Analysis of Cellular Adhesion on Substrate Containing Multi-Walled Carbon Nanotube (MWCNTs)

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Statement of Purpose: The promises of the use of carbon nanotubes (CNTs) for biomedical purposes to run into some difficulties. Despite evidence of cytotoxicity of CNTs, there are also a large number of publications of studies of biomaterials based on CNTs that support the idea of biocompatibility of the CNT. There are investigations of successful interactions between materials based on CNTs and neuronal cells, osteoblasts, fibroblasts, antibodies, immune system, "DNA and drug delivery", among others [1]. Results from our group showed that the purity of CNTs without amorphous carbon or metal debris was enough to get biocompatibility of cells tested [2]. The structures of aligned MWCNT revealed that a slightest contact between the structures of cells and nanotopography is crucial to ensure the efficiency and bioactivity in the growth and spreading of cells without producing cytotoxic effects. Therefore, adhesion to the substrate being a crucial point for the proliferation and survival of these cell types, in this work, we proposed to study the ability of cell adhesion in the first contact of the cells to different types of substrates containing vertically aligned MWCNT. In order to generating important information on their properties for future use of these structures in the biomaterial manufactures.

Methods: VACNT films were produced as a thin film, using a microwave plasma chamber at 2.45GHz on Ti substrate (10x10x1mm) with Ni or Fe catalyst. Superhydrophilic VACNT composites were obtained by the incorporation of oxygen-containing groups using a pulsed-direct current plasma reactor with an oxygen flow rate of 1 sccm, at a pressure of 85 mTorr, -700 V and with a frequency of 20 kHz [3]. The cells used were fibroblasts extracted from embryo of 13.5 days of development from transgenic “green mouse” by digestion, because they are more sensitive and close to the living organism. For the evaluation of amount of the cells adhered on the substrate were used a blue fluorescence dye that has nucleus affinity (DAPI) and evaluated fluorescence microscopy (FM) after 24h of culture. Counting was performed in the program ImageJ and statistics with Graphpad Prism 5. Also the scanning electron microscopy (SEM) analysis were employed to investigate the structural surface characteristics of the substrates within Ni or Fe catalyst.

Results: We produced different substrates from two types of catalysts (Fe and Ni) and having distinct characteristics of hydrophobicity through the treatment or not of plasma with O_2. Comparing the results of preferential adhesion of all substrates as shown in Figure 1, we found that a variation in susceptibility of primary fibroblasts adhesion by increasing extent, indicating Fe as the worst substrate adhesion and FeO2 as the best and closest efficiency of Ti adhesion (Control).

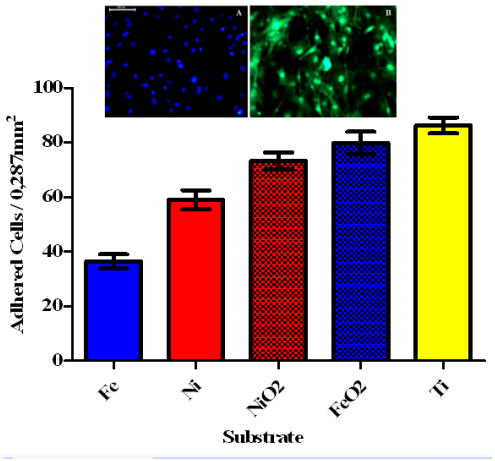

Figure 1: Upper pictures of embryo fibroblasts on MWCNT by fluorescence microscopy (blue:DAPI and green: natural fluorescence from green fluorescent protein). Graphic of cellular adhesion among different substrates of MWCNT.

In Figure 2 showed the surface of substrate containing CNT produced by Fe and Ni catalyst respectively.

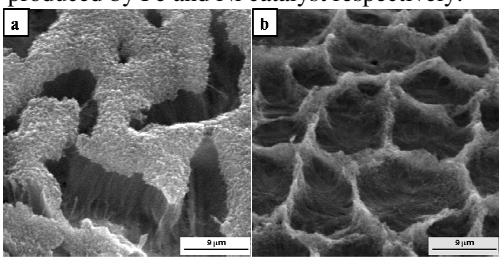

Figure 2: SEM of substrate produced by Fe (a) and Ni(b) catalyst.

Conclusions: We produced 4 different substrates containing MWCNT with increasing susceptibility to adherence of the cells that may be used for different proposes. These characteristics were mainly due tree factors, the wetability caused by plasma O_2 treatment, the density of carbon nanotubes per mm^2 and the topography of substrate surface. The surface produced by Ni catalyst seemed to avoid the cell slip through the substrate when compared with Fe catalyst. What can explain the lower effect for Ni with plasma O_2 treatment and the better efficiency of adherence on the Fe treated surface. All or some of them may have good features for biomedical interests.

References:

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