Microcontact Printing of Poly (L-lysine) Using PDMS Stamps for the Adhesion and Patterning of Neurons

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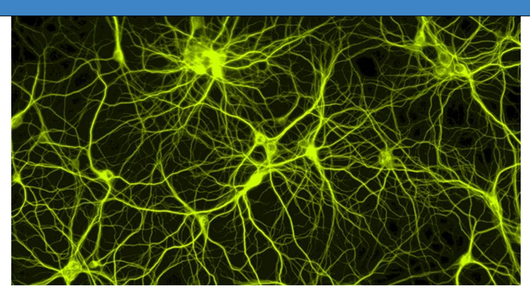
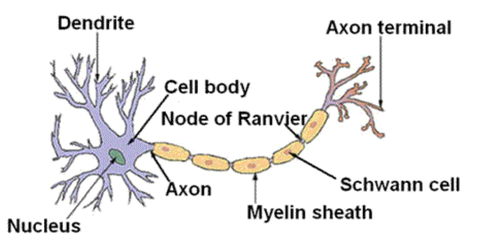
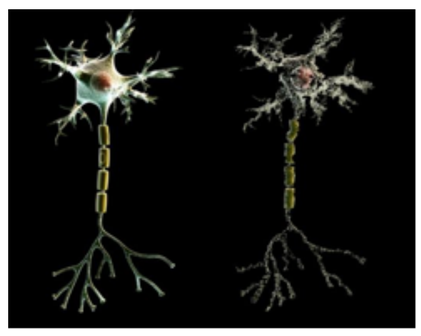
Abstract

One method that facilitates the high throughput of microcontact printing arrays of proteins for the adhesion of neurons is a micropatterning technique using Polydimethylsiloxane (PDMS) stamps. This technique was used as an aid to achieve patterning of neurons and growth of neural extensions (dendrites and axons). The PDMS was made from a mixture of Sylgard 184 PDMS base and PDMS curing agent in a ratio of 10:1 by mass. These stamps were cured in a polyurethane master mold with pillar relief patterns of the desired arrays on a hot plate at 70 degrees Celsius. The stamp included raised circular pillars, in 32x32 array patterns that varied in pitch and diameter. Once the stamps were cured, they were cut out and treated with Poly (L-lysine) (PLL), a cell adhesion promoter solution, pillar side up. Once dried, a residual layer of PLL remained and was then deposited on a glass substrate by applying force to the PDMS stamp on the substrate pillar side down. A glass-backed PDMS stamp proved to achieve the best deposited patterns of PLL. Also, in conjunction with the glass backing, 50-200 gram weights were used as the stamping forces to allow for a more even distribution of force in an attempt to prevent stamp deformation. Stamped substrates were then placed in culture wells, and hippocampal rat neurons were introduced with media into each well. After an incubation period, neurons preferentially anchored to the stamped PLL spots on the substrates. Neurite growth between neurons appeared between 5-10 DIV.

The Behavior of Neurons







Why Study Neurons?

-One *billion* people suffer from some form of ND

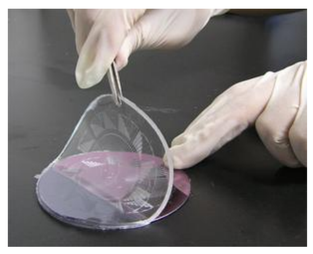
-Europe spent *$194 million* in 2004 alone on palliative care

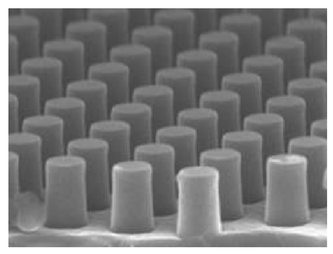
-Effective care is largely unavailable to many suffering with NDs

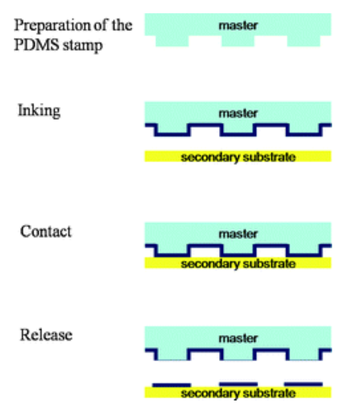
The World Health Organization (WHO, 2007)

Microcontact Printing

This process of microprinting employs the use of a Polydimethylsiloxane (PDMS) elastomer stamp to pattern Poly (L-lysine) in array geometries on a glass substrate.

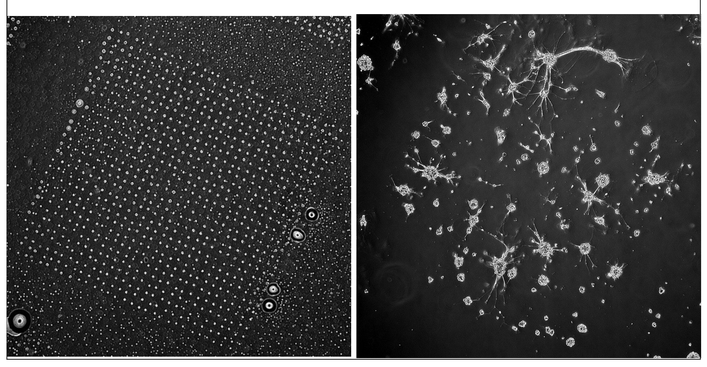






The pillar geometries on the PDMS stamp are treated with Aminopropyltriethoxysilane (APTES) to make the stamping surface more hydrophilic. Then PLL is deposited onto the treated surface.

Once dried, there is a residual layer of PLL that is deposited onto a glass substrate when stamped. Since PLL is a cell-adhesion promoter, neurons preferentially adhere to the PLL "spots", which makes quantification of neurite growth possible.



Results

25 µm pitch

35 µm pitch

Unmodified PDMS Stamp 9 DIV, force of finger, 0.5 mg/ml PLL

-Large overgrowth areas

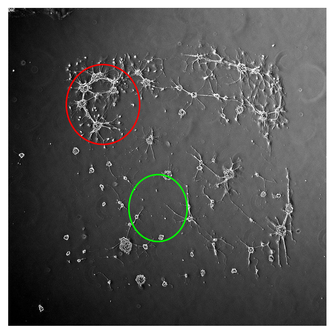
-PLL absent areas

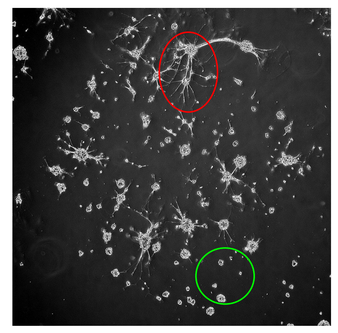
-Non-uniform distribution of force causing stamp deformation

Results

25 µm pitch

35 µm pitch





Unmodified PDMS Stamp 12 DIV, 200 gram weight applied, 0.5 mg/ml PLL

-More defined pattern

-Less overgrowth

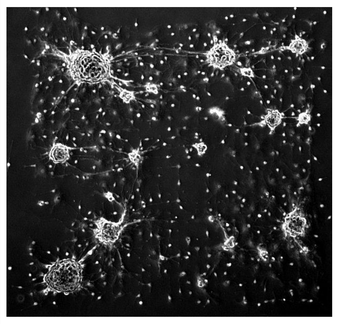
-"Patchy" neuronal growth

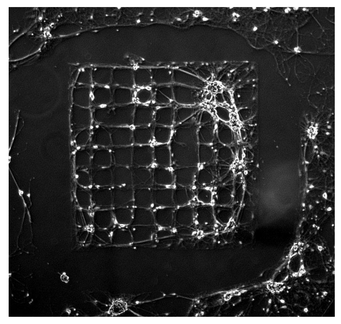
-Neuronal overgrowth still at some PLL spots

-Both indications of pillar deformation

25 µm pitch

"Window" neurites





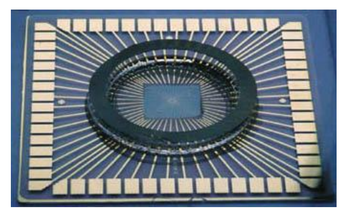
Glass-backed PDMS Stamp 9 DIV, 50 gram weight applied, 0.5 mg/ml PLL

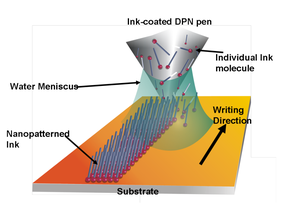
-Still some overgrowth, may be due to not completely drying stamp

Continuing the Research

Further work will include using these stamping methods to grow neurons onto multielectrode arrays (MEAs) to quantify electrical behavior of healthy neurons compared with damaged or disordered neurons.

Also, utilizing atomic force microscopy (AFM) to directly deposit PLL onto the substrate might prove to be more reproducible and automatic than µ-contact printing with PDMS stamps.





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