Supplementary Material

# Supplementary Data

## Metabolic activity assay: MTS methodology

An NG108-15 neuronal cell line (mouse neuroblastoma x rat glioma hybrid, cancerous. ECACC, United Kingdom) was seeded in vitro onto the bioactive to evaluate its effects on metabolic activity. 500 neuronal cells were seeded on the bioactive surface with Dulbecco’s Modified Eagle’s Medium (DMEM, Sigma, United Kingdom) supplemented with 10% foetal bovine serum v/v (FBS, PAN Biotech, Germany), 1% L-glutamine v/v (200 mM, Sigma, United Kingdom), 1% penicillin-streptomycin v/v (P/S, 10,000 units penicillin / 10 mg/mL streptomycin, Sigma, United Kingdom), 0.25% amphotericin B v/v (250 µg/mL, Sigma, United Kingdom), and incubated at 37℃ and 5% CO2 for a total time of 7 days. Culture media was changed at day 2 to serum free media (DMEM supplemented with 1% L-glutamine, 1% P/S, 0.25% amphotericin B). At this moment, for control surfaces, serum free media was also supplemented with NGF, BDNF or NGF plus BDNF at concentrations of 1 pg/mL, 1 ng/mL, 10 ng/mL, 100 ng/mL, and 1 µg/mL. For surfaces with immobilised NGF, BDNF or NGF plus BDNF, serum free media with no growth factors was used.

CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, United Kingdom) was preformed to evaluate the effect of the bioactive surfaces on metabolic activity. This assay contains MTS, which is a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] and an electron coupling reagent (phenazine ethosulfate, PES). Both come together in the same solution, which is used directly onto the samples. At day 7, 20% of CellTiter 96® AQueous One Solution Cell Proliferation Assay solution (v/v) was added directly to the samples and incubated at 37 ℃ and 5% CO2 for 4 hours. After the incubation period, the absorbance was read at 490 nm with Bio-Tek ELx800 absorbance microplate reader and KC junior software 1.41.8 (Bio-Tek Instruments, USA).

## Cell attachment assay: Crystal violet methodology

PCL scaffold, PCL Air scaffold, PCL + NH2+ scaffold and PCL + NH2++ Heparin scaffold were tested to evaluate if NG108-15 neuronal cells adhered to them. Firstly, NG108-15 neuronal cells (1,500 cells) were seeded on each scaffold, which were placed inside a 24 well plate, with culture medium (DMEM supplemented with 10% FBS v/v, 1% L-glutamine v/v, 1% P/S v/v and 0.25% amphotericin B). The samples were incubated for 3 days at 37℃ and 5% CO2. Then, the samples were fixed with 3.7% formaldehyde (Sigma-Aldrich, United Kingdom). The scaffolds were then transferred to a new 24 well plate. A 0.2% crystal violet solution (w/v Sigma, United Kingdom, in 10% (v/v) ethanol (Fisher Scientific, United Kingdom)) was added to each sample and incubated for 10 minutes at room temperature. Then, the samples were rinsed one time with PBS. After, a 10% acetic acid solution (v/v Fisher Scientific, United Kingdom; in dH2O) was added to each sample and used to elute the stain. The eluate was transferred to a 96-well plate, in triplicate, and the absorbance read at 630 nm using a Bio-Tek ELx 800 absorbance microplate reader and KC junior software 1.41.8 (Bio-Tek Instruments, USA). Three independent tests were performed, each sample was tested in triplicate.

# Supplementary Figures and Tables

## Supplementary Figures

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Supplementary Figure 1. Secondary antibody control groups A) PCL + NH2+ scaffold, and B) PCL + NH2+ + Heparin + Immobilised BDNF scaffold. Scalebar= 500 µm.

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**Supplementary Figure 2.** Metabolic activity of NG108-15 neuronal cells, measured with MTS assay at day 7, cultured onto different bioactive surfaces with NGF, BDNF or NGF plus BDNF at different concentrations. A) NGF was present in culture medium in TCP, NH2+ and NH2+ + Heparin (controls). NGF was immobilised on NH2+ + Heparin + Immobilised NGF (test). B) BDNF was present in culture medium in TCP, NH2+ and NH2+ + Heparin (controls). BDNF was immobilised on NH2+ + Heparin + Immobilised BDNF (test). C) NGF plus BDNF was present in culture medium in TCP, NH2+ and NH2+ + Heparin (controls). NGF was immobilised on NH2+ + Heparin + Immobilised NGF plus BDNF (test). Two-way ANOVA statistical analysis was performed with Tukey procedure of multiple comparisons (\*p<0.05). Three independent tests were performed. Each condition was done in triplicate.



Supplementary Figure . Crystal Violet assay to evaluate NG108-15 neuronal cell adhesion to bioactive surface on electrospun PCL scaffold. Assuming that all the NG108-15 neuronal cells adhere to TCP (100%), the adhesion of PCL scaffolds decreased by 18%, however, it was not significantly different with respect to TCP. Furthermore, PCL Air scaffolds, PCL + NH2+ scaffolds and PCL + NH2++ Heparin scaffolds increased their adhesion properties by 47%, 76% and 121% respectively in comparison to TCP. One-way ANOVA with Tukey’s multiple comparison test. \*p< 0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001.