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Supporting Information of the Manuscript

Biocompatible MWCNT Scaffolds for Immobilization and Proliferation of *E. coli*

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Experimental Part

1. Preparation of MWCNT-CHI buffered suspensions: Chitosan solutions (1 wt.%) were prepared by dissolving chitosan flakes (0.5 g) in 50 mL of an aqueous solution of acetic acid (0.05 M, pH 5.5). MWCNTs were functionalized by refluxing 500 mg of MWCNTs in 25 mL of nitric acid (14 M) at 130 ºC for 6 hours. After cooling, the functionalized-MWCNTs were repeatedly washed with distilled water until complete nitric acid removal, and left to dry. Functionalized-MWCNTs (90 mg) were dispersed in 1.5 mL of chitosan solution (1 wt.%), sonicated and stirred.

2. ISISA processing for scaffolds preparation: The suspensions (1 mL) were collected into insulin syringes and dipped at a constant rate of 5.9 mm/min into a cold bath maintained at a constant temperature of –196 ºC. The unidirectionally frozen samples were freeze-dried using a ThermoSavant Micromodulyo freeze-drier. The resulting monoliths kept both the shape and the size of the insulin syringes (in this particular case) and of any container where the suspensions might be collected prior freezing.

3. Preparation of bacteria-glucose-alginate beads: Sodium alginate was dissolved in a diluted minimum medium (1/10) for a sodium alginate content of 2 wt. %. Glucose (20 wt. %) was also added to the alginate solution. The glucose-alginate beads loaded with *E. coli*-GFP were prepared by addition of 1 mL of M9 minimal salts medium containing bacteria (500 µL of the above cell suspension to 500 µL of diluted minimum medium) to 9 mL of the glucose-alginate solution. After stirring for 10 minutes, the mixture was dropped into a gently stirred solution of $CaCl_2$ at a volumetric ratio of 1/5 using a syringe equipped with a needle of 0.45 mm. After stirring for 10 minutes, the resulting beads (of ~1 mm diameter) were washed with 50 mL of distilled and deionized water, before use. The number of beads added to the MWCNTs-CHI suspension was 20, to have ~10^6 bacteria/mL. Temperature was maintained at 4 ºC during the whole process to avoid bacteria growth.

4. Bacteria 3D Cultures: The experiment represented in Scheme Ib was done by soaking the MWCNT scaffold into a suspension of bacteria in culture medium (~10^6 bacteria/mL), while that represented in Scheme Ic was done by simply soaked into the culture medium (bacteria is already immobilized within the scaffold structure). The culture medium was composed of 25 mL of M9 minimal salts medium supplemented with 50 mM sodium citrate and 30 mM glucose (as carbon source). Sodium citrate is not a carbon source for *E. coli* but allows for alginate beads liquation. For this purpose, scaffolds were maintained in culture medium at 4 ºC for 60 minutes (bacteria growth is negligible at 4 ºC). The amount of alginate beads was fixed to have ~10^6 bacteria/mL in the buffered suspension after bead dissolution. Thereafter, the flasks were incubated at 37 ºC for 24 hours under gentle stirring to allow for bacterial growth.

5. Bacteria plate cultures: Bacteria were spread onto Petri dishes containing LB growth media solidified with 1.5 wt.% agar. Finely ground MWCNT scaffolds, PVA scaffolds (as negative control) and PVA scaffolds containing CFX (as positive control) were deposited on the centre of the solidified agar. Plates were incubated for 24 hours at 37ºC and the formation of an halo (indicative of growth inhibition) around the deposited scaffolds was directly visualized. All measurements were performed in triplicate.

6. Samples Characterization: Sample morphologies were investigated by scanning electron microscopy (SEM) using a Zeiss DSM-950 instrument. Confocal fluorescence microscopy was performed with a Radiance 2100 (Bio-Rad) Laser Scanning System on a Zeiss Axiovert 200 microscope. The confocal fluorescent micrographs of MWCNT scaffolds shown in Figure 3 were taken from the external surface of the scaffold up to a depth of 32 μm. The confocal fluorescent micrographs of MWCNT scaffolds shown in Figure 4 were taken on the internal surface of sliced samples with different deep in focus (20, 40, 60 and 80 μm). The averaged number of bacteria per millimeter squared was obtained after counting 4-5 images slicing the sample at different positions and focus depths. Images shown in figures 3 and 4 are representative of such a set of images.