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# CLAMPS Substrates Support Ex Vivo Axon Extension from the Hypothalamus in Brain-on-Chip Platforms

Over the past 17 years, nearly 600 publications have explored microfluidic platforms or tissue chips for localized drug perfusion and electrophysiological monitoring of organotypic brain slices. However, only a few studies report actual axonal projections from these slices. Achieving axonal outgrowth in microfluidic systems has remained difficult and is often deemed infeasible in peer review. Among successful cases, the hippocampus is key—its structure offers a natural edge, allowing dissection with minimal damage and preserving axonal architecture. To date, no other brain region has demonstrated ex vivo axonal extension in organotypic slices. The hypothalamus, essential for physiological homeostasis, poses unique challenges due to its complex architecture and lack of defined axonal exit zones. Magnocellular neurons from this region are particularly resistant to survival and outgrowth in standard culture.

In this work, we present a microfluidic and surface chemistry strategy enabling axonal extension from hypothalamic organotypic brain slices. Our custom surface formulation, called CLAMPS (Cell-Like-Adhesion through Matrix-Polymer Substrates), combines peptide epitopes with carbon-based aliphatic polymers to create a transparent, molecular-scale substrate. CLAMPS in microfluidics rescues magnocellular neurons from failure-to-thrive conditions in dishes and microfluidic cultures.

We demonstrate that CLAMPS is necessary and sufficient to support magnocellular neuron growth in monoculture compared to standard conditions ($p < 0.0001$, one-way ANOVA with multiple comparisons). CLAMPS-based microfluidics also sustain hippocampal organoids and retain brain slice integrity in vitro.

Microfluidic devices were fabricated at CNMS-NRL (CNMS2024-B-02677) using SU-8 photolithography and PDMS molding. Chambers are 300–400 µm tall, with 50 µm channels supporting axonal conduit formation and localized perfusion. Axonal outgrowth from hypothalamic biopsy punches was confirmed in 5-week-old brain slices cultured for 10 days, then immunolabeled for axonal tau.

## Topical Area

Biology and life sciences

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