix. Adsorption of albumin proteins on the perfluoropolymer regions renders the surface cytophobic. In a second method, a three-dimensional micro-fluidic system, fabricated from PDMS is tested as a way to guide neural growth through total confinement and for cell placement. Both of these methods are tested for alignment and compatibility on a commercially available micro-electrode array (MED64). Biological culture and imaging techniques are considered. © 2002 Elsevier

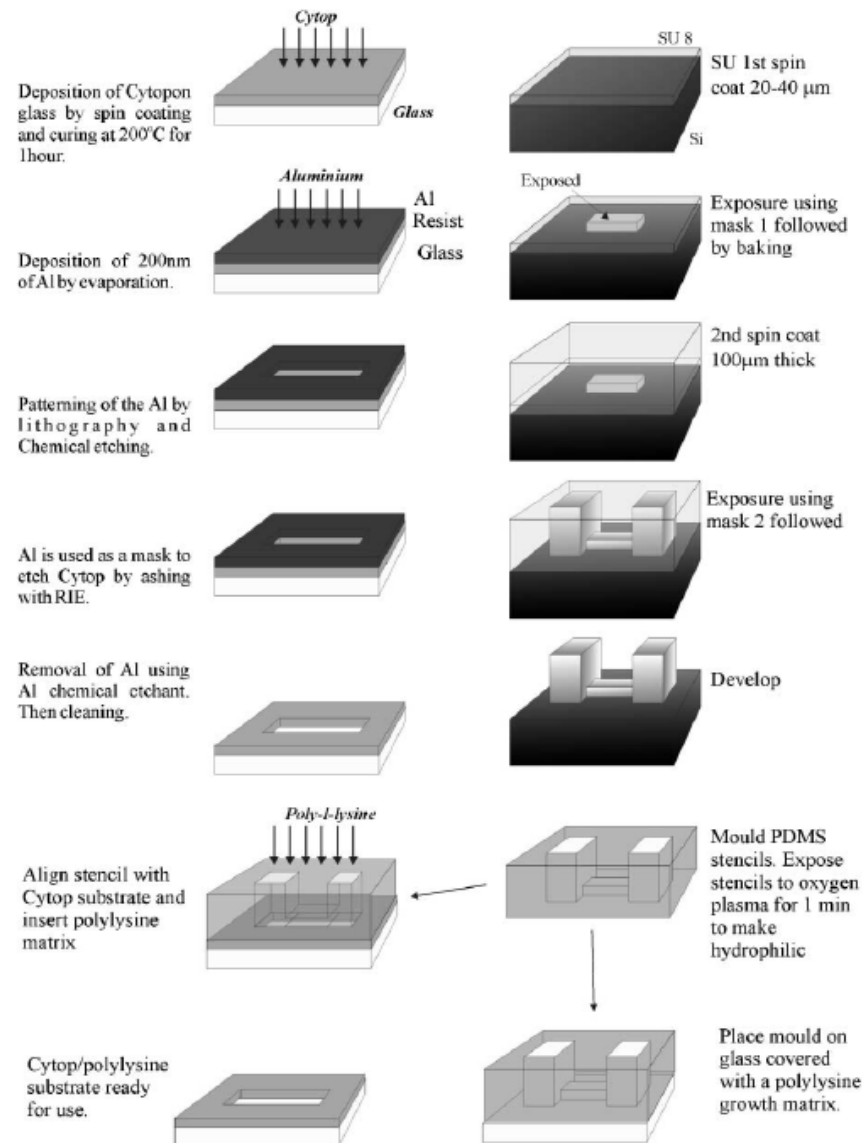


Fig. 1. Fabrication of the Cytop/poly-L-lysine structures on glass substrates. Initially Cytop structures are made on the glass substrate. Then a PDMS stencil is made using an SU-8 molding process. Finally, the PDMS stencil is aligned to the glass/Cytop substrate and a poly-lysine coat is formed through immersion in poly-lysine solution.

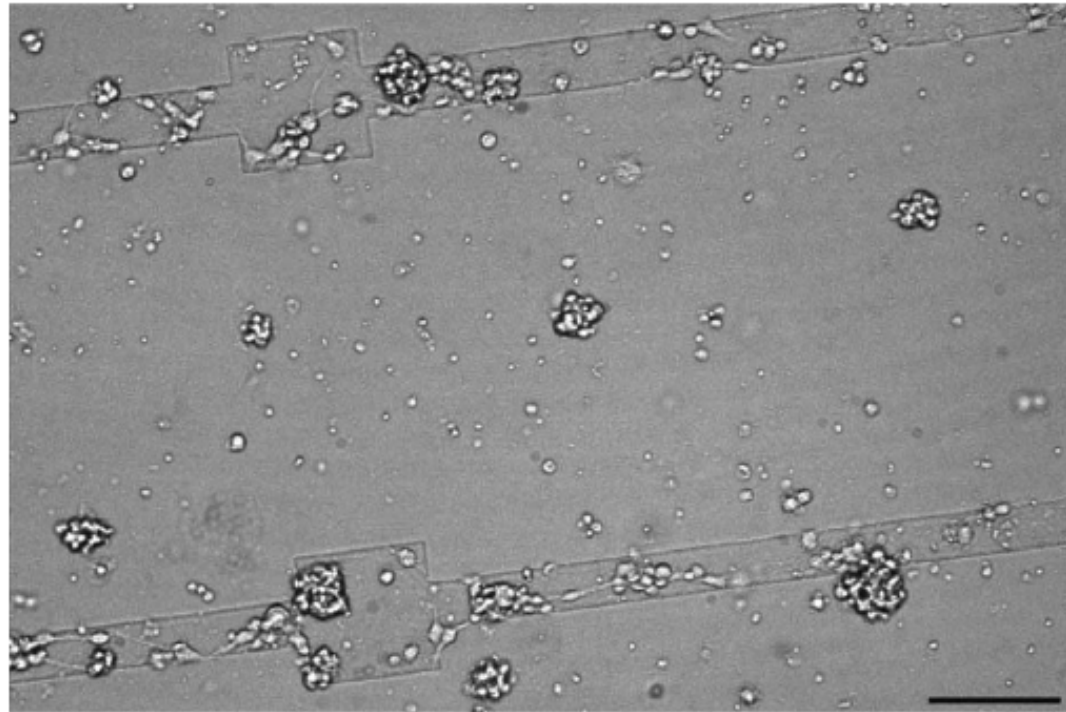


Fig. 2. Phase contrast micrograph of neurons patterned on 50 nm path structures. Note no growth of neurons on the Cytop. Picture is taken in vitro and the large round cell clumps are floating dead cells. Scale bar is 100  $\mu\text{m}$

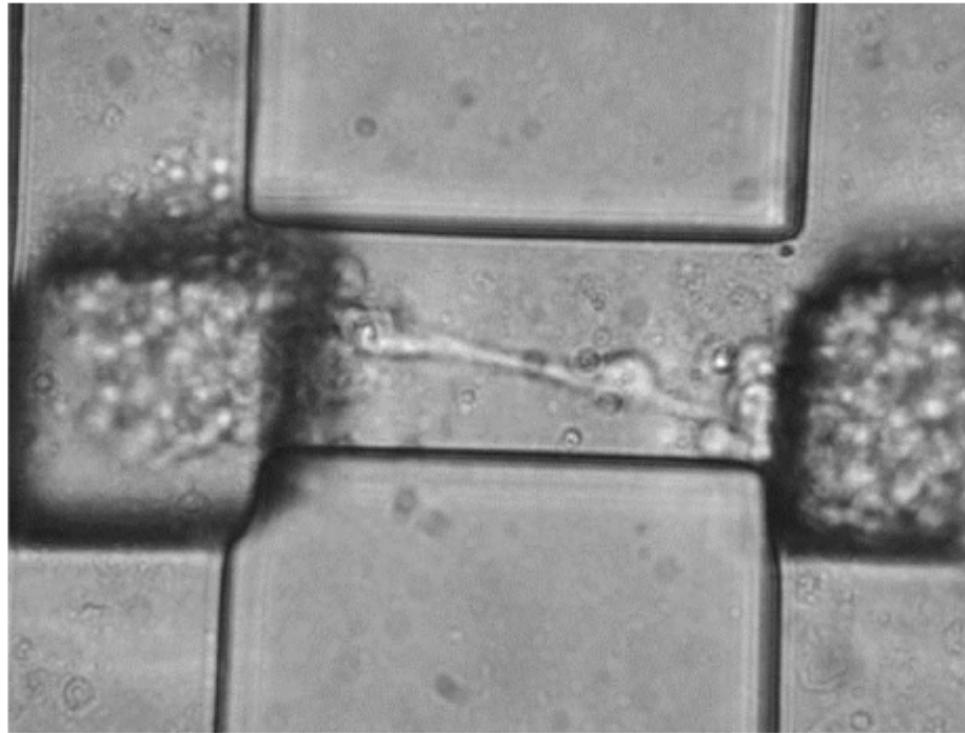


Fig. 3. Differential interference contrast image of primary neural cells and axon formation in a micro-fluidic channel after 48 h of growth. The channel paths are 40  $\mu\text{m}$  wide. The two squares at either end are the vertical entrances for the cells.

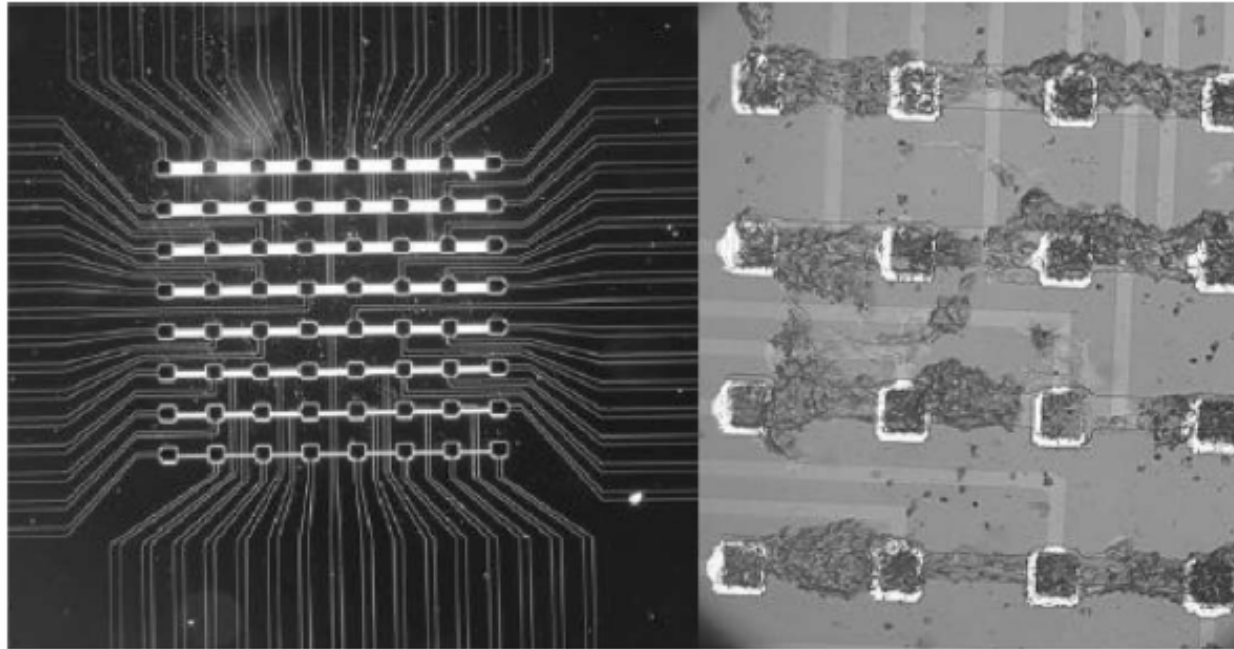


Fig. 4. Patterning on the electrode chip. Cytop perfluoropolymer patterning in the dark field image on the MED64 can be seen to the left and a bright field micrograph of a fixed cell culture patterned on top of the electrodes can be seen on the right.

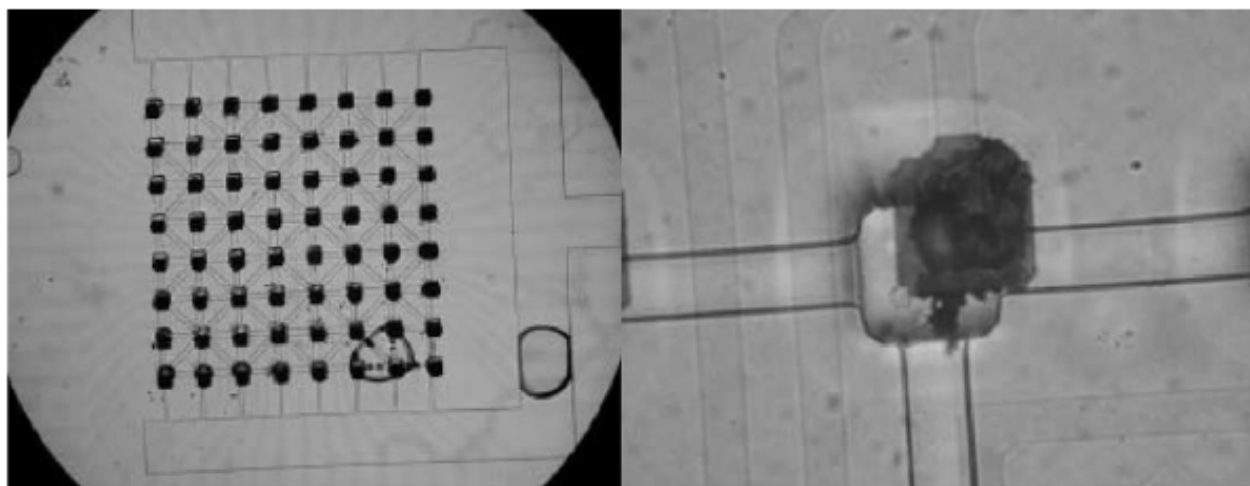


Fig. 5. Alignment of the PDMS micro-fluidic array on top of a MED64 micro-electrode array. Each vertical hole ( $50\text{ }\mu\text{m}$  wide) in the PSMS membrane is aligned to each of the 64 micro-electrodes (black squares). On the left one cell can be observed next to the electrode aligned with the micro-fluidic channels.

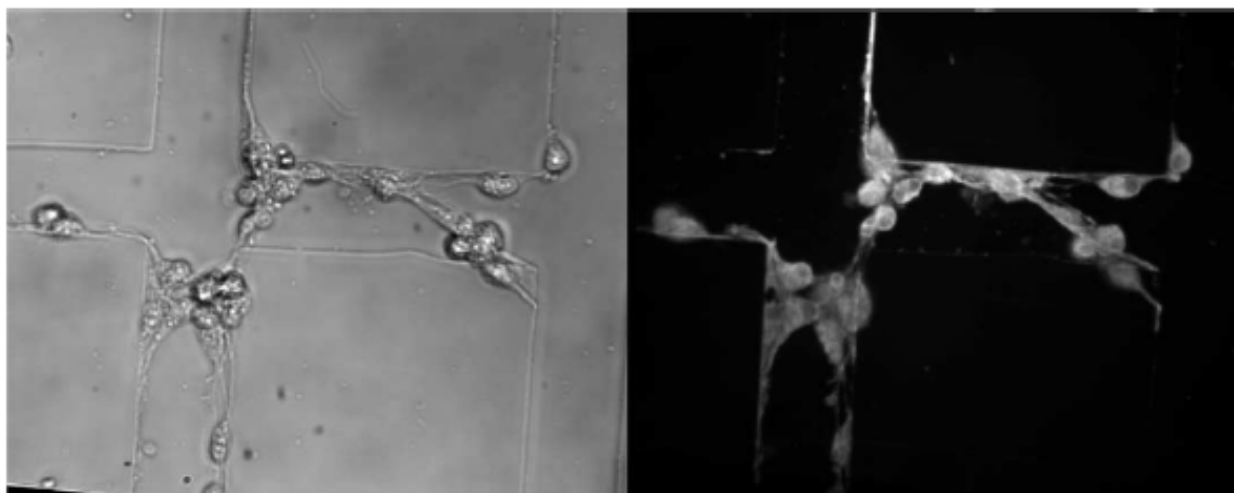


Fig. 6. Differential interference microscopy and fluorescent imaging through TAU-GAP antibody tagging. In neural patterning, extensions often grow along the interface of the walls and growth substrate. Contrast microscopy is not able to distinguish axons in this position due to diffraction along the interfaces. Fluorecent microscopy is less affected by diffraction and thus allow the identification of the neural extensions. Neural path-widths are  $40\text{ }\mu\text{m}$ .