## **Supplementary Information**



Mask I Mask II 1500um spacing 400um Diameter/20um width 400um Diameter/20um width 100um Gap 150um Dot C C C 0 C С 0 0 0 0 0 0 C C С С C 0 С C C 0 0 0 0 C 0 0 0 0 0 C 0 0 С С (b) (c) **Capture dot** Gap 50 Loop (d) Digital microscope system UV light meter OBT UV light source 

Figure S1. The overview images (AutoCAD format) of the two matched masks and the used UV system. Mask Images of (a) Mask I and (b) Mask II. Mask I is composed of gapped loops. The dimension of these gapped loops was 20  $\mu$ m wide, 400  $\mu$ m in diameter, and 100  $\mu$ m gap wide; there was a 6 × 6 loop matrix patterned with the same loops (the distance between two loops is 1500  $\mu$ m). The dimension of patterns in mask II was capture dots with diameter of 150  $\mu$ m, which were designed to spatially match mask I. (c, d) Illustration of dimensions of the GelMA ring's loop and capture dot. (e) UV system images: UV light source (Omnicure<sup>®</sup> S2000) used for photo-polymerization of hydrogels was manufactured at EXFO Photonic Solutions Inc. (Ontario, Canada). A Light meter (Omnicure<sup>®</sup>) was used to measure power and irradiance from the UV light source, ensuring a reliable and controlled UV output. Images were taken using a digital microscope system (KEYENCE, VH-Z500R).



**Figure S2.** Poission distribution of single cell capturing efficiency in GelMA rings. This cell capture event is Poissonian, where the probability mass function is given by:  $f(k; \lambda) = \lambda^k e^{(-\lambda)} / k!$  ( $\lambda > 0$ , k = 0, 1, 2, ...). "k" in this study represents the number of cells captured in one GelMA hydrogel ring, and it indicates capturing no cell, single cell and multiple cells in a GelMA hydrogel ring when k is equal to 0, 1 and other positive integers, respectively. " $\lambda$ " is the expected value of the cell capture event, which in this study equals to the product of cell concentration times volume of GelMA hydrogel capture dot. "e" is the base of natural logarithm (e = 2.71828...). Thus, if the diameter of GelMA hydrogel capture dot is kept constant, the " $\lambda$ " value is only linear to the cell concentration. The probabilities of capturing single cell, where k is equal to 1, can be plotted under various cell concentrations by Poisson distribution, shown as Supplementary Figure 2S. The Poisson distribution reaches maximums when x-axis is 0.375 and 0.85 (shown in gray rectangle filling), indicating an optimal cell concentration for high sing cell capturing efficiency should range from 0.375 to  $0.85 \times 10^6$  cells/ml with the capture dot diameter from 100 µm - 150 µm.



**Figure S3. Typical phase contrast images of proliferating 3T3 cells in GelMA rings. (a)** shows a monoclonal 3T3 population after 3 weeks of *in vitro* culture (IVC). The dashed area in **(a)** was enlarged in **(b)**, showing that these 3T3 cell fully crowded the GelMA hydrogel in 3D.



Figure S4. Typical fluorescence images of axonal growth of the captured single neuron cultured in 3D GelMA ring. (a-f) Axonal growth process at different time points of *in vitro* culture (IVC) from 8 hour until week 3. These images were from different samples due to no further culture after staining. (g) shows the phase contrast image of a growing axon, and its soma in the dashed area was enlarged in (h).



Figure S5. *In vitro* culture of rat cortical neuron on polylysine-coated Petri dish shows the axonal circle using a  $20 \times$  objective. Axonal circle was occasionally observed from normally cultured neurons on Petri dish. Neural axon seems to form a synapse with its own cell body, i.e., autapse.