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Multi-Walled Carbon Nanotubes for Detecting Metabolites

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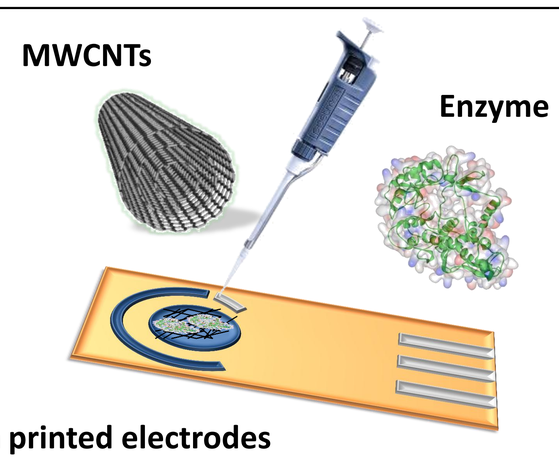
Fabrication of effective and more stable devices for metabolite detection is required for accurate medical diagnosis. Nanostructured electrodes with multi-walled carbon nanotubes (MWCNTs) are proven to increase the active area and to provide an enhanced biomolecule electrocatalysis. MWCNTs seem also to mimic a "friendly" environment for enzymes immobilized by physical adsorption. Protein engineering allows the production of tailor-designed enzymes for their integration into biosensing platforms.
MWCNTs have been integrated by drop casting onto graphite screen printed electrodes and the electrochemical detection of various metabolites has been preformed



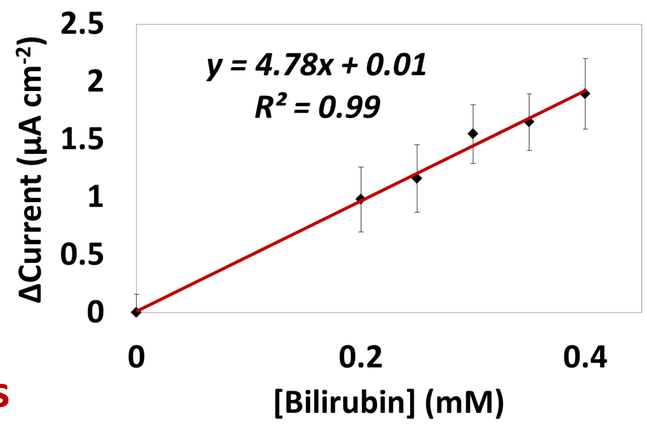
Superior sensing performance of MWCNT-based electrodes

Bilirubin measurements with and without MWCNTs

Electrode	Sensitivity [µA mM^{-1} cm^{-2}]	Detection limit [µM]	R^2
Graphite	5.5 ± 0.9	57.0 ± 9.0	0.89
MWCNT-Graphite	83.4 ± 1.9	8.0 ± 0.2	0.99

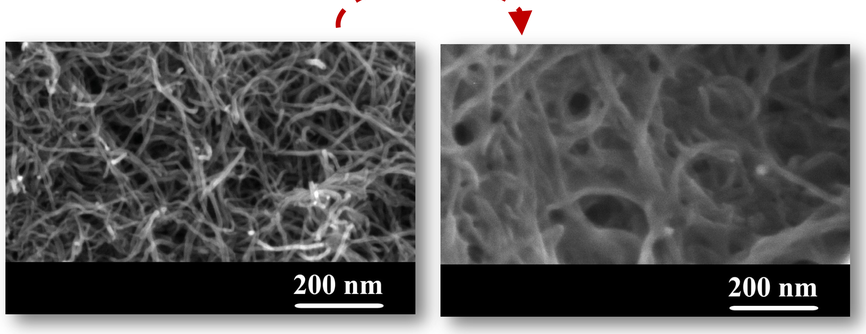
MWCNTs improve the sensing parameters of one order of magnitude and allow bilirubin detection even in presence of normal albumin concentrations

Bilirubin detection in the critical concentration range for neonatal children in presence of albumin



Physically adsorbed enzyme onto MWCNTs

Cytochrome P450 immobilization onto MWCNTS



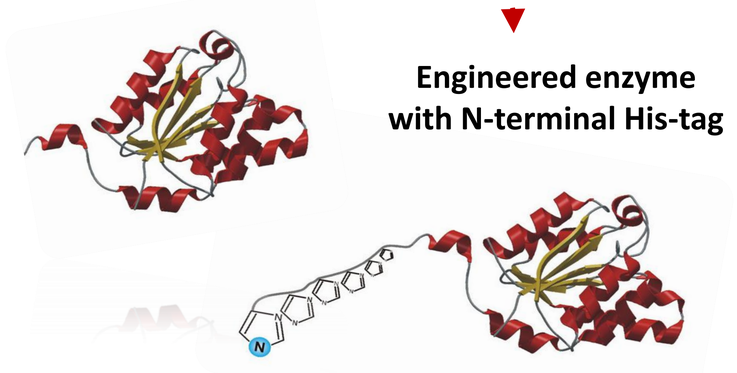
MWCNT diameter doubling means that each tube is surrounded by a single cytochrome P450 monolayer which enables the detection of arachidonic acid

Arachidonic acid detection

Sensitivity [µA mM^{-1} cm^{-2}]	Detection limit [nM]
1140 ± 92	389 ± 31

Engineered enzymes incorporated onto the MWCNT-sensors

Wild type enzyme



The engineered lactate oxidase improves catalysis and selectivity for the substrate and remains active up to 50 days after the immobilization

L-Lactate sensing performance using three different enzyme biosensors including *drop cast* MWCNTs

Lactate oxidase	Sensitivity [µA mM^{-1} cm^{-2}]	Detection limit [µM]	Linear range (mM)
Engineered*	35.6 ± 6.2	30 ± 6	0-1
Wild type (purified)*	18.8 ± 6.8	110 ± 21	0-0.8
Commercial enzyme*	26.6 ± 5.8	58 ± 21	0-1

** Aerococcus viridans*

Conclusions

- The advantage of the MWCNT use has been demonstrated by detecting bilirubin even in presence of albumin, the bilirubin carrying protein
- The physical adsorption of an enzyme has been proved by immobilizing a cytochrome P450 onto MWCNTs for sensing arachidonic acid
- A chemically engineered lactate oxidase has shown higher activity and stability over other enzymes providing an ideal basis for further improvement of next generation lactate sensors

Publications
1. Taurino et al. Sensors and Actuators B, 2011, 160 (1), 327-333
2. Taurino et al. IEEE Sensors Journal, Accepted
3. Taurino et al. Surface Science, 2012, 606 (3), 156-160
4. Taurino et al. 4th IEEE IWASI Conference , 2011, 90-93, Savelletri di Fasano, Br, Italy

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