# Mechanisms of internalization, and sub-cellular localization, of bare and bovine serum albumin-stabilized carbon nanotubes in human alveolar lung epithelial A549 cells *in vitro*

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## Introduction and aim of the study

Due to the unique combination of physical-chemical properties of carbon nanotubes (CNTs) they have been successfully applied in pharmacy and medicine. However, there has been conflicting data reported regarding their safety and biocompatibility, especially concerning their cellular binding, routes of internalization, and intracellular trafficking.

The aim of our study was to characterize nonfunctionalized and carboxylated (COOH) bovine serum albumin (BSA) – stabilized multi-walled CNTs (MWCNTs) and to elucidate their internalization and fate in adenocarcinomic human alveolar basal epithelial cells (A549) *in vitro.*

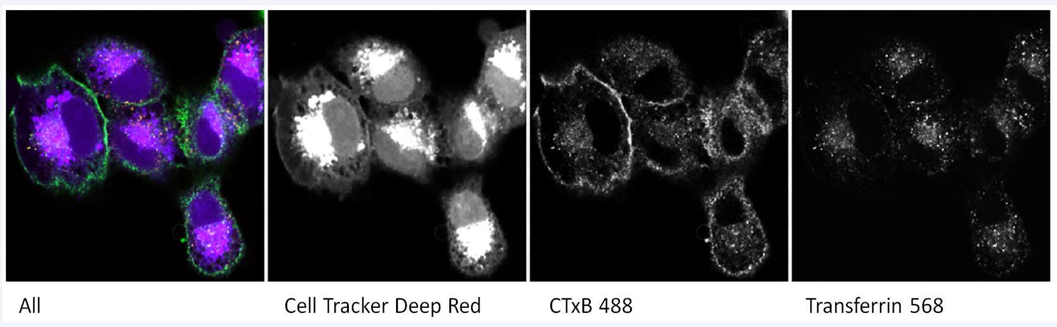
## Methods

**Preparation of BSA-stabilized MWCNTs suspensions:**
1 mg/ml stock dispersions were prepared in glass vials by prewetting nonfunctionalized MWCNTs and COOH-MWCNTs powder in 30 µL ethanol (96% purity) followed by dispersion in 0,036 w/v % BSA solution during 15 minutes of probe sonication (400 W, 25% amplitude).

**Characterization of MCNTs:**

- Transmission electron microscopy (TEM):
  Jeol 1200EX 80Kv Max system
- Atomic force microscopy (AFM):
  Park Systems XE-100 Advanced Scanning Probe Microscope
- Differential centrifugal sedimentation (DCS):
  CPS Instruments Inc., 8 w/w % - 24 w/w % sucrose gradient, 20.000 rpm
- Zeta-potential measurements:
  Malvern Zetasizer

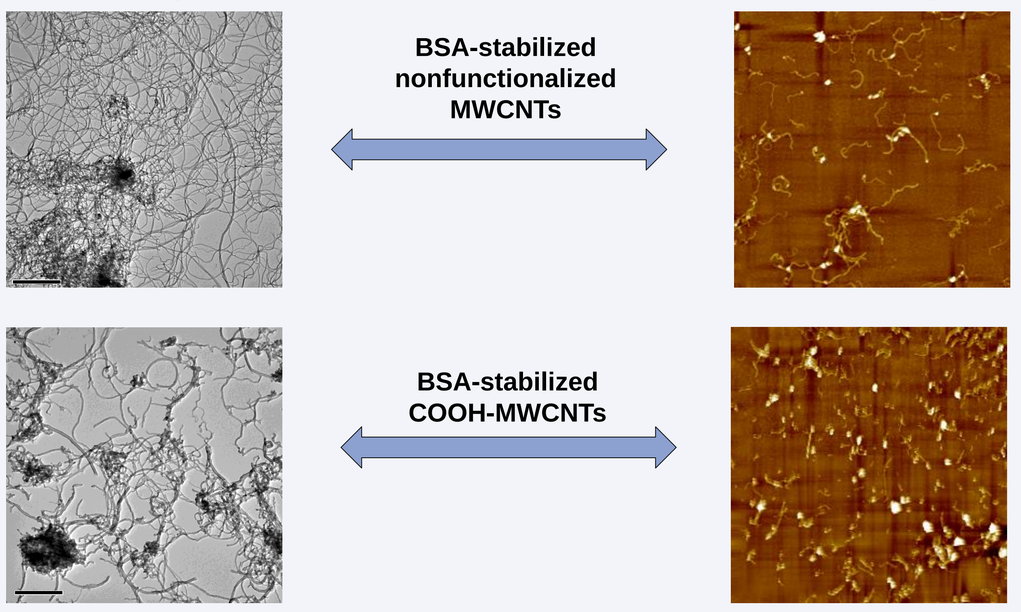
**MWCNTs internalization in A549 *in vitro*:**
To assess the role of clathrin-mediated and caveolin-dependent endocytosis in the internalization of MWCNTs in A549, cells were transfected with specific siRNAs against Caveolin-1 and AP-2 (protein involved in clathrin-mediated endocytosis). Decrease of the internalization of MWCNTs was studied by confocal microscopy with reflectance based detection of MWCNT (inverted Zeiss LSM 710).

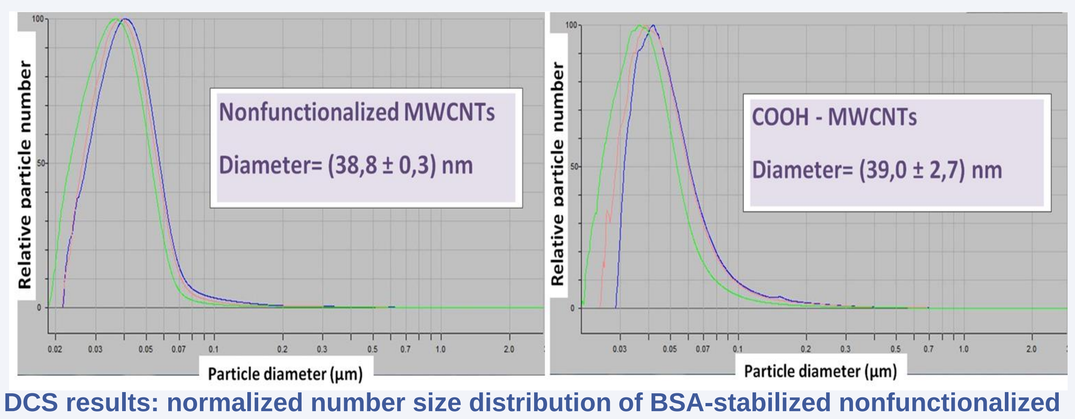



**Confocal images of preliminary control experiments: A549 cells were incubated in solutions of fluorescently labeled transferrin (clathrin-mediated endocytosis) and CTxB (caveolin-dependent endocytosis) under similar experimental conditions as used for the experiments with MWCNTs.**

## Results

**TEM images** **AFM images**

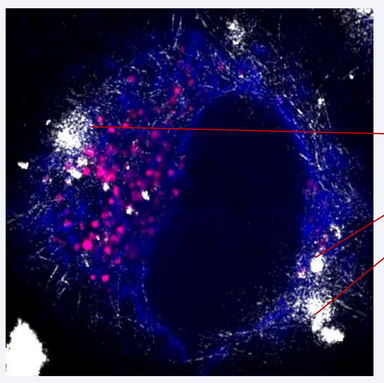



**DCS results: normalized number size distribution of BSA-stabilized nonfunctionalized MWCNTs (left) and COOH-MWCNTs (right).**

**ξ-potential velues**
nonfunctionalized MWCNTs:
(-15,3 ± 1,7) mV

COOH-MWCNTs:
(-28,2 ± 0,4) mV

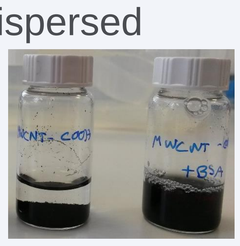


**COOH-MWCNTs subcellular localization in A549 (white: COOH-MWCNTs)**

**COOH-MWCNTs**

## Outputs

- By using BSA as a dispersing agent we were able to obtain fairly stable dispersions of both nonfunctionalized MWCNTs and COOH-MWCNTs. However, further optimization of dispersion protocol to obtain reproducible and dispersed MWCNTs suspensions is required.




**Left: COOH-MWCNTs dispersion in water**
**Right: COOH-MWCNTs dispersion in 0,036 w/v % BSA solution**

- For MWCNTs internalization in A549 cells, control experiments with transferrin and CTxB showed effective knockdown of target endocytosis proteins (caveolin-1 and AP-2) was demonstrated. Analysis of impact on MWCNT uptake underway!

## Other highlights / expertise gained / collaborations

- Training in confocal fluorescence microscopy
- Opportunity to present my research to the UoB team
- Experienced a different research environment and culture

## Benefits of TA

- Relevant information gained on the very recent approaches in nanomaterial characterization
- Broadened methodological skills in assessment of bio-nano interactions
- Expanded my research network and experienced another research environment

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