The modulation of osteogenic cell function by MWCNT surface characteristics

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Statement of Purpose: The basic constitution of the carbon nanotube is the covalent bond between individual carbon atoms, as occurs in planar graphite. This is one of the strongest chemical bonds found in nature. The tensile strength of carbon nanotubes is one of several characteristics that make them attractive for potential use in a mechanical or structural context. As this may extend into the area of biomaterials, a methodical general characterisation of the interaction of MWCNT with cells is desirable. MWCNT could potentially impact on cell behaviour, whether or not this is an intended consequence. As such, it would be important to elucidate the effects of these surfaces on the various parameters used to measure cell function. Various efforts to address this issue have been limited by issues such the poor substrate design (leading to MWCNT detachment), a limited number of replicates being used, the use of a limited number of assays within the study (meaning results could not be compared or corroborated).
Another feature of studies to date has been the short period of time for which the cultures have been maintained, limiting the strength of conclusions that could be drawn from such work. This study addresses these issue to provide a comprehensive characterisation of the in vitro interactions of an osteogenic cell line with MWCNT, where the latter provides a surface on which the cells are cultured.

Methods: Surfaces were prepared by heat pressing liquid dispersed MWCNT onto a HDPE surface. Within the 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 variable that changed between the groups was the surface on which cells were cultured i.e. ≥90% MWCNT surfaces, ≥95% 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 a standard cell culture surface. The cultures were maintained for 14 days in total, with cells being harvested at pre defined times points of 24 hours, 72 hours, 7 days and 14 days

Results:
Metabolic activity was comparable between the control surfaces and the non carboxylated surfaces at day 7, with the level of metabolic activity on the carboxylated MWCNT significant less than the other groups. These differences were also observed at day 14.

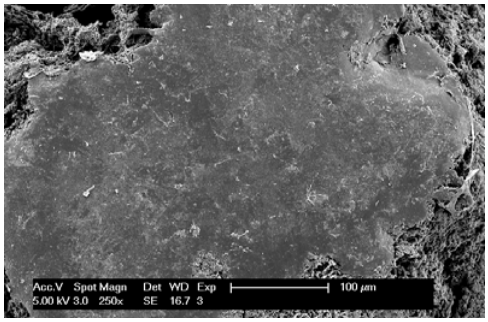

Figure 1. MG63 cell on a ≥90% MWCNT surface after 72 hours in culture.

Cell proliferation followed the patterns observed with metabolic activity. The carboxylated MWCNT were associated with a significantly lower level of cell proliferation during the first 7 days of culture although this differences lost statistical significance beyond 7 days in culture.

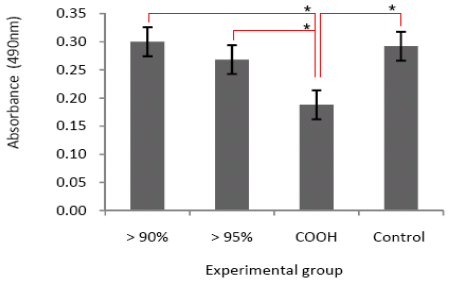

*Figure 2. Cell metabolic activity on the experimental surfaces and control groups as measured by the MTS assay after 7 days in culture (*p<0.05)*

The expression of alkaline phosphatase, as a measure of differentiation was comparable between the non carboxylated surfaces and the control surfaces. Measures of cell stress, overall, did not indicate any MWCNT surface being excessively harmful.

Conclusion
In conclusion, a new and reproducible technique for preparing MWCNT surfaces for cell culture has been demonstrated. It has also been demonstrated that MWCNT surfaces provide a surface comparable (in terms of effect on cells) to conventional cell culture surfaces, with the modifications based on purity levels having a limited impact, although chemical modification (such as the presence of a carboxyl group) can be associated with significant impairment in cell function