Supporting Information

Materials and Methods

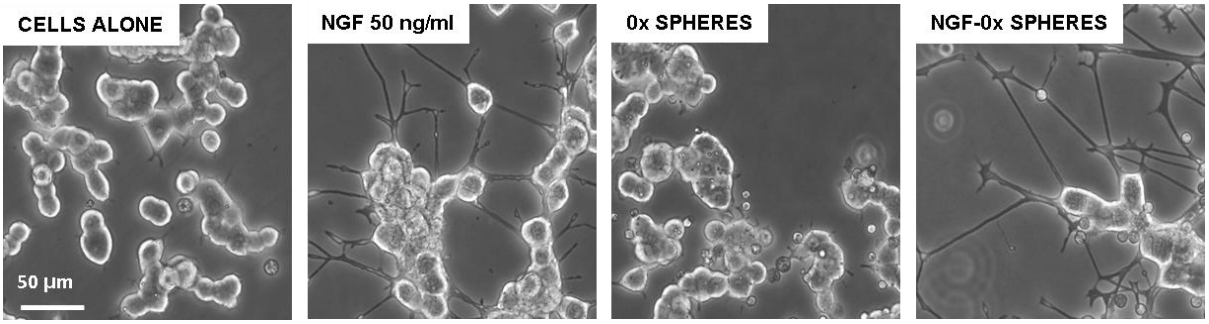
Fabrication of collagen hollow-spheres

Collagen hollow-spheres were fabricated according to the method previously described [21, 33]. Briefly, commercially available 4.5 µm polystyrene beads (0.5 g) (Spherotech Inc., Illinois, US) were spun down for 15 min at 7,000 rpm, supernatant was removed and spheres were dried in the oven (37^{o}C) overnight. Spheres were dispersed in 20 ml of sulphuric acid and sonicated for several min until solution was homogenous. Spheres were negatively charged by 18 hours incubation with sulphuric acid at 40^{o}C. Then, the sphere solution was centrifuged at 6,000 rpm and supernatant was discarded. Spheres were washed twice with 80% ethanol and once with water before final re-suspending in the distilled water. Following sulfonation, beads were resuspended in 0.1M acetic acid and mixed with 5 mg/ml of collagen solution at a weight ratio of 1:7 (collagen: beads). The mixture was stirred for 2 hours at RT to allow coating. Solution was centrifuged for 5 min at 7,000 rpm to separate collagen-coated beads from free collagen. Then, spheres were washed with PBS and re-constituted in PBS buffer. To generate cross-linked spheres free amino groups of collagen were cross-linked using 8a15kSG PEG at a weight ratio 1:1 (1x) or 1:2 (2x) (collagen: cross-linker). Cross-linker was first dissolved in PBS solution at 40 mg/ml and then mixed with collagen spheres. Cross-linker-sphere solution was incubated at RT for 1 hour. Then, the polystyrene template was removed by three washes (5 min, 3,500 rpm) with 20% (v/v) tetrahydrofuran (THF) to produce hollow spheres. Non-cross-linked spheres (0x) were produced in a similar manner, except that the polystyrene template was removed just after coating step. Hollow spheres were washed three times with 20% ethanol to ensure removal of any remaining THF and then stored in 70% ethanol at 4^{o}C to prevent potential contamination.

Primary DRG preparation

Female Sprague-Dawley rats were mated with males of the same strain and females and allowed to gestate to embryonic (E) day 16. At E16 the pregnant mother was euthanized and the embryos were removed by hysterectomy. DRGs were dissected and stored in cold Hanks buffered salt solution (HBSS). DRGs were washed once in fresh HBSS prior to purification. Whole embryonic DRGs were incubated in 25 ng/ml trypsin at 37°C for 15 minutes. DRGs were centrifuged at 1000 *g* for 10 minutes to pellet the cells. The pellet of cells was washed three times in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% FBS, 0.7% L glutamine, 1% Pen/Strep, 10 ng/ml nerve growth factor, 0.02M FrdU and 0.8M uridine culture medium. The DRG pellet was resuspended in culture medium, triturated and dissociated cells were counted using a haemocytometer. Embryonic DRG cells were seeded in 96 well plates at 4000 cells/well or in 24-well plates at 100,000 cells/well.

Results



Supporting Figure 1: The bioactivity of released NGF assessed on PC12s following treatment with NGF-loaded non-cross-linked (0x) collagen spheres.