The unpredictable protein corona interaction with Multiwall Carbon Nanotubes and a versatile functionalization technique

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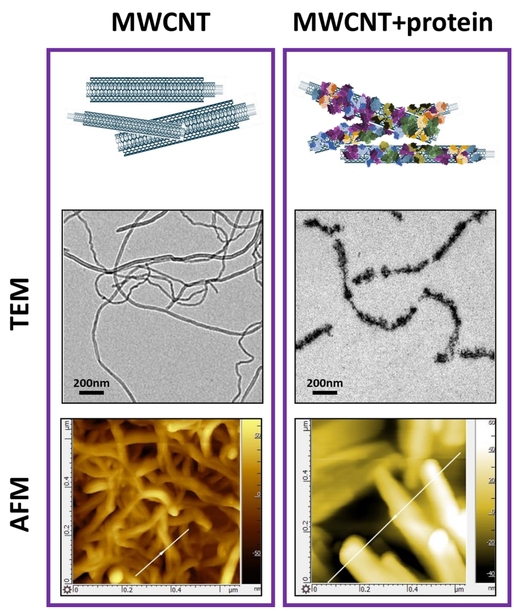
COST ACTION CA 17140 NANO2CLINIC CANCER NANOMEDICINE - FROM THE BENCH TO THE BEDSIDE

INTRODUCTION

The intrinsic physicochemical properties of multiwall carbon nanotubes (MWCNTs) such as elemental composition, resilience, thermal properties, surface reactivity, and in particular the ability to capture biomolecules on their surface make them the undisputed interest in biotechnology. The protein's interaction with MWCNTs creates a biological coating that endows them the ability to interact with some cell receptors, penetrate membranes or interfere with cell biomechanics, so controlling the biocorona is pivotal in MWCNTs nanobiotechnology. But some of these proteins unfold, triggering an immune response that unpredictably changes the biological activity of CNTs. For this reason, the control of the biocorona is fundamental in the nanobiotechnology of CNTs.

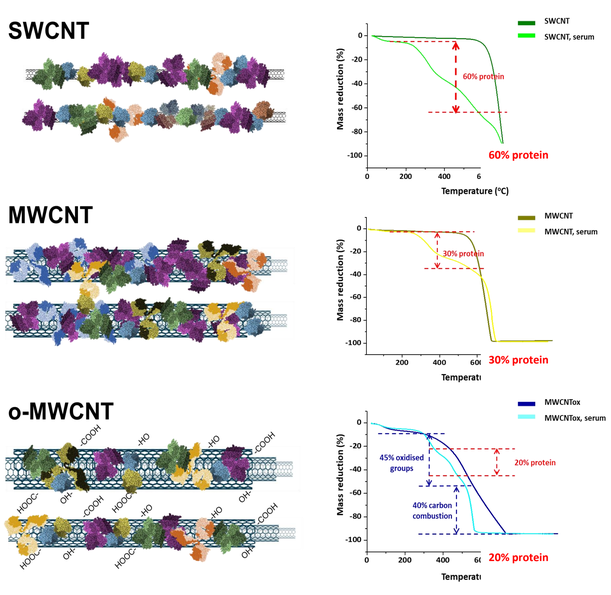
RESULTS

Morphological changes



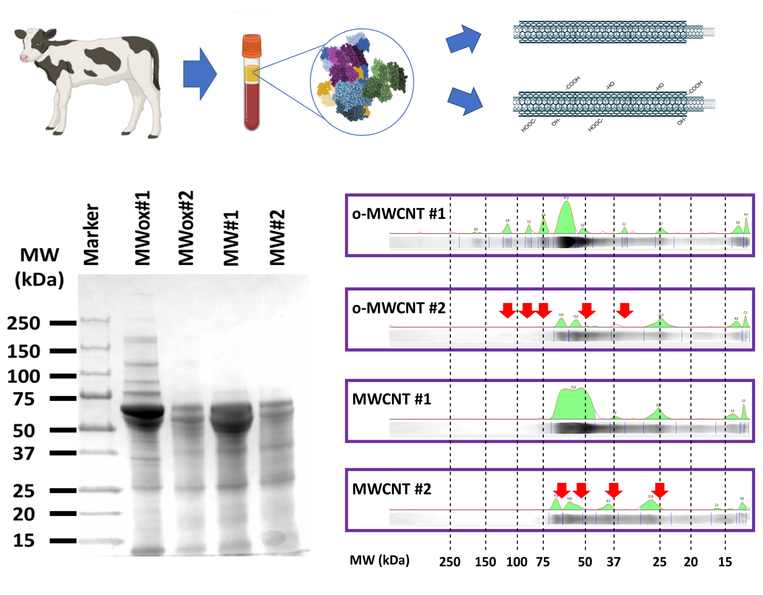
Functionalized MWCNTs significantly increase their diameter upon functionalization with proteins. TEM and AFM images of identical MWCNTs before (left) and after (right) serum functionalization. A significant enlargement of the nanotube diameter is observed upon protein coating.

Amount of protein absorbed



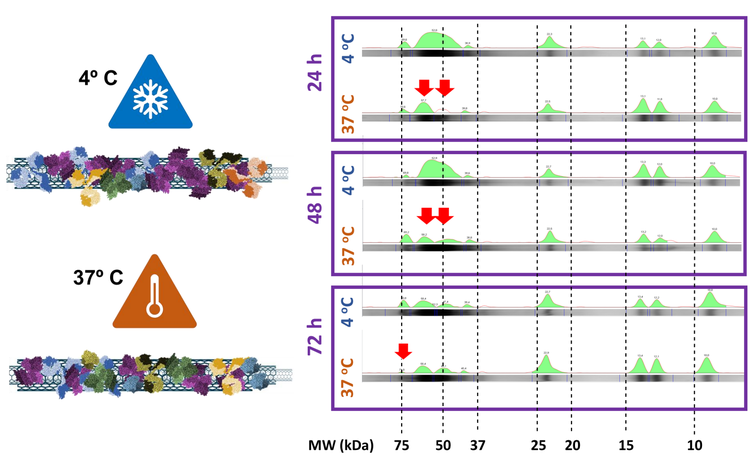
Thermogravimetric analysis (TGA) of SWCNTs, pristine MWCNTs, and o-MWCNTs. The calculated percentages of the final mass of the functionalized nanotubes corresponding to the biomolecular coatings are indicated in red.

CNTs type



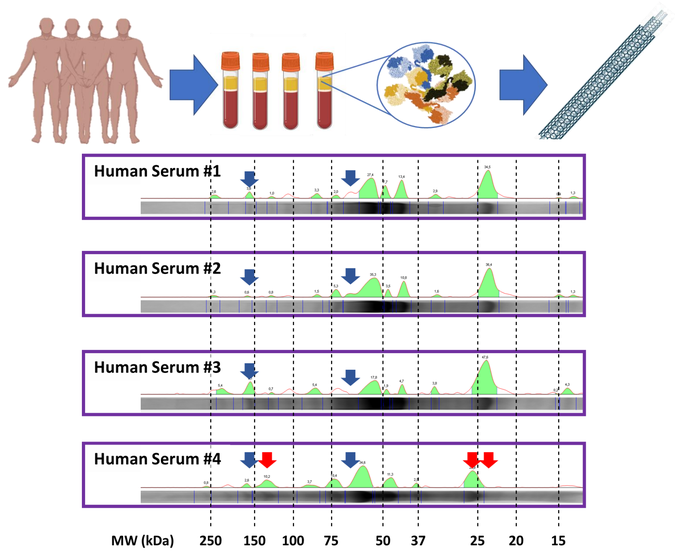
Biochemical landscapes of o-MWCNTs and MWCNTs functionalized with the same bovine serum. The qualitative and semi-quantitative protein landscapes (green profiles) were calculated from the SDS-PAGE protein analysis. These profiles demonstrate how identical protein components interact very differently with each nanotube. Arrows indicate some of the most divergent protein peaks.

Temperature and time



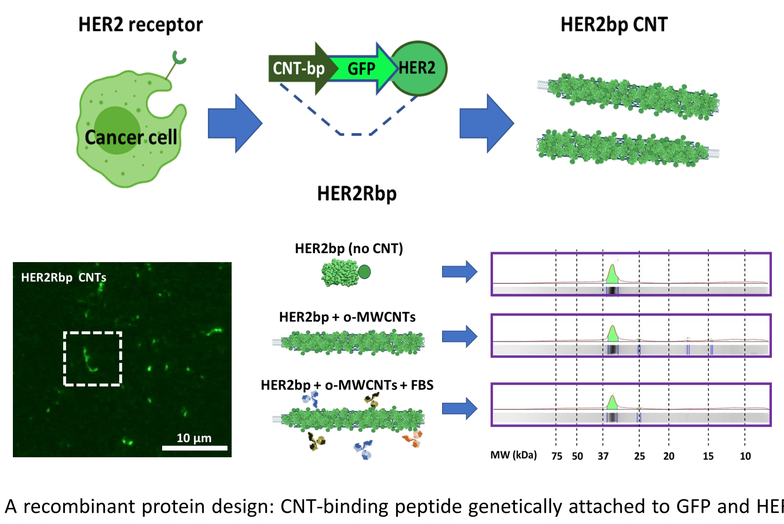
The protein landscapes obtained upon MWCNTs incubation with bovine serum at 4 ºC or 37 ºC during 24, 48, and 72 h. SDS-PAGE analysis reveals how the same protein components interact very differently with the same nanotube as a function of temperature and time. Arrows indicate some of the most divergent protein peaks.

Different human serum



Biochemical landscapes of MWCNTs functionalized with 4 different human sera. The biochemical landscapes reveal how the protein components of human sera interact differently with the same nanotube. Arrows indicate some of the most divergent protein peaks. Some proteins are completely absent in some of the samples (blue arrows) while others display significant changes in their affinity for the nanotube (red arrows).

Design a predictable protein coating for nanotubes



A recombinant protein design: CNT-binding peptide genetically attached to GFP and HER2 binding peptide. Confocal microscopy image of fluorescent HER2bp-functionalized CNTs. Fluorescence indicates the GFP in the protein is not denatured upon attachment. Protein landscapes of the purified HER2bp (top); the protein stripped from the HER2bp functionalized nanotubes (middle lane), and the total protein stripped from the functionalized nanotubes after incubation with serum. The presence of a single band demonstrates how the synthetic protein prevents unwanted biofouling of serum proteins on the nanotube.

CONCLUSIONS

Understanding and controlling the biomolecular coating on CNTs is the first step in nanobiotechnology and nanomedicine to be able to predict the biodistribution and effects of CNTs. This step is critical to producing reliable nanotherapies and nanovectors for in vivo applications. The protein corona endows CNTs with a biological identity that enables their intermingling with local components and participation in exquisite complementary molecular interactions. These proteins determine the interaction of nanotubes with biological components, such as receptors, membranes, cytoskeletal filaments, etc., so understanding their variation and controlling their nature is essential in in vivo contexts. For this reason, we have developed a genetic engineering method to produce a stable custom-designed biocorona on nanotubes that allows predetermining the nature of the CNTs, and thus improve the biodistribution and effects of CNTs and CNT-based nanostructures.

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