Supplementary information

Graphene–Based Neuron Encapsulation with Controlled Axonal Outgrowth

Koji Sakai, Tetsuhiko F. Teshima, Hiroshi Nakashima and Yuko Ueno

Supplemental Experimental

Supplementary Figure S1

Supplementary Figure S2

Supplemental Video 1 Self-folding of graphene/parylene-C films
Supplemental Video 2 Neuron encapsulation within graphene/parylene-C micro-rolls
Supplemental Video 3 Calcium response to glutamate in encapsulated HEK cells expressing GluR1
Supplemental Video 4 Time-lapse observation of neuronal behaviour within micro-rolls
Supplemental Video 5 Calcium response to glutamate in encapsulated hippocampal neurons
Supplemental Video 6 Synchronous spontaneous activities in hippocampal neurons

Supplementary Experimental

Characterisation of micro-roll

To characterise the micro-roll, we obtained time-lapse images of folded film and measured the geometrical features (thickness, curvature and pore diameter) and Raman spectra of the micro-roll. The time-lapse image of folded film was obtained using an optical microscope (ECLIPSE TE2000, Nikon) with a charge-coupled device camera (DP73, Olympus). The thickness of parylene-C was measured using a surface profiler (Alpha step IQ, KLA-Tencor) with a resolution of 0.1 nm. The curvature radius of the micro-rolls was defined as the distance between two edges of a micro-roll in images
obtained using an optical microscope (ECLIPSE TE2000) with a charge-coupled device camera (DP73). The images for measuring the pore diameter were obtained by differential interference contrast microscopy in SD-OSR (Olympus). The pore diameter was obtained by circle fitting using ImageJ. The spectroscopic characterisation before and after folding was performed using a Raman spectrometer (in Via Qontor, Renishaw). A laser-diode continuous-wave laser ($\lambda = 532$ nm) was used as the excitation light source.

**Scanning electron microscopic observation of neuron-laden micro-roll**

We used a scanning electron microscope (SEM) to observe axons attached to the pores on a micro-roll surface. We prepared a sample with a neuron-laden micro-roll for the SEM imaging. The sample was rinsed with phosphate buffered saline and then fixed with 2 wt% glutaraldehyde (Wako) for 30 min. The sample was dehydrated with a series of ethanol/water mixtures of increasing ethanol concentration (30 - 99%) and then immersed in tert-butyl alcohol (Wako). The sample was lyophilised with a freeze drier (FS-2030, EYELA). The fixed sample was sputter coated with gold (Ion Sputter E-1030; Hitachi) and observed with SEM (S4300, Hitachi).

**Calcium imaging for monitoring cellular activity**

We performed calcium imaging to visualise responses to a pharmacological stimulation and spontaneous activity. A sample with a cell-laden micro-roll was loaded with 4 $\mu$M Fluo-8 AM (Abcam) and 0.05% Pluronic F-127 (Thermo Fisher Scientific) for 30 min at 37 °C to monitor the intracellular $\text{Ca}^{2+}$. After the dye loading, the sample was rinsed twice with a recording medium. A phosphate buffered saline solution was used for the recording media for HEK cells. The recording media for primary hippocampal neurons was a HEPES buffered saline solution (adjusted to pH 7.2 with NaOH) of the following
composition: 148 mM NaCl, 2.8 mM KCl, 10 mM HEPES, 10 mM glucose, 2 mM CaCl2 and 2 mM MgCl2. To record spontaneous neuron activity, we removed Mg\(^{2+}\) from the HEPES buffer to induce synchronous N-methyl-D-aspartate receptor-mediated activity. The fluorescent images were obtained using a confocal fluorescence microscope (LSM 510, Carl Zeiss). The fluorescent intensity of individual cells was measured in ROIs that were manually chosen using ImageJ. The fluorescent intensity trace was normalised as \(F/F_0\). \(F_0\) is the initial level of the trace.
Supplementary Figure S1 Classification of three-dimensional structures of the micro-rolls. (a) Schematic images of single-roll and double-roll configurations. (b) Schematic images indicating the definition of the number of windings, $n_w$ corresponding to the micro-roll structures.
**Supplementary Figure S2 Entire Raman spectra.** Raman spectra of a SiO$_2$ substrate, a pristine parylene-C film on the SiO$_2$ substrate, a monolayer graphene on the SiO$_2$ substrate, and monolayer graphene that attach to the parylene-C film before and after folding. The spectrum of parylene-C film shows peaks at 1335 cm$^{-1}$ and 1610 cm$^{-1}$. There are the characteristic Raman peaks at 2675 cm$^{-1}$ (2D band), 1585 cm$^{-1}$ (G band) in the monolayer graphene.