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

Mirian Michelle Machado[1], Anderson Oliveira Lobo[2,3], Sandra Cristina Ramos[3], Evaldo José Corat[2,3] and Marcus Alexandre Finzi Corat[1].

[1]*CEMIB-Unicamp, Campinas/SP, Brazil.* [2]*Instituto Tecnologico de Aeronautica, Sao Jose dos Campos, SP, Brazil.*
[3]*Instituto Nacional de Pesquisas Espaciais, LAS, Sao Jose dos Campos, SP, Brazil.*

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 in the first contact to spreading on different surfaces of substrates containing vertically aligned MWCNT, evaluating the tension of adhesion fibers of cytoskeleton by actin formation. 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 spreading of the cells adhered on the substrate were used a program ImageJ to calculate the area of cell surface on contact of substrate and statistics was performed by Graphpad Prism 5 program. The actin fiber was stained with rhodamine phalloidin and the nuclei were observed as blue fluorescence due to DAPI that has nucleus affinity and images were generated by fluorescence microscopy (FM) after 24h of culture.

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 we showed that no substrate had the same efficiency of cellular spreading as much as the Ti without carbon nanotubes. Despite that, when we compare the substrates by catalyst origin, we might observed mainly in the Fe substrate we had significantly improving of cellular spreading (Figure 1).

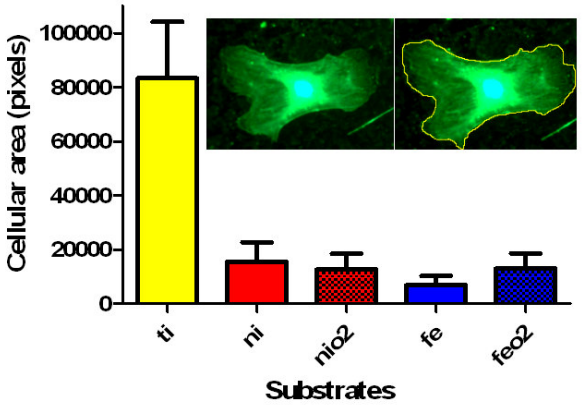

Figure 1: Upper pictures of embryo fibroblasts on MWCNT by fluorescence microscopy showing the example of area calculated (green: natural fluorescence from green fluorescent protein). Graphic showed of cellular spreading among different substrates.

Furthermore, only in Fe substrate containing MWCNT was possible to observe organized actin fibers with tension appearance (Figure 2- top line). Assembling of Focal Adhesion Kinase (FAK), usually formed in focal adhesion complex in the cell, appears as red dots mainly in the substrate treated with plasma O_2 (Figure 2- bottom line).

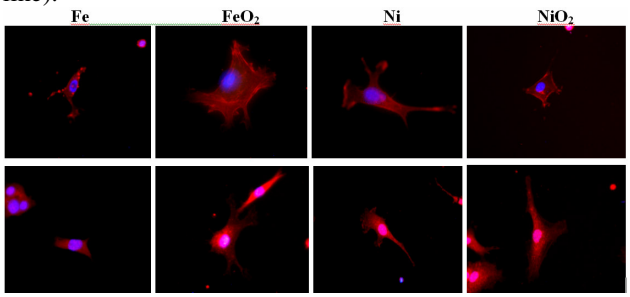

Figure 2: FM of actin fibers (top line) and Fak (bottom line) in red and nuclei in blue.

Conclusions: We produced 4 different substrates containing MWCNT but none had as good spreading in 24h of culture than Ti substrate, being the Fe catalyzed substrate worst likely because your high hydrofobicity. But when they received treatment of Plasma O_2 they had better results what were evidenced by marking the actin and FAK proteins, which are proteins involved mainly with cellular adhesion properties of the cells. The contrast of improvement was more evident between Fe and FeO_2 substrates. Good goals for tracking the first steps of cell adhesion on MWCNTs substrates that might be used for biomaterials manufature.

References:

1. Smart SK, Carbon 2006; 44: 1034-1047.
2. Lobo AO, Materials Science and Engineering C 2008, 28: 532–538.
3. Estroff LA, Hamilton AD. Chem. Mater 2001;13:3227.