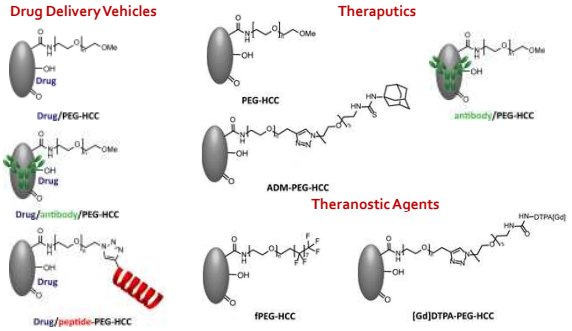
# *Nanotechnology: What Is It and Why Does the Clinician of 2015 Need to Know About It?*

*James M. Tour*
*Department of Chemistry, Rice University*
*www.jmtour.com*

RICE

Smalley Institute

## Variations on a Theme




Samuel, E. L. G.; Marcano; D. C.; Berka, V.; et al. *Proc. Nat. Acad. Sci.* **2015**, *112*, 2343–2348.
Samuel, E. L. G.; Duong, M. T.; Bitner, B. R. et al. *Trends Biotechnol.* **2014**, *32*, 501-505.
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Bitner, B. R.; Marcano, D. C.; Berlin, J. M. et. al. *ACS Nano* **2012**, *6*, 8007–8014.
Sharpe, M. A.; Marcano, D. C.; Berlin, J. M. et. al *ACS Nano* **2012**, *6*, 3114–3120.
Sano, D.; Berlin, J. M.; Pham, T. T. et al. *ACS Nano* **2012**, 6, 2497– 2505

## PEG-HCCs are non-toxic

- no toxicity in cell culture
- no toxicity *in vivo*
  - no signs of discomfort, fatigue, or weight loss
  - no toxicity to heart, lungs, spleen, kidneys, liver, or brain
  - unchanged renal and liver markers
  - normal hematology
- PEG-HCCs accumulate mostly in the spleen, liver, and kidneys; are excreted through the urine and bile duct
- blood half-life is 2 to 3 hours after i.v. injection; 27 hours after s.c. injection

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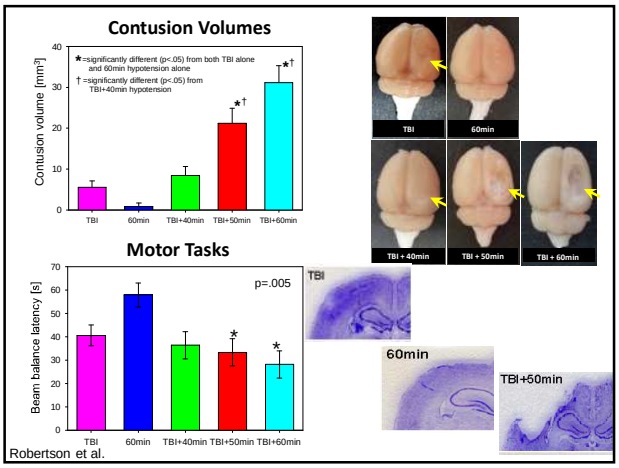
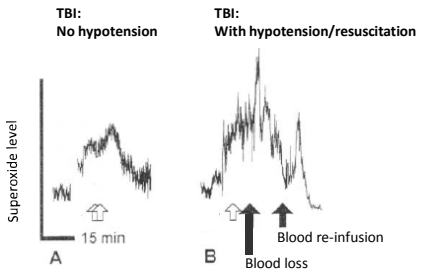



Robertson et al.

## *Oxidative Stress During TBI*




⇧ = existing antioxidant therapy: PEG-SOD

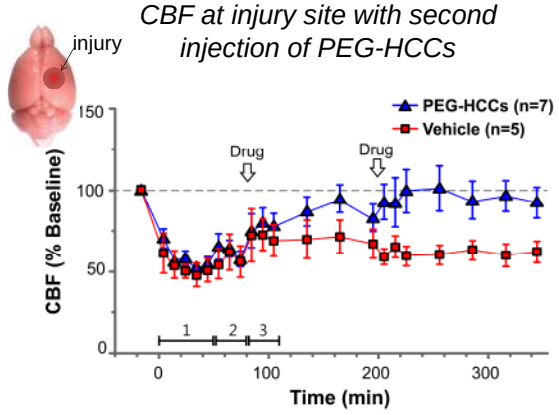
Fabian, DeWitt & Kent, JCBF Met 1995

## *TBI Model*

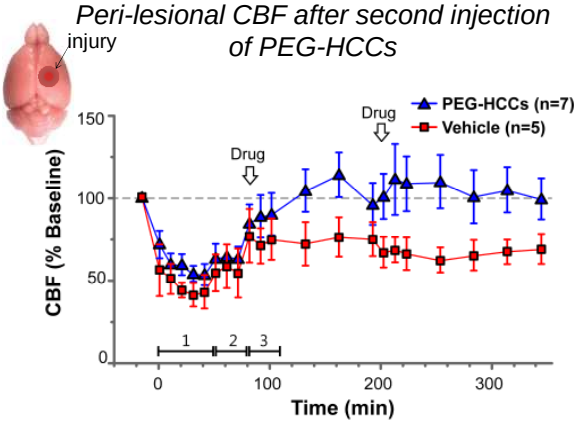

| Injury and hemorrhage (50 minutes) | Prehospital (30 minutes) | Definitive care (30 minutes) | Test outcome (2-6 h) |
|---|---|---|---|
| • Ventilate (air)<br>• Mild TBI<br>• Withdraw blood to MAP 40 mmHg | • Ventilate (air)<br>• Initial resuscitation-saline MAP ≥ 50 mmHg | • Give drug<br>• Ventilate with 100% oxygen<br>• Reinfuse blood<br>• Saline to MAP ≥ 60mmHg | • 0-6 h: Monitor cerebral hemodynamics<br>• 6 h: Determine superoxide (SO) and nitric oxide (NO) levels |

Chart: Adapted from Claudia Roberson, MD

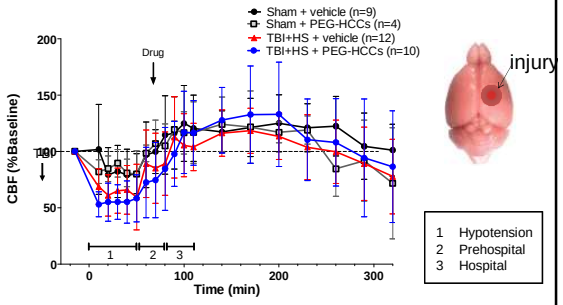
## *CBF at injury site with second injection of PEG-HCCs*




## *Peri-lesional CBF after second injection of PEG-HCCs*




## *No significant difference in CBF contralateral to TBI after drug treatments*



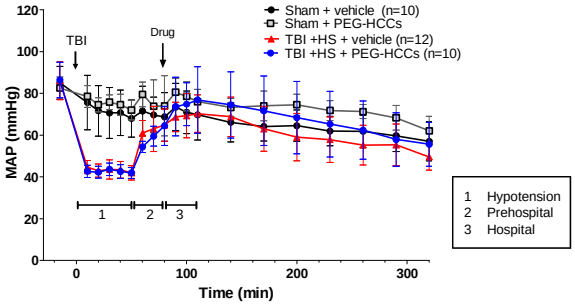




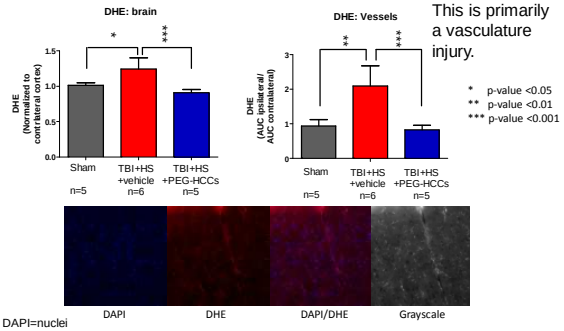
1 Hypotension
2 Prehospital
3 Hospital

Blood flow measured at on cortex contralateral to injury site (side opposite of the injury).
Control for reperfusion injury caused by hypotension period.
Mean + standard deviation

## *No significant difference in mean arterial pressure (MAP) after PEG-HCC treatment*




## *PEG-HCC treatment reduces staining for superoxide (SO) anion in the brain and cerebral vessels*




This is primarily a vasculature injury.

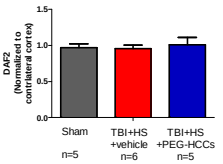
* p-value <0.05
** p-value <0.01
*** p-value <0.001

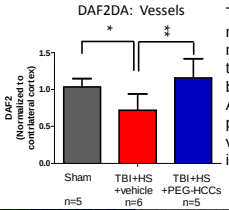



DAPI=nuclei
DHE= superoxide staining (dihydroethidium)
AUC= area of the curve (used to quantify DHE fluorescence in a cross section the vessels)

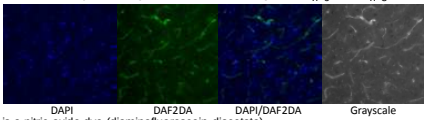
## *PEG-HCC treatment increases staining for nitric oxide (NO) in cerebral vessels*

DAF2DA: Brain
no significant differences




There is minimal injury in mTBI unless there is low blood pressure. Again, this primarily a vasculature injury.



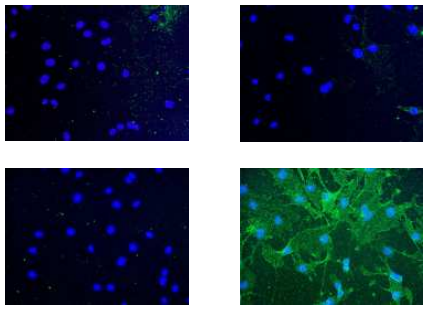

DAF-2DA is a nitric oxide dye (diaminofluorescein diacetate)
AUC= area of the curve (used to quantify DAF2DA fluorescence in a cross section the vessels)
DAPI = nuclear stain
Grayscale = same image as the DAF-2DA (just easier to see the vessels which are white on this image)

## *In Vitro Targeting for TBI: Rapid Cell Binding*

PEG-HCCs anti-p-selectin/PEG-HCCs

p-selectin-
(-histamine)

p-selectin+
(+histamine)

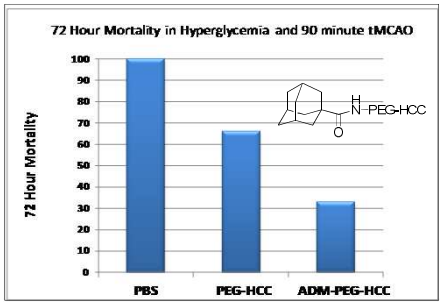


By simply mixing the PEG-HCCs with an antibody against p-selectin, we demonstrated that the construct was targeted to brain endothelial cells induced to upregulate p-selectin (by treatment with histamine). Similar upregulation of p-selectin occurs rapidly following trauma.

## *Preliminary Testing of PEG-HCCs in a Stroke Model*

- *Model of transient middle cerebral artery occlusion with a thread inserted at the base of the brain to restrict blood supply through the carotid artery*
- *To mimic clinical administration of a recanalization therapy, the thread is removed at a clinically relevant time point (90 min)*
- *For this study, the most severe version of the model was tested which is performing the occlusion in a rat that has been made hyperglycemic with a pancreatic toxin, streptozotocin*
- *PEG-HCCs or adamantane (ADM)-linked PEG-HCCs were administered in hyperglycemic rats with a 90 min transient middle cerebral artery (tMCA) occlusion*

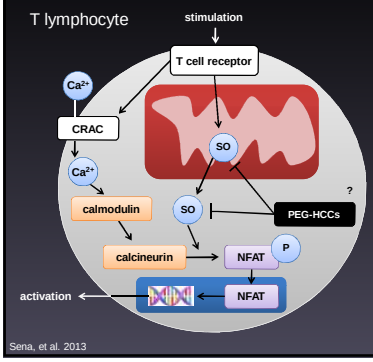
## *Preliminary Results: Mortality (n = 16 rats)*




*Vehicle treated rats have **100%** mortality with transient middle cerebral artery occlusion*
*Treatment with PEG-HCCs at the time of recanalization reduced mortality to **66%***
*Treatment with ADM-PEG-HCCs further reduced mortality to **33%***

PEG-HCCs for the treatment of multiple sclerosis and rheumatoid arthritis

## Superoxide (SO) is required during T cell activation

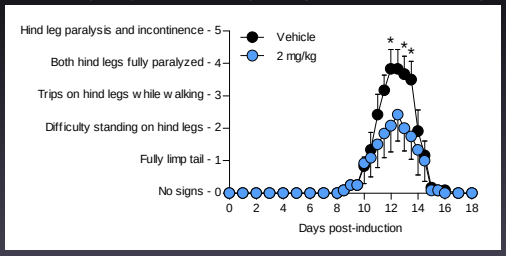



Stimulating T cells induces the production of mitochondrial SO, which is necessary in a signaling pathway responsible for the activation of T cells. Pathogenic T cells have their SO-mediated pathway for activation inhibited by PEG-HCC. The SO pathway is a necessary pathway to complement the Ca pathway.

Sena, et al. 2013

## PEG-HCCs treat paralysis in an acute rat model of multiple sclerosis

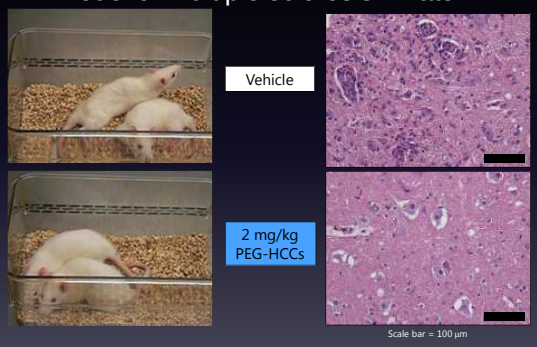
Treated every 3 days subcutaneously starting at the onset of clinical signs




*N* = 6 rats/group; **P* < 0.05

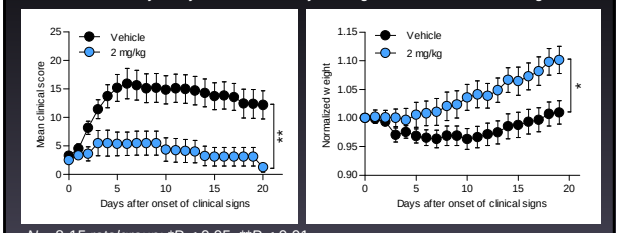
4 = wheelchair-equivalent; 2 = cane-equivalent

## PEG-HCCs reduce the severity of an acute model of multiple sclerosis in rats




## PEG-HCCs reduce disease severity in a rat model of rheumatoid arthritis

Treated every 4 days subcutaneously starting at the onset of clinical signs




*N* = 8-15 rats/group; **P* < 0.05, ***P* < 0.01

Scoring for each rat: 1 point per swollen/red finger or toe, 1 point per mid-foot digit or knuckle, 5 points per wrist or ankle

## Conclusions

- PEG-HCCs are not generalized immunosuppressants unlike most current treatments
- PEG-HCCs treat rat models of autoimmunity
- The selective uptake of PEG-HCCs by T cells can be utilized as a novel targeted therapy

## *Further Mechanistic Features*

Turnover numbers (moles of consumed $O_2^{\bullet-}$/moles of PEG-HCCs) were as high as a dramatic ~1.3 million or 87,000 $s^{-1}$ (confirmed >20,000 $s^{-1}$ by stop-flow) which is an order of magnitude higher than most efficient single-active-site enzymes, and suggests that a PEG-HCC could possess multiple catalytically active sites, underscoring the advantage of a nanoparticle over a small molecule or enzyme. Furthermore, 2.4 nM of PEG-HCCs are able to scavenge 2.8 μM of $O_2^{\bullet-}$ and 53.7 μM of $^{\bullet}OH$, while being inactive toward $NO^{\bullet}$ and $ONOO^-$ (peroxynitrite increases if excess $O_2^{\bullet-}$ reacts with $NO^{\bullet}$, but we scavenge $O_2^{\bullet-}$).

## *Why does the clinician of 2015 need to know about nano?*

Nano-based drugs can have remarkable capacity and kinetics that are not seen with small molecules or enzymes– hence they are a complementary class of therapeutic agents

Mission Connect Mild Traumatic Brain Injury Consortium, Dept. of Defense (W81XWH-08-2-0141, W81XWH-08-2-0143)
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**Disclosure: J. M. Tour is a shareholder in Acelerox LLC and Avid Chemotherapeutics LLC which both seek to commercialize the PEG-HCCs. This is managed by the Office of Compliance, Rice University**

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Lizanne Nilewski
William Sikkema
Dr. Jason Mann
Dr. Almaz Jalilov
Dr. Daniela Marcano
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