

## Electronic Supplementary Information for

### **Exploring the dark side of MTT viability assay of cells cultured onto electrospun PLGA-based composite nanofibrous scaffolding materials**

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## **Additional XTT experimental details:**

### **Materials**

XTT sodium salt (Sodium 2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) was purchased from Sangon Biotech (Shanghai, China) Co., Ltd. Activation Reagent PMS (phenazine methosulfate) was purchased from Beijing Biodee Biotechnology Co., Ltd (Beijing, China).

### **XTT formazan preparation**

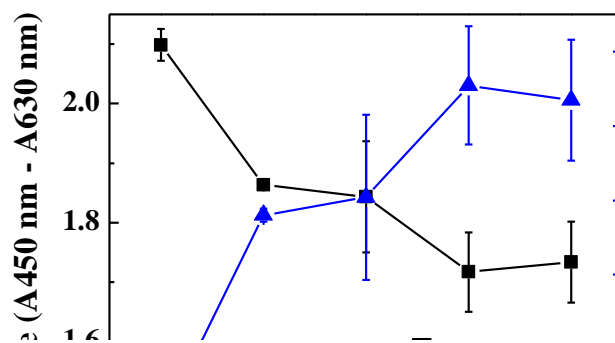
XTT (6.6 mM) was dissolved in pre-heated (60 °C) DMEM medium. PMS (22 mM) was dissolved in PBS buffer. Solutions of XTT and PMS were mixed with a 1:1 volume ratio. Similar to MTT formazan preparation, the XTT formazan used for adsorption onto electrospun nanofibers was prepared by adding XTT solution into viable L929 cells. In brief, L929 mouse fibroblasts were cultured in a 6-well TCP for 3 d and then XTT solution was added. After 2 h incubation to form XTT formazan in viable cells, soluble XTT formazan was obtained. The XTT formazan solution was diluted with PBS buffer with an absorbance (A<sub>450 nm</sub>- A<sub>630 nm</sub>) of around 2.0.

### **XTT formazan adsorption**

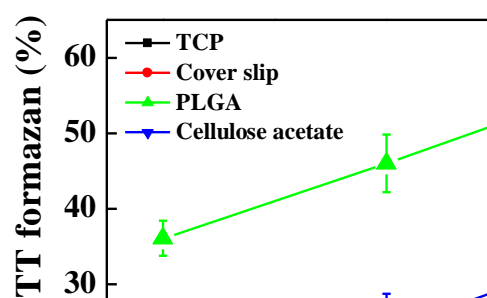
The samples for XTT formazan adsorption includes TCP, cover slip, electrospun PLGA nanofibers, and CNTs(3%)/PLGA nanofibers. The adsorption experiment was performed according to the procedures similar to MTT formazan adsorption assay. XTT formazan (400 µL) was added onto all substrates in a 24-well plate, followed by 2 h incubation in a shaker (37 °C). Then the XTT formazan solution was transferred into a 96-well plate and the OD values (A<sub>450 nm</sub>- A<sub>630 nm</sub>) were measured.

**Table S1.** OD values measured by MTT assay for negative control samples without cell culture.

| Samples   | Cover slips   | PLGA          | HNTs(3%)/PLGA | HNTs(5%)/PLGA | CNTs(3%)/PLGA | CNTs(5%)/PLGA | TCPs          |
|-----------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| OD values | 0.129 ±0.0021 | 0.135 ±0.0032 | 0.145 ±0.0077 | 0.148 ±0.0081 | 0.156 ±0.0051 | 0.158 ±0.0043 | 0.129 ±0.0032 |



**Figure S1.** Adsorption of XTT formazan onto different substrates after incubation of 2 h.



**Figure S2.** MTT formazan adsorption onto different substrates at different time point.