Carbon nanotube carpets as substrates for programming stem cell differentiation

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Statement of Purpose: Stem cells have the unique capability of self-renewal as well as being able to differentiate into diverse cell types.[1,2] The ability to control and program differentiation of stem cells can provide understanding of the role of stem cells in diseases and generate renewable sources of cells for regenerative medicine. This ability, however, is limited by our nascent understanding of how surrounding cues and signaling pathways direct stem cell differentiation. The aim of this study was to elucidate the co-operative effects of diverse biophysical signals on stem cell fate and function. To accomplish this goal we developed "intelligent platforms" using carbon nanotubes (CNTs) to evaluate the effects of matrix stiffness, roughness, and compliance on stem cell differentiation. The elastic characteristics of CNTs offer attractive opportunities for the production of substrates with full controllable mechanical properties (by varying tube length, diameter and density). In this talk, I will present the effects of CNT carpets on the attachment, growth and differentiation of stem cells to multiple lineages.

Methods: CNT carpets were formed through a thermal chemical vapor deposition (CVD) process. The length of CNTs was controlled by varying either the thickness of catalyst layer or the growth time. The packing density of the CNTs was controlled by changing the duration of the hydrogen exposure during the CNT growth. CNT carpets were sterilized by immersion in ethanol, rinsed with DI water, and exposed to UV light for half. Next, CNT carpets were seeded with human mesenchymal stem cells (hMSCs) at a density of 50,000 cells/sample. Cells were marinated in a humidified incubator at 37°C with 5% CO_2. The morphology of hMSCs cultured on the CNT carpets was assessed at predetermined time points using scanning electron microscopy (SEM). Immunofluorescent staining in conjunction with calorimetric assays was used to evaluate the differentiation of hMSCs on CNT carpets. Briefly, safranin-O, mayoD1 and oil-red staining were used to evaluate chondrogenic, myogenic and adipogenic differentiation of hMSCs. Stained samples were examined using confocal laser scanning microscope (CLSM) and phase contrast microscope (Nikon Eclipse TE200).

Results: CNT carpets were composed of dense arrays of aligned and discrete hair-like nanofibers arranged normal to supporting substrate in a "nail-bed" configuration (Fig. 1a-b). The diameters and lengths of the individual CNTs range between 15-40 nm and 50-320 nm respectively. Top view of CNT carpets showed assembly of CNT bundles due to capillary forces (Fig. 1a).

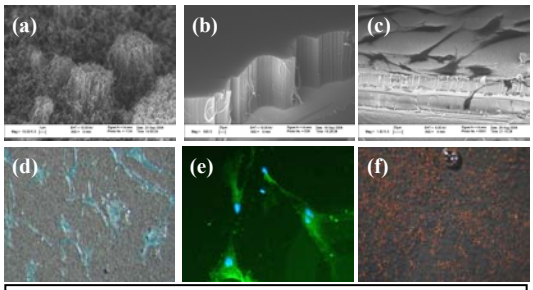

Figure 1. (a) SEM image of CNT carpet (top view). (b) SEM image of CNT carpet (side view). (c) SEM image of hMSCs on CNT carpet at day 7. (d-f) Chondrogenic, myogenic and adipogenic differentiation of hMSCs on CNTs.

We found that the CNT carpets support the attachment and growth of hMSCs. Cell proliferation studies indicated that cell number increased during early stages and reached a plateau between day 18-20 (results not shown). Subsequently, we evaluated the differentiation of hMSCs to multiple lineages as cultured on CNT carpets. Results showed hMSCs to be able to undergo chondrogenic, myogenic and adipogenic differentiation (Fig 1d-f).

Conclusions: In summary, CNT carpets support the adhesion, growth and differentiation of hMSCs multiple lineages. At present, the authors are evaluating the mechanical properties of CNT carpets with various fiber diameters, lengths and packing density. We are using the CNT carpets with controlled stiffness, roughness and compliance to understand the underlying mechanisms by which microenvironmental cues control differentiation. This is the first study to examine the effect of CNT nanocarpets on hMSC and as such is important for new and emerging technologies in drug delivery, tissue engineering, and regenerative medicine.

References

1) Lutolf MP and Hubbell JA. *Nat. Biotechnol.* **2005**; 23(1):47-55.
2) Khademhosseini A, Langer R, Borenstein J and Vacanti JP. *Proc. Natl. Acad. Sci. USA* **2006**, 103 (8):2480-87.