

Engineering Biocompatibility and Assembly in Carbon Nanotube Electrodes Using the Physicochemical Properties of Chitosan

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1. Introduction

Biomaterials and medical devices constitute a \$100 billion industry that improves the life of millions of people. Devices and implants are used in applications as diverse as blood vessel replacements, catheters, contact lenses, hip joints, ventricular assist devices... The biocompatibility of these prostheses is dictated by their surface properties (their interfaces) and by the local mechanical environment they induce. Nanotechnology has the potential to transform biomaterials by tuning their properties at the nanoscale so as to unlock the body's innate powers of organisation and self-repair. Our aim is to understand the basic science of "biocompatibility" and use it to design novel materials tailored for their specific function.

Here we investigate the basic principles to construct body implantable electrodes that fulfill the following characteristics: biocompatibility (= controlled interface and mechanical properties), and defined hierarchical structure (from nano to micro to mm). We study the formation of nanocomposites of carbon nanotubes with the biopolymer chitosan. Chitosan is a linear biocompatible [1] and bioactive polysaccharide (Fig. 1) extracted from the shells of shrimps that has applications in agriculture, water filtration, self-healing materials..., in medicine it has been used to clot blood, for transdermal drug-delivery, tissue engineering, etc. Chitosan is an amphiphilic cationic polyelectrolyte that adopts different conformations and interactions depending on the balance between hydrophilic and hydrophobic interactions [2]. Here we make composites that combine the conductive and mechanical properties of single walled carbon nanotubes (SWNTs) and the biocompatibility and tunable self-assembly properties of chitosan.

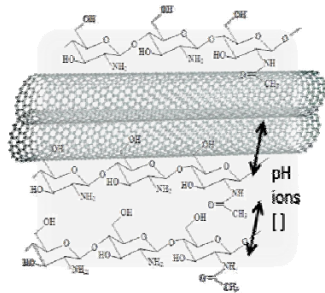


Fig. 1 SWNT-chitosan composite structure depends on the physico-chemical interactions of amphiphilic chitosan with hydrophobic nanotubes which are dependent on pH, ions present in solution and chitosan/SWNT concentration.

The composites structure, composition, interface and mechanical properties are analysed using amplitude modulated atomic force microscopy (AM-AFM), multifrequency

AFM and force spectroscopy in buffered saline solutions.

2. Results and discussion

Interaction of chitosan with individual SWNTs: effect of nanotube length

SWNT were acid treated in a mixture of $\text{HNO}_3:\text{H}_2\text{SO}_4$ (1:3) as described elsewhere [3] for 1 ½ h. This resulted in "short" nanotubes of 25-300 nm in length (analysed using AFM). A mixture of these "short" nanotubes and chitosan (10000:1 in weight) (Sigma 75-85% deacetylated 190-310 kDa ~200-300 nm molecular length determined with AFM) was stirred overnight in sodium acetate buffer with 1 % acetic acid (pH 4) and then sonicated. The pH was chosen to maximize the interaction of single molecules of chitosan with individual SWNTs (ref) This results in homogeneous functionalisation and solubilisation of individual "short" SWNTs with more than one molecule of chitosan (Fig.2 a). "Long" SWNTs of >1 μm in length were treated in the same way with chitosan but this treatment resulted in a different interaction mechanism where the chitosan molecules attach to long SWNT dissolving yet connecting and aligning them Fig 2 (b).

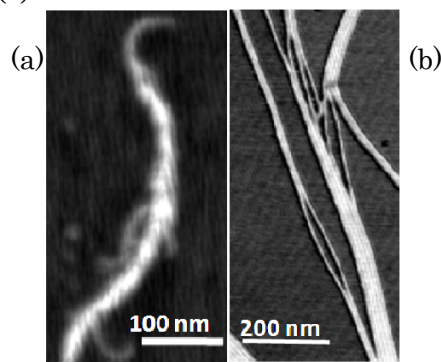


Fig. 2 AM-AFM images of (a) "short" acid treated SWNT coated with several visible chitosan molecules. (b) long SWNTs connected aligned through chitosan interactions.

Nanostructured physically cross-linked network of SWNT-chitosan composites

A concentrated solution of short nanotubes prepared as described above of SWNT:chitosan (2:1) was drop-casted on mica, dried in vacuum and rehydrated with a pH 7 buffer and imaged in solution (Fig. 3 (b)); a chitosan-only hydrogel layer prepared in the same conditions is shown for reference

in panel (a). The (b) layer is compact and the chitosan nanotube interactions produce a local structure of aligned SWNTs, shown in the inset of (b). Fig. 3 (c) shows a monolayer formed in the same way using long ($> 1 \mu\text{m}$) SWNTs. The nanotubes are aligned and the chitosan molecules act as “glue” between them. Fig. 4 (d) shows the corresponding multifrequency AFM 2-eigenmode phase image (the contrast correlates with mechanical properties) where the mica, chitosan and nanotubes are clearly identified.

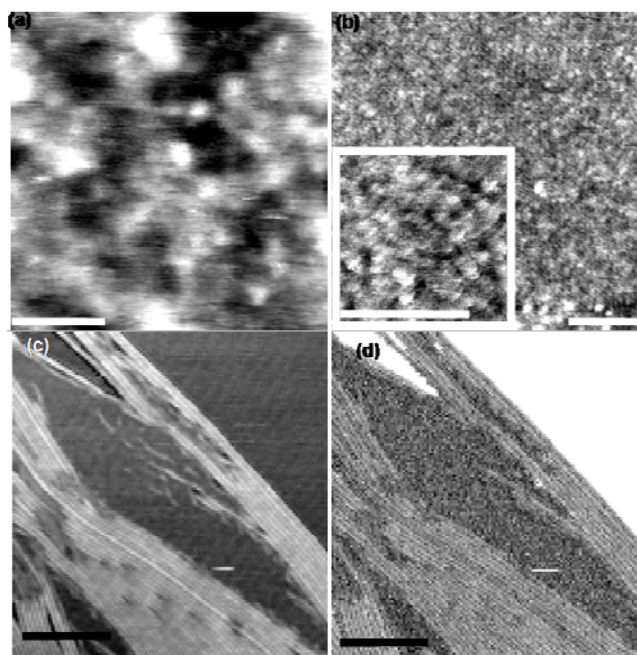


Fig. 4 AM-AFM in pH 7 40 mM MOPS buffer, 5 mM MgCl_2 of (a) a chitosan-only layer; (b) a layer of short SWNTs coated with chitosan, inset shows detail of the alignment of the SWNTs. (c) is a multifrequency AFM height image of long SWNTs-chitosan composite aligned and assembled on a surface of mica, (d) is the corresponding 2-eigenmode phase where mica (white), SWNT (light grey) and chitosan (dark grey) can be clearly distinguished.

The interface with the liquid

The biocompatibility of these surfaces depends crucially on the interface with the liquid; ideally the nanocomposite would have an interface as close as possible to that of biocompatible chitosan. Fig. 5 shows the results of the force spectroscopy comparing a pure chitosan layer, a pure SWNT layer and a SWNT-chitosan layer (all the layers are casted on mica and a mica surface is used for reference). Force curves are used to assess the interface with the liquid [4] (pH7; 40 mM MOPS 5mM MgCl_2) and the stiffness of the layer using the AFM tip as an indenter. The SWNTs do not stick well to the underlying mica and the AFM tip interacts with them at long distance from the surface. The chitosan layer electrostatic/steric interactions are longer than mica ($\sim 15 \text{ nm}$) and the same longer range interaction is detected in the nanotubes/chitosan composite. The different slopes in the indentation region for SWNT/chitosan and chitosan-only layers are due to the higher elasticity modulus of the nanocomposite as compared with the chitosan hydrogel.

3. Conclusions

Short ($\sim 50\text{-}300 \text{ nm}$) SWNT can be individually coated, solubilised and dispersed with chitosan molecules that wrap them through hydrophobic interactions. Layers of physically cross-linked short SWNT-chitosan nanocomposite can be deposited on hydrophilic surfaces (mica) and remain stable in physiological pH solutions. These layers have a higher stiffness than chitosan-only hydrogels while presenting a similar interface with the liquid. The composite presents a controlled nm-scale structure with aligned SWNT: this composite is a good candidate for a biocompatible material where mechanical properties can be tuned using nanotubes of different stiffness and dimensions (e.g. multi-walled carbon nanotubes).

Longer, $> 1 \mu\text{m}$ SWNTs present a different interaction with chitosan, which produces micron-scale alignment of physically connected SWNTs. This provides a novel intriguing possibility of producing biocompatible electrodes. The long range order of the SWNTs structures is expected to produce excellent conductive properties that are currently under investigation.

In conclusion, we have shown that by controlling the pH, the salt concentration, the concentrations of both chitosan and SWNT and the lengths of the SWNT a variety of nanostructured composites can be produced that may have applications as e.g. drug delivery systems, or biocompatible implantable electrodes.

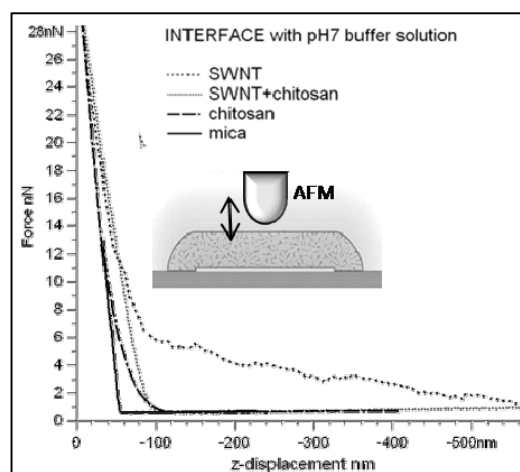


Fig. 5. Force spectroscopy. The AFM tip is approached to the different surfaces and the deflection of a calibrated AFM cantilever (0.5 N/m SiNi TR800 Olympus) is used to calculate the force of the interactions first in the liquid at the interface and then during the indentation of the layers.

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References

- [1] E. Khor, L.Y Lim, *Biomaterials* 24 (2003) 2339.
- [2] A. Domard, *Carbohydrate Polymers* 84 (2011) 696.
- [3] H. Burch, E. Brown, S. Contera, et al. *J Phys Chem C*, 112 (2008) 1908.
- [4] K. Voitchovsky, S. Contera, et al. *Biophys J*. 90 (2006) 2075.