SUPPORTING INFORMATION

Axon-First Neuritogenesis on Vertical Nanowires

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Methods

Chemicals. Silicon tetrachloride (SiCl$_4$, 99.998%) and ammonium fluoride (NH$_4$F, ~40% in water) were purchased from Aldrich. Hydrofluoric acid (HF, 32-52% in water) was purchased from Acros. Acetone and isopropyl alcohol were purchased from Shachun. Si(111) wafers were purchased from LG Siltron.

Cell Culture. Primary hippocampus from E18 Sprague-Dawley rat was dissociated in Hank's Balanced Salt Solution (HBSS) using a fire-polished Pasteur pipette. The cell suspension was centrifuged for 2 min at 1000 rpm, and a cell pellet was resuspended in Neurobasal media (Gibco) supplemented with B-27 (Invitrogen), 2 mM GlutaMax (Gibco), 12.5 μM L-glutamic acid (Sigma), and penicillin-streptomycin (Gibco). Dissociated cells were seeded at the density of 500-200 cells/mm$^2$ on a poly-L-lysine-coated coverslip or vg-SiNWs. The adhesive coating was not necessary in case of vg-SiNWs. Cultures were maintained in an incubator (5% CO$_2$ and 37 °C), and a half of media was replaced with fresh culture media without L-glutamic acid supplement every 3-4 days. This study was approved by IACUC (Institutional Animal Care and Use Committee) of KAIST.

Time-Lapse Imaging. At 7 hours in vitro, neurons on poly-L-lysine-coated coverslips or vg-SiNWs were fluorescently labeled using a 200-nM NeuO (from Prof. Chang at NUS, Singapore) in Neurobasal media (Invitrogen) for 5 min. The substrates then immersed in the fresh media containing 10 mM of HEPES (Sigma) before being placed in a live cell imaging chamber (Live Cell Instrument), which was installed on the stage of an upright fluorescence microscope (BX61WI, Olympus). Subsequently, neurite outgrowth was monitored by an Olympus 20× immersion type objective with EMCCD (iXon, Andor) with 5-min intervals for 24 h.

Calcium Imaging. Oregon Green BAPTA-1 (OGB-1, Sigma) was used as a Ca$^{2+}$ indicator for measuring neural activity in an optical way. Neuron-cultured samples were incubated under buffered artificial cerebrospinal fluid with the following composition—25 mM NaHCO$_3$, 25 mM d-glucose, 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH$_2$PO$_4$, 1 mM MgCl$_2$·6H$_2$O, and 2 mM CaCl$_2$·2H$_2$O —with 2 μM of OGB-1 in pluronic F-127 20% solution/DMSO (Sigma) for 30 min at 37 °C. After incubation, the samples were washed twice with fresh buffered artificial cerebrospinal fluid and stabilized for 30 min at 37 °C. Fluorescence intensity dynamics was measured by using a microscope (BX51; Olympus) with FITC filter cube and a 20× immersion objective (Olympus). Time-lapse images were recorded through sCMOS Neo (Andor Technology) with ~30 Hz of frame rates. Raw intensity values were extracted by using customized software implemented by Matlab, and relative changes of fluorescence intensity to baseline ((F-F$_0$)/F$_0$) were regarded as neuronal calcium signals.

Instruments and Characterizations. Neuronal growth on the prepared substrates was investigated by field-emission scanning electron microscopy (FE-SEM; Hitachi S-4800). Before FE-SEM imaging, the cultured substrates were coated with platinum (thickness: 10 nm). Fluorescence micrographs of neuron cultures were obtained using Olympus BX51M (Olympus) equipped with a CCD camera (DP71; Olympus). From the images, the lengths of
major neurites and the numbers of neurites were measured with Neuron J plugin in Image J software (NIH).

**Preparation of Au-coated substrates.** A Si(111) wafer substrate was first cleaned with acetone and isopropyl alcohol, and then dried using a flow of nitrogen. The substrate was subsequently dipped into a buffered HF solution (a mixture of HF solution (9% in water) and NH₄F solution (32% in water)) for 4 min to remove the native oxide layer, followed by rinsing with water. The prepared Si(111) wafer was then coated with a 1-nm-thick Au film by e-beam evaporation, the catalytic activity of which was utilized for vapor-liquid-solid (VLS) growth of nanowires. Next, the substrate was diced into small pieces with dimensions of 1 cm (width) × 1 cm (length).

**Synthesis of vg-SiNWs.** Vertically grown silicon nanowires (vg-SiNWs) were synthesized by chemical vapor deposition (CVD) method in a 12-inch horizontal tube furnace (Lindberg/Blue M) equipped with a 1-inch diameter quartz tube, as shown in Figure S1. Prior to the synthesis, the quartz tube was evacuated and flushed repeatedly with 10% H₂ gas (high purity, 99.999%) in order to minimize oxygen contamination. Having loaded the Au-covered Si(111) substrate on the center of the tube furnace, the temperature was increased to a growth temperature (860 °C) with a rate of 10 °C/min under a flow of 100 sccm carrier gas and 750 sccm dilution gas. The carrier gas flowed through the bubbler containing SiCl₄ as a Si source, and the dilution gas flowed directly into the reaction quartz tube. After 10 min of growth, the quartz tube was slowly cooled to room temperature in order to avoid adsorption of gaseous HCl, a byproduct of the decomposition of SiCl₄ during the growth.

**Synthesis of patterned vg-SiNWs.** The patterned vg-SiNWs were obtained by photolithography. Briefly, a Si(111) wafer was first washed with acetone and isopropyl alcohol for several times. The substrate was then spin-coated with a positive photoresist (AZ GXR-601). After a masked irradiation of intense light, the exposed regions of PR were chemically developed. Then, a 1-nm-thick Au film was deposited by e-beam evaporation on the PR-patterned substrate. By dipping into acetone, a patterned Au film was formed. The growth process of vg-SiNWs was identical, as mentioned above.

**Characterizations.** Surface morphologies of synthesized vg-SiNWs were characterized by field-emission scanning electron microscopy (FE-SEM, JEOL JSM-7600F) at an acceleration voltage of 15 kV with energy-dispersive X-ray spectroscopy (EDX) for chemical analysis. The single-crystalline structure of vg-SiNWs was analyzed by transmission electron microscope (TEM, JEOL 2010). The samples for TEM analysis were made by depositing an ethanolic solution of detached SiNWs, prepared by sonication of a vg-SiNW substrate in ethanol, onto holey carbon 300 mesh copper grids (Structure Probe). X-ray diffraction (XRD) patterns of the vg-SiNWs were obtained by using a Rigaku diffractometer (D/MAX-1C) with a monochromatic beam of Cu Kα radiation.

**Mechanism of the growth: SEM analyses of vg-SiNWs.** Figure S2 describes the growth process of vg-SiNWs. As the temperature of the substrate increased, the Au film started to crack, resulting in liquid-phase Au nanodroplets of various sizes. These Au nanodroplets
then migrated over the surface to form Au nanoparticles (AuNPs) by the two well-known processes: coalescence and Ostwald ripening. In the process of coalescence, two AuNPs merged into a single, larger AuNP, reducing the total interfacial energy of the system, whereas in Ostwald ripening process, Au atoms were removed and transferred from one AuNP to another by evaporation or surface diffusion, increasing the irregularity in the size-distribution of the AuNPs. Upon reaching the reaction temperature, vapor-phase Si precursors were introduced into the liquid-phase AuNPs by carrier gas (10% H₂ in Ar), and the single crystals of Si were formed by precipitation from Au-Si alloy. The lattice of the precipitated Si crystal matched that of the Si(111) substrate, which supported epitaxial growth of SiNWs along the [111] direction. The prepared vg-SiNWs were analyzed by SEM (Figure S2b). The averaged diameters of AuNPs and vg-SiNWs were 13±8 nm and 72±8 nm, respectively (Figure S2c). The broader size-distribution of vg-SiNWs relative to AuNPs was due to the agglomeration of AuNPs at high temperature. The averaged inter-distance between adjacent vg-SiNWs was less than 1 μm, which resulted in the adhesion and development of neurons without being penetrated by the nanowires.

**TEM and XRD analyses of vg-SiNWs.** TEM analysis showed that the synthesized nanowires were highly single-crystalline (Figure S3a-c). The XRD patterns of the vg-SiNWs synthesized from a 1-nm-thick Au film exhibited a major (111) peak at 28.58° (Figure S3d).
**Figure S1.** Tube furnace for the synthesis of vg-SiNWs. Schematic diagram of the furnace used for the synthesis of vg-SiNWs.

**Figure S2.** Growth mechanism of Au-catalyzed vg-SiNWs. (a) Schematic of the growth mechanism of vg-SiNWs. (b) SEM image (tilted for 20°) of vg-SiNWs after the VLS growth. The scale bar is 5 μm. (c) Diameter-distributions of AuNPs formed from Au film, and resulting vg-SiNWs.
Figure S3. Characterizations of vg-SiNWs. (a) Low-magnification TEM image of a vg-SiNW detached from the substrate. (b) High-resolution TEM image taken from the area denoted in a. (c) An SAED pattern indexed for cubic Si crystalline. (d) XRD pattern of vg-SiNWs.

Figure S4. Directional accumulation of F-actins within the somata. Immunostained hippocampal neurons on vg-SiNWs (3 hours in vitro). Target: β-tubulin-III (red); F-actin (green). The scale bar is 40 μm.
References


