In Vitro Evaluation of Osteoblast and Fibroblast Response to Nano-phase Fillers

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Introduction: It is hypothesized that the use of nano-phase fillers within a polymer matrix will improve the mechanical properties, compared to the homogeneous polymer and a composite with standard micron-sized reinforcement, to a level suitable for orthopaedic load-bearing biomaterial applications. However, research regarding the *in vitro* biocompatibility of nano-dimensioned materials is often conflicting. Several authors have found decreases in cell viability and proliferation when grown in the presence of a range of nano-phase materials, including carbon nano-tubes and hydroxyapatite and titania nano-particles (Peters K. J Mater Sci: Mater Med. 2004;15:321-325 & Monteiro-Riviere NA. Toxicol Lett. 2005;155:377-384). It was also established that nano-particles were internalized into the cells causing cytoskeletal dysfunction (Moller W. Toxicol Appl Pharmacol. 2002;182:197-207). Conversely, other studies have shown improved cell viability, adhesion and function when grown in the presence of similar nano-materials (Gutwein L. Biomaterials. 2004;25:4175-4183). The aim of this study was to assess the cellular response of fibroblasts and osteoblasts to precipitated nano-particulate $CaCO_3$, nano-crystalline titania and graphitic nano-fibers measured in terms of cell density, cellular activity and differentiation and cell morphology. Also taken into account were any differences in cell response to particulate size.

Methods: Precipitated nano-particulate $CaCO_3$ (48 nm ± 2.4) was produced by carbonation of calcium hydroxide and EDTA (1 wt %) in methanol. Micro-particulate (termed conventional) $CaCO_3$ (> 1 μm) was used as received from Fisher Scientific. Nano-crystalline titania particles (6 nm ± 0.7) were fabricated using sol-gel and conventional titania (> 500 nm) produced by annealing the nano-phase version. Graphitic nano-fibers (155 nm ± 13) were synthesized using chemical vapor deposition and conventional graphite (> 1 μm) used as supplied from Arthur Branwell & Co (Epping, UK). Primary human osteoblasts (HOB; PromoCell) and murine fibroblasts (Swiss, 3T3; ECACC) were seeded onto Thermanox slides and grown under standard conditions (37 °C/5 % CO_2) for 48 hours. The culture media was then replaced with particle supplemented media at three particle concentrations (1000, 5500, 10000 μg ml^{-1}) for a further 2 or 6 hours (particle free media was used as a positive control). Cells were then fixed, stained with propidium iodide and density determined by counting in 22 random fields using fluorescence microscopy. Cell metabolic activity was determined using an alamar blue assay over 1, 4 and 7 days, followed by a DNA assay. HOB differentiation was assessed with an alkaline phosphatase assay over 4, 7, 10 and 14 days. The morphology of both cell types after subjection to the above conditions was analyzed using SEM.

Results & Discussion: All of the assays indicated that cell density (**figure 1**), activity, differentiation and morphology (**figure 2**) for either cell type and over all the time periods were not significantly affected by the presence of either nano or conventional $CaCO_3$. It is hypothesized that this occurs due to the presence of additional Ca^{2+} ions, which has been found to improve cellular differentiation and proliferation (Maeno S. Biomaterials. 2005;26:4847-4855). Titania particles of both sizes had a negligible effect on cell responses as measured by the range of assays. However, adverse responses to graphite particles were detected, since by 7 days cell density and activity had significantly decreased, with those cells that stayed adhered having a rounded morphology, indicating cell death (**figure 2**).

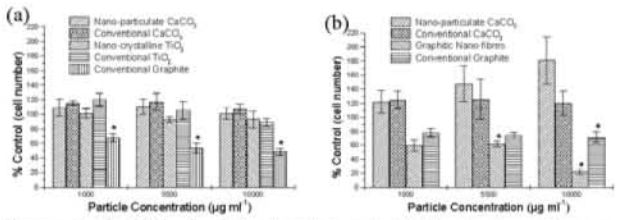

Figure 1. Variation in (a) osteoblast and (b) fibroblast number on Thermanox slides with different quantities of particles added to the culture medium after 6 hours.

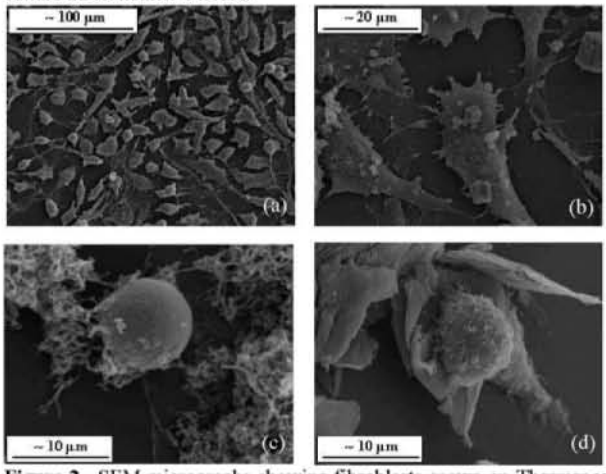

Figure 2. SEM micrographs showing fibroblasts grown on Thermanox slides after 7 days with (a) 100 μg ml^{-1} nano-particulate $CaCO_3$, (b) 10,000 μg ml^{-1} conventional $CaCO_3$ (c) 5500 μg ml^{-1} graphitic nano-fibers and (d) 1000 μg ml^{-1} conventional graphite.

Conclusions: This study demonstrated that precipitated nano-particulate $CaCO_3$ and nano-phase titania did not have an adverse effect on cell behavior. Differences in particle size did not alter the cell response, with the cell death caused by graphite indicating that particle chemistry has more of an influence than size.