Novel biocompatible chitosan decorated single-walled carbon nanotubes (SWNTs) for biomedical applications:theoretical and experimental investigations

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Cytotoxicity assay method

Caco-2 cells were seeded in 96-well plate and incubated for 2 days in culture medium (Media 199, Invitrogen, supplemented with FCS (10 %) and penicillin / streptomycin (50 μg/mL) at 37 °C in air (95 %) : CO_2 (5 %). Media was removed and cells were incubated for 2 h with polymer (500 μL) dissolved in Media 199, at concentrations of 0.1, 1 and 10 mg/mL. SDS (10 mg/mL) was used as positive control and culture media as reference for 100% cell viability. Media and test solutions were then removed after treatment time and cells washed with PBS. Freshly prepared MTT (200 μL) in Media 199 (0.5 mg/mL), without any additions, was added and cells were incubated for 1 h at 37 °C. Subsequently, MTT solutions were removed from the wells and DMSO (400 μL) was added to dissolve formed formazan crystals. After homogeneous agitation (10 min), the absorbance was read at 584 nm.

Table A NMR spectra analysis for NBSC, NPSC.

NBSC		NPSC	
Chemical shift / ppm	Assignment	Chemical shift / ppm	Assignment
0.9 - 1.1	-NHCH$_2$(CH$_2$)$_2$CH$_3$	1.1	-NHCOCH$_2$CH$_2$(CH$_2$)$_{12}$CH$_3$
1.3 - 1.5 1.5 - 1.8	-NHCH$_2$(CH$_2$)$_2$CH$_3$	1.2 - 1.3	-NHCOCH$_2$CH$_2$(CH$_2$)$_{12}$CH$_3$
		1.2 - 1.4	-NHCOCH$_2$CH$_2$(CH$_2$)$_{12}$CH$_3$
2.0 - 2.2	-NH-CO-CH$_3$	2.0 - 2.1	-NH-CO-CH$_3$
		2.5	-NHCOCH$_2$CH$_2$(CH$_2$)$_{12}$CH$_3$
2.9 - 3.4	2H in C2	3.0 - 3.1	2H in C2
3.7 - 3.8	-NHCH$_2$(CH$_2$)$_2$CH$_3$		
3.6 - 3.9 3.9 - 4.1	2H in substituted C6	3.6 - 3.7	2H in substituted C6 (overlapping with next peak)
4.1 - 5.1	HOD and all other H	4.3 - 4.6	HOD and all other H
5.1 - 5.2	1H in C1	4.8 - 4.9	1H in C1
		8.4	-NHCO-

Tukey Kramer: **p <0.01; ***p < 0.001 compared to NOSC

Characterisation of chitosan derivatives

i) FT-IR results

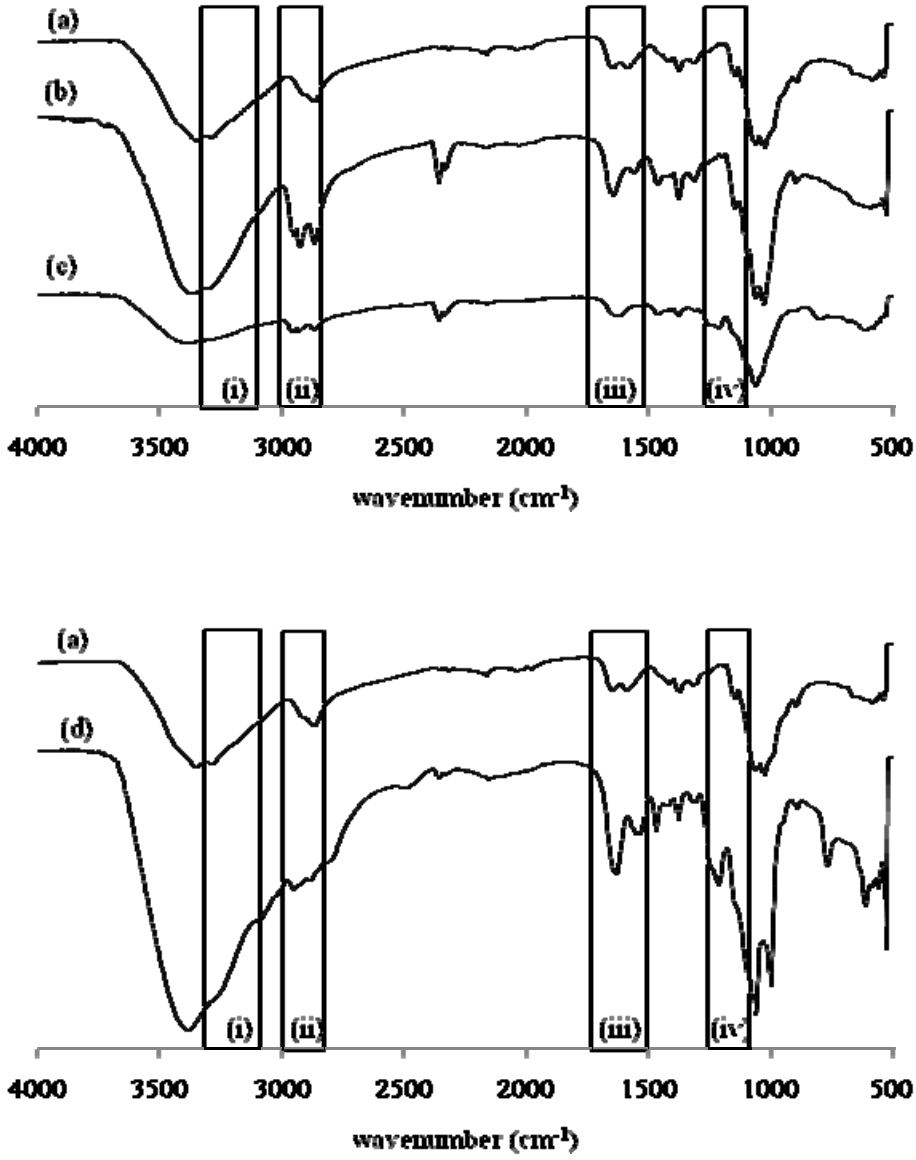

Fig. A ATR spectra of (a) chitosan, (b) *N*-butyl chitosan, (c) *N*-butyl-*O*-sulphate chitosan and (d) *N*-palmitoyl-*O*-sulphate chitosan.

ii) X-RD analysis

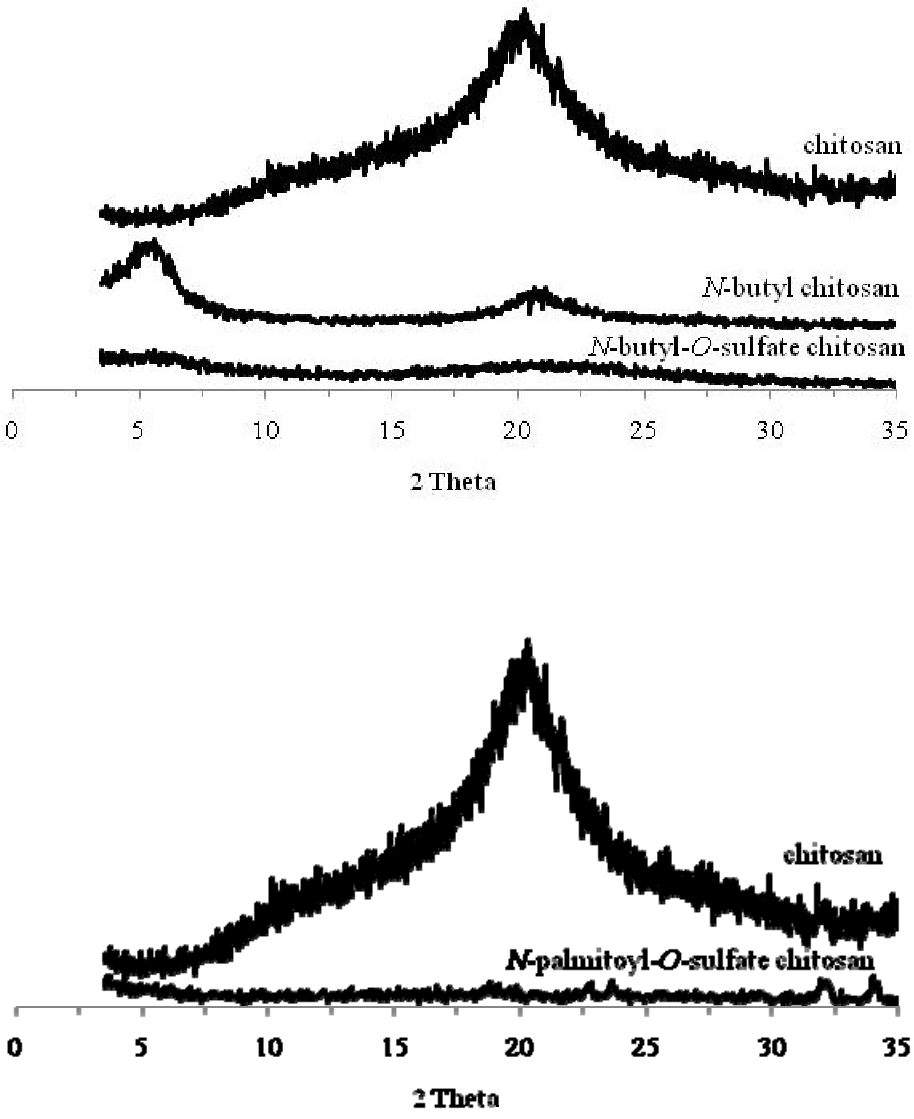

Fig B XRD patterns of chitosan and its derivatives.

Atomic Force Microscopy (AFM) studies

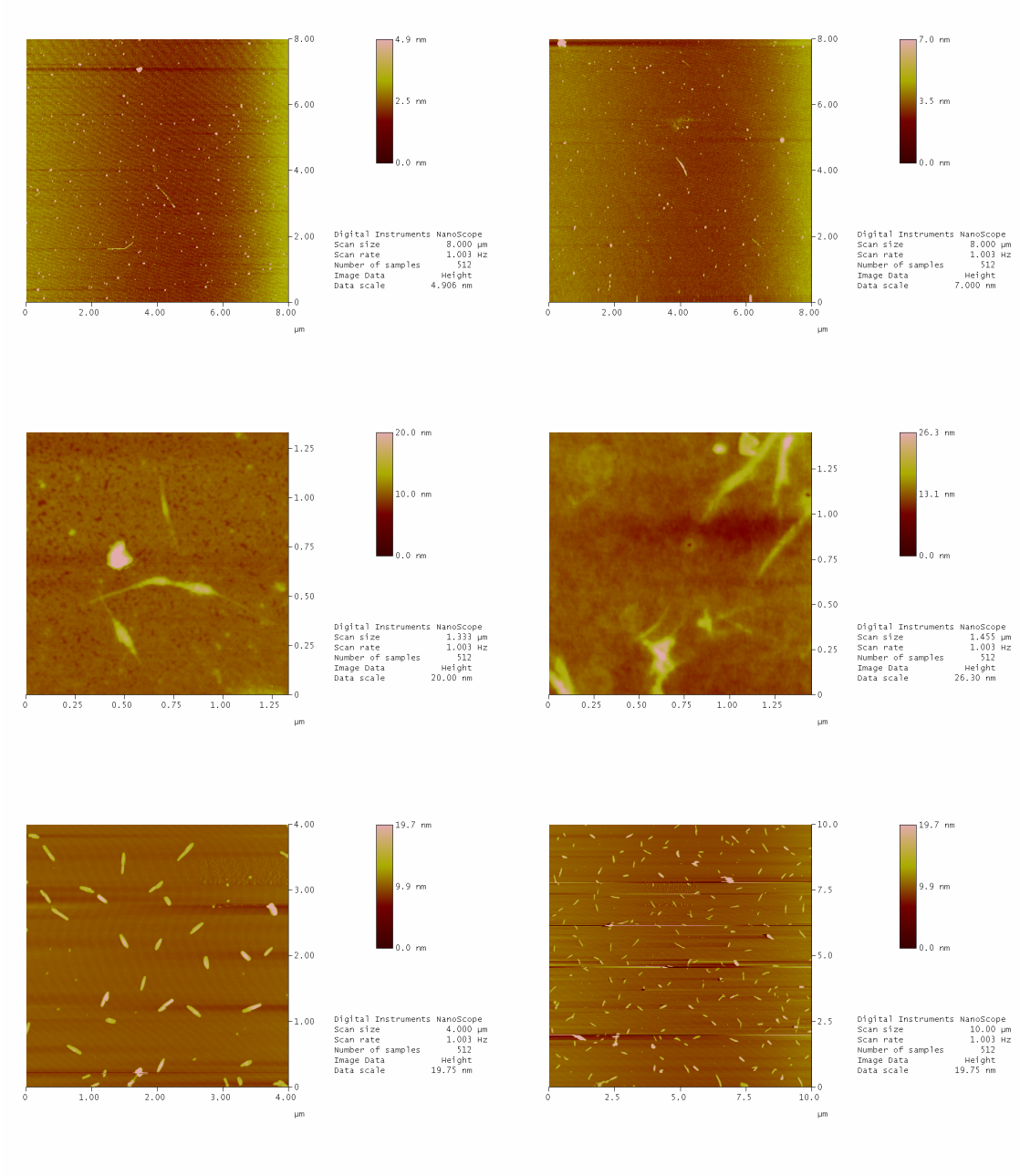

Fig. C AFM topography images of SWNTs coated with NBSC (top panels), NOSC (middle panels) and NPSC (bottom panels).