

# A fast flexible inkjet method for patterned neuron culture

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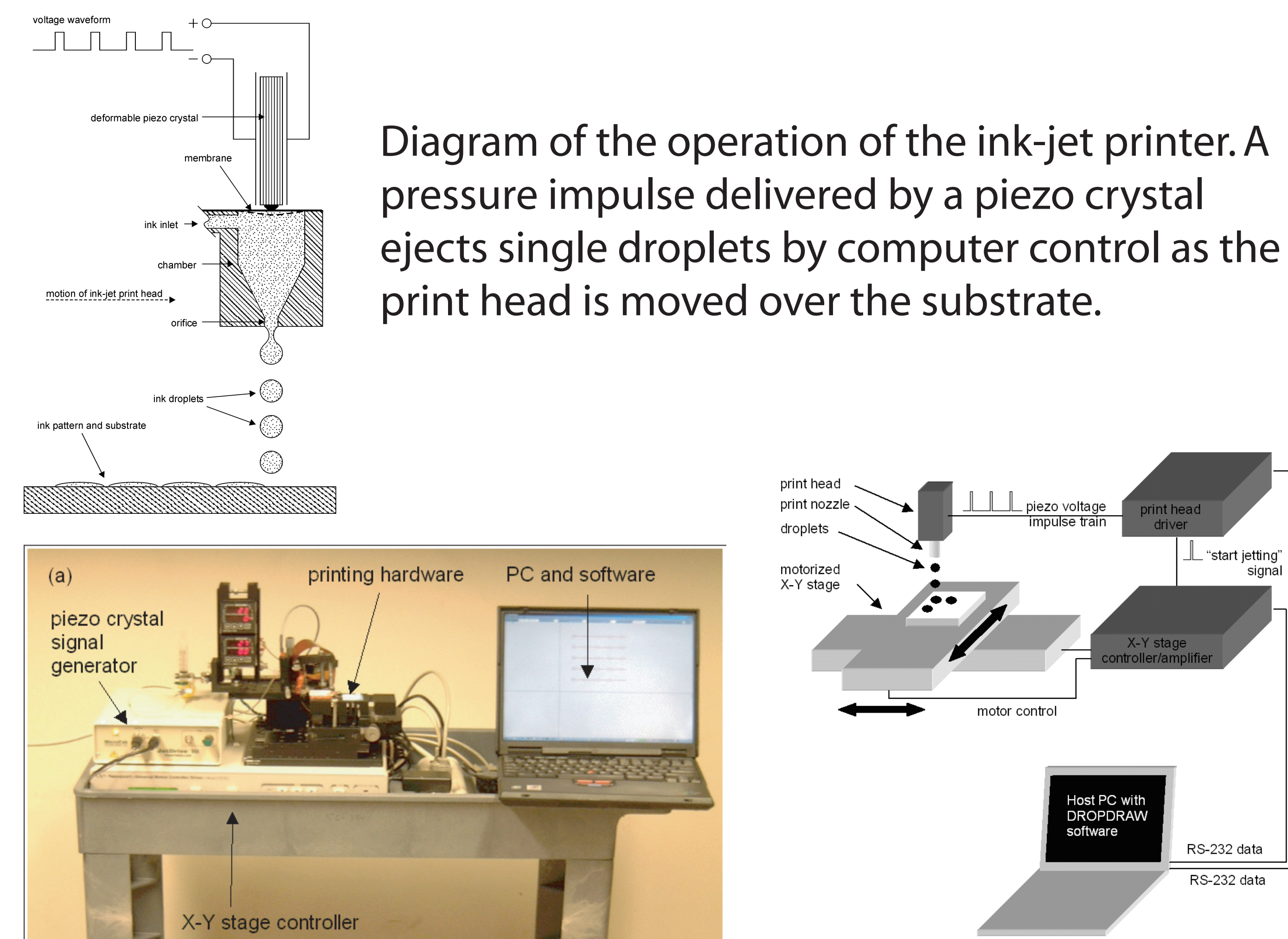
## Introduction

We present an ink-jet printing technique that allows precise micropatterning of cell adhesion substrates. Compared with traditional photolithography or recent soft lithographic techniques like PDMS stamping, our ink-jet method allows faster and less expensive fabrication of new patterns.

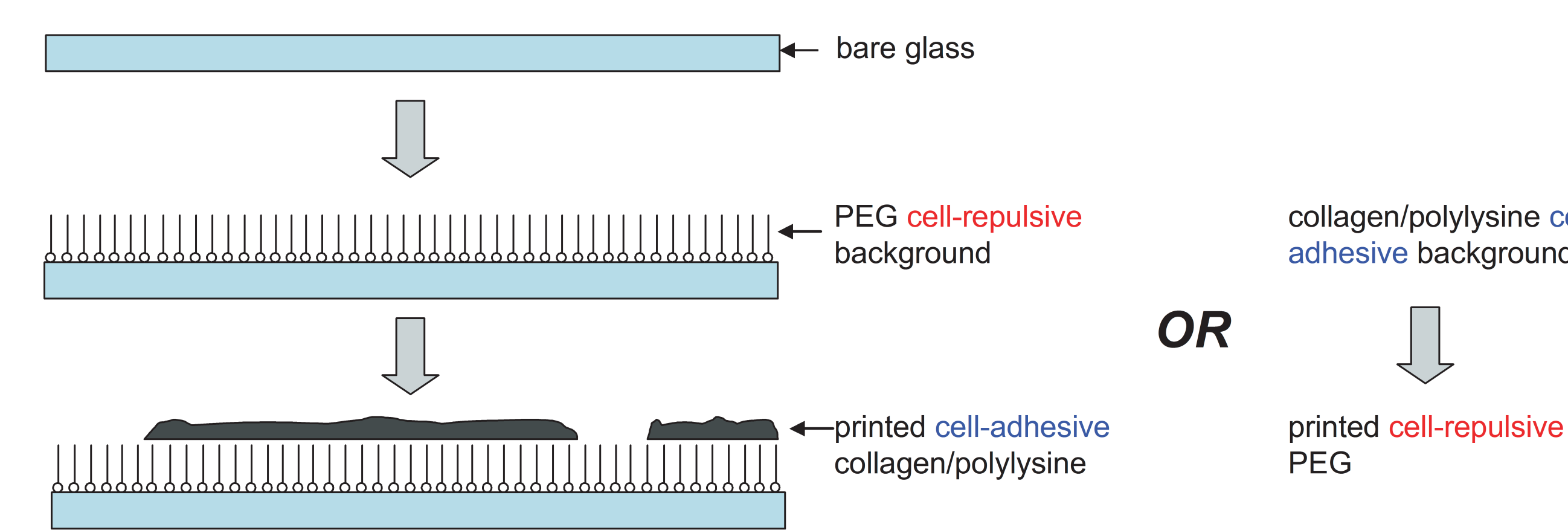
Disassociated rat hippocampal culture grown at low densities on our inkjet-printed surfaces show:

- Excellent adherence to the pattern over extended time periods
- Normal electrophysiological properties
- Similar distributions of immunocytochemical markers for synapses and inhibitory cells as found in unpatterned, low-density controls

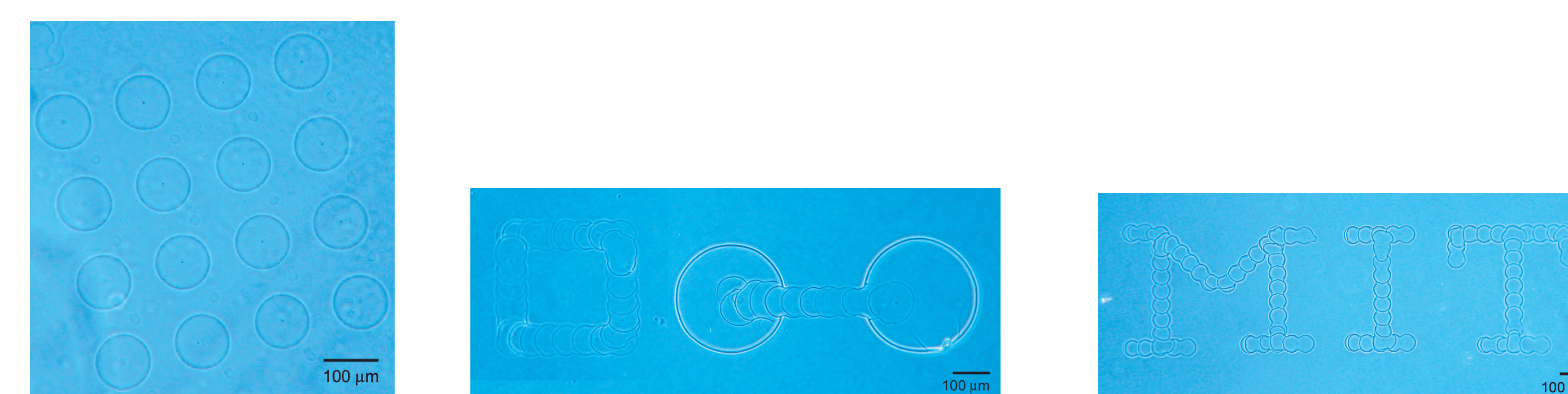
## Methods



## Patterning

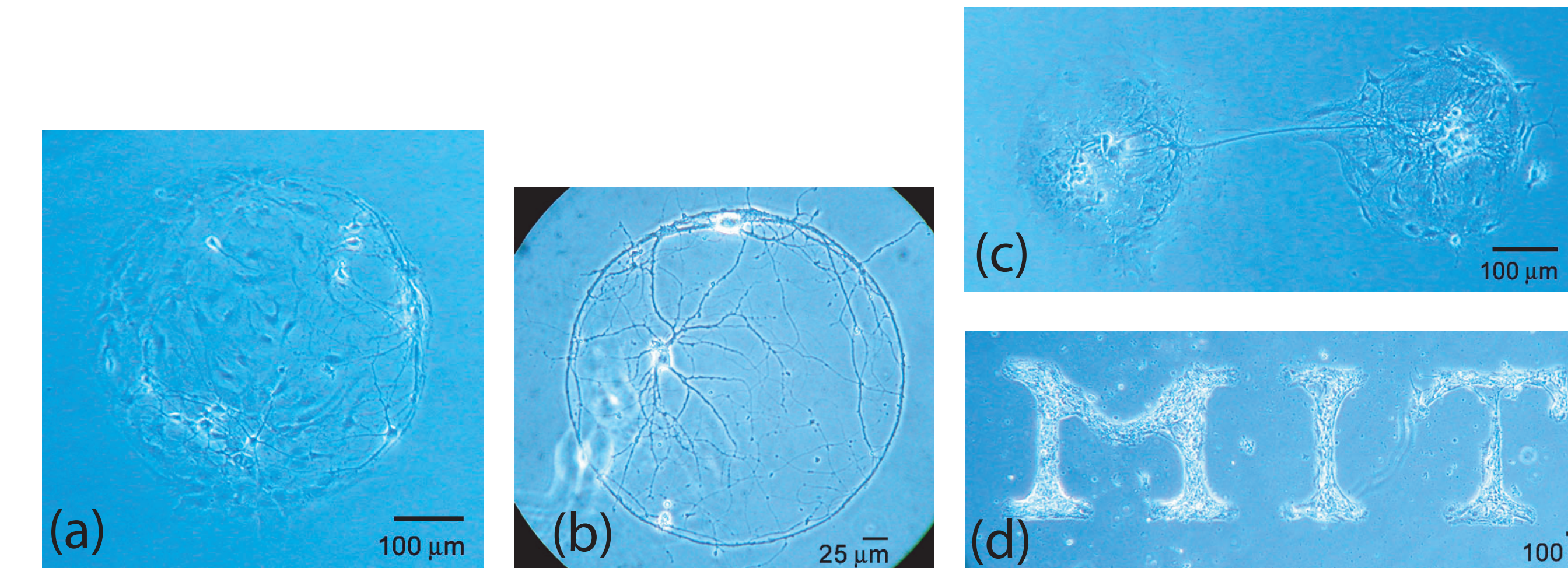


The flexible surface chemistry allows for printing in either positive or negative relief. This flexibility is possible because the adhesion and repulsion molecules have the same functional/reactive group.

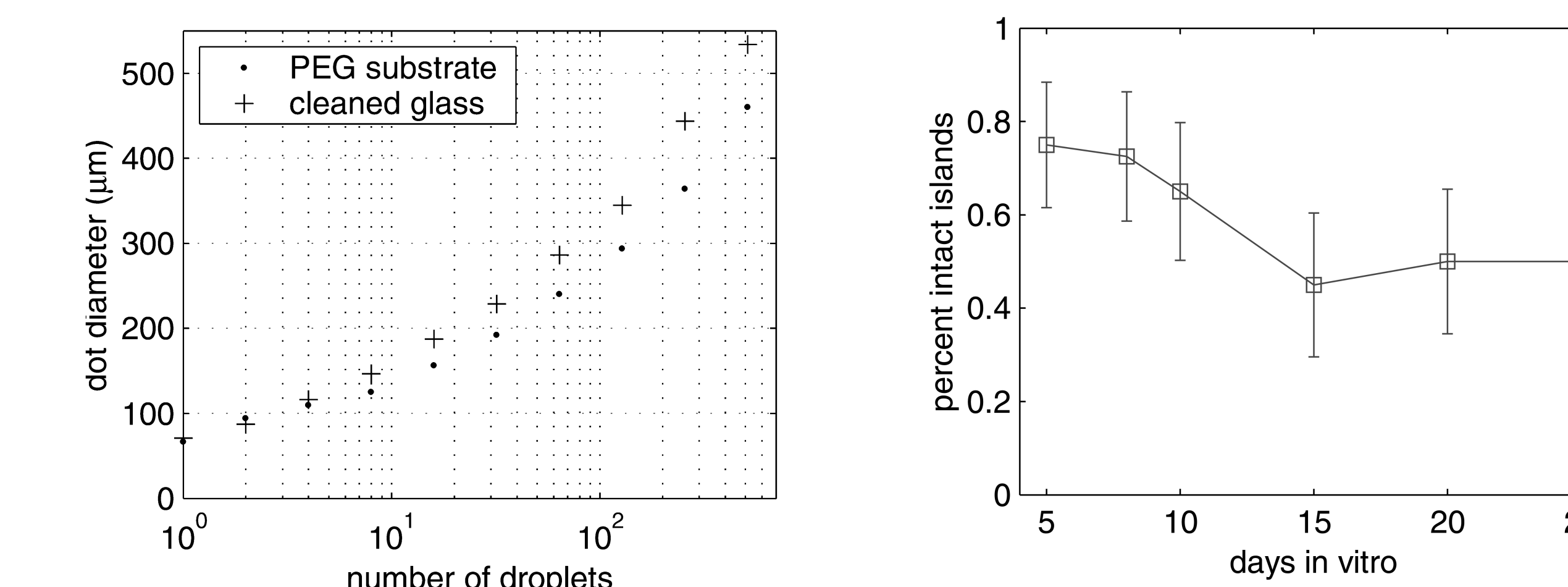


Printed patterns can include micro-islands (left) and connected micro-islands and arbitrary shapes (middle and right).

## Results

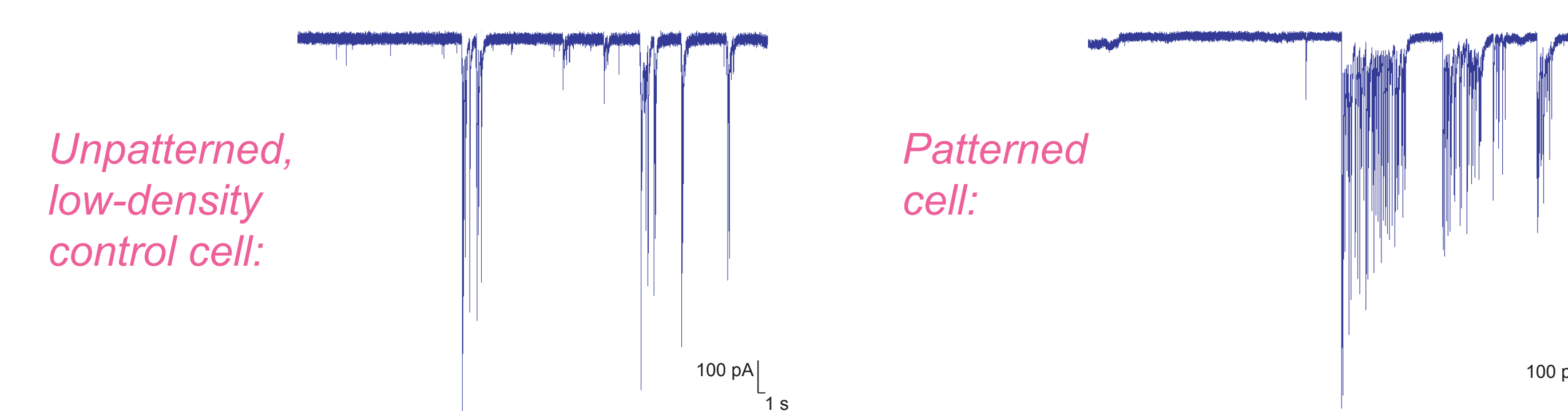


P1 hippocampal neurons patterned on micro-islands with glia (a), without glia (b), connected micro-islands (c), and arbitrary shapes (d).

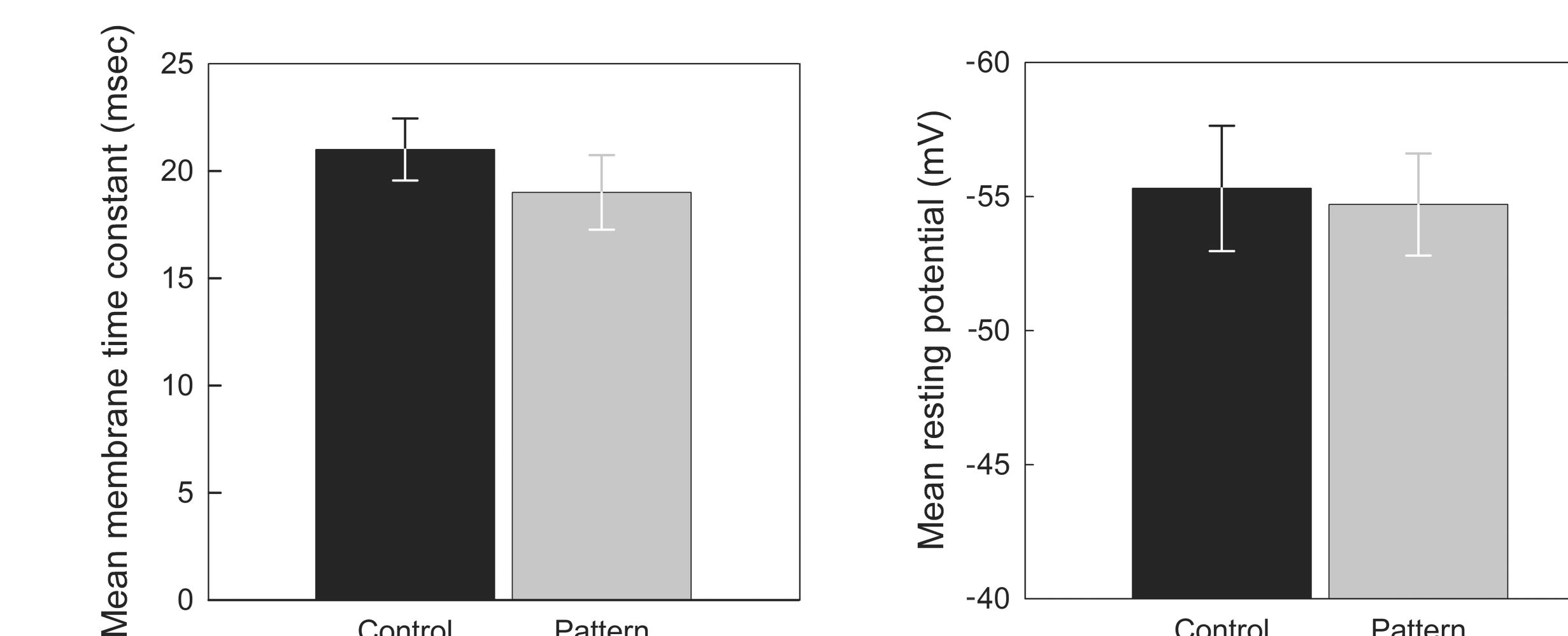


Micro-islands can be sized according to the number of droplets deposited.

50% of islands remain intact after 25 days *in vitro* (DIV).

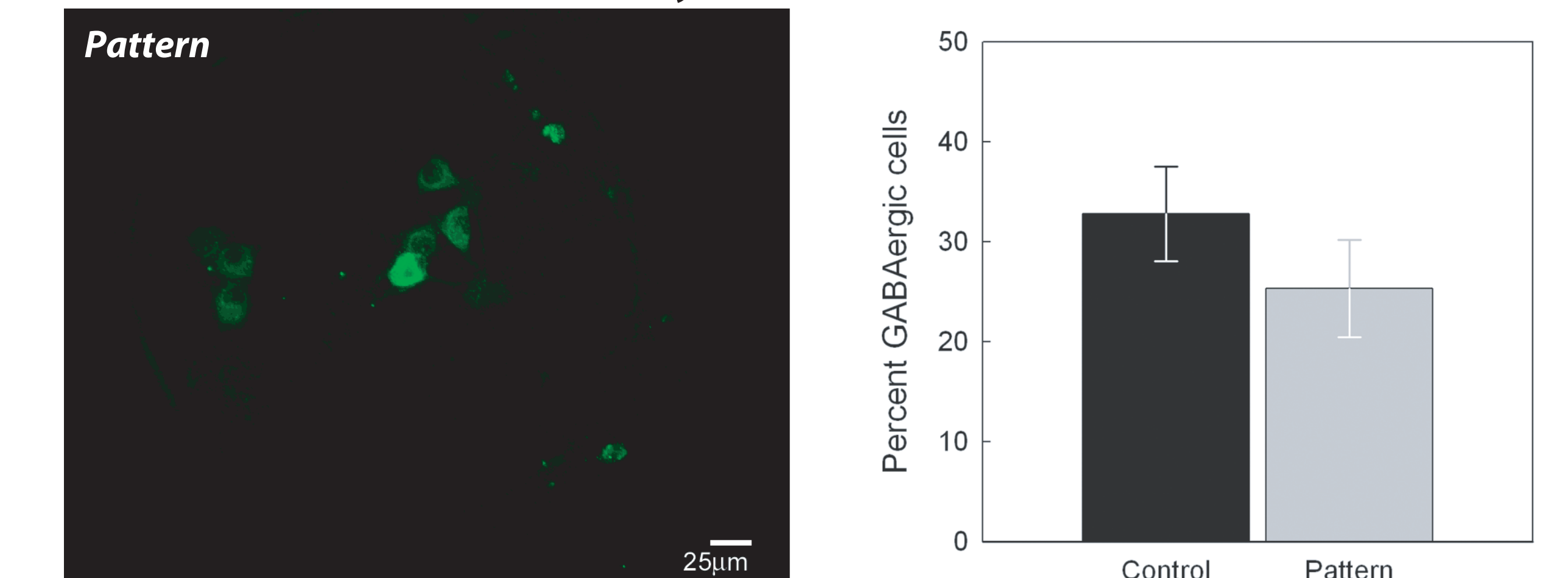


Synaptic activity traces from voltage-clamp recordings at DIV 10-12.



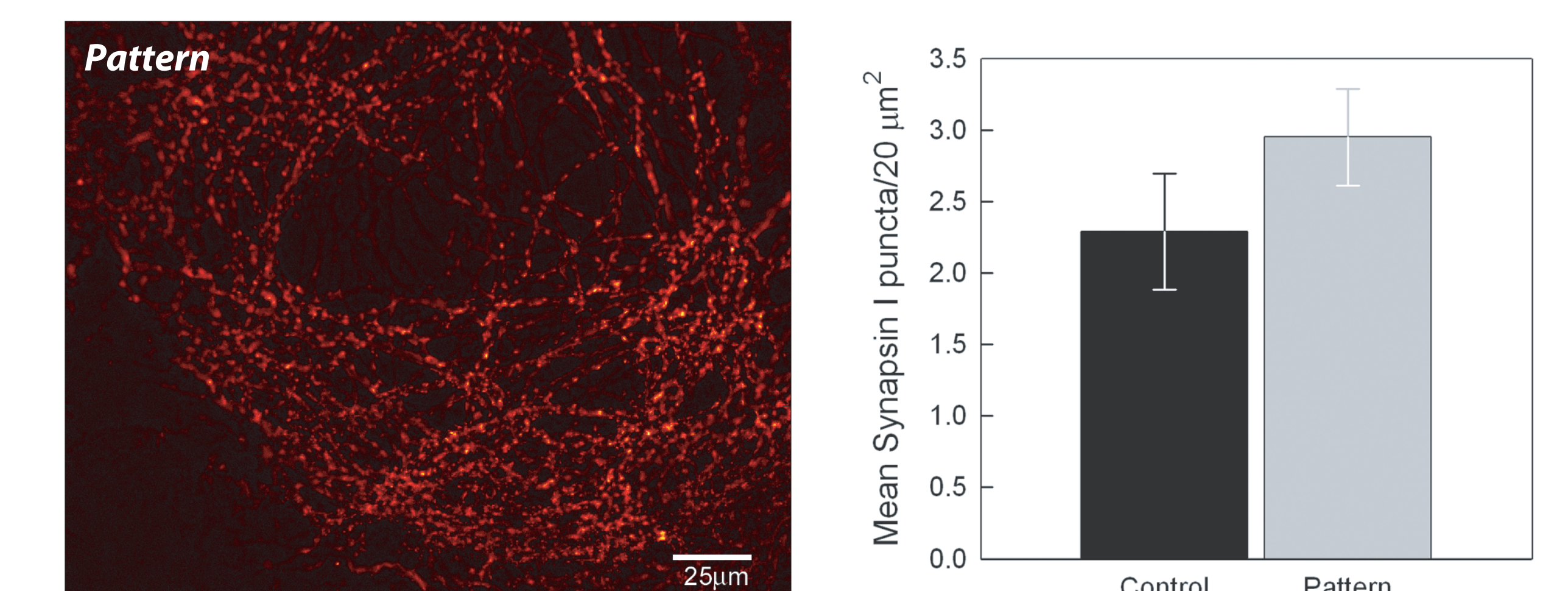
Similar passive membrane properties and resting potentials are found in patterned and unpatterned low-density control cells.

• Immunostain for inhibitory cells (GABA):

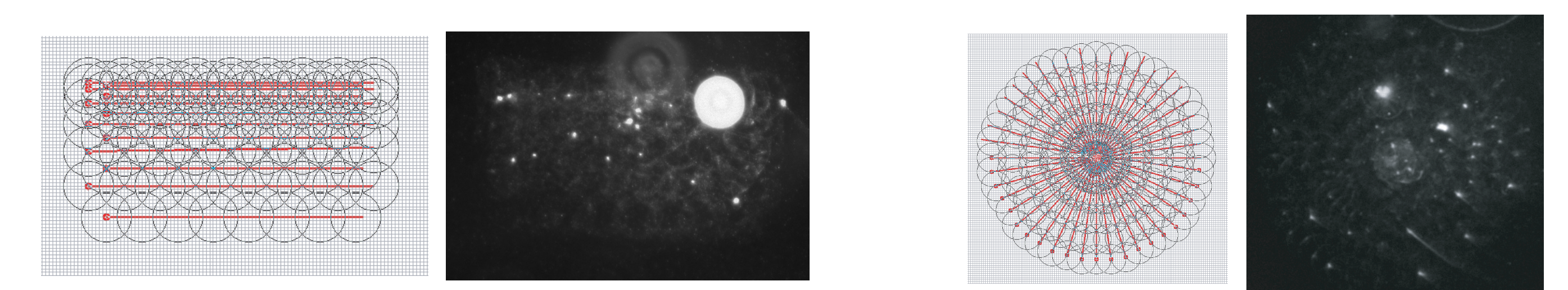


Patterned cultures show a lower percentage of inhibitory cells (25%) than low-density control cultures (33%) but are not significantly different ( $p < 0.05$ ).

• Immunostain for excitatory and inhibitory synapses (Synapsin I):



Synaptic density is not significantly different ( $p < 0.05$ ) between patterned cultures (2.95 synapses/20 $\mu\text{m}^2$ ) and low-density control cultures (2.29 synapses/20 $\mu\text{m}^2$ ).



FUTURE WORK: Gradients of laminin for developmental study of axon guidance. Linear gradient (left) and circular gradient (right) shown by laminin immunostain adjacent to CAD software representation.

## Summary

- We designed an ink-jet printer to programmably arrange chemical factors.
- Patterned neurons exhibited:
  - healthy electrophysiological properties
  - spontaneous activity
  - adherence to patterns for extended periods of time
  - normal immunocytochemical characteristics
- The ability to print gradients and other shapes suggest the use of ink-jet printing for further studies in neuron-substrate interaction and arrangement of neural circuits.

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