Assessment of Carbon Nanotube Toxicity using 3D Protein Matrices

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Introduction: As the fields of biomaterials and nanotechnology converge and advance toward the clinic, there is an increasing need to understand how nanoparticles interact with cells and tissues. Work in our laboratory has focused on developing 3D protein hydrogels as models for assessing the effects of nanoparticles on cell growth rate, function and viability. In the present study, we have used quantification of DNA amount as well as metabolic activity to assess the proliferation and function of human dermal fibroblasts in 3D collagen Type I matrices in the presence and absence of functionalized single-walled carbon nanotubes (SWNT).

Methods: Constructs were created by combining human dermal fibroblast cells (HDFb), carboxylated SWNT, culture medium, fetal bovine serum and acid-solubilized bovine collagen (Type I). Collagen fibrillogenesis was initiated by raising the pH and temperature to physiological levels. Constructs were cultured for seven days, with media being changed every third day of incubation. After one week of culture, the cell number of each construct was assessed using either a Hoechst dye-based DNA assay or an XTT tetrazolium salt assay for metabolic activity. In order to test the effects that carbon nanotubes had on cell proliferation, SWNT were added to the gels, the culture media, or both. Control samples consisted of 3D gels that were completely free of SWNT, as well as 2D HDFb cultures seeded on top of collagen gel substrates.

Results: Fig. 1 shows images of 3D collagen Type I constructs with varying SWNT loading. Constructs became visibly darker as SWNT content increased. Fig. 2 shows cell number as assessed by metabolic function of HDFb in 2D and 3D culture under conditions in which the SWNT were in the gel, in the culture media, or in both. In 2D cultures on collagen substrates (panel A), exposure to SWNT caused a decrease in cell number. The effect was dose-dependent when SWNT were embedded in the gel, but was more pronounced when SWNT were also in the culture medium. In 3D cultures (panel B), cell number at the conclusion of the study was significantly lower in constructs containing SWNT than in controls. Interestingly, 3D constructs exposed to SWNT only in the media saw a smaller decrease in cell number as compared to constructs that had SWNT embedded directly in the gel. Presence of SWNT in the gel caused a marked decrease in cell number, which was similar to constructs exposed to SWNT in both the gel and media. Analysis of DNA content (not shown) confirmed these trends.

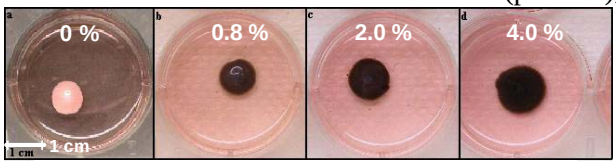

Fig. 1: Collagen disks with varying CNT loading.

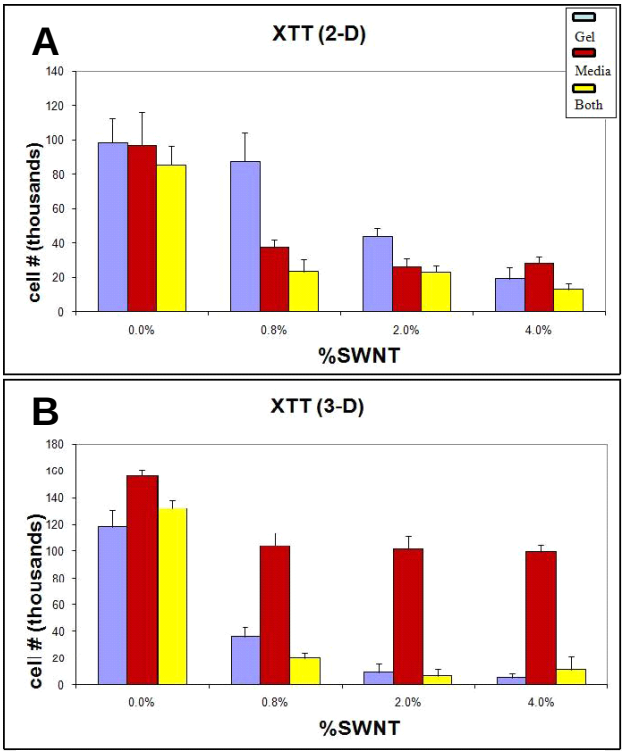

Fig. 2: XTT metabolism assay results for 2D and 3D cultures.

Conclusions: These results demonstrate that exposure to SWNT can affect fibroblast metabolism and growth. Our approach of incorporating nanoparticles into the cellular environment in different ways shows that cellular response to these particles is dependent upon the presentation of the SWNT in 2D and 3D. The use of defined 3D protein matrices has potential for studying the interactions between cells, proteins and nanoparticles. Better information about nanoparticle toxicity and effects on cell function is critical in moving nanobiomaterials into clinical use.