Controlling neuronal cell responses via LASER-fabricated 3D micro/nanostructured and patterned substrates

Anthí Ranella
Model of complex 3D structure of extracellular matrix (ECM) and cell-ECM interactions.

Biomaterials, 2010, 31 (17), 4639
Science is built of facts the way a house is built of bricks, but an accumulation of facts is no more science than a pile of bricks is a house — Henri Poincaré
How do cells interact with micro/nanostructures of well-defined sizes, at the molecular level?

Cell response dependence on tunable topography and defined chemistry
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Laser based fabrication of biomimetic scaffolds

I. Ultrafast laser micro/nano structuring
   Hierarchical micro/nano structuring
   Dr. Emmanuel Stratakis

II. 3-D nano-structuring using multi-photon polymerization
    Sub-diffraction limit structuring
    Dr. Maria Farsari

III. Single pulse UV laser irradiation of biopolymers
    Micro porous foam structuring on natural biopolymers
    Dr. Alexandros Selimis
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Ultrafast laser micro/nano structuring

...a simple but effective method to fabricate silicon micro/nano structures over a large area with superior control of structure geometry and pattern regularity.

fs Laser irradiation of Si in a reactive gas atmosphere...

...can produce quasi-periodical structures exhibiting double scale roughness (Si spikes)

superior control of structure geometry and pattern regularity
Tailoring the Wettability of Solid Surfaces

As microroughness $\uparrow$ Surface Hydrophobicity $\uparrow$

Tailoring the Morphology of Solid Surfaces

As roughness ↑
- Spikes' height ↑
- Interspike distance ↑
- Spike density ↓

Spike preferred orientation

Whereas at the lower laser fluences the spikes don't seem to exhibit a preferred orientation, as the laser fluence increases, the spikes present a striking parallel aligned orientation.
Microconical silicon substrates as cell culture platforms

Therefore, the suggested topography could be described as **semiperiodical discontinuous** (arrays of oriented microcones) comprising an **anisotropic feature** (elliptical cross-section).
Microconical silicon substrates as cell culture platforms

The simplicity of the irradiation process offers the possibility of patterning areas with different degrees of roughness on the same culture substrate.
Neuron-like cell line
- PC12 cells

Primary cultures
- Schwann cells
- Sympathetic neurons (Superior cervical ganglia)
- Sensory neurons (Dorsal Root Ganglia)
PC12 cells
(Pheochromocytoma cells)

“The cells tended to form clumps composed of 5-20 cells”

Greene & Tischler 1976
PC12 cells
(Pheochromocytoma cells)

In the presence of Nerve Growth Factor (NGF) they obtain the phenotype of sympathetic neurons (they develop processes, have varicosities and become electrically excitable)

PC12 cells are a useful model for the study of neuronal differentiation at cellular & molecular level

Greene & Tischler 1976
Effect of surface roughness on PC12 cell growth

PC12 cells were grown on all three roughness types, while sharing the same morphological characteristics, including the relatively small and rounded shape cluster formation.

Among the different non coated roughness substrates, PC12 cells seemed to prefer the low roughness structures. MCs surfaces were largely preferred as compared to flat ones (2- to 8-fold higher proliferation while flat surfaces could not support cell growth after 7 days of culture.)
Effect of surface roughness on PC12 cell growth in the presence of NGF

The PC12 cells growing on the low and mid roughness MCs could differentiate towards the neuronal cell lineage, showing increased, flattened cellular body, sprouting neuritic processes.

Effect of surface roughness on PC12 cell differentiation

Fluorescence microscopy images

Actin
Tubulin

Low Roughness  Medium Roughness  High Roughness

Differential response-
No differentiated PC12 cells on highly rough Si surface!
Effect of surface roughness on PC12 cell growth in the presence of NGF

Differentiated cells (%): Cells with Neurites/total cells

$L_{neurite}$: The mean length of the longest neurite per cell

PC12 cell line

A correlation between the geometrical characteristics of the topographical features of the surfaces and the (respective) cell responses

How do primary cells of the nervous system respond to the underlying surface topography?
In vitro experiments with cells

Neuron-like cell line
- PC12 cells

Primary cultures
- Schwann cells
- Sympathetic neurons (Superior cervical ganglia)
- Sensory neurons (Dorsal Root Ganglia)
Primary cells on μ-patterned Si substrates

Schwann cells

All three micro-patterned Si substrates could equally well support the growth of Schwann cells

Culture medium: DMEM + 1% FBS
Culture time: 7 DOC

C Simitzi, P Efstathopoulos, et al. 2015 Biomaterials 67:115-128
All three micro-patterned Si substrates could equally well support the growth of Schwann cells.

**Schwann cells**

- Culture medium: DMEM + 1% FBS
- Culture time: 7 DOC
- Coating: Collagen solution

Although very few neurons could grow on the flat Si substrates, all micropatterned Si substrates did support extended neuronal outgrowth.

**Sympathetic neurons**

- Culture medium: RPMI + 1% FBS + 100 ng/ml NGF
- Culture time: 7 DOC

C Simitzi, P Efstathopoulos, et al. 2015 Biomaterials 67:115-128
Schwann cells on µ-patterned Si substrates

Remarkably, there is a trend for preferred outgrowth orientation on mid and high roughness substrates.
Sympathetic neurons on µ-patterned Si substrates

Topography-dependent axonal outgrowth pattern:
Axons on the low roughness substrates were shown to grow randomly, whereas axons on medium and high roughness substrates followed a parallel alignment growth pattern.

C Simitzi, P Efstathopoulos 2015 Biomaterials 67:115-128
Topographic guidance of neural cell outgrowth

- The importance of surface roughness over nerve cell outgrowth and network formation is emphasized.

- Axonal outgrowth pattern was dependent on the underlying topography.

- Preferred Schwann cell outgrowth orientation towards the substrates with increasing roughness.
**In vitro experiments with cells**

**Neuron-like cell line**
- PC12 cells

**Primary cultures**
- Schwann cells
- Sympathetic neurons (Superior cervical ganglia)
- **Sensory neurons (Dorsal Root Ganglia)**
Dorsal root ganglia (DRG) are collections of sensory nerve bodies, their axons and Schwann cells located posterolateral to the spinal cord.

These can be isolated from embryonic mice and grown in culture, allowing one to follow the process of axonal myelination.
Whole dorsal root ganglion explants

Low Roughness
Isotropic Cell Outgrowth

High Roughness
Anisotropic Cell Outgrowth

S100: Schwann Cells NF: Neurons

The trend for preferred orientation of cell migration and axonal outgrowth is enhanced as the surface roughness increases.
Spatial relationships between axons & non-neuronal cells

S100: Schwann cells
Neurofilament: Axons
To-Pro: Cell nuclei
Spatial relationships between axons & non-neuronal cells

C Simitzi, P Efstathopoulos 2015 Biomaterials 67:115-128
Schwann cell migration and axonal outgrowth on micropatterned Si surfaces

- The plasticity of the Schwann cells and their processes allowed them to create a “carpet”
- This glial cell “carpet” served as a substrate for the outgrown neurites

Schwann cells were guided by the underlying topographical features of the micropatterned silicon surface

Neurons were, in turn, outgrown on top of them
Co-culture of Schwann cells & SCGs neurons on µ-patterned Si substrates

Neurite Outgrowth Is Directed by Schwann Cell Alignment in the Absence of Other Guidance Cues!
Conclusion

- **PC12 cell line**
  - The cell response (outgrowth & differentiation) was influenced by the substrate topography.

- **Primary ganglion cell cultures**
  - The micropatterned substrates can support ganglion explant nerve cell outgrowth, without the need for coating.
  - The micropatterned substrates can support outgrowth and network formation of dissociated ganglion neurons.
  - There is a trend for preferred outgrowth orientation of Schwann cell on mid and high roughness substrates.

  a correlation between the geometrical characteristics of the topographical features of the surfaces and the (respective) cell responses.
Laser based fabrication of biomimetic scaffolds

I. Ultrafast laser surface’s modification
   Hierarchical micro/nano structuring

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3-D nano-structuring using multi-photon polymerization

1-photon absorption

2-photon absorption

3-D nano-structuring using multi-photon polymerization

Laser source, Ti:Sapphire:
\[ \lambda = 800 \text{ nm}, \tau_{\text{pulse}} < 20 \text{ fs}, \text{repetition rate} = 75 \text{ MHz} \]
3-D nano-structuring using multi-photon polymerization

Photopolymer
Biocompatible, non toxic, non-biodegradable, Hybrid (organic/inorganic)
3-D nano-structuring using multi-photon polymerization

Polylactide-based biodegradable photopolymer

3-D nano-structuring using multi-photon polymerization

Schwann cells (SW10)

NEURO2A
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Process followed:

1. Biopolymer film preparation
2. UV laser treatment
3. Surface foam structure formation
4. Cell attachment and growth
Biopolymer foam-like scaffolds casted on glass

Gelatin

Chitosan

Collagen
Chitosan foam-like scaffolds casted on different substrates:

- aluminum
- silicon
- quartz
- glass
Conclusion

The cell response (outgrowth & differentiation) was influenced by the substrate topography.

The micropatterned and 3D bridge-bearing substrates can support outgrowth and network formation of dissociated ganglion neurons.

There is a trend for preferred outgrowth orientation of Schwann cells.

↓

a correlation between the geometrical characteristics of the topographical features of the surfaces and the (respective) cell responses
Thank you for your attention!