SWNT Composites for Tissue Engineering Applications: Characterization and Cell Interactions

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Introduction: Tissue Engineering aims to replace damaged tissues and organs with an engineered product consisting of cells, a 3D scaffold in the shape of the tissue, and growth factors to promote regeneration. However, mimicking conditions with respect to desired tissue type is a challenge. In these investigations Single Walled Carbon Nanotubes (SWNT) were dispersed within collagen hydrogels to assess in vitro cell – SWNT matrix interactions with possible applications for nerve tissue engineering. The objective of these investigations was to design a modified 3D matrix with improved SWNT dispersion characteristics, whilst maintaining biological compatibility. Towards this goal, hydrogels with dispersed SWNT were seeded with PC12 neuronal cells to assess *in vitro* cell-matrix interactions. By improving SWNT dispersion materials may be developed with enhanced electrical and mechanical properties that may have important implications for wound healing and nerve tissue engineering.

Methods: Two preparations were assessed; Albumin-coated SWNT (using the surfactant 'Sodium Cholate'), and SWNT alone. Prepared SWNT's were dispersed within collagen hydrogels and assessed. SEM analysis was used to determine the microstructure of collagen/SWNT matrix.

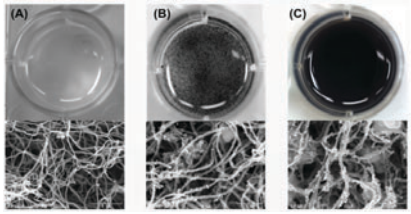

Figure 1. A) Collagen B) Collagen+ albumin coated SWNT C) Collagen + albumin coated SWNT dispersed in sodium cholate and dialyzed. SEM images showing collagen gels with 5% SWNT at magnification (20kx).

Compression analysis was performed using Instron Universal Testing Machine, at a constant rate of 0.3%/s over 150-200 s duration. PC12 (pheochromocytoma) cells from the mouse adrenal gland were cultured within, and on the hydrogel composits. Cell metabolic activity, viability, and proliferation was assessed at days 3 and 7, using the Alamar blue and trypan blue assays. Real-time PCR analysis of relative gene expression assessed the expression of p53, mdm2 (apoptotic) and cyclin e, cyclin b, cdk1, pcna (cell cycle regulator). Statistical significance was calculated at $p < 0.05$, all samples were at $n = 3$ unless stated otherwise.

Results:

Surfactant-aided dispersion significantly increased SWNT dispersion (Figure 1) and electrical conductivity. Raman Spectroscopy of the SWNT constructs' displayed a shift at the breathing mode to 280 cm^{-1} due to collagen matrix surrounding around SWNT.

PC12 cells were seeded on and within the composite biomaterials. Cells were seen to migrate into the collagen gels, with total cell density increasing over time (Fig. 2). At day 7 metabolic activity values of the collagen controls were in a similar range to the SWNT constructs, however day 3 collagen controls showed higher metabolic activity.

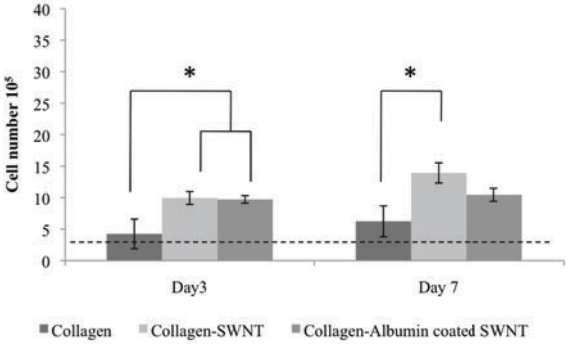

Figure 2. Cell density at Day 3,7, PC12 cells seeded inside of the gels. Cell seeding density is shown with a dashed line ($2.5x10^5$ / disc).

Relative gene expression of seven genes was investigated in this study. Other than PCNA, an example DNA clamp protein involved in DNA synthesis, which was down regulated at day 3 in the Collagen –SWNT sample, all other genes assessed were not significantly different than controls.

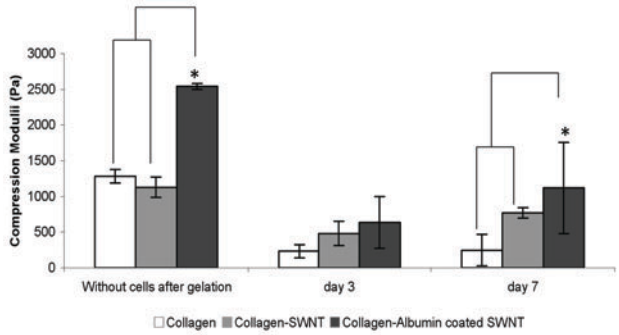

Figure 3. Compression Modulus; Values decreased more than 50% at day 3, and increased again at day 7. No statistical variation was noted between the different preparations at day 3, however, samples displayed a significant increase in material stiffness at day 7 except collagen controls, with respect to collagen.

Conclusions: The main finding of this study is that cellular function is maintained when cells are cultured in the presence of SWNT composite hydrogels. Using albumen to enhanced SWNT dispersion resulted with a stiffer and more conductive material whilst maintaining biocompatibility. Incorporation of SWNT into the collagen hydrogel matrix accelerated the gelling time.