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Cytocompatibility and Mechanical Strength of Hydroxyapatite Reinforced with Multi-Walled Carbon Nanotubes

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Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) composition is similar to the mineral phase of bone and is an excellent material to use in bone replacement materials. Clinically, it has been used in dental and orthopaedic applications in bulk form as filler, and as a coating for more than 30 years [1]. However, its poor mechanical properties (natural brittleness and unsatisfactory strength of HA) prevented it from being used in clinical applications, under load-bearing [2].

Since carbon nanotubes (CNTs) entered the world materials stage [3], their mechanical properties have been praised as some of the best present. CNTs possess outstanding mechanical properties and chemical stability which resulted from their cylindrical graphitic structure, and carbon is one of the known fundamental basics in the development of life on the planet Earth [4]. Their strength and stiffness, combined with their small size and large interfacial area suggest they may have great potential as reinforcing agent for HA [5]. Thus, HA composite coatings reinforced with CNTs might have tailored properties, including high strength and good bioactivity [6]. In order to obtain an acceptable mechanical reinforcement, the surface of CNTs must be functionalized to improve the dispersion of individual CNTs in the ceramic matrix, and also to induce perfect interfacial bonding between CNTs and HA, which could be ultimately responsible for an efficient load-transfer mechanism [7,8].

In the case of CNT-based biomaterials, particular nano toxicity issues must be considered. Recent reviews highlighting nano toxicological challenges with CNTs are available [9-11]. If CNTs are to be included into composite substances for medical applications, evidence of their potential bioactivity and toxicity is necessary. Depending on the dissolution rate of the matrix material in vivo, CNTs could be released into the body, possibly inducing a harmful response. The presence of CNTs may have no damaging effects and could even enhance its bioactive properties. The response of multi-walled carbon nanotubes (MWCNTs) to human lung epithelial cells, osteoblast-like cells and primary osteoblast cells showed that these cells attached and survived on MWCNTs, although the proliferation effect is not much. They suggested that the dimensions and spacing of CNTs may be the key to determine subsequent cell spreading and proliferation [12]. Other studies have shown that functionalising CNTs can improve their aqueous dispersibility and biocompatibility [13-15]. The aim of this study is to investigate the mechanical properties and the cytotoxicity of the HA composites, incorporated with different types of MWCNTs.

The cytotoxicity and mechanical properties of a novel multi-walled carbon nanotube reinforced hydroxyapatite composite, using different types of MWCNTs (MWCNTs, 95% purity, MWCNTs-OH, 99.9% purity) are presented. Compressive strength tests and cell culture experiments with human CCD-18Co fibroblasts were performed. Briefly, HA powder was mixed with de-ionized water, 15 wt.% bovine serum albumin (BSA) and 0.5 wt.% of hydroxylated MWCNTs (MWCNTs-OH, purity 99.9%), MWCNTs (MWCNTs, purity 99.9%) and MWCNTs (MWCNTs, purity 95%), respectively, to produce HA/MWCNTs-OH/BSA and HA/MWCNTs/BSA composites. The paste was packed into a cylindrical stainless steel mold (diameter=6 mm, length=12 mm), and stored in a Gyro-Rocker Incubator (Model: S170) at 37°C and 97% humidity for 24 h. Then, the samples were taken out and dried at room temperature. Viability of human fibroblast cells (CCD-18Co) was assessed using the MTT assay. To test the cytocompatibility of the HA/MWCNT/BSA samples which is in powder form (to increase the surface area of the particles, in order to cover the cells completely), the viabilities of the cells were determined. CCD-18co cells were seeded at a density of 1.5×10^5 cells per well in 96-well plates. The test substance was diluted with media to the desired concentrations of 6.25, 12.5, 25, 50, 100 and 200 µg/ml from the stock. Into each well containing the cells, 100 µl of test substance of various concentrations was added, and 100 µl of medium (as the control) was added to the cells. The mitochondrial respiratory activity of the fibroblasts treated with the composite was determined colorimetrically, using MTT assays [16].

Figure 1 shows the effect of HA composites on CCD-18Co fibroblast cells, as measured by MTT assay. Cytotoxicity study indicates that in low concentrations of tested samples, both composites show better biological response in promoting cell growth compared to higher concentrations. However, according to the results, HA/MWCNT-OH/BSA composites were not only non-toxic in different concentrations between 6.25-200 µg/ml, but also at low concentration (6.25 µg/ml) of test samples, it indicated a proliferative effect on CCD-18Co cells. As shown in figure 1, at low concentrations (6.25 µg/ml), HA/MWCNT-OH/BSA showed approximately 200% cell viability; thus, this composite had a greater effect on the viability of the cells. All quantitative data are expressed as mean ± deviation, and the level of statistical significance was defined as $p<0.05$.

In the last study [17], cytotoxicity of calcium phosphate composite (CPC/MWCNT-OH/BSA) was studied. The percentage of CCD-18Co cell viability was reduced from 102% to 66%, by increasing the concentration from 6.25-200 µg/ml. Figure 2 shows the effect of CPC/MWCNT-OH/BSA and HA/MWCNT-OH/BSA composites on viability of CCD-18Co cells. It indicates that a cell proliferative effect of the HA/MWCNT-OH/BSA composites is significantly higher than the CPC/MWCNT-OH/BSA composite, especially in a low concentration of 6.25 µg/ml.

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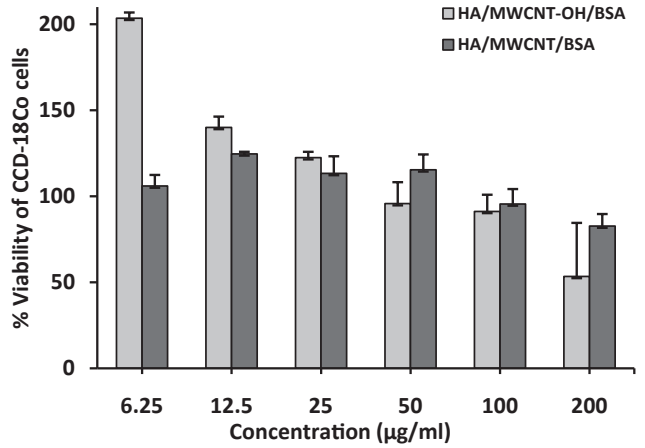

Figure 1: Effect of HA composites on CCD-18Co fibroblast cells as measured by MTT assay. (Data are presented as the means ± 2 standard deviations, n = 3).

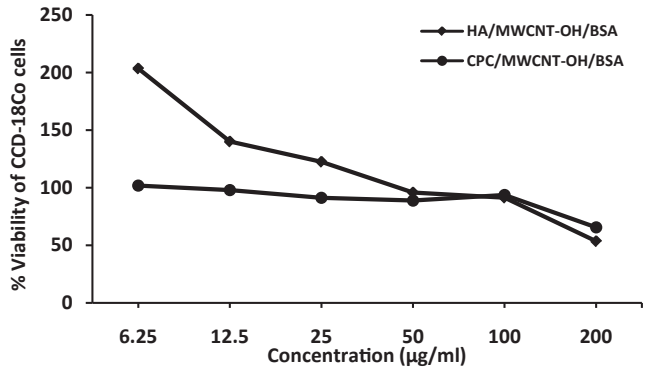

Figure 2: Effect of CPC/MWCNT-OH/BSA and HA/MWCNT-OH/BSA composites on viability of CCD-18Co cells (Data are presented as the means ± 2 standard deviations, n = 3).

The compressive strength of composites was tested using an Instron 3367 universal testing machine, with crosshead speed of 1.0 mm/min. Results showed that the HA/MWCNT-OH/BSA composite had higher compressive strength (20 MPa), compared to HA/MWCNT/BSA with compressive strength of 13 MPa. Compressive strength of trabecular bone is in the range of 2-12 MPa, and for cortical bone it is in the range of 100-230 MPa. The compressive strength of new composite is in the range of cortical and trabecular bone, thus, it has potential for being used as the bone replacement material.

In this study, HA/MWCNT/BSA composites prepared with various types of MWCNT (MWCNT-OH, 99.9% purity and MWCNT with 95% purity). The novel HA/MWCNT-OH/BSA composites showed favourable cytocompatibility with high compressive strength (20 MPa), and it is therefore, considered an attractive bone replacement material. The biological effect of the compound in cell proliferation hints potential wound healing effect, which adds further benefit to this composite. Further studies are required to find the mechanism of affecting cells by these composites, and more research is needed for increasing the compressive strength of composite to make it applicable in clinical purpose, under load-bearing.

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