Interaction of carbon nanotube material with rat skin by 21 Tesla MRI

Rakesh Sharma[*,***], K Shetty[*], R Liang[**], CJ Chen[***]
[*]Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Tallahassee, FL
[**]Departments of Industrial and Manufacturing Engineering, and [***]Center of Nanomagnetics and Biotechnology, FAMU-FSU College of Engineering, Tallahassee, FL 32310

ABSTRACT

Carbon nanotubes are emerging as potential tools in drug delivery nanocarriers to target specific cell types and carbon cages filled with metal molecules may improve MRI microimaging. The present paper reviews the current state of art on detection of possible hazards of carbon nanotubes with limitations to use of them as potential biomedical material for their bio-applications. The present report demonstrates the possibility of 30 nm sized carbon nanotubes penetrating in skin tissue very fast during microimaging MRI experiments within 2-3 minutes because of its small size and easy penetration across barrier. It indicates that some types of nanoparticles might pose as tiny toxin to tissue before it is used as nanocarrier, chemotherapeutic nanosphere. The carbon nanotubes showed damage to epidermis and hair across the skin. By histology, main damage was at the level of membrane and nucleus at different epidermis, dermis layers. These features suggest the thorough experimental validation to exclude any chance of carbon nanotubes or nanoparticles before their acceptance as potential tools in health nanotechnology.

Key words: *Carbon nanotubes, MRI microimaging, skin, nanotechnology*

1 INTRODUCTION

Carbon nanotubes (CNT) interact with tissues quickly and get entry across membrane inside the cells and molecular assemblies. Recently, CNT has got attention for their wider applications in both technology and biomedical research as cited in table 1. However, the knowledge of CNT interaction with biological tissues and events of CNT-tissue interface is less known despite of excitement of CNT nanotechnology wider applications [1].

2 MATRIALS AND METHODS

High resolution 3D FLASH T1 weighted MRI was performed in a 21.1T scanner using a Rf birdcage R 15/900 coil (Bruker Biospin) and PARAVISION 3.2 software at NHMFL. The MRI microimaging was performed before and after placing 10 nm CNT in glass capillaries at different intervals of 2, 4, 6 hours using scan parameters: TR/TE/flip angle = 750ms/ 4.18ms/25°, FOV/matrix size/spatial resolution = 2.6×3.4 cm/ 256×256/0.15 mm, and the inversion time (TI approximately 250 ms) set to null normal skin. Epidermis and hair follicle were measured by delineation of areas on images as previously described elsewhere [3].

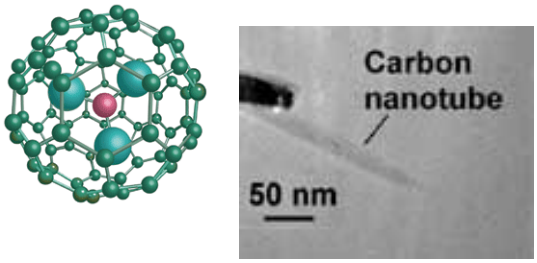

Figure 1: (on left) The carbon nanotube assembly is shown as buckyball to demonstrate as potential carrier of bioactive molecules for application in nanotechnology.

Figure 1: (on right) Transmission electron microscope image of a multiwalled carbon nanotube material attached to tip of tungsten.

3 APPROACH OF MRI MICROIMAGING AT SKIN-CNT INTERFACE

At our laboratory, we have attained the capability of 900 MHz or 21T MRI microimaging for achieving up to 15 micron spatial resolution [2]. Increased awareness of skin health and CNT-tissue interaction may be answered by skin magnetic resonance microimaging (MRM) as quick skin microscopy. The novelty of the present study was to achieve fast 3D FLASH spin-lattice relaxation (T1) weighted images of real time skin-CNT interface and predict the effect of CNT on different skin features with possibility of measurement.

4 RESULTS

The ex vivo MRI 3D FLASH images showed axial, sagittal and coronal images. The coronal images are shown in Figure 1. The images showed distinct morphological and structural features of 3 skin layers. The ex vivo excised skin MRI of the ventral abdomen skin showed epidermis, dermis, hair follicle, sebaceous oil gland as shown in Figure 2.

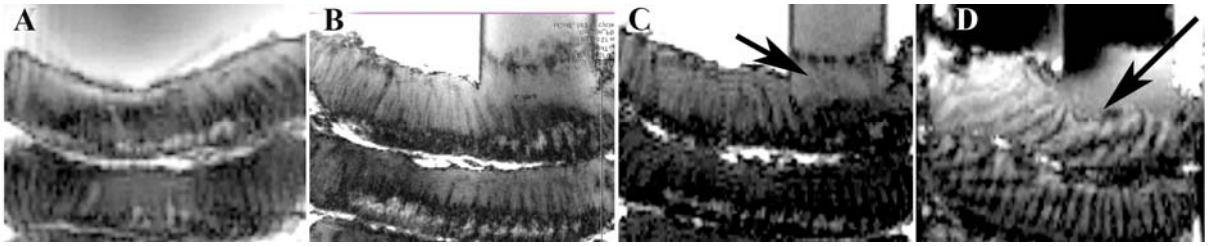

Figure 2: The skin microimaging at 21 Tesla MRI using fast 3D FLASH technique shows control without carbon nanotubes (A); carbon nanotube sample in tube placed for 3 minutes on skin top (B); after 15 minutes carbon nanotube sample in tube placed on skin top (C); after 6 hours carbon nanotube sample stayed on top of the skin (D). Notice the slow damage to epidermis and dermis by carbon nanotubes caused shown by arrow.

Table 1: The table represents potential bio-applications of carbon nanotubes based on tissue-carbon nanotube interaction.

Use in nanotechnology	Particle	Application
Tactile Sensors	Skin sensitive nanofilms	Surgery
Buckyballs	Carbon cages filled with metal molecules	MRI diagnostics
Single-shot Chemotherapy	Nanospheres	Cancer targeting
Tracking Flow in the Brain	Tiny Calcium sensor	Brain mapping Nano-optics
Optical nanotubes	Nanotubes	
Tiny Pumps	Diagnostic nanochips	Blood tests
Nano Particles	Drug delivery carriers	Drug therapy
Nanotubes Neuron-triggers	Small electrodes	Retinal implants
Nano bullets	Drug filled anticancer drugs	Ovarian Cancer
Nanomedicine	Modeling software	Nanoparticle design
Nanowires in the Brain	Brain implants	Cheap and safe
Sensitive Nano Test	Nanotechnology in heart attack, AD	Genetics
Tiny Toxins	Toxicity and free access in tissues	Tracking Toxicity

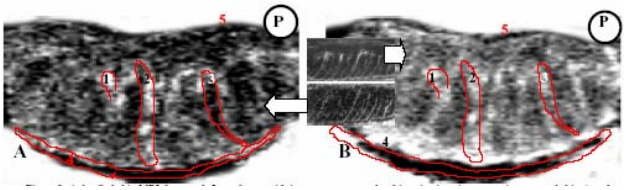

Figure 3: A detailed skin MRI features of hair, dermis and epidermis 1-5 are shown with insert to represent the skin microimaging power in untreated skin (panel A). For comparison, phantom P filled with water was placed. The CNT post-treatment reduced the visibility of skin structural features (panel B) due to damage to epidermis and hair follicle at the CNT-tissue interaction.

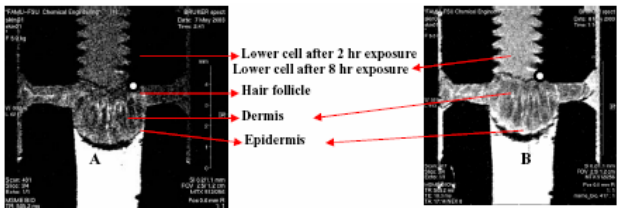

Figure 4: The figure shows distinct epidermis, dermis and hair follicles in post treatment skin images by using two different methods of contrast T1 weighted (panel A), T2 weighted (panel B) after 6 hours to analyze MRI signal intensities as shown in Table 1 as guide to compare the MRI signal intensities.

Table 2: The table represents the display of different MRI signal gray scale intensities on control normal untreated skin as dark hypointense, gray, isointense, bright hyperintensities at low +, moderate ++ and highest +++ levels. Depending upon different weighting schemes of T1-weighted, T2-weighted and proton density weighted images, the skin features of hair, epidermis, dermis and sebaceous gland showed up with different signal intensities at different weighting schemes due to their different proton environments.

Skin MRM features	T1 weighted	T2 weighted	Proton density weighted
Hair follicle	gray	brighter;++	dark
Epidermis	isointense	brighter;+++	brighter;++
Dermis-reticulum	gray	gray	brighter;+
Dermis-papillary	light gray;++	dark gray	brighter;+
Sebaceous gland	isointense;+	hypointense;+	brighter;++
Stratum corneum	brighter;++++	Dark-gray	isointense

Table 3: The table represents post CNT-skin contrast to get information of different skin features on MRM proton density weighted images at TE=15 ms and TR=1500 ms. Notice the distinct MRI signal intensities of different skin features after CNT and epidermis thickening or broken, bizarre and non-continuous appearance shown in Figure 1(panels B-D).

Skin MRM features	Before CNT	Post CNT
Hair follicle	dark	gray
Epidermis thickening	gray; SET = 100 µm	bright; SET = 240 µm
Dermis	gray	bright
Sebaceous gland	dark	bright
Glycolipid	brighter	isointense
Stratum corneum	brighter	gray

The CNT-skin interface showed consistent damage to skin tissue on MRI microimages shown by arrows in Figure 2. The hair follicles remained intact while epidermis membrane and dermis vasculature was badly damaged. The skin features were distinct and measurable. However, the skin features showed different signal intensities on MRI images at different T1, T2 or proton density contrast settings as shown in Table 2. The features were measurable to calculate the size of rat skin features at CNT-skin interface with distinct features after CNT exposure as shown in Table 3 and Figures 3 and 4.

4 DISCUSSION

The CNT material is now widely accepted in various bio-applications as shown in Table 1. Its increasing utility depends on the minimal damage caused by CNT. To accomplish it, skin viability changes at skin-CNT interface provides clue to answer the status of CNT as potential nanotube applicable both in biomedical use and technology. Skin epidermis and hair follicle are indicators of skin viability and represent the surrogate markers of CNT induced skin effect at CNT-skin interface. The MRI signal intensity depends on a number of parameters of protons such as proton density, T1 and T2 relaxation times, bulk motion, diffusion, magnetic susceptibility as main determinants. The cellular distribution of water changes in presence of CNT diffusion across the skin-CNT interface. The Due to change in water or total proton density associated with water physical state at interface is likely to alter MRI signal intensities of epidermis, dermis, and subcutaneous tissue. These changes reflect as contrast and brightness in MRI images. In our choice of 3D FLASH the pulse repetition times and short data collection times (echo times) were short to generate T1 weighted images.

In present ex vivo skin MRM study of CNT-interface, the features of MRM visible skin tissue showed positive correlation with CNT effects as contrast enhancement and epidermis thickening (% SET). However, MRM might overestimate the % SET and hair follicle tissue, especially in the skin epidermis layers. The epidermis is most sensitive and viable layer due to its diffusion properties and it showed the attenuated MRM signal intensity in post-CNT treated skin epidermis layer regions. It displays greater attenuation of MRM signal after 8 hours of exposure perhaps due to disrupted epidermis in the inferior segments. After CNT exposure, epidermis may become dysfunctional and showed up bizarre or thickened on MRM.

In skin, MRM displays different characteristics of each skin layer viz. epidermis, dermis, sebaceous gland and hair follicle. However, the functional epidermis and hair follicle assessment in present study after CNT exposure had uncertainty and limitations. The assessment was validated and it was not homogenous. There are some recognized limitations in the present study. Our rat skin sample size was relatively small and limited.

5 CONCLUSION

Carbon nanotubes exhibit potentials of nanoparticle based imaging of skin structures. These can be potential imaging contrast agents for cell trafficking, metabolic events for real-time analysis.

6 REFERENCES

[1]. N Jonge, Y Lamy, K Schoots, TH. Oosterkamp, *Nature* 420, 393-395(2002)
[2]. R Fu, WW Brey, K Shetty, P Gor'kov, S Saha, et al. *J. Magn. Reson.*, 177, 1-8 (2005)
[3]. R Sharma, BR Locke. 21T MRI microscopy of rat skin. *Proceedings. ISMRM* (2006), Abs 2001