# MULTI-WALLED CARBON NANOTUBES USED AS CHITOSAN REINFORCEMENT ENHANCE OSTEOBLAST CELL RESPONSE OF THE COMPOSITE

**Anthoula Kroustalli, Antonia Moutzouri, George Athanassiou, Despina Deligianni**
*Laboratory of Biomechanics and Biomedical Engineering, Department of Mechanical Engineering and Aeronautics, University of Patras, Greece*

## Introduction

The main goal in the design process of engineered bone substitutes is to achieve physicochemical, mechanical and architectural properties that will mimic the native tissue and enhance cell response. Composite biomaterials have been explored for the preparation of bone tissue engineering scaffolds, since they possess improved characteristics compared with single components [Gardin et al, 2012]. Chitosan (CS) is a natural biopolymer that has been shown to be biocompatible and exhibit favorable osteogenic and osteoconductive properties [Di Martino et al]. To enhance its mechanical strength, multi-walled carbon nanotubes (MWCNTs) have been proposed as reinforcement filling [Kroustalli et al, 2012]. The aim of this study was to investigate osteoblast response to MWCNTs reinforced chitosan membranes in terms of cell proliferation, differentiation and cytotoxicity.

## Methods

Two different kinds of thin films made of CS and MWCNTs reinforced CS (MWCNT/CS) were used. CS films were prepared from crab shells chitosan powder (Sigma) by a solution evaporation method. The MWCNTs used in this work were obtained from Nanothinx, (purity 98,3%). Human osteoblastic cell response was investigated by determining cell proliferation, ALP enzyme activity, total protein content and cytotoxicity at 1, 3 and 7 days of cell culture on the prepared substrates. Plastic culture dish substrate was used as control.

## Results

The results of intracellular total protein content, ALP enzyme activity (Fig. 1) and proliferation, of the cells cultured on CS and MWCNT/CS were comparable to control at 1 and 3 days but they were significantly higher than control at 7 days ($p<0.05$). Moreover, the response on MWCNT/CS was also significantly higher than that on neat CS at 7 days ($p<0.05$). Regarding the cytotoxicity results, both CS and MWCNT/CS demonstrated higher levels of toxicity at all time points tested compared to control, with the composite exhibiting the higher values.

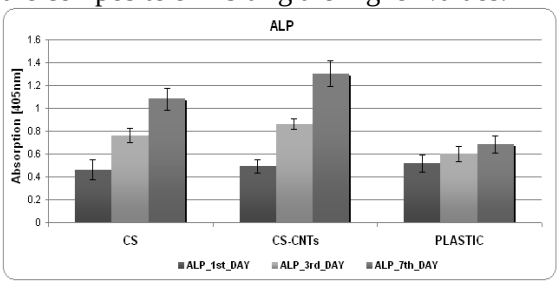


Figure 1: ALP activity expression on the various substrates.

## Discussion

Successful bone tissue engineering relies not only on the differentiation of cells in contact with the implant materials, but also on the proliferation capacity of the cells so as to obtain a 3D matrix. Although the reinforcement of chitosan with MWCNTs in this study seems to slightly impair the biocompatibility of neat chitosan, the level of toxicity has not affected the cell growth, suggesting that it has not reached considerably high toxic levels. The results of this study indicate that the combination of MWCNTs and chitosan may be used for implants with favourable osteoconductive and ostoinductive properties. Enhanced osteoblast activity has also been reported in the literature with the use of scaffolds of MWCNTs and CS [Jayachandran et al, 2010]. Further understanding of the interactions between cells and such promising composites are needed so as to elucidate possible mechanisms of toxicity, as well as to assess the specific material and surface properties that support adhesion, proliferation and differentiation.

## References

Di Martino *et al*, Biomaterials, 26: 5983–90, 2005.
Gardin *et al*, Int. J. Mol. Sci., 13:737-57, 2012.
Jayachandran *et al*, Mar. Drugs, 8, 2252-66, 2010.
Kroustalli *et al*, J Biomechanics, Vol: 45, Sup 1 , P S48, July 2012.