Carbon nanotubes in nanomedicine: Synthesis, Functionalization and Characterization

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INTRODUCTION

Carbon nanotubes (CNTs) have unique physical and chemical properties that open attractive possibilities in many applications. INASMET is developing applications of CNTs in the biomedical field. A thorough understanding of the CNT synthesis parameters and an effective purification process is needed to improve the biocompatibility of synthesized CNTs. The three main applications addressed at INASMET are CNT polymer nanocomposites to obtain scaffolds for tissue engineering, CNT nanozirconia composites for prostheses fabrication and functionalization of CNTs for biosensor devices.

SYNTHESIS

Multi Wall Carbon Nanotubes (MWNTs) were synthesized by Chemical Vapour Deposition (CVD) of methane with two different catalysts:
- Ni on SiO_2/Al_2O_3 (average diamete 16nm) and
- Ni nanoclusters on a Si wafer (average diameter 100nm)

The optimal operation conditions for a high yield process have been obtained by means of an experimental design.

Optimal operation conditions for the synthesis of CNTs:

Reaction time	*10 min*
Temperature	*1000 °C*
Catalyst mass	*0.5 g*
Ratio H_2/CH_4 in gaseous stream	*1*

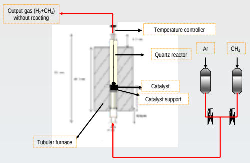


Figure 1. *Scheme of CVD reactor.*

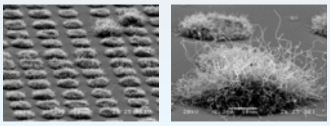


Figure 2. *SEM image of synthesized CNTs on Si wafers*

The Raman spectrum is consistent with Multi Wall Carbon Nanotubes

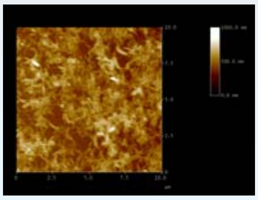


Figure 3. *AFM image of synthesized CNTs.*

PURIFICATION

The obtained product not only contains CNTs but also catalyst particles, amorphous carbon and graphite like particles as sub products. A thorough multistep acid cleaning is required to purify the sample.

Table 1. *Analytical results of catalyst content before and after the purification process.*

Elements	Raw CNTs (%)	Purified %
NiO	40,4	4,5
Al_2O_3	4,5	0,13
SiO_2	3,9	0,19

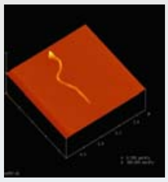


Figure 4. *AFM image of a purified isolated CNT.*

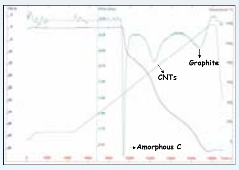


Figure 5. *Thermogravimetric analysis to determine the % of carbon nanotubes, amorphous carbon and graphite like particles in the sample.*

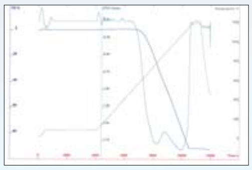


Figure 6. *TGA graph of purified material.*

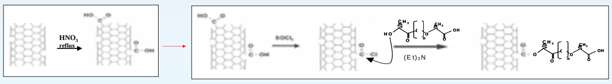
PLLA-CNT Nanocomposites

CNTs nanocomposites have been proposed as a possible new orthopaedic implant material due to their mechanical, electrical and cytocompatibility properties. Moreover, nanotubes increase osteoblast (bone-forming cell) adhesion and functions. But their applications in materials are limited by dispersion difficulties.

We have fabricated carbon nanotubes covalently functionalized with one of the biodegradable polymer more often used in bone regeneration, the poly(L-lactic acid) (PLLA), through ester bond to provide a good CNT dispersion.

FUNCTIONALIZATION

- Oxidation with nitric acid to create carboxilyc group
- Treatment with thionylchloride to obtain a acylchloride groups
- Attachment of PLLA, through ester bond



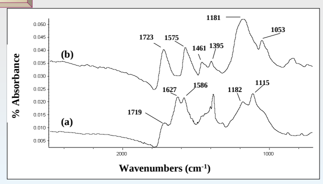
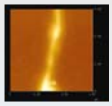


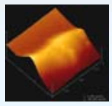
Figure 7. ***FTIR*** *spectrum:*

(a) *SWNT-COOH*
ν ***(C=O)*** *at 1719 cm^{-1},*
ν ***(C-O)*** *at 1182 cm^{-1},*
ν ***(C-O-H)*** *at 1115 cm^{-1}.*
ν ***(C=C)*** *frequency at 1586 and 1627 cm^{-1}*

(b) *SWNT-PLLA*
ν ***(C=O)*** *at 1723 cm^{-1} ester bands*
ν ***(C-O)*** *at 1181 and 1053 cm^{-1}*
ν ***(C=C)*** *at 1575 cm^{-1}*



Figures 8 and 9. *2D (up) and 3D (down)* ***AFM*** *images of an isolated SWNT with PLLA around.*



CNT-NANOZIRCONIA Composites

Zirconia ceramics are widely used as femoral heads, but case studies show that delayed failure can occur in vivo. MWNT/ZrO_2 nanocomposites are studied with the aim to reach a higher resistance to crack propagation.

We have coated MWNT with the ceramic material to obtain a homogeneous dispersion of CNTs and a good wettability of the CNT surface with the matrix, so the properties are actually improved.

MATERIALS AND METHODS

MWNTs coated with Nanozirconia were obtained by hydrothermal synthesis.

- ✓ $Zr(OH)_4$ aqueous solution in teflon autoclave at 240° C
- ✓ Dried in a stove at 70° C for one day.

Figure 10. *Stainless steel teflon autoclave*

XRD, FTIR and RAMAN: nanocrystalline zirconia, with a mix of tetragonal and monoclinic phases has been obtained, with an average grain size of 30 nm

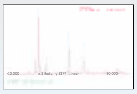


Figure 11. *XRD pattern*

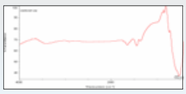


Figure 12. *FTIR spectrum*

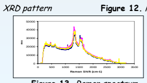
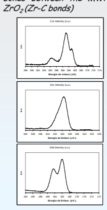


Figure 13. *Raman spectrum*

Figure 14. ***XPS*** *spectra results show bonds between the MWNTs and the ZrO_2 (Zr-C bonds)*



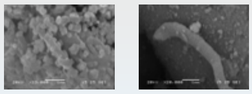


Figure 15. *SEM images of nanozirconia coated CNTs*

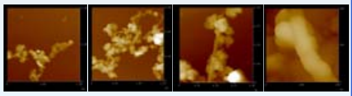


Figure 16. *Atomic Force Microscopy (AFM) images of nanozirconia coated CNTs*

BIOSENSORS

In vitro cytotoxic effect of purified CNT samples was investigated, based on the ISO 10993-5 Standard.

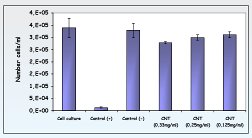


Figure 17. Flow citometry quantitative results.

Three different concentrations of CNT (0.33 mg/ml, 0.25 mg/ml and 0.125 mg/ml) in contact with Hela cells (ATCC CCL-171) were cultured during 48 h to study a possible toxicity in the cells.

Quantifying the number of cells by flow cytometry (using 7-Amino-Actinomycin D), the results confirmen a non-cytotoxic effect in any CNT concentrations.

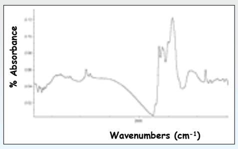
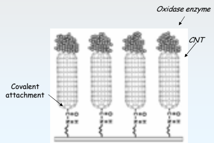


Figure 18. *FTIR spectrum of CNTs functionalized with amine groups.*

At the moment, our work is focused on the further modification of CNTs by coupling biomolecules to their surface. We are studying the functionalization of CNTs by a new process functionalization. We have achieved low damage CNTs with a high amino group.

The goal is the covalent attachment of the biomolecules at the tip of CNTs.



CONCLUSIONS

- Multi Wall Carbon Nanotubes (MWNTs) are synthesized by Chemical Vapour Deposition (CVD) process and purified with a multistep acid treatment.
- FTIR and AFM results show the presence of poly(L-lactic acid) grafting to the CNT and Infrared Spectroscopy results have demonstrated chemical modification via an esterification reaction.
- A CNT/Zirconia nanocomposite with C-Zr bonds has been obtained. The process is able to control the texture and the grain size of the zirconia synthesized
- Although an effect of the concentration of CNTs in the biocompatibility tests has been observed, the concentrations of CNTs tested (0.33 mg/ml, 0.25 mg/ml and 0.125 mg/ml) are non-cytotoxic
- A thorough study in the functionalization of carbon nanotubes is being carried out to achieve a covalent attachment between CNTs and specific enzymes.

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