Apatite coated MWCNT provide a biomimetic surface for the culture of osteogenic cells

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Statement of Purpose:

The ultra-structure of bone consists of two main components: the mineralised component and the organic collagenous component. The latter acts as a template onto which calcific deposition can take place. A parallel exists dimensionally between collagen and multi walled carbon nanotubes (MWCNT). If, therefore, apatite could be deposited onto MWCNT, the result could potentially be a material with a similar ultra-structure to bone. This could offer a new approach to the tissue engineering of bone, in the quest to find better and more robust ways of filling bone defects. The advantage offered by such a structure would be the magnitude increase in tensile strength afforded by the replacement of collagen with MWCNT. An important aspect of in the assessment of an osseous material in which the type I collagen would be replaced by dimensionally similar MWCNT would be the response of cells to the new material, in this case MWCNT with an apatite surface layer. The present study demonstrates the generation of apatite coated MWCNT, as a potential avenue of bone tissue engineering and characterises the response of osteogenic cells cultured on these surfaces.

Methods:

MWCNT were dispersed in an organic solvent and then embedded into a HDPE surface by a pressure aided thermal process. The resulting MWCNT surface then had apatite deposited onto it by a modification of the process outlined by Kokubo [1]. Within the main study, four experimental groups/surfaces were outlined; these groups were used to study metabolic activity, cell proliferation, cell differentiation and cell stress. Each of the preceding parameters were measured by MTS, CyQuant, Alkaline Phosphatase (ALP) expression and Lactate Dehydrogenase (LDH) levels respectively.

Within the broad experimental groups that have been outlined, the only real variable that changed between the groups was the surface on which cells were cultured i.e. ≥90% purity MWCNT surfaces, ≥95% purity MWCNT surfaces, carboxylated MWCNT surfaces and the control surfaces which were comprised of tissue culture plastic, to allow relative comparisons to be made both between the individual MWCNT surfaces and between the individual MWCNT surfaces and a standard cell culture surface.

The cultures were maintained for 14 days in total, with cells being harvested at pre defined time points of 24 hours, 72 hours and 14 days.

Results:

After seven days in culture, no significant difference existed between the experimental groups.

This changed beyond seven day, with significant differences emerging, which showed the carboxylated surfaces to be least compatible with cell metabolic activity.

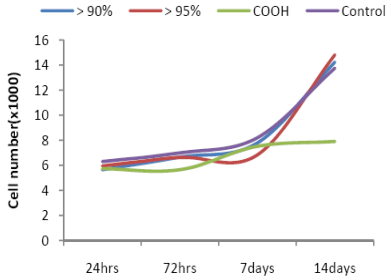

Figure 1. Cell proliferation observed over a 14 day period of culture

This pattern was repeated when cell proliferation was evaluated. For the first seven days in culture, there was no significant different between the surfaces in terms of cell proliferation; however, after day 7, differences did emerge and these differences between the surfaces trended towards statistical significant but did not reach significance. The carboxylated surfaces were associated with the lowest level of alkaline phosphates expression, a measure of osteogenic differentiation. No consistent differences existed between the experimental surfaces when the levels of cell stress were measured.

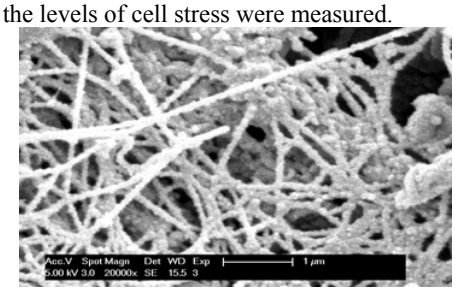

Figure 1. Scanning electron micrograph of apatite coated MWCNT.

Discussion

The present study demonstrates a unique and reproducible method of depositing calcium phosphate based apatite on MWNCT, replicating the principle on which bone ultra-structure is based. It is also demonstrates that these surfaces can be used to culture osteogenic cells.

The presence of an apatite surface coating reduced functional differences between the cells being cultured on different surfaces for the first 7 days in culture; a point that merits further study is an elucidation of the mechanisms underlying the changes in cell behaviour observed after 7 days, although it could be related dissolution of the apatite coating layer.

Reference(s)

[1] Kokubo, T., Takadama, H. (2006) How useful is SBF in predicting in vivo bone bioactivity? Biomaterials, 27 (15), 2907-15.