Advancing Regulatory Science

Insight into How Carbon Nanotubes Cause a Thrombus: Activation of Platelets Through "Store-Operated" Calcium Entry

CBER study provides data critical to developing effective techniques for evaluating carbon nanomaterial biocompatibility with blood

"Carbon Nanotubes Activate Store-Operated Calcium Entry in Human Blood Platelets" ACS Nano 5(7):5808-5813 (2011)

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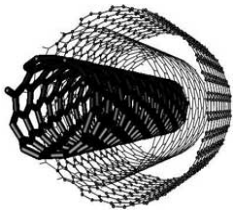
Carbon Nanotubes: Medical Promise & Regulatory Challenge



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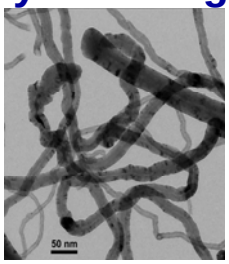
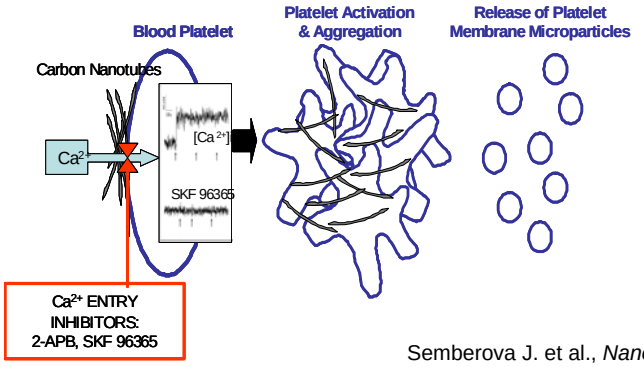
Carbon Nanotubules



Carbon nanotubes (CNTs) represent a major advance in nanotechnology and may have numerous medical applications. CNTs are increasingly being developed for use in diagnostic biosensors, drug delivery nanosystems, intravascular imaging nanoprobes, and other devices that contact blood. Recent research suggests, however, that CNTs in the blood can cause thrombus formation by activating platelets. Derangements of platelet activity can cause thrombosis, a leading cause of death and disability in the developed world. CBER scientists in OBRR are clarifying the molecular basis for platelet activation by CNTs. Such knowledge will help FDA develop techniques to predict whether a specific CNT-based drug, biologic, or medical device poses a potential threat of thrombus formation.

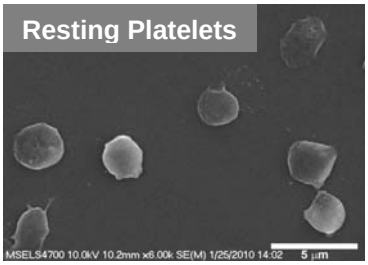
A Link Between Calcium Inflow and Platelet Activation



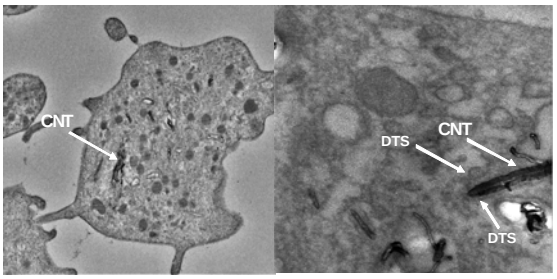
Semberova J. et al., *NanoLetters* 2009

Calcium ions are required for blood clotting. Normally, the flow of extracellular Ca^{2+} into cells occurs through a specific channel in the plasma membrane. This flow occurs in response to a decrease in the concentration of Ca^{2+} in intracellular organelles, such as the endoplasmic reticulum. In platelets, the organelle storing Ca^{2+} is the dense tubular system. The mechanism by which Ca^{2+} loss from such storage organelles controls the influx of extracellular Ca^{2+} is called "store-operated Ca^{2+} entry" (SOCE). Previous work by OBRR scientists (Semberova J. et al., NanoLetters 2009) provided the following insights into CNT activation of platelets:

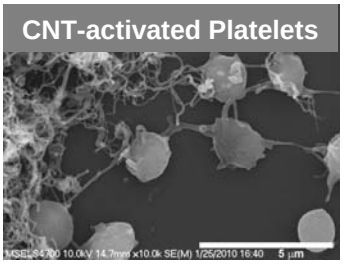
- Various CNTs induce human platelets to aggregate and release of platelet membrane microparticles
- CNT-induced platelet aggregation is dependent on the influx of extracellular Ca^{2+}, a process that is sensitive to Ca^{2+} entry inhibitors



CBER Scientists Visualize the Effect of CNTs on Platelet Activation



TEM showed that CNTs penetrate the platelet membrane and interact with the dense tubular system (DTS).



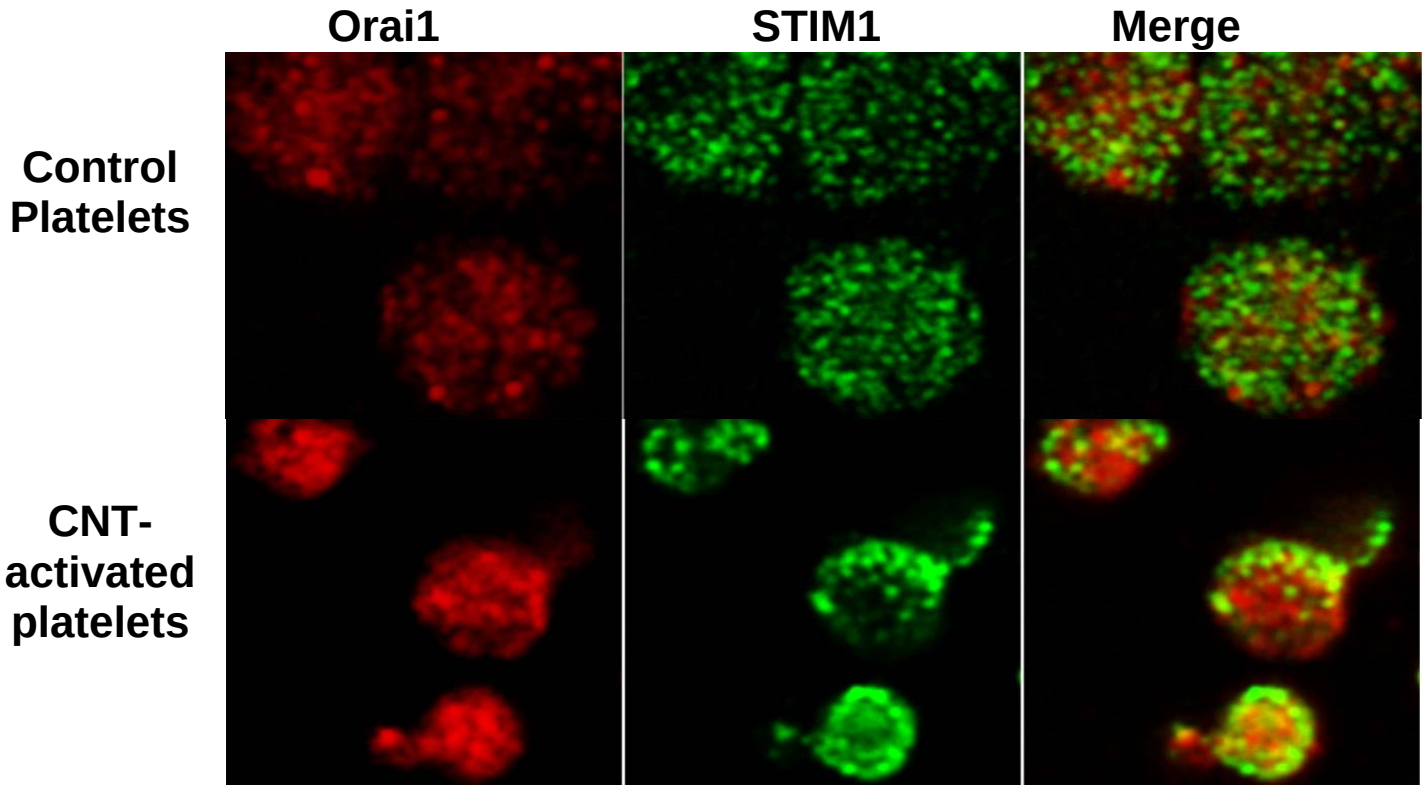
Summary of the Study Results

Recent work by OBRR scientists further clarified the molecular mechanism of CNT activation of platelets involving SOCE. The two major protein structures that control SOCE are:

- Orai1: molecules that span the plasma membrane.

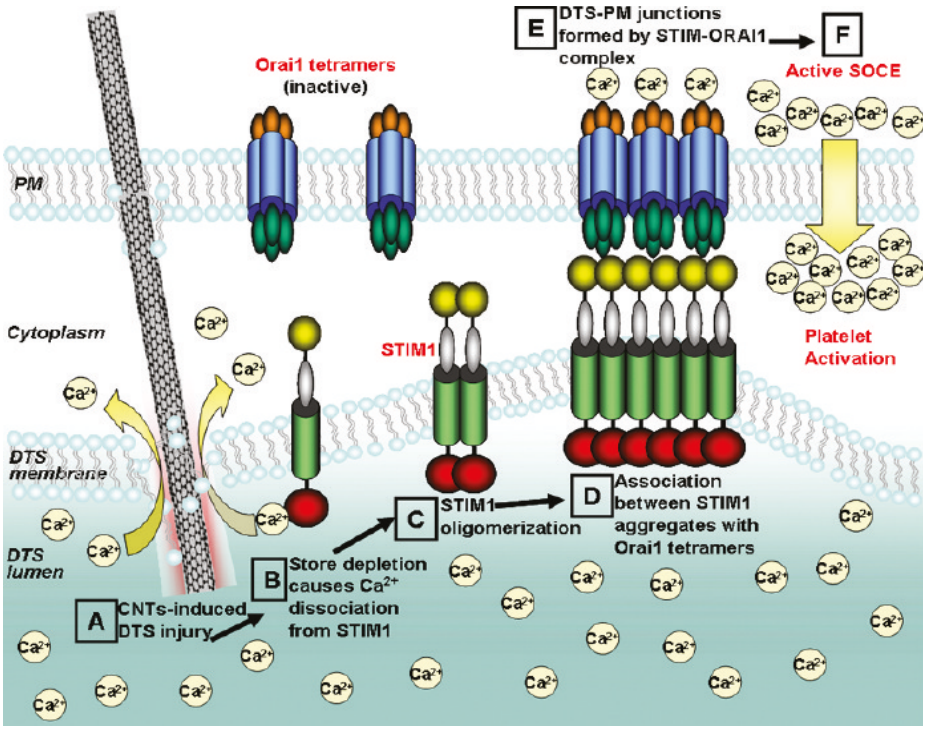
- STIM1: molecules found mainly in the dense tubular system membrane that sense the loss of Ca^{2+} from this organelle. After sensing Ca^{2+} loss, STIM1 molecules form oligomers and complex with Orai1 molecules. The Orai1 and STIM1 complex form the basic SOCE unit inducing extracellular Ca^{2+} influx.

- CNTs penetrate the platelet plasma membrane without causing discernible damage to the membrane.
- CNTs that penetrate the platelet membrane interact with the dense tubular system, leading to depletion of their Ca^{2+} content.
- Depletion of these Ca^{2+} stores is accompanied by the oligomerization and clustering of STIM1 and Orai1, which indicates activation of SOCE.



Confocal microscopy of platelets treated with CNTs. STIM1 aggregate and co-localize with Orai1 following Ca^{2+} release from the dense tubular system, as shown by the yellow color in the **"**merge**"** panel.

Model of a Mechanism of CNT-Induced Platelet Activation by CBER Scientists



These findings explain the molecular mechanisms by which CNTs induce platelet activation.

This new knowledge will be critical to developing techniques that FDA reviewers can use to evaluate the biocompatibility of carbon nanomaterials with blood, thereby ensuring the safety of approved CNT-based medical products.