Analysis of cardiac repolarization as a tool for the noninvasive assessment of cardiovascular system exposure to engineered nanomaterials: Pilot Observations

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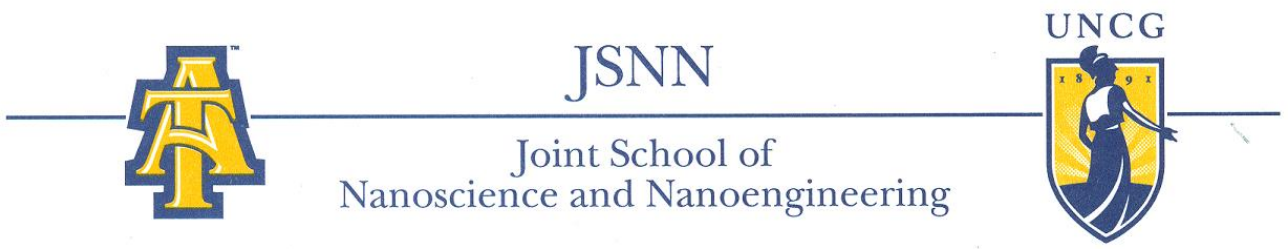


Current Challenges

- Recent findings have demonstrated that just a moderate pulmonary exposure to carbon nanotubes may trigger an oxidative vascular damage which, in turn, may significantly accelerate the formation of atherosclerosis and atherosclerotic plaques

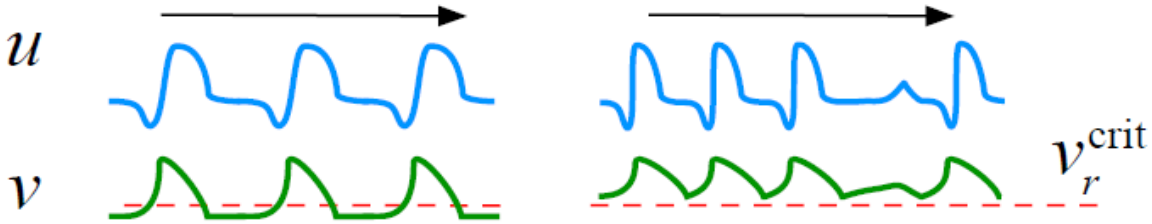
- Although these findings established the importance of biochemical and immunological markers for identifying nanotube induced oxidative stress and vascular damage, the association of such exposure with noninvasive electrophysiological factors is not understood

- Even if monitoring of cardiovascular system using electrophysiological measurements is one of the most robust biomedical tools, it is still not adapted for environmental health and safety studies

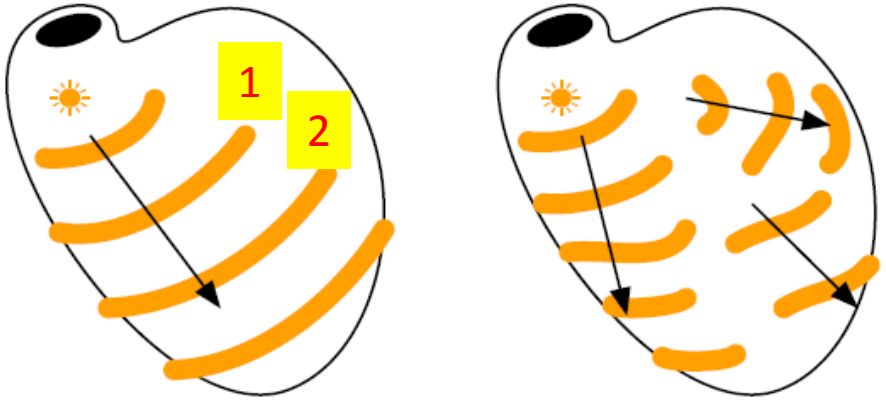
- Development of the non-invasive electrographic predictors of toxic effects on human cardiovascular system is of a critical importance for public health

The central hypothesis of our study is that the dynamics of cardiac repolarization can be utilized for the non-invasive systemic evaluation of cardiovascular nanotoxicity

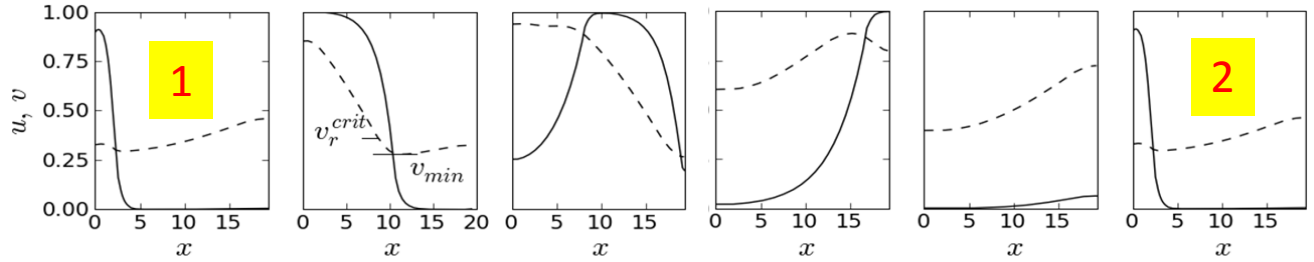
Repolarization, Reserve of Refractoriness (RoR) and Stability of Propagation



Action potential, u, and the level of cardiac refractoriness/repolarization, v.



$v_{min} < v_r^{crit}$ $v_{min} > v_r^{crit}$



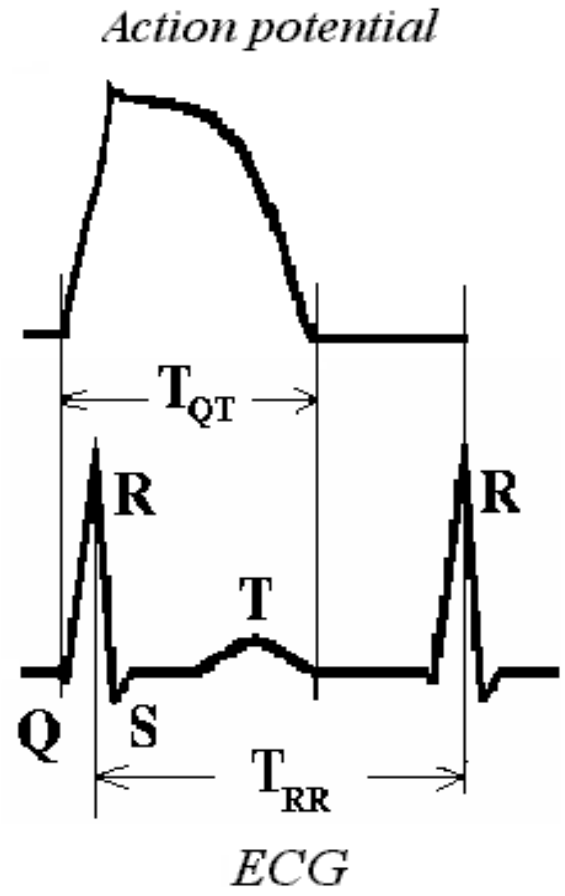
Stable propagation is in place when

$$RoR = (v_r^{crit} - v_{min}) > 0$$

Pending US Patent application, J.M. Starobin et al. No. 14/130,363; 2012; PCT/US2012/044672 filed on 12/31/2013

Solving Reaction-Diffusion Model to Calculate RoR

Action potential, u , reflects itself as the ECG signal at the body surface

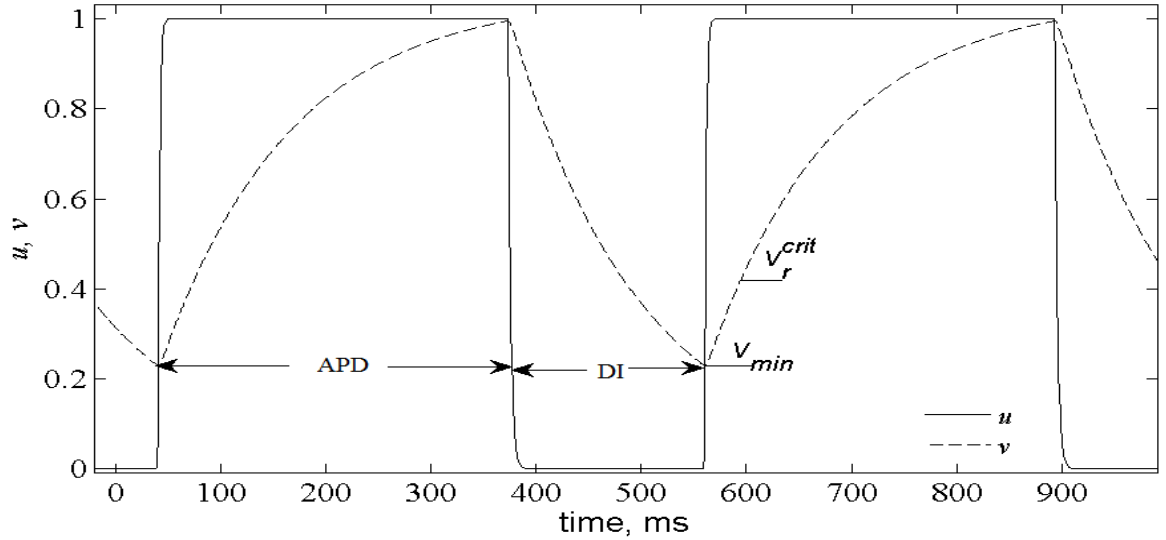


Our method uses a two-variable Chernyak-Starobin-Cohen (CSC, Eqs. 1) reaction-diffusion model, which is analytically solvable and offers a robust criterion for the stability of an excitation wave in an excitable cable, RoR > 0

$$\frac{\partial u}{\partial t} = \frac{\partial^2 u}{\partial x^2} - i(u,v) + P(x,t)$$
$$\frac{\partial v}{\partial t} = \varepsilon(\zeta u + v_r - v) \qquad (1)$$

At fixed values ς and v_r APD (QT/Θ), DI (RR-QT)/Θ and RoR can be found from Eqs. (2), where Θ is a scaling parameter equal to 1ms.

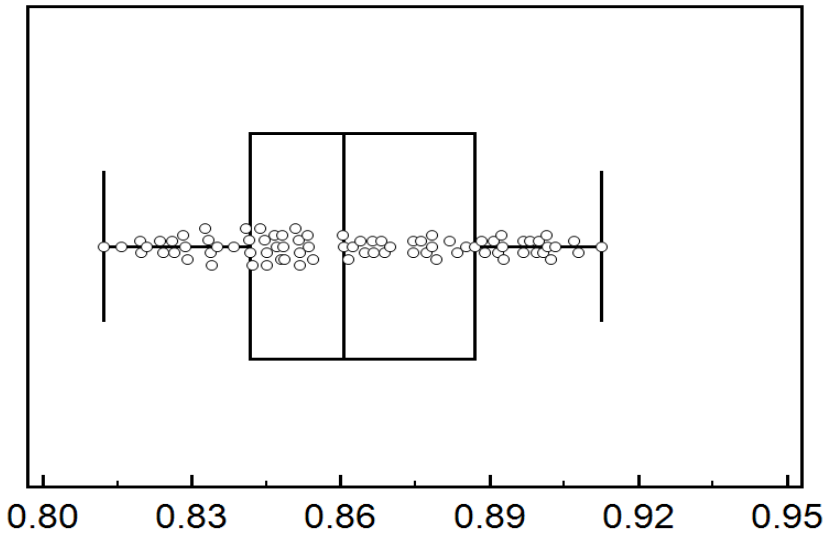
$$APD = \frac{1}{\varepsilon}\ln\frac{\zeta + v_r - v_{\min}}{\zeta + v_r - 1};\ DI = \frac{1}{\varepsilon}\ln\frac{1 - v_r}{v_{\min} - v_r};\ RoR = \frac{v_r^{crit} - v_{min}}{v_r^{crit}} \qquad (2)$$



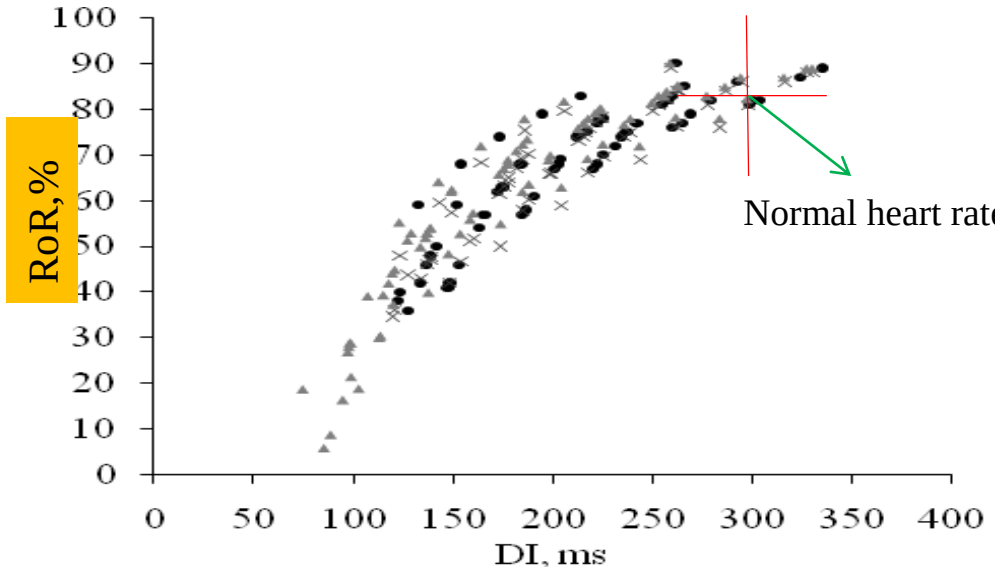
Solving Reaction-Diffusion Model to Calculate RoR in Patients – Pilot Study

funded by American Heart Association, S.F. Idriss et al. Feasibility of non-invasive determination of the stability of propagation reserve in patients. In: *Proceedings Computing in Cardiology*, 2012, Krakow, Poland, 39, 353-356.

Clinical measurements were performed at Duke University Medical Center after obtaining IRB consent on 15 patients of either genders and different ethnic origins, 13-22 years old. All patients were undergoing electrophysiology (EP) testing and ablation for supraventricular arrhythmias. All had structurally normal hearts with normal ventricular function and without known or suspected ventricular arrhythmias.

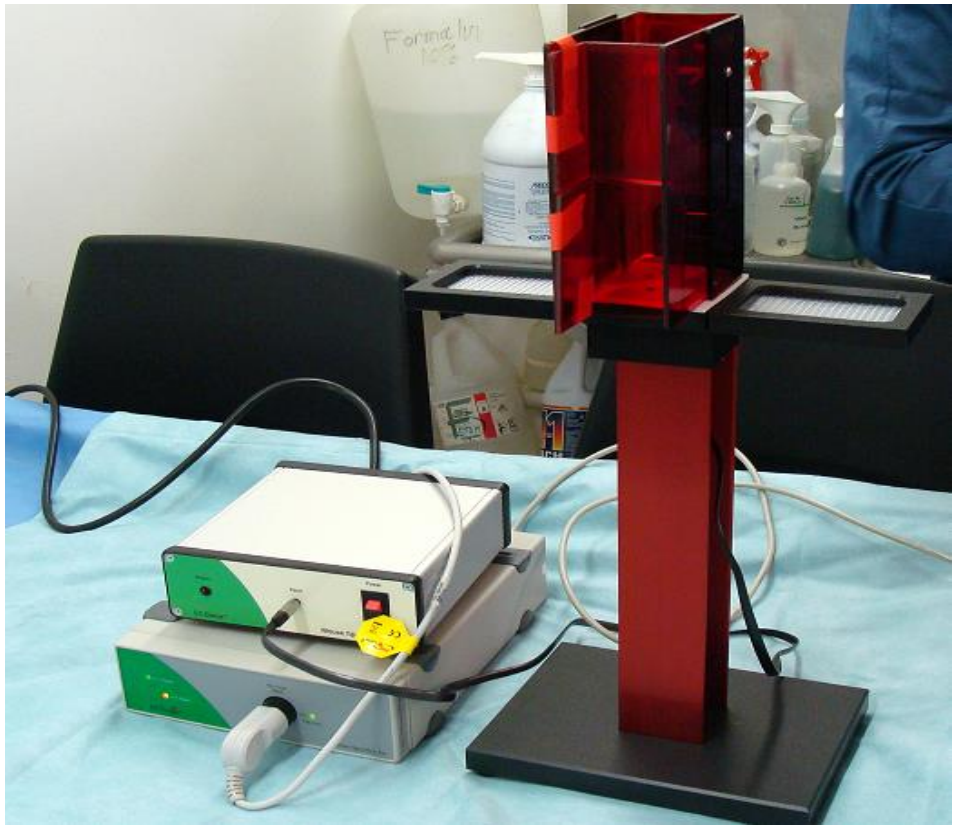


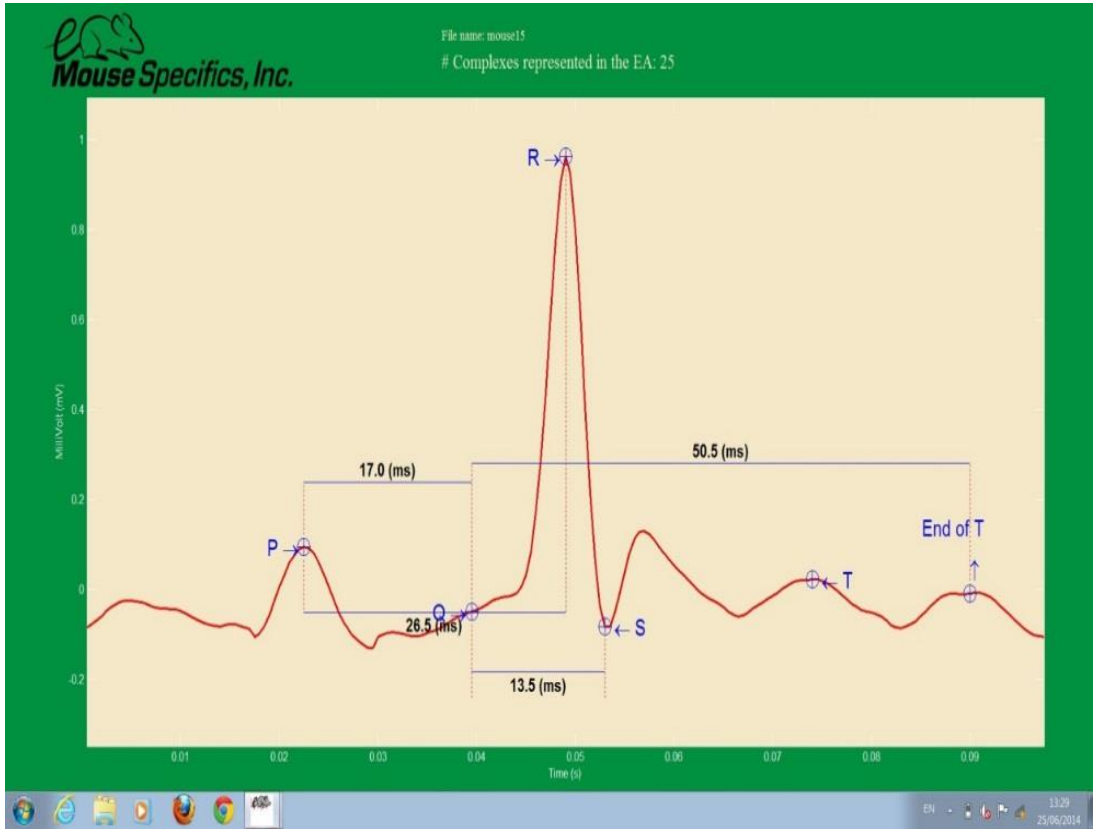
A box plot of the QT_{RV}/QT_{II} ratio of intracardiac and surface QT intervals. The median value 0.8606 is approximately equal to the cos(30°). The 95% confidence interval lies between 0.8491and 0.8697.



Dependence of *RoR* on the diastolic interval DI for all patients and all pacing plateaus. Black dots (●) represent the invasively-measured RoR_{RV}, symbols (x) represent the noninvasive approximation RoR_{app}, and triangles (▲) represent RoR'_{app} computed using the singular limit Eqs. (2).

Non-Invasive ECG System for Small Animals





Left Panel - ECGenie system with its electronics and mice ECG measurement chamber.
Right Panel - Example of QT and RR interval detection performed by ECGenie software.
Advantages: Rapid, non-invasive and longitudinal exposure assessment

Pulmonary Exposure to Engineered Nanomaterials

Goal: To demonstrate the ability to make reliable non-invasive ECG measurements and assess reserve of refractoriness in mice exposed to

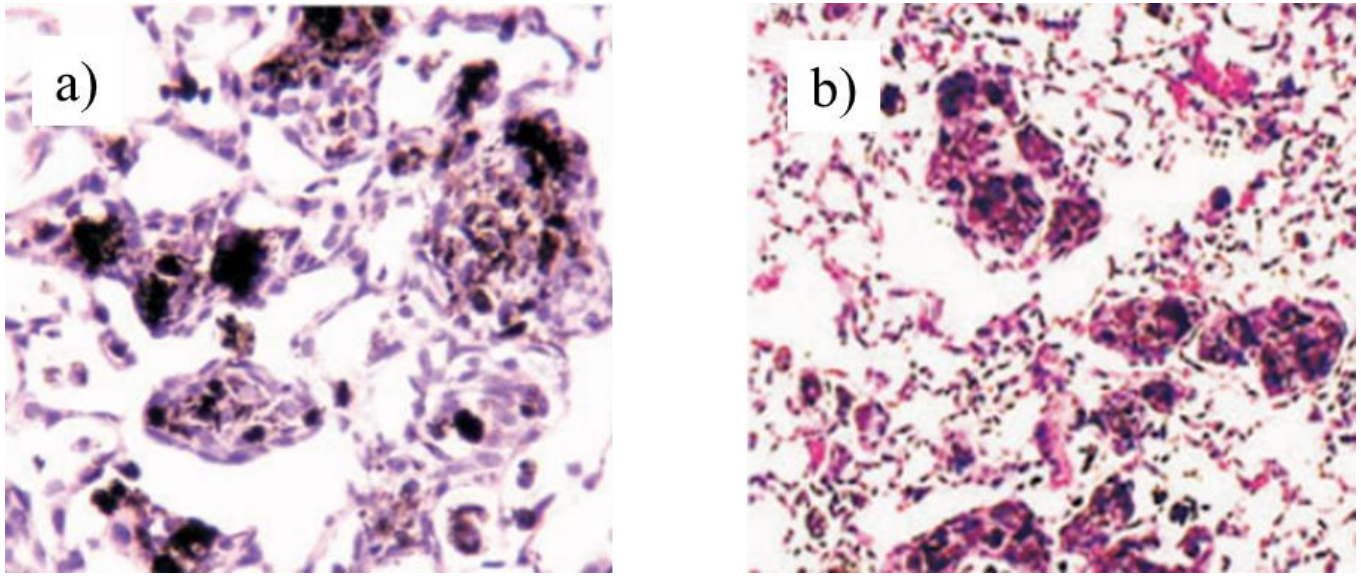
- SWCNTs at normal or elevated stress level (with dobutamine)
- Ceria NPs at normal or elevated stress level (with dobutamine)

Concentrations tested – Normal and elevated stress level

- 0, 40 and 400 µg/mouse dosages of SWCNTs
  - 1-2 nm by 1-3 µm long >90% SWCNT **(**Nanostructured and Amorphous Materials, Inc)
  - Non-ionic copolymer, pluronic F68 in PBS was used as a dispersant to obtain an uniform dispersion of SWCNTs
- 0, 20 and 200 µg/mouse dosages of Ceria NPs
  - 1% ceria dispersion - 150 nm in diameter in water (100-1000x)
  - Ceria dispersions used are the “model” Ceria CMP slurries
- To obtain elevated stress levels
  - Increasing dosages 40, 160 and 320 µg/kg of clinical grade dobutamine, a b1-adrenergic agonist, were intraperitoneally administered to conscious mice to elicit the cardiac response observed during exercise

Pulmonary Toxicity of Single-Wall Carbon Nanotubes (SWCNT)

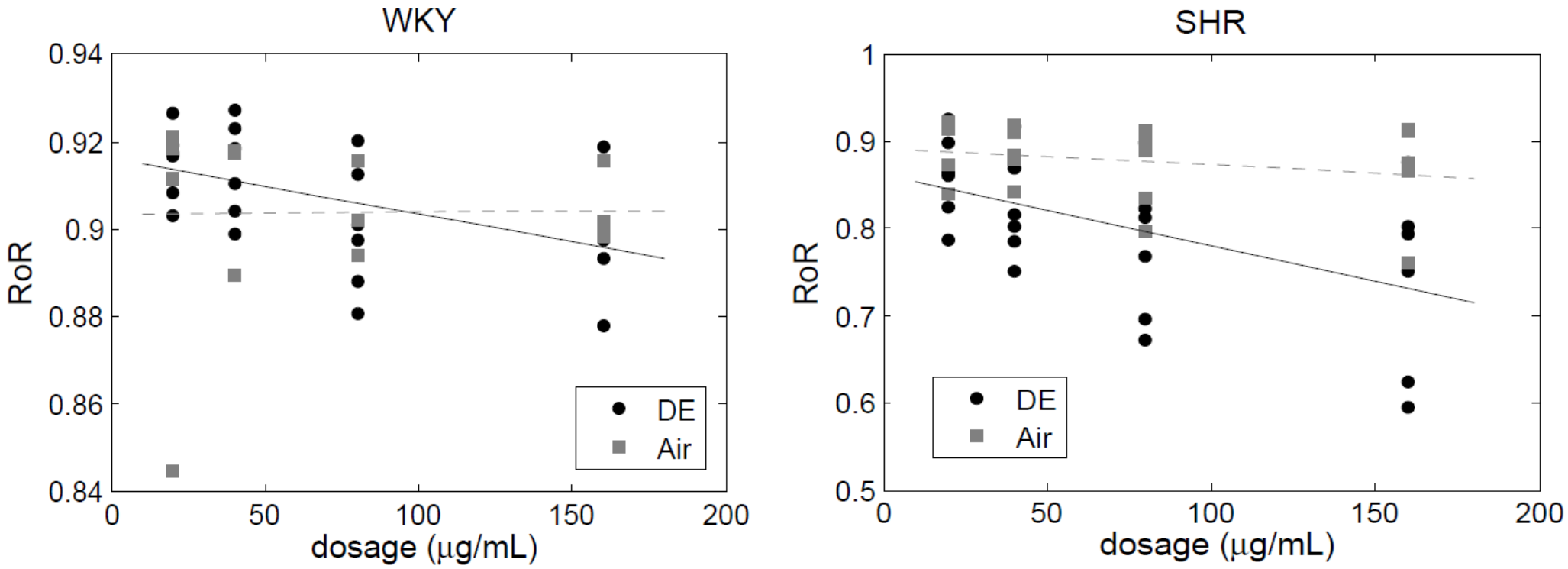
- Previous studies have shown that higher concentrations (200 - 500 µg/mouse) of SWCNTs delivered via intratracheal instillation cause carbonaceous particles accumulation in the lungs or have killed the mice after 4 to 7 days.



Lung tissue from mice instilled with 500 µg of a test material per mouse. Mice were euthanized 7 days after the single treatment (a) and 90 days after the single treatment (b); picture reproduced from C.W. Lam et al. Toxicological Sciences 77, 126-134 (2004)

Diesel Exhaust Worsens Cardiac Conduction Instability in Dobutamine-Challenged Spontaneously Hypertensive Rats

In our previous study *(Hazari et al. 2015)* we found that a single exposure to diesel exhaust (whole body exposed to 150 μg/m^3 for 4 hours) potentiated only minor decrease of RoR in Wistar-Kyoto normotensive (WKY) rats (left panel). However, during the same dobutamine challenge spontaneously hypertensive (SHR) rats revealed a tangible almost on the order of magnitude higher decrease of RoR (right panel).

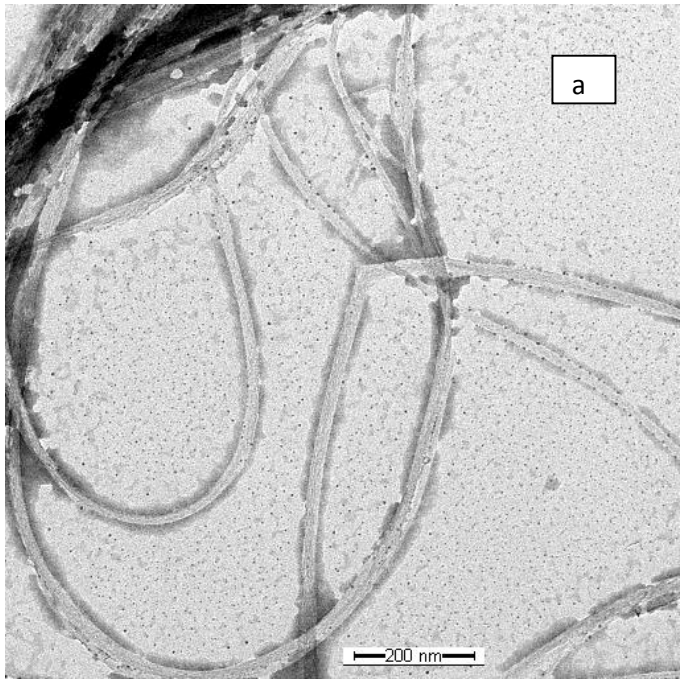


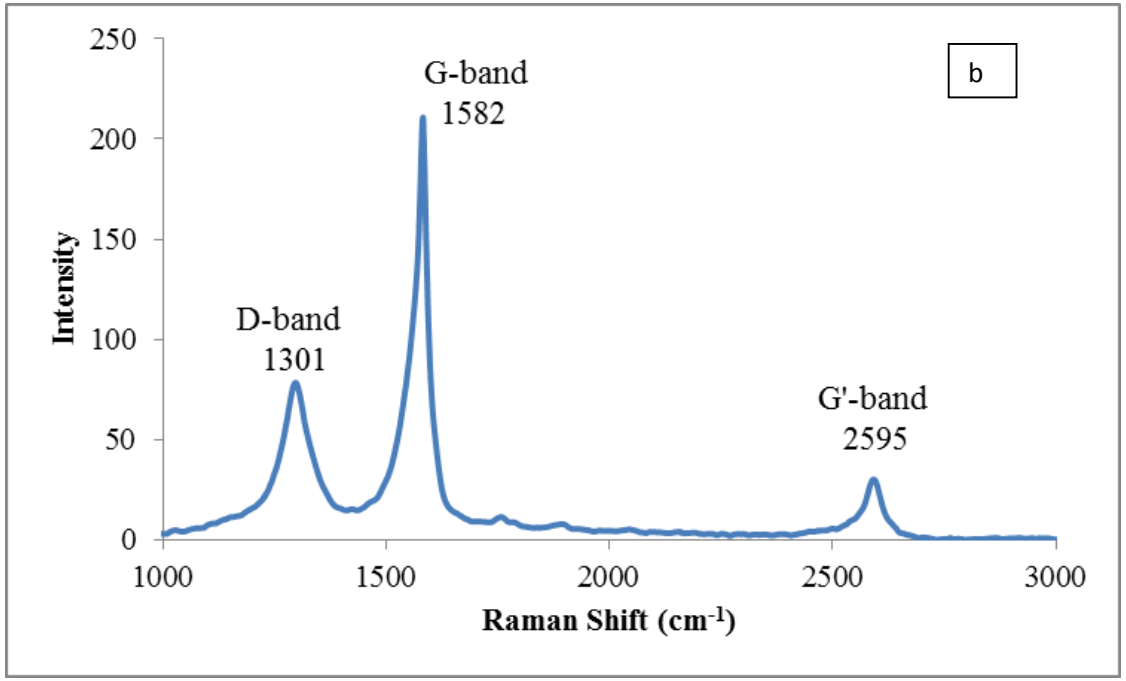
M.S.Hazari, J.Lancaster, J.M.Starobin et al. Diesel exhaust worsens cardiac conduction instability in dobutamine-challenged spontaneously hypertensive rats, Journal of Toxicology, Submitted in 2015

Experimental Sequence

- Female CD-1 Charles River mice (22±2g) 40-49 days age were used for pulmonary exposure experiments
- It was found that one day is sufficient for acclimatization of mice to the ECGenie system to allow reliable data recording.
- Day 0: Baseline ECG recordings on pre-exposed test and control mice
- Day 0: Mice were randomly assigned to the following exposed and control groups:
  - SWCNT groups: low concentration (n=5), high concentration (n=5), and no-exposure/control (n=5) groups
  - Ceria NPs groups: low concentration (n=5), high concentration (n=5), and no-exposure/control (n=5)
  - Lightly anaesthetized animals were exposed via intratracheal instillation to either SWCNTs dispersed in 50 µl phosphate buffered saline (PBS) or Ceria NPs in model ceria CMP slurries
- Day 1, 3 and 7 after SWCNT and Ceria instillation, ECG recordings were performed in all groups of mice.
- After completion of ECG recordings all mice were euthanized with isoflorane followed by cardiac puncture to collect blood, lungs, trachea and heart for ex vivo analyses
- Postmortem 0.5-1 ml blood samples were collected to evaluate blood lactate dehydrogenase (LDH) and differential cell counts to assess toxicity of exposure.
- Organs of the mice (trachea, lungs, heart) were harvested and stored at -80^{0}C for immunohistology and ultrastructural characterization.

Characterization of SWCNTs

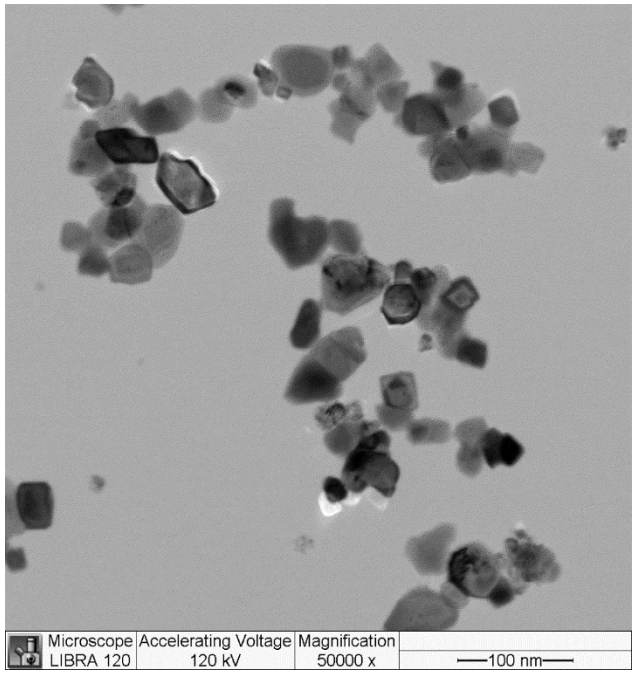




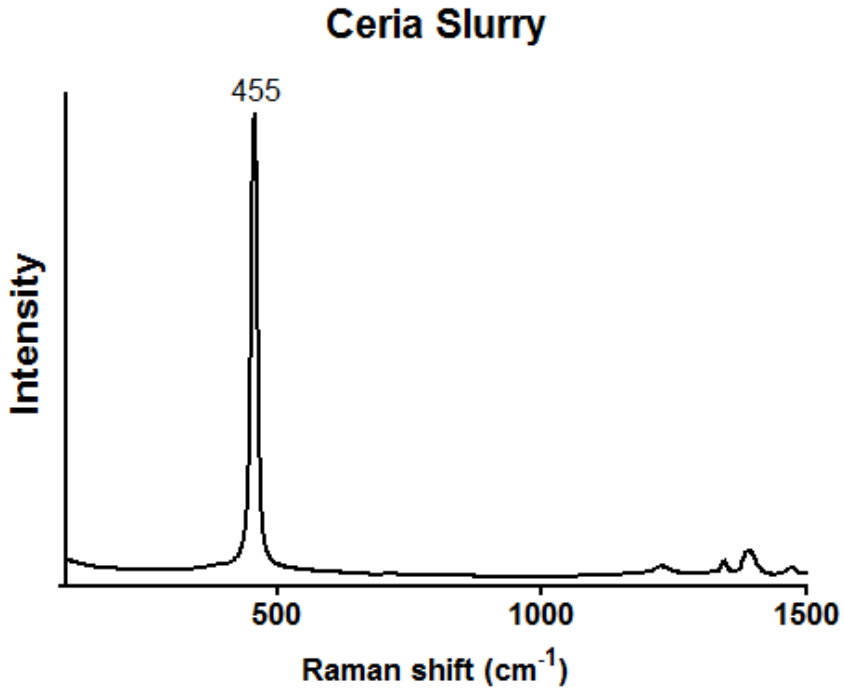
(a) Transmission electron microscope image of CNTs dispersed in 1% pluronic F68 and (b) Confocal Raman spectrum of the SWCNTs dispersion

- SWCNTs (Nanoamor) were dispersed in PBS with 0.1% pluronic F68 (Sigma-Aldrich) by sonication for 2 hours.
- TEM image indicates the dispersion of SWCNTs.
- Raman spectrum on SWCNT dispersion indicates the presence of D, G and G' bands which are typical for the SWCNTs.

Characterization of Ceria NPs in Model CMP Slurries



TEM of Ceria NPs in model ceria CMP slurry



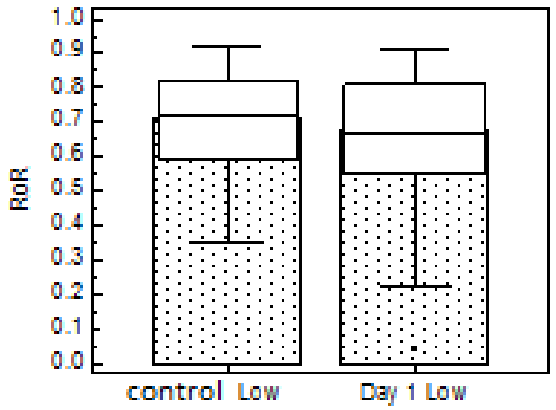
Raman spectrum of model ceria CMP slurry

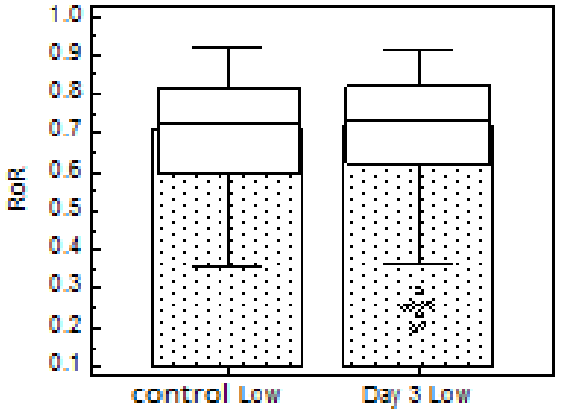
- 1% Ceria NPs in model CMP slurry of pH (3-4) and size (60-100 nm)
- BET surface area – 16.98 m^2/g

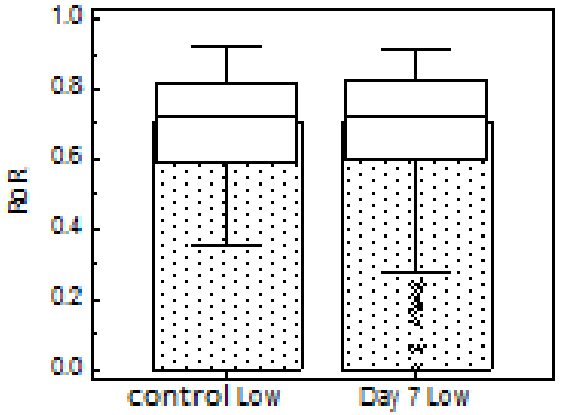
T-test Comparison of RoR Dynamics for Post-CNT Exposure without Dobutamine

(40-low and 400-high, µg/mouse)

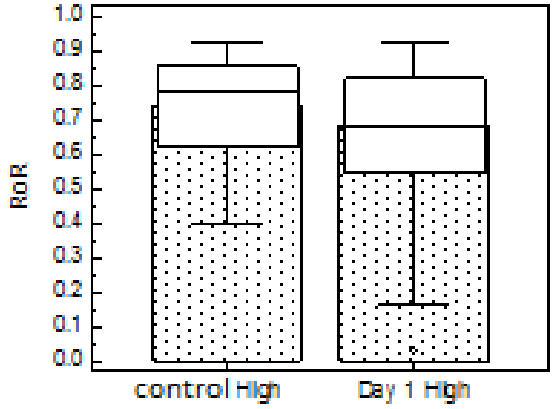
Low (40µg/mouse)

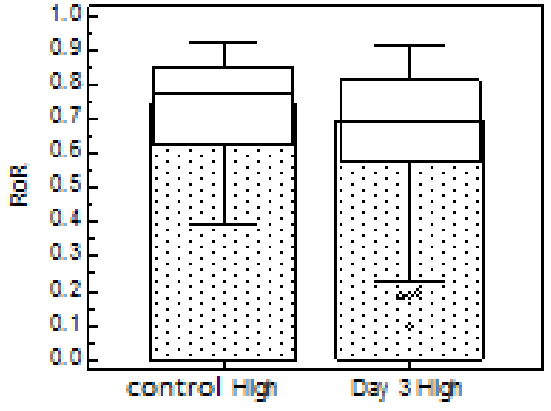


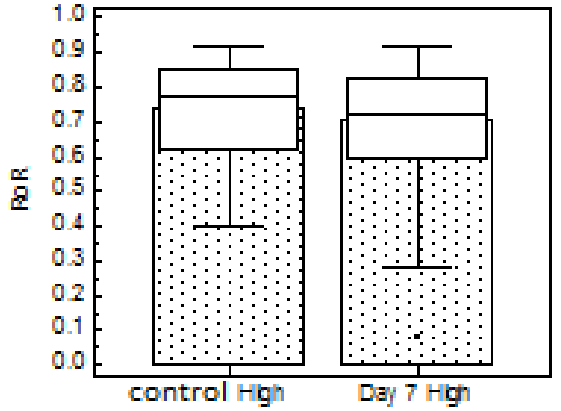




High (400µg/mouse)







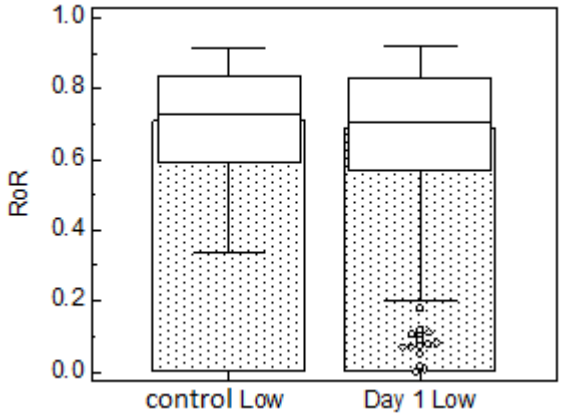
Group	RoR change from Control (%)	p-value
Day 1 Low	-4.2	<0.0001
Day 3 Low	1.69	0.0794
Day 7 Low	-0.54	0.5861
Day 1 High	-7.84	<0.0001
Day 3 High	-6.56	<0.0001
Day 7 High	-4.81	<0.0001

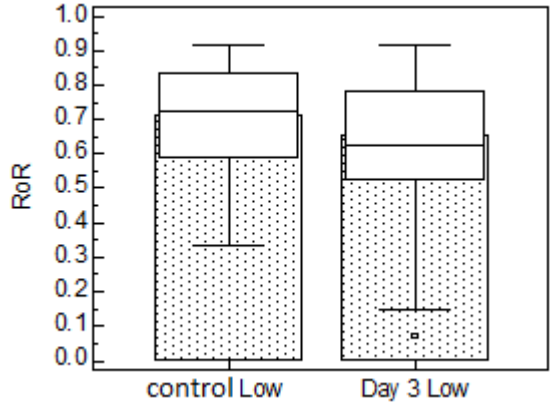
- Low dosage of CNT affects RoR only on the 1st day and diffuses away after that
- High dosage of CNT results in consistent, however relatively small, decrease of RoR through out all 7 days

T-test Comparison of RoR Dynamics for Post-Ceria Exposure without Dobutamine

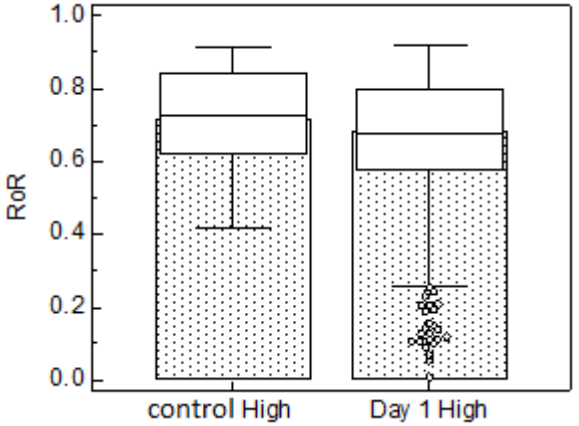
(20-low and 200 –high, µg/mouse)

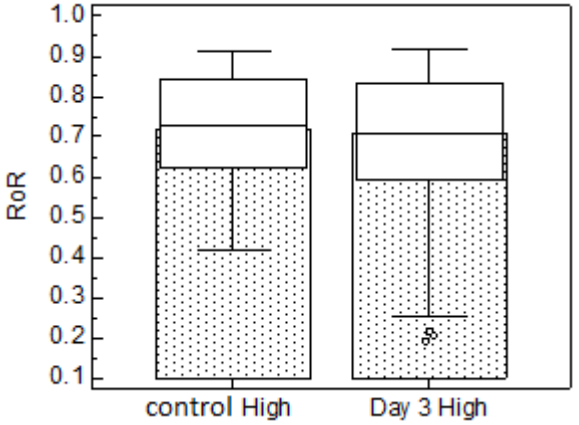
Low (20µg/mouse)





High (200µg/mouse)





Group	RoR change from Control (%)	p-value
Day 1 Low	-2.99	0.0101
Day 3 Low	-8.04	<0.0001
Day 1 High	-4.88	<0.0001
Day 3 High	-1.73	0.0285

- Unlike CNT both low and high dosages of Ceria slightly decrease RoR values on day one and day three

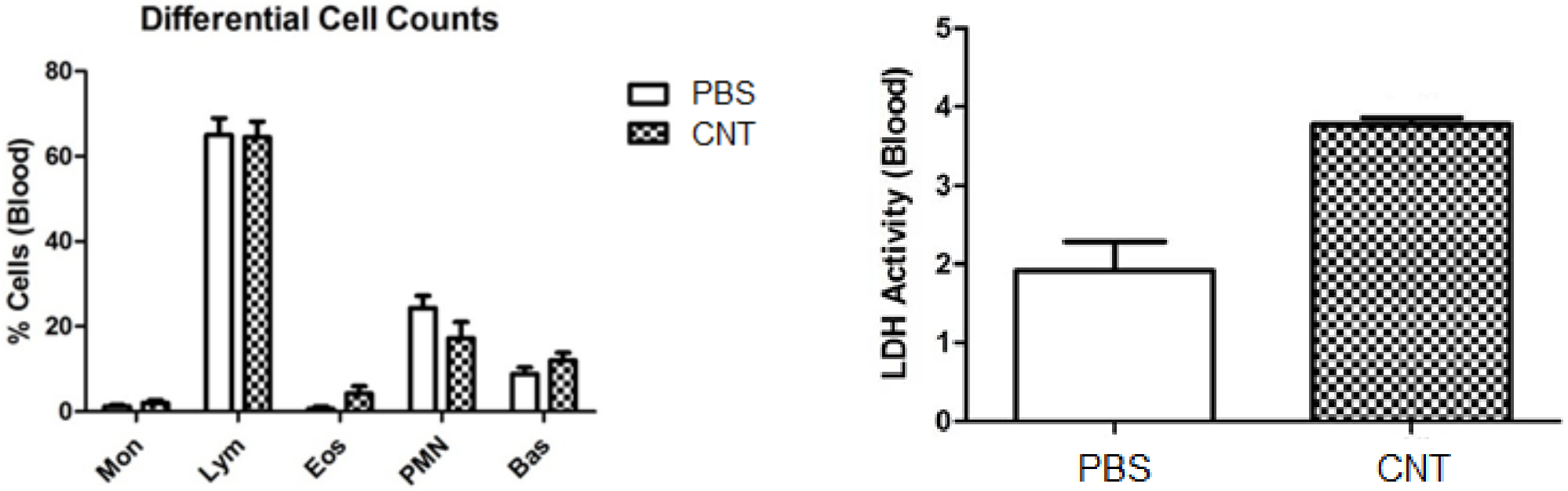
Heart Rate Dynamics for Post-Ceria and CNT Exposure with Dobutamine Stress (Work in progress)

Initial experiments with dobutamine at 40, 160 and 320 μg/kg administered to each mouse intraperitoneally did not elicit the increase of heart rate.

Work is currently in progress with higher dosages (1- 3 μg/g mouse body weight,) of dobutamine to stimulate sufficient increase (~30-50% of baseline) of heart rate to mimic physiological stress [M.S.Tang, 2007]

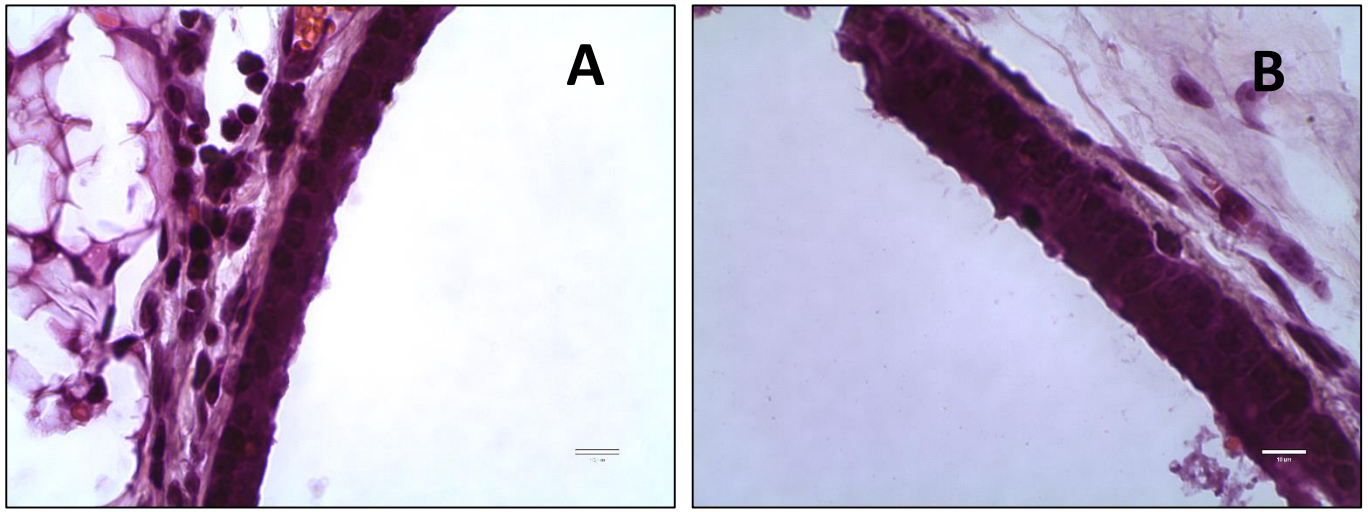
M.S.Tang, T.Ramunddal, M.Lindbom et al. Native cardiac reserve predicts survival in acute post infarction heart failure in mice, Cardiovascular Ultrasound 2007, 5:46

Comparative Blood Tests for PBS and SWCNT Exposure without Dobutamine: Pilot group n=5

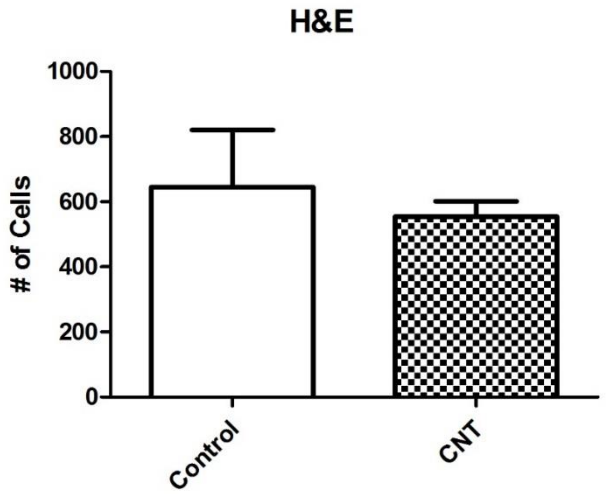


- **Left Panel** – differential blood cell counts for monocyte (Mon), lymphocytes (Lym), eosinophils (Eos), neutrophils (PMN) and basophils (Bas)
- **Right Panel -** assessment of serum blood lactate dehydrogenase (LDH) increase (T-test, P=0.0025)
- Since LDH is insufficiently specific marker of myocardium injury, especially in the absence of electrocardiographic changes, this may indicate some cellular damage related to lungs or kidney rather than to myocardium

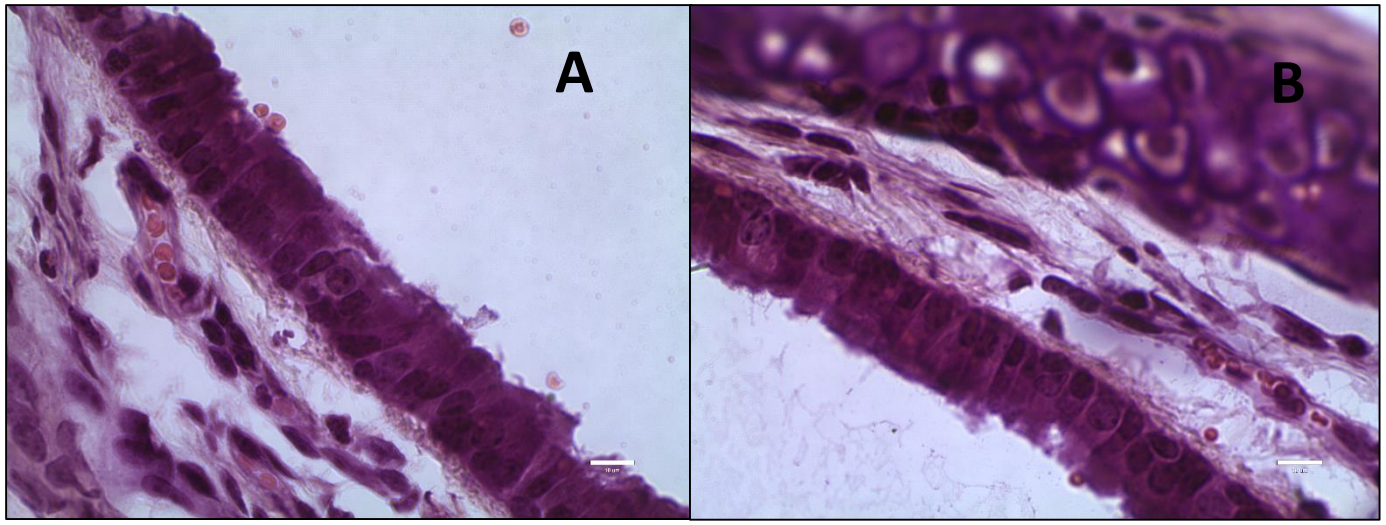
Preliminary immunohistochemical analysis



A and B Trachea of control mice (#2) stained for H&E. Mag 1000x.



There were no differences in the number of cells present in the tracheal epithelium of mice treated or not with carbon nanotubes. Two-three slices from each mouse were counted and averaged. N=3.



A and B Trachea of mice treated with carbon nanotubes (#7) stained for H&E. Mag 1000x.

Conclusions

We found that:

- Exposure to different concentrations of ceria caused more pronounced decrease of reserve of refractoriness and stability of cardiac propagation than exposure to corresponding dosages of SWCNT
- Toxic influence of SWCNT caused decrease in reserve of refractoriness as well as the increase of blood level of lactate dehydrogenase in a pilot subgroup of mice

Work in Progress and Future Directions of Research

- Work in progress on biochemical and tissue analyses for all mice to investigate immunohistological markers and for ultrastructural characterization of tissue samples
- Work in progress to investigate the influence of stress induced by dobutamine
- Examine effects of SWCNT/Ceria exposure to mice with cardiac deficiencies such as atherosclerosis, hypertension and/or arrhythmias

Acknowledgements

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- Joint School of Nanoscience and Nanoengineering for continuous support of our project
- SRC/Cabot for model ceria CMP slurries