Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary information

Graphene –Based Neuron Encapsulation with Controlled Axonal Outgrowth

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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 (λ = 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 μM Fluo-8 AM (Abcam) and 0.05% Pluronic F-127 (Thermo fisher Scientific) for 30 min at 37 °C to monitor the intracellular 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.

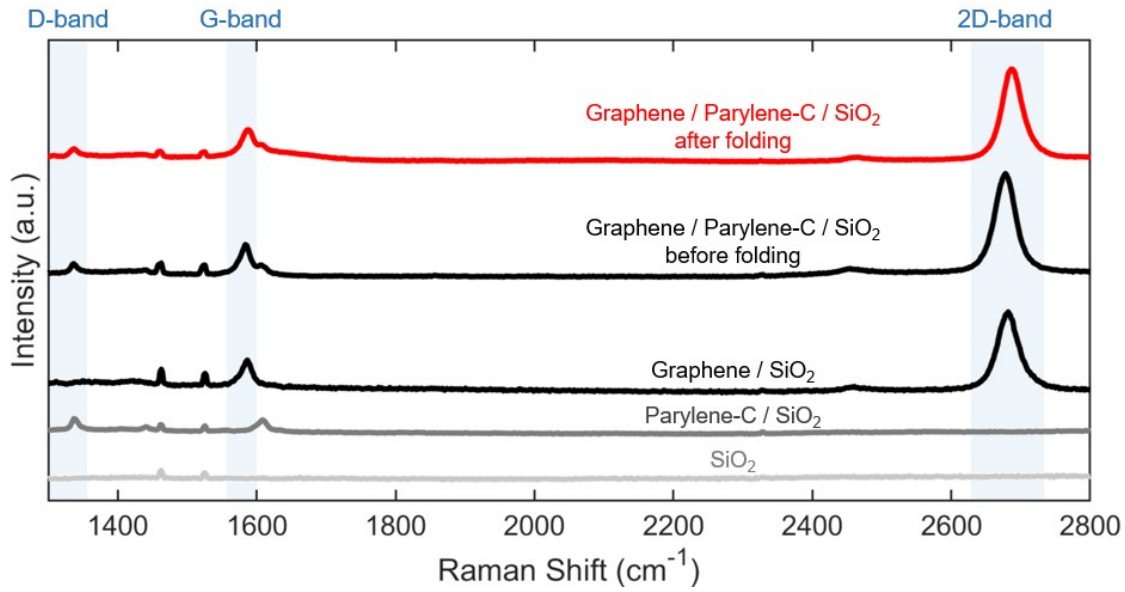
a

Configuration	Cross section of micro-roll
Single roll	
Double roll	

b

Number of windings, n_w	Cross section of micro-roll
$n_w < 1$	
$1 < n_w < 2$	
$2 < n_w$	

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.