

Supporting Information

Differentiation properties of 3D scaffolds nanostructured with multi-walled carbon nanotubes on human induced pluripotent stem cells

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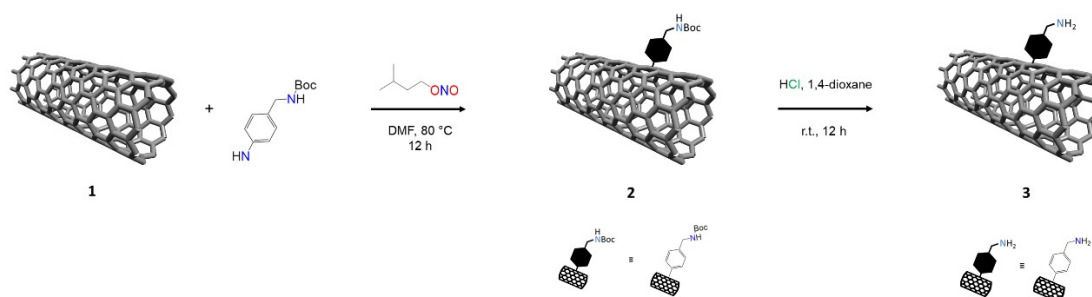


Figure S1. Schematic representation of Tour multistep reaction for covalent functionalization of MWCNTs. For schematic purposes SWCNT surfaces are depicted instead of MWCNTs.

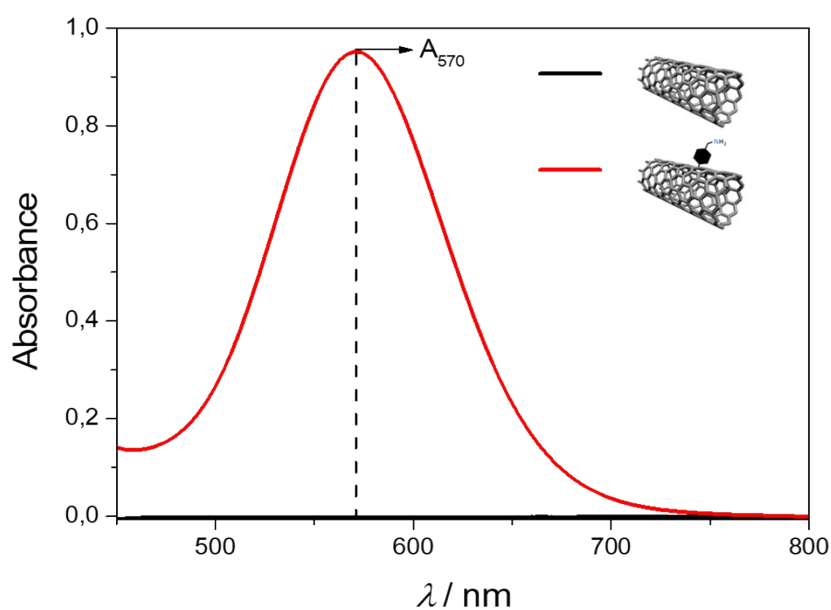


Figure S2. Kaiser Test on non-functionalized MWCNTs (black trace) and MWCNTs following the introduction of the benzylamino group (product **3**, red trace). Only the second material resulted positive to the test with the formation of the Ruhemann complex from the typical absorption band at 570 nm (black dotted line).

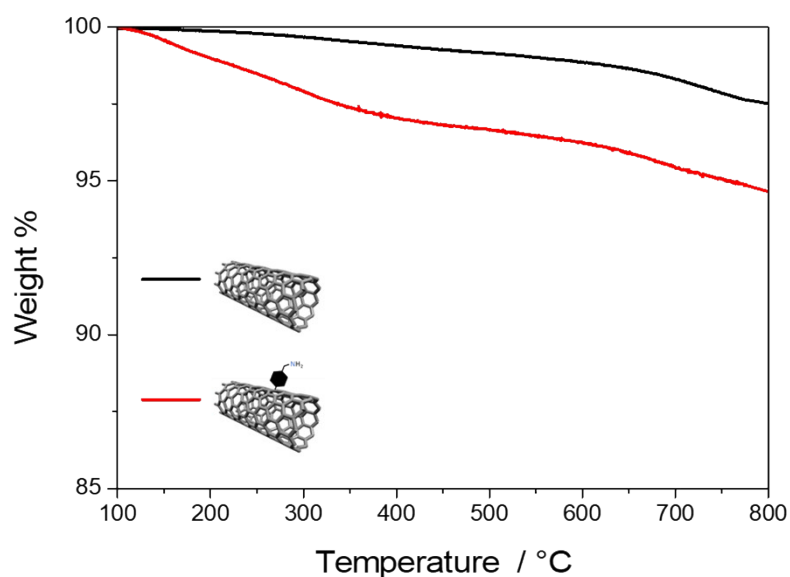


Figure S3. Thermogravimetric analysis of MWCNT (black curve) and product **3** (red curve). TGA curves of both samples were recorded under N_2 atmosphere (TGA Q500, TA Instruments). Heating program: pre-heating to 100 °C followed by 10 °C min^{-1} up to 800 °C. The weight loss at 500 °C corresponds to the decomposition of benzylamine functional groups (Bosi et al., 2015 Sci Rep, 5, 9562) and was used to calculate the functionalization degree (FD) according to: $FD (\mu mol g^{-1}) = (WP \times 10^4) / MW$, where WP is the weight loss percentage and MW is the molecular weight of the functional group. The resulting FD value is 331 $\mu mol g^{-1}$.

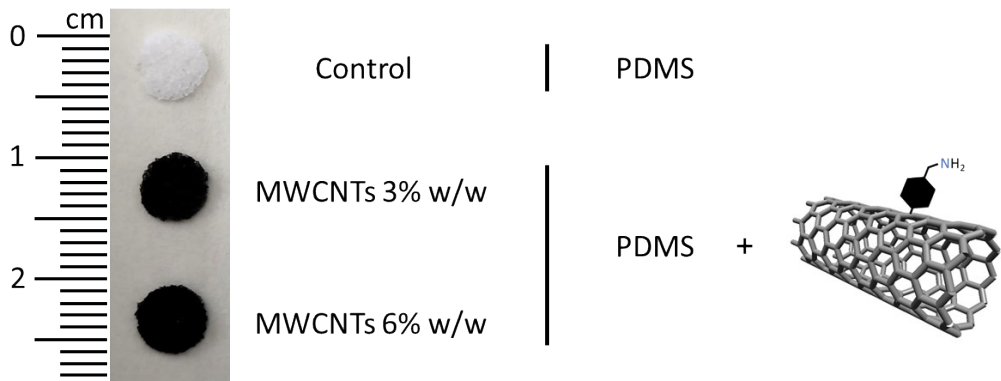


Figure S4. Picture of the 3 types of scaffolds: the control, made of PDMS only, and the two types of nanostructured scaffolds differing for the concentration of MWCNTs: 3% w/w and 6% w/w. For schematic purposes SWCNT surfaces are depicted instead of MWCNTs.

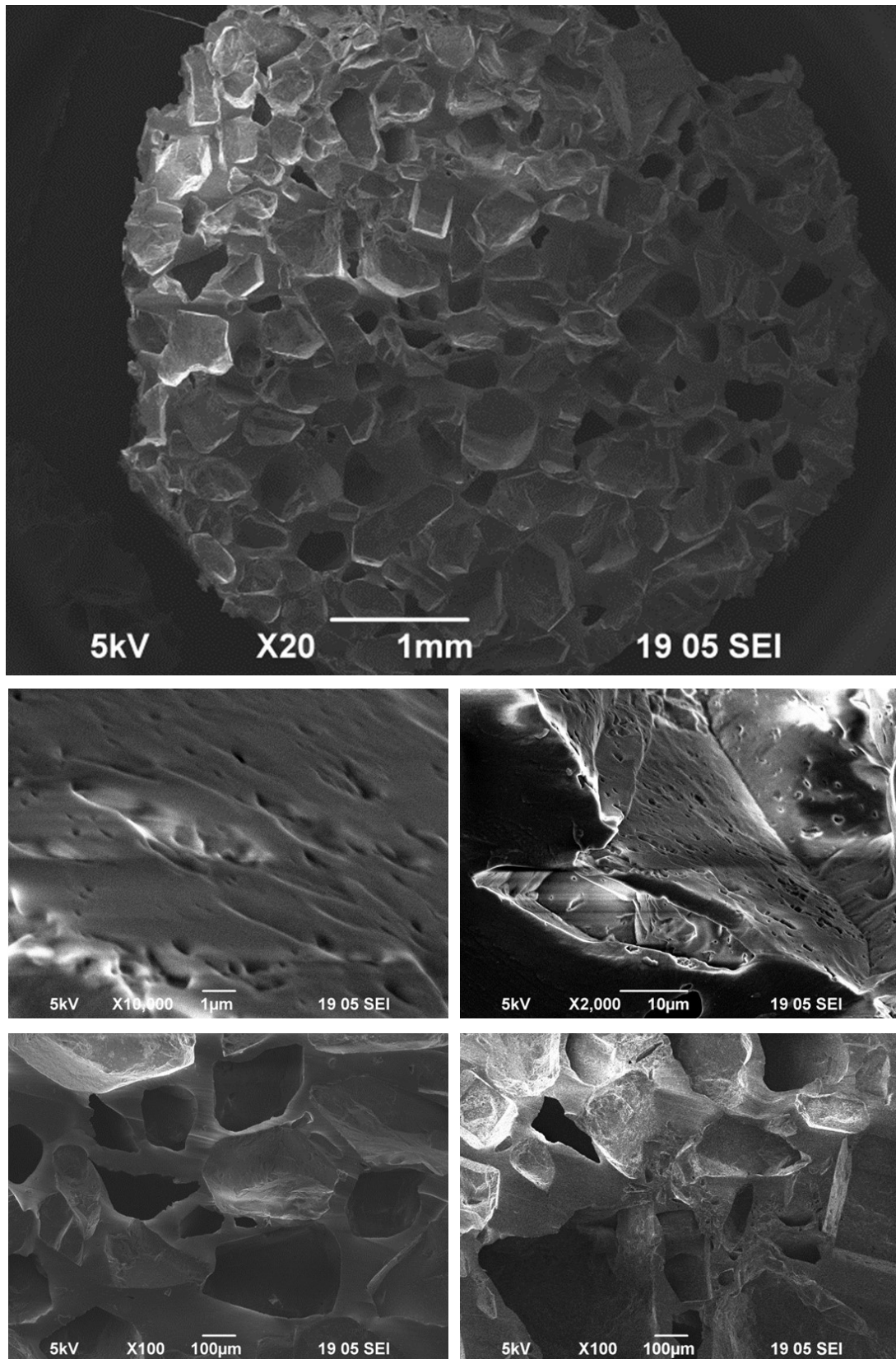


Figure S5. Representative SEM images of 3D scaffolds produced with sugar grain sizes of 250-600 μm and only PDMS. Reported magnifications are indicated by scale bar: 1 mm, 1 μm, 10 μm or 100 μm.

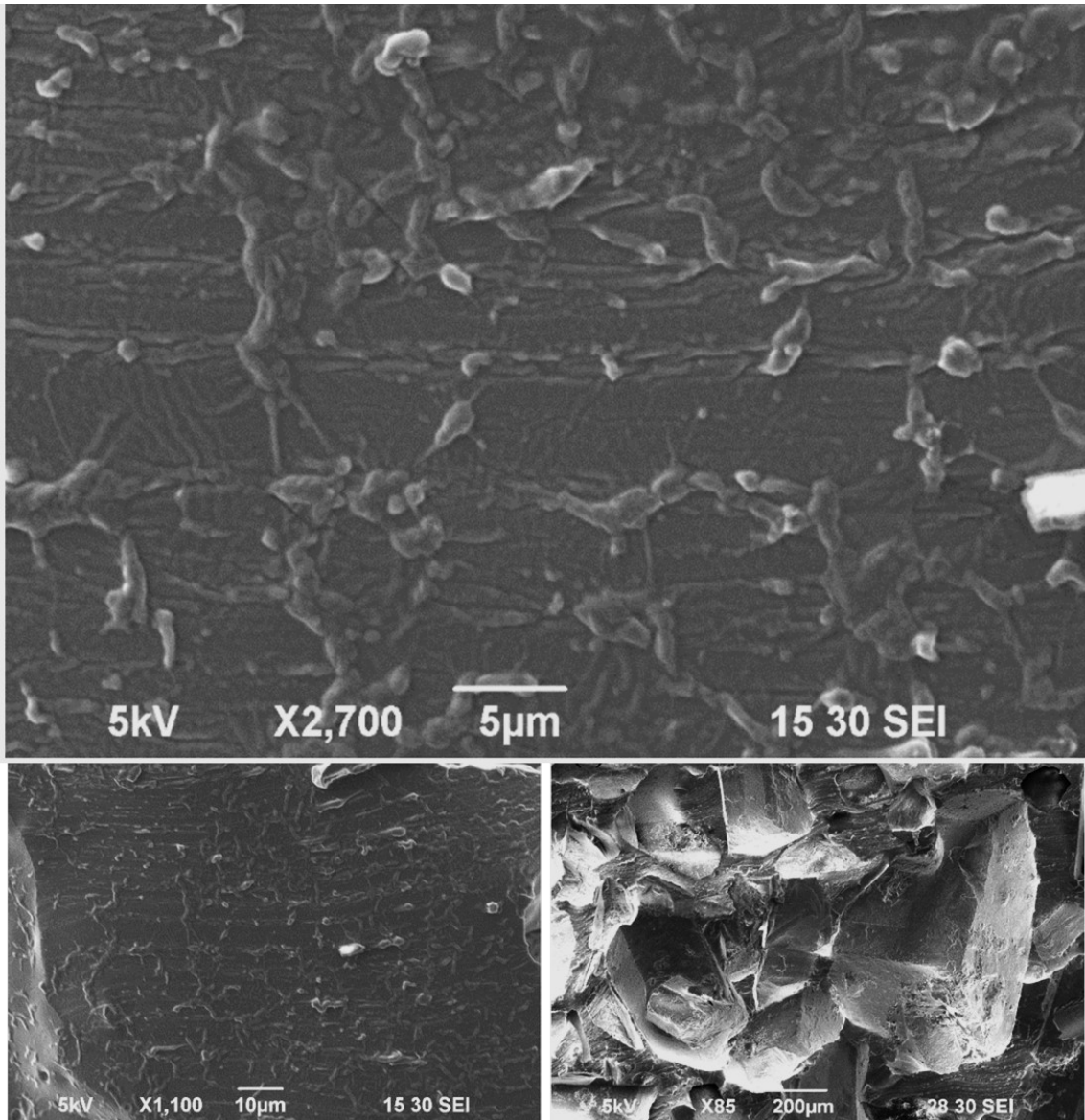


Figure S6. Representative SEM images of 3D scaffolds produced with sugar grain sizes of 100-250 μm and only PDMS. Reported magnifications are indicated by scale bar: 5 μm , 10 μm or 200 μm .

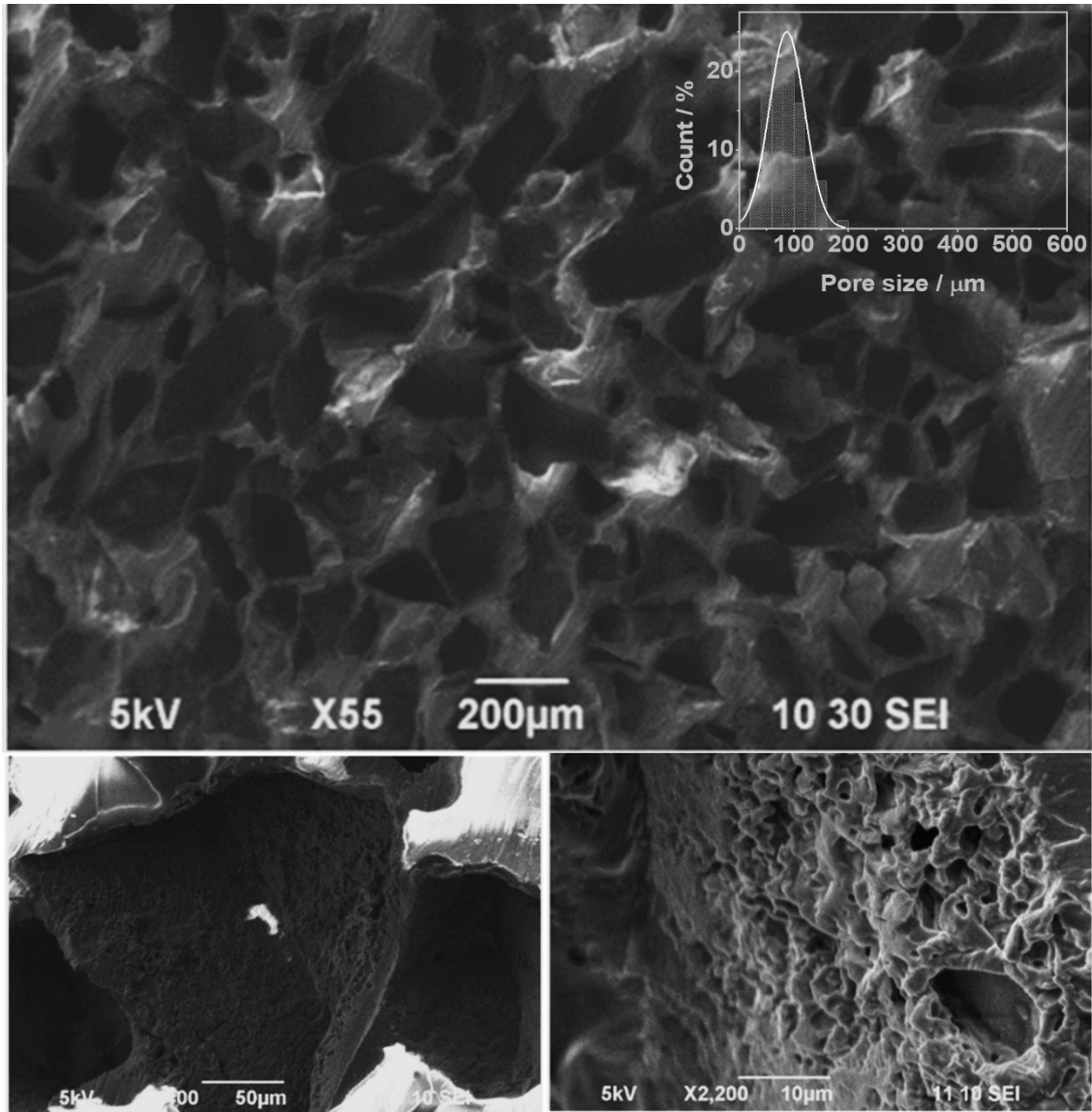


Figure S7. Representative SEM images of 3D scaffolds produced with sugar grain sizes of 100-250 µm and by PDMS in presence of MWCNTs at 6% w/w. Reported magnifications are indicated by scale bar: 200 µm, 50 µm and 10 µm. Inset: pore size distribution determined from SEM images by manual ImageJ analysis (n = 100 pores), showing an average pore size of 87 ± 32 µm, in agreement with the designed porosity range.

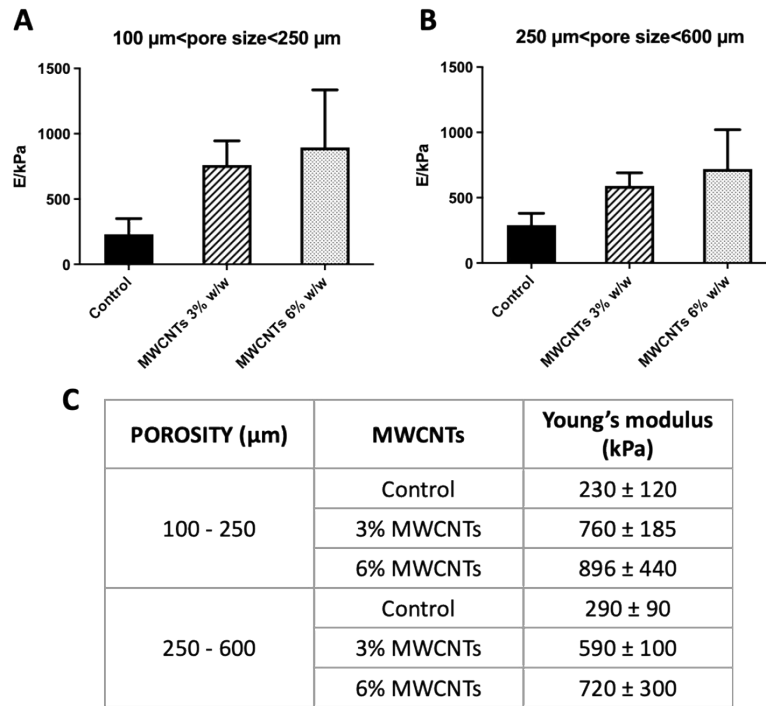


Figure S8. Young's modulus data of the 3D MWCNT-nanostructured (3% or 6% w/w) PDMS scaffolds with 100-250 μm (A) or 250-600 μm pore size range (B) and relative data (C).

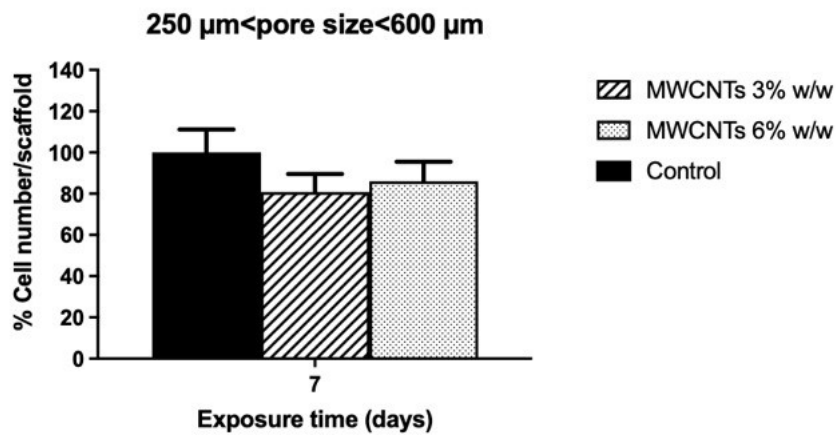


Figure S9. Effects of 3D MWCNT-nanostructured (3% or 6% w/w) PDMS scaffolds with 250-600 μm pore size range on the number of cells adhered to nanostructured scaffolds after 7 days of contact. Cell count is represented by the mean \pm SE of at least 3 independent experiments. Statistical differences: two-way ANOVA and Bonferroni post-test.

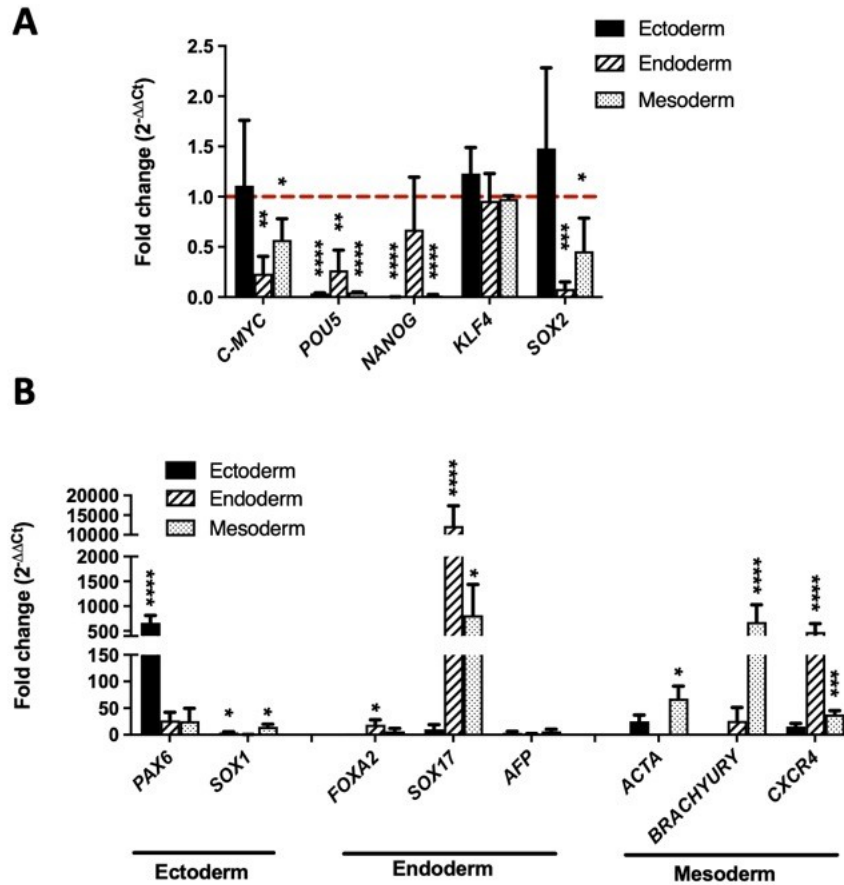


Figure S10. Gene expression in iPSC after selective differentiation towards ectoderm, endoderm and mesoderm germ layers using the StemMACSTM Trilineage Differentiation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). (A) Effects on the expression of gene markers of stemness. (B) Effects on the expression of gene markers of the 3 embryonic layers. Gene expression values were calculated as fold change ($2^{-\Delta\Delta Ct}$) versus control iPSC maintained under standard culture conditions (red line) and are the mean of 3 experiments \pm SE. Statistical analysis: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$ (two-way ANOVA and Bonferroni post-test).