Electronic Supplementary Information

Electrically Conductive MEH-PPV:PCL Electrospun Nanofibers for Electrical Stimulation of Rat PC12 Pheochromocytoma Cells

Rajiv Borah[1], Ganesh C. Ingavle[2], Susan R. Sandeman[2], Ashok Kumar[1,], Sergey V. Mikhalovsky[2]*

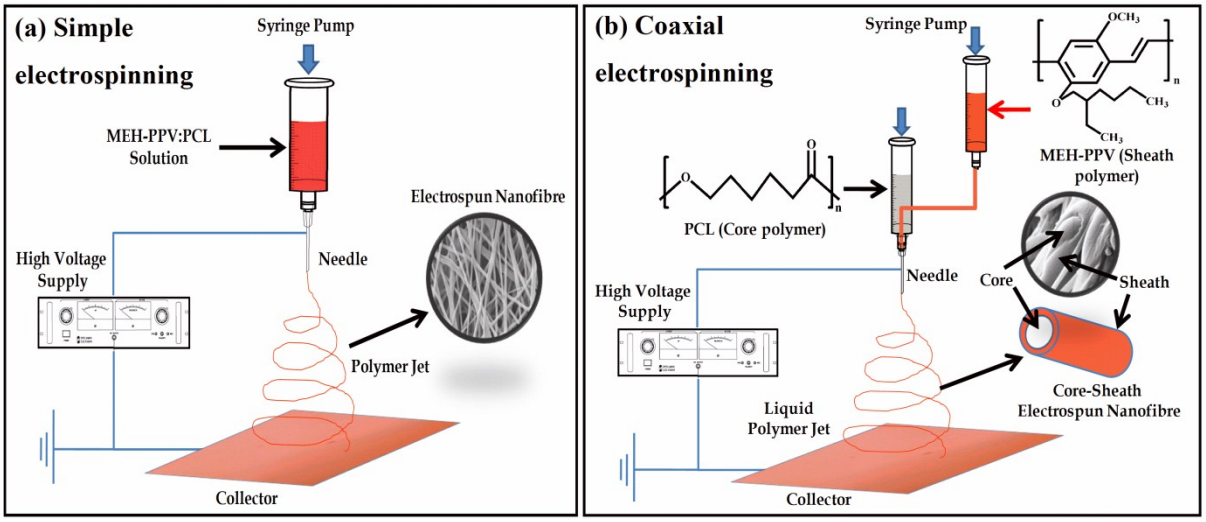

Figure S1. Schematic representations showing electrospinning of MEH-PPV:PCL by (a) simple electrospinning process and (b) coaxial electrospinning process.

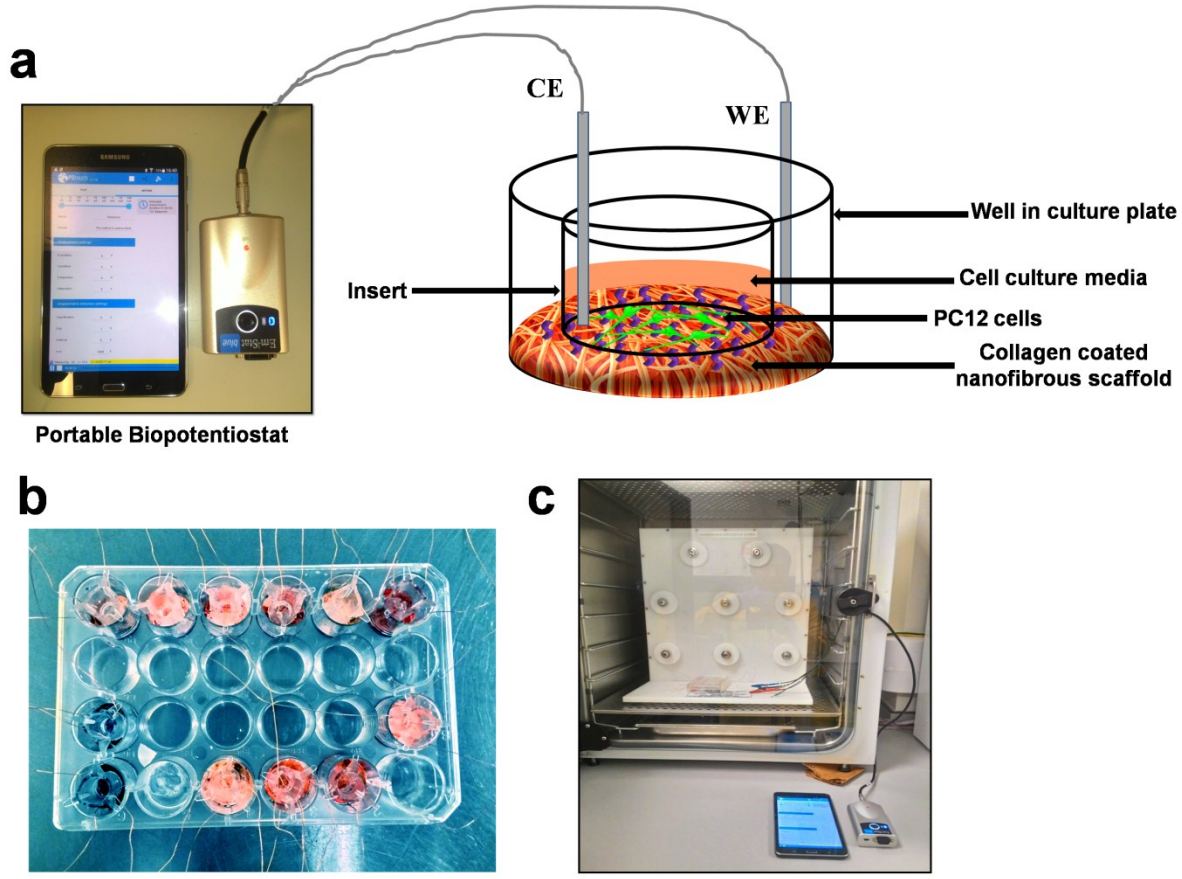

Figure S2. (a) Schematic illustration of the electrical stimulation experiment using a custom made electrical stimulation set up ; (b) photograph of self made cell culture plate with different electrospun MEH-PPV:PCL meshes (orange colur) fixed on it for electrical stimulation experiment; (c) photograph of the electrical stimulation experiment in situ.

Mechanical properties

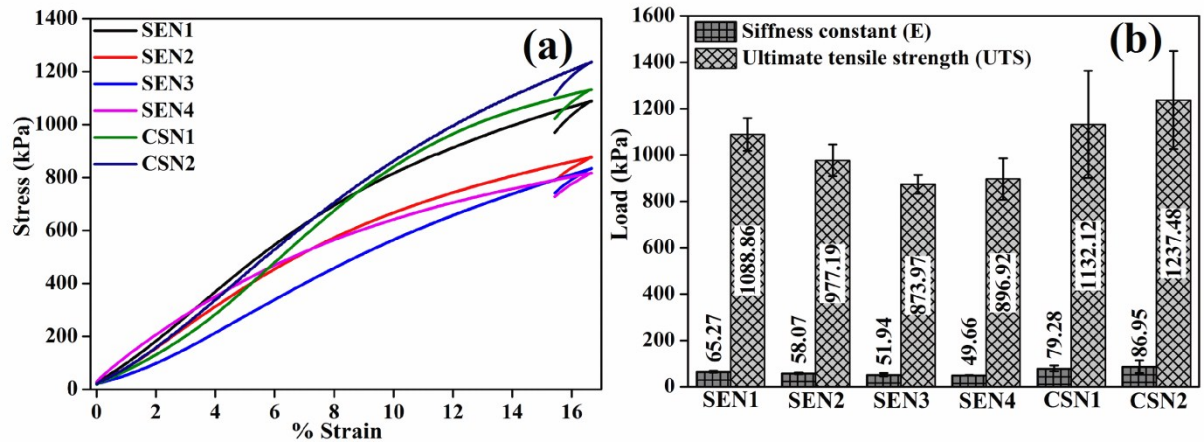

Figure S3. (a) Stress *vs* Strain curve of electrospun meshes as indicated; (b) Comparison of Young Modulus or stiffness constant (*E*) and ultimate tensile strength (UTS) of different electrospun meshes. Data were expressed as Mean ± S.D (n=3).

The tensile testing indicates the strength and elasticity of the film, which can be reflected by the stiffness constant (*E*) and ultimate tensile strength (UTS). It is suggested that scaffolds for normal cell function should be strong but flexible. Stress *vs.* strain curves were measured for all the electrospun nanofibre scaffolds and are presented in Figure S3. Mechanical properties, especially the Young's modulus or stiffness constant (*E*) and UTS of the scaffolds were derived from these curves and are presented in Figure S3(b). The stiffness constant (*E*) of SEN1, SEN2, SEN3 and SEN4 was measured to be 65 ± 5 kPa, 58 ± 4 kPa, 52 ± 7 kPa and 50 ± 3 kPa, respectively. The UTS of SEN1, SEN2, SEN3 and SEN4 was found to be 1089 ± 71 kPa, 977 ± 69 kPa, 874 ± 46 kPa and 897 ± 89 kPa, respectively. The stiffness constant (E) of CSN1 and CSN2 was measured to be 79 ± 14 kPa and 87 ± 27 kPa, respectively. The ultimate tensile strength of CSN1 and CSN2 was measured to be 1132 ± 231 kPa and 1237 ± 212 kPa, respectively. These results are consistent with previous reports that thicker nanofibres have moderately higher stiffness constant.[1] The exact mechanical properties of MEH-PPV in non-woven nanofibrous mats have not been investigated prior to this study. However, the increase

in the stiffness constant (*E*) with increase in fibre diameter can be correlated with the strong junctions in thicker fibres.[1] The lower value of the stiffness constant (*E*) of SEN2, SEN3 and SEN4 as compared to SEN1 and core-sheath nanofibre mat (CSN1 and CSN2) is also believed to be due to the presence of beaded fibres in the formers. The core-sheath nanofibre mats of CSN1 and CSN2 have a higher stiffness constant (*E*) and UTS due to a larger fibre diameter and core-sheath morphology.

Live/dead assay. The live/dead assay of 3T3 fibroblasts and PC12 cells on the electrospun meshes was performed using ethidium homodimer-1 (EthD-1, Thermo Fisher Scientific, UK; staining dead cells), calcein AM (Thermo Fisher Scientific, UK; staining live cells) and 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI, Molecular Probes, UK; staining nucleic acid).[2,3] For PC12 cells, the electrospun meshes were coated with Collagen I prior to experimentation. The 3T3 cells were seeded on the electrospun meshes at a concentration of 1×10^4 cells/well in a 48 well plate for the live/dead assay for 7 days. The PC12 cells were seeded on the collagen coated electrospun meshes at a concentration of 1×10^5 cells/well in a 48 well plate for 7 days. The scaffolds seeded with cells were washed with sterile PBS thrice prior to staining and transferred to a new culture plate. Cells were stained with a solution of 4 μM EthD-1, 2 μM calcein AM and DAPI in PBS and were then incubated for 30 min at room temperature before evaluation using confocal microscopy.

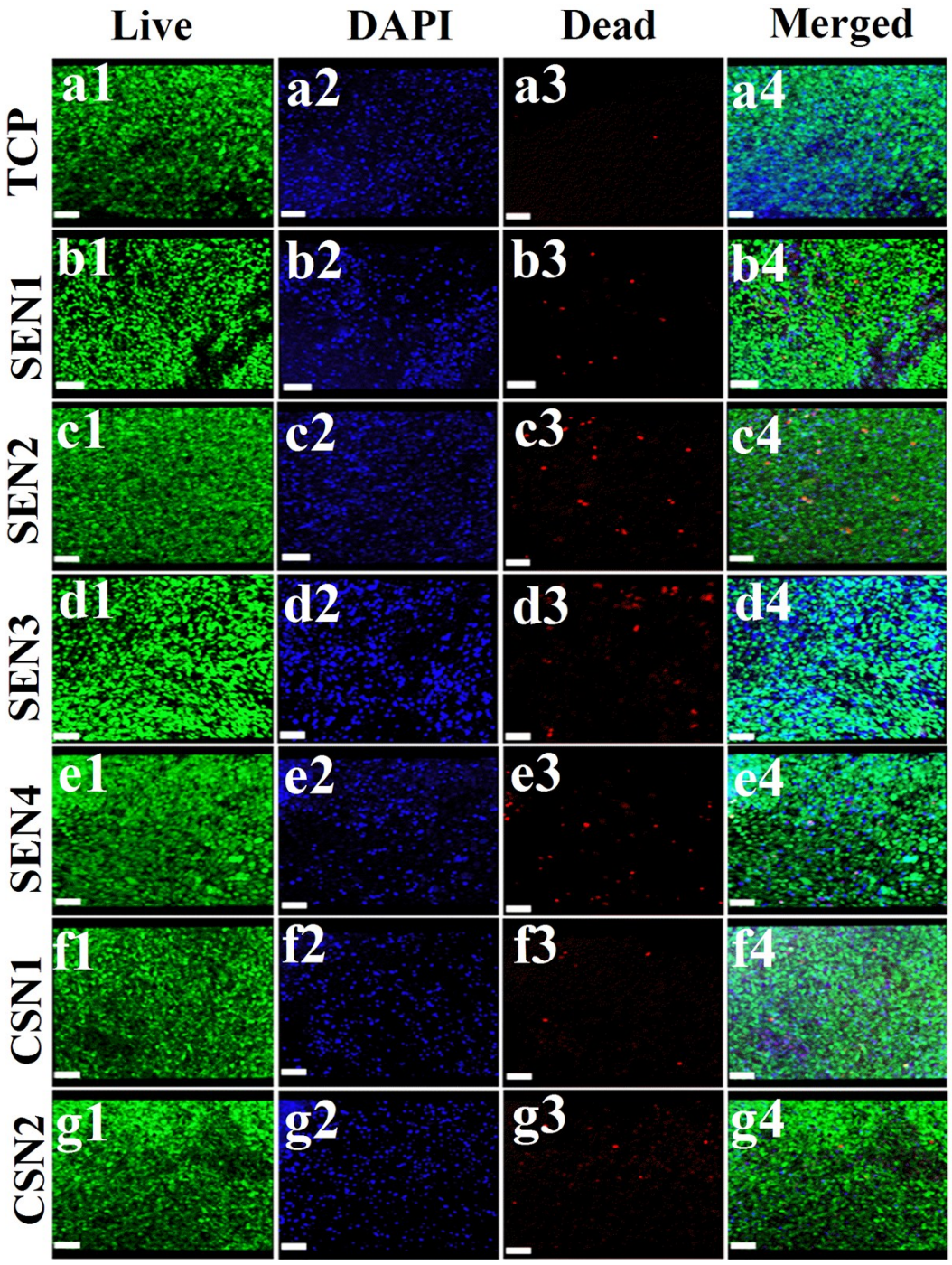

Figure S4. Cell viability assay of 3T3 fibroblasts on different electrospun MEH-PPV:PCL meshes (SEN1:a1-a4; SEN2:b1-b4; SEN3:c1-c4; SEN4:d1-d4; CSN1:e1-e4 and CSN2:f1-f4) after 7 dyas of culture in direct contact by live/dead staining using EthD-1 (staining dead cells), calcein AM (staining live cells) and DAPI (staining nucleic acid). Live cells were stained with green, deead cells were stained with red, and nucleic acids were stained with blue. (Scale bar = 100 μm)

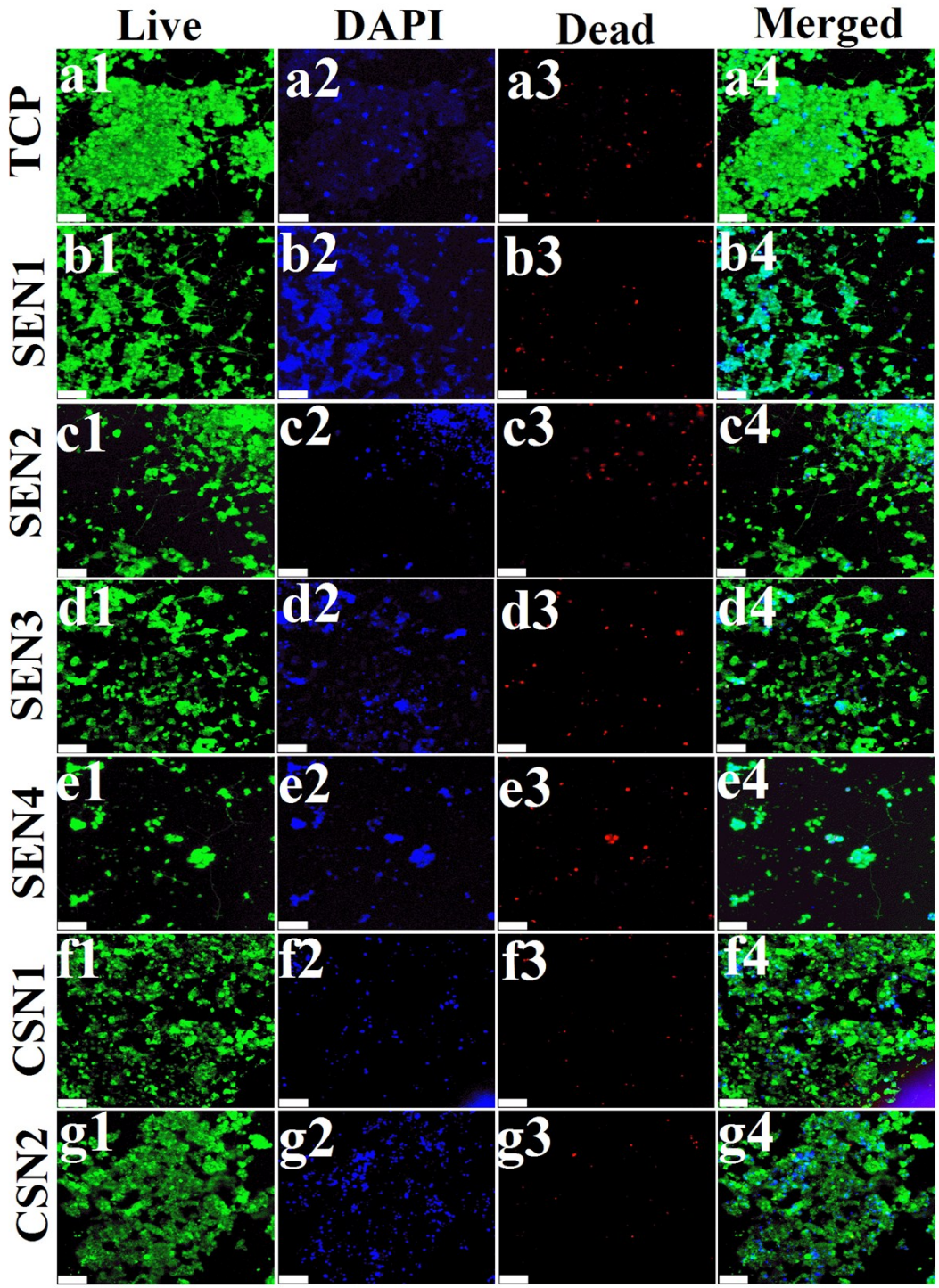

Figure S5. Cell viability assay of PC12 cells on different electrospun MEH-PPV:PCL meshes (SEN1:a1-a4; SEN2:b1-b4; SEN3:c1-c4; SEN4:d1-d4; CSN1:e1-e4 and CSN2:f1-f4) after 7 dyas of culture in direct contact by live/dead staining using EthD-1 (staining dead cells), calcein AM (staining live cells) and DAPI (staining nucleic acid). Live cells were stained with green, deead cells were stained with red, and nucleic acids were stained with blue. (Scale bar = 100 μm)

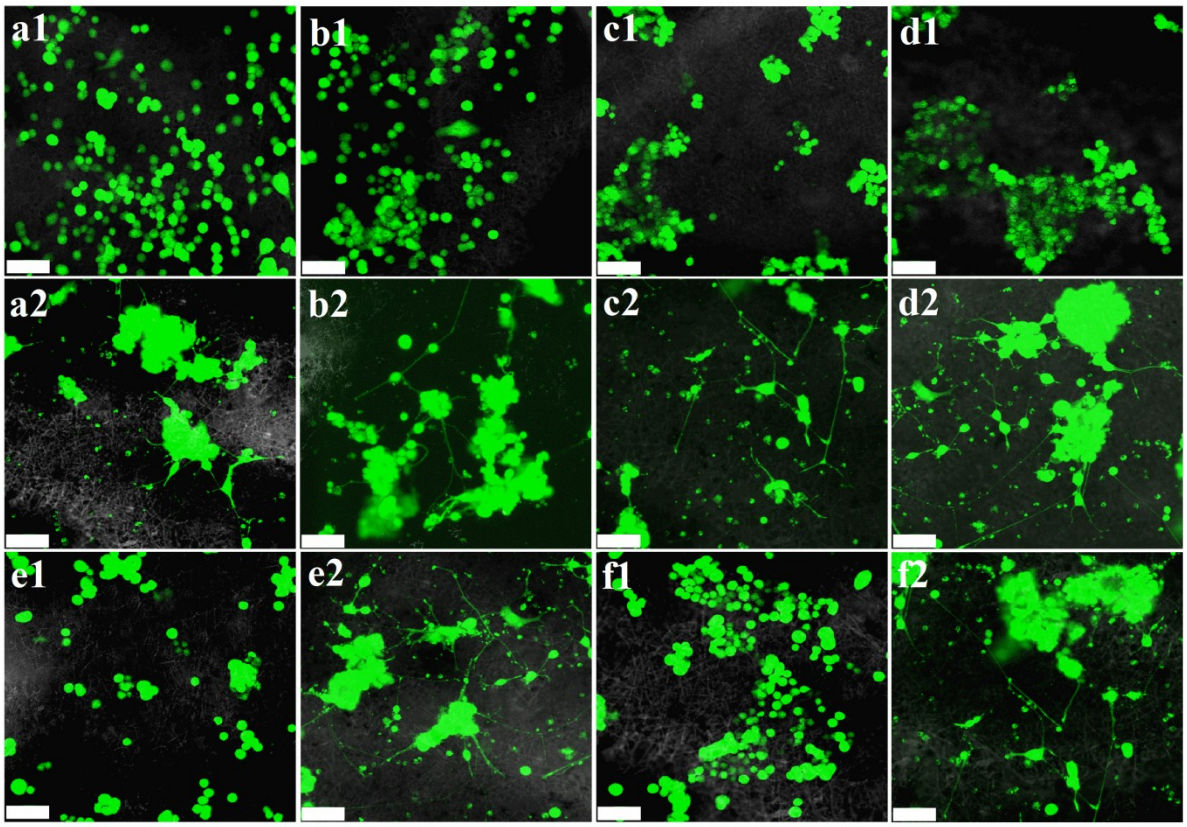

Figure S6. Confocal images with phase contrast overlay of beta (III) tubulin immunostained PC12 cells cultured in normal cell culture media in absence of NGF for 7 days on the various blended electrospun meshes under no electrical stimulation (a1-SEN1, b1-SEN2, c1-SEN3, d1-SEN4, e1-CSN1, and f1-CSN2) and under electrical stimulation of 500 mV/cm for 2h/day for 3 consecutive days (a2-SEN1, b2-SEN2, c2-SEN3, d2-SEN4, e2-CSN1, and f2-CSN2) [Scale bar = 75 μm]. Electrical stimulation for 2 h/day for 3 consecutive days causes morphological changes of the PC12 cells, which is evidenced by the characteristics neuronal marker tubulin protein expression in the microtubule cytoskeleton. This observation is in agreement with the findings of Kimura *et. al.*[4]

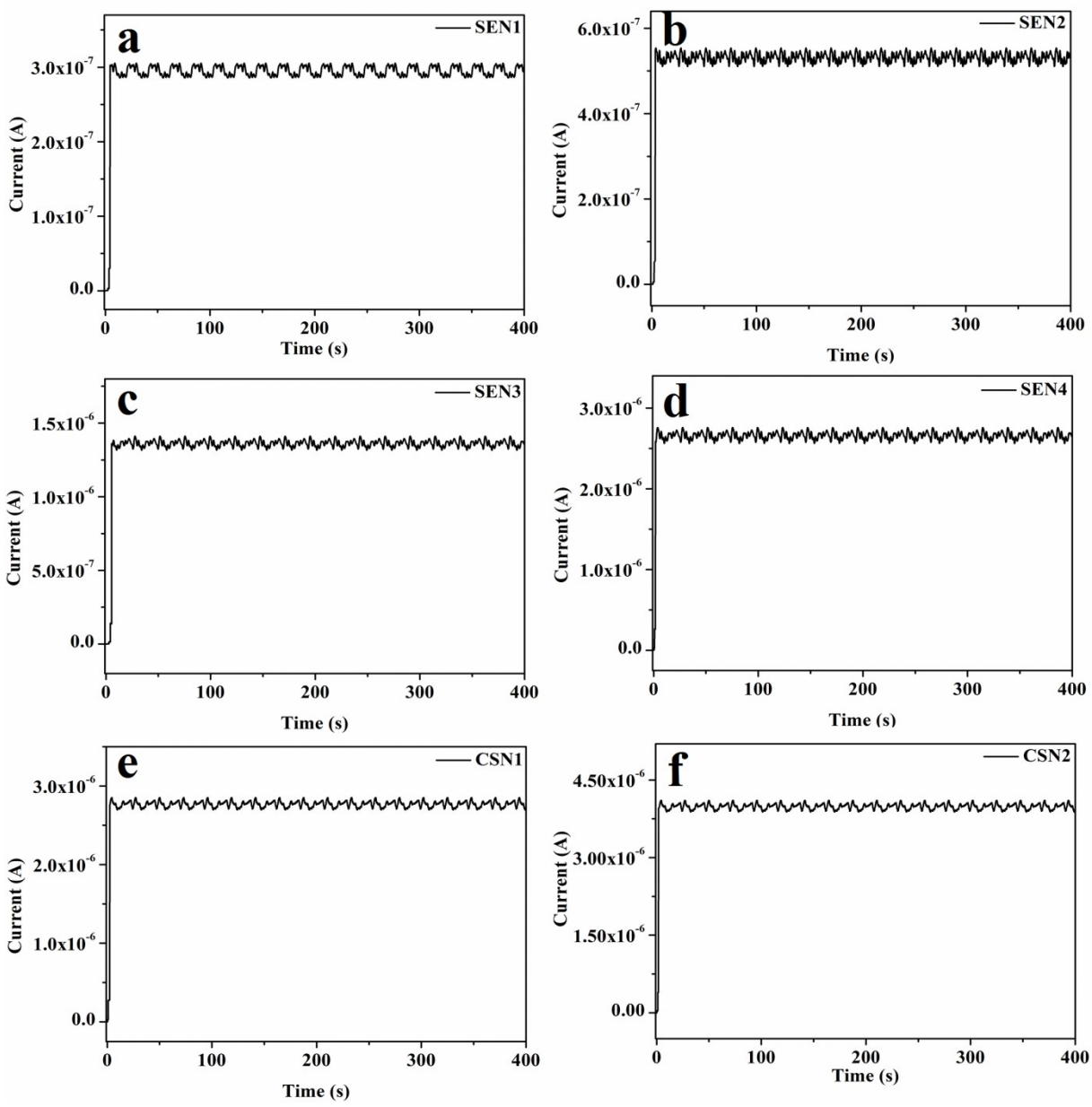

Figure S7. Current signal recorded (upto 400 s) during electrical stimulation of PC12 cells through conductive MEH-PPV:PCL nanofibres through (a) SEN1, (b) SEN2, (c) SEN3, (d) SEN4, (e) CSN1 and (f) CSN2. A constant potential of 500 mV/cm for 2 h was applied in chronoamperometric technique in pulsed mode (pulse duration 1 ms).

Statistical analysis of electrically stimulated neurite formation and neurite outgrowth on the different electrically conductive MEH-PPV:PCL nanofibres

It has been observed that under electrical stimulation the percentage of neurite bearing cells, neurite per cell, neurite length per cell and median neurite length are different on the different MEH-PPV:PCL nanofibres. The data analysis using two-way ANOVA with replication reveals the statistical difference in the pecentage of neurite bearing cells under stimulated condition between SEN1 *vs* SEN4, SEN1 *vs* CSN1, SEN1 *vs* CSN2, SEN2 vs SEN4, SEN2 *vs* CSN1, SEN2 *vs* CSN2, SEN3 *vs* CSN1, SEN3 *vs* CSN2, SEN4 *vs* CSN1, and SEN4 *vs* CSN2 at $p<0.01$ [Figure 10 (g)]. Nonetheless, the percentage of neurite bearing cells under electrical stimulation is statistically significant at $p<0.05$ between SEN1 *vs* SEN3 and SEN2 *vs* SEN3, whereas there are no statistical differences between SEN1 *vs* SEN2 ($p=0.55$), SEN3 *vs* SEN4 ($p=0.95$), and CSN1 *vs* CSN2 ($p=0.60$) [Figure 10 (g)].

The neuite per cell is also significantly different at $p<0.01$ between SEN1 *vs* SEN4, SEN1 *vs* CSN1, SEN1 *vs* CSN2, SEN2 *vs* CSN1, SEN2 *vs* CSN2, SEN3 *vs* CSN1, and SEN3 *vs* CSN2, whereas the neurite per cell is statistically significant at $p\leq0.05$ between SEN2 *vs* SEN4 and SEN3 *vs* SEN4 [Figure 10 (h)]. In contrast, there are no significant differences in neurite per cell between SEN1 vs SEN2 ($p=0.19$), SEN1 vs SEN3 ($p=0.18$), SEN2 *vs* SEN3 ($p=0.89$), SEN4 *vs* CSN1 ($p=0.39$), SEN4 vs CSN2 ($p=0.82$), and CSN1 vs CSN2 ($p=0.58$) [Figure 10 (h)].

Furthermore, under electrical stimulation condition, the neurite lenght per cell is statistically different $p\leq0.01$ between SEN1 *vs.* SEN4, SEN1 *vs.* CSN1, SEN1 *vs* CSN2, SEN2 *vs.* CSN1, SEN2 *vs.* CSN2, SEN3 *vs.*CSN1 and SEN3 *vs.* CSN2, while the statistical significance exists at $p<0.05$ between SEN1 *vs.* SEN2, SEN1 *vs.* SEN3, SEN2 *vs.* SEN4, and SEN3 *vs.* SEN4 [Figure 10 (i)]. However, the neurite length per cell under electrical stimulation is not statistically different between SEN2 *vs.* SEN3 ($p=0.36$), SEN4 *vs.* CSN1 ($p=0.13$), SEN4 *vs.*CSN2 ($p=0.50$)and CSN1 *vs.* CSN2 ($p=0.52$) [Figure 10 (i)]. Besides, the

median neurite length under electrical stimulation is statistically differfent between SEN1 *vs.* SEN4, SEN1 *vs.* CSN1, SEN2 *vs.* SEN4, SEN1 *vs.* CSN2, SEN2 *vs.* CSN1, SEN2 *vs.* CSN2, SEN3 *vs.*CSN1, and SEN3 *vs.* CSN2, only ($p<0.05$) [Figure 10 (j)].

References

(1) Z. Wang, Y. Cui, J. Wang, X. Yang, Y. Wu, K. Wang, X. Gao, D. Li, Y. Li, X. L. Zheng, Y. Zhu, The effect of thick fibers and large pores of electrospun poly (ε-caprolactone) vascular grafts on macrophage polarization and arterial regeneration. *Biomaterials,* **2014,** *35(22)*, 5700-5710.

(2) Q. P. Pham, U. Sharma, A. G. Mikos, Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue Eng.* **2006,** *12(5),* 1197-1211.

(3) M. Borjigin, C. Eskridge, R. Niamat, B. Strouse, P. Bialk, E. B. Kmiec, Electrospun fiber membranes enable proliferation of genetically modified cells. *Int. J. Nanomed.* **2013,** *8,* 855.

(4) K. Kimura, Y. Yanagida, T. Haruyama, E. Kobatake, M. Aizawa, Gene expression in the electrically stimulated differentiation of PC12 cells. *J. Biotech.* **1998,** *63(1),* 55-65.