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the influence of surface area and chemistry

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ROS Activity in Carbonaceous Nanoparticles: The Influence of Surface Area and Chemistry

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Conclusions

Cross-validation of ROS assays

- Robust agreement between ROS production across EPR_{CPH} , acellular and cellular $DCFH_2-DA$ assays (Figure 1), and hemolytic activity (Figure 2).
- Nanographene had stronger $DCF_{ACELLULAR}$ response compared to EPR_{CPH} .
- EPR (dry state, no spin probe) measures free electrons, probably inside particle, with no clear relationship to biologically relevant ROS production.

ROS production and surface properties

- Low ROS production for sp^3 -hybridized (diamond) nanomaterials.
- ROS production of sp^2 -hybridized nanomaterials mainly driven by surface area but,
- ROS production was modulated (4-fold) by surface composition and significantly enhanced by sulfur oxide moieties at the carbon surface.
- Linear model based on XPS surface composition to predict ROS surface production ($R^2 = 0.99$, Figure 3).

Background

- IARC classifies Carbon Black in Group 2B, "possible human carcinogen".
- Surface area is an effective dose metric to describe production of reactive oxygen species (ROS) and both in vitro and in vivo response and recent in vivo studies suggest a direct route of genotoxicity through ROS production and oxidative stress.
- But, specific surface area alone cannot explain all variation in ROS production from carbon nanomaterials.

Aims

- Cross-validate intrinsic ROS and surface-related ROS production of multiple assays.
- Link ROS production and physicochemical properties of the carbon nanomaterials.
- Assess ROS vs shape toxicity pathway by evaluation towards Hemolytic Potential.

Methods

Intrinsic ROS:

- Electron Paramagnetic Resonance (EPR) at room temperature (295K) and 77K liquid nitrogen temperature (localized states).

Surface-related ROS production:

- EPR with CPH spin probe (EPR_{CPH}) – 10 min incubation
- Acellular ROS Production using $DCFH_2-DA$ ($DCF_{ACELLULAR}$) - 3h incubation
- Cellular ROS Production ($DCFH_2-DA$) in A549 epithelial cells ($DCF_{CELLULAR}$) - 3h incubation

Physicochemical characterization

- Brauner-Emmett-Teller (BET) - specific surface area (SSA)
- CHNSO combustion elemental - bulk elemental composition
- X-ray photoelectron spectroscopy (XPS) – surface elemental and chemical composition

Cytotoxicity

- Hemolytic Potential assay in sheep red blood cells (SR0051B)

ROS Production

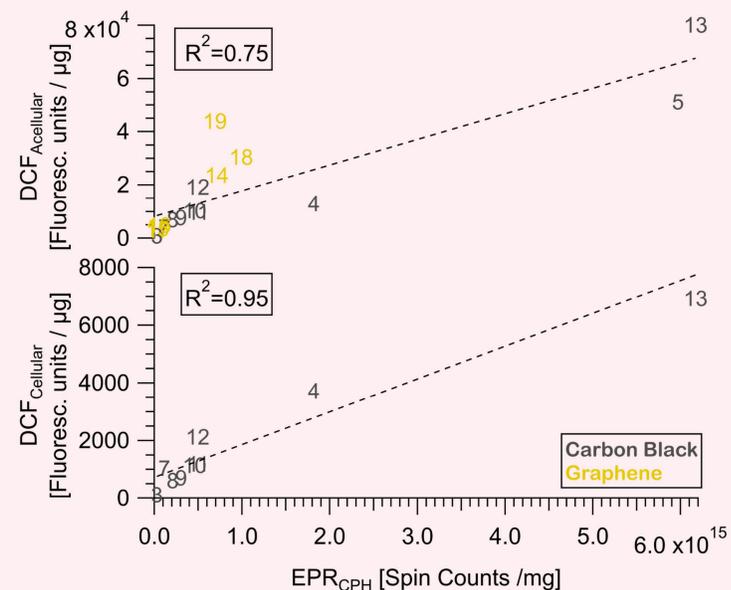


Figure 1. EPR_{CPH} versus $DCF_{ACELLULAR}$ (top) and $DCF_{CELLULAR}$ (bottom) and linear regression slopes (dashed lines).

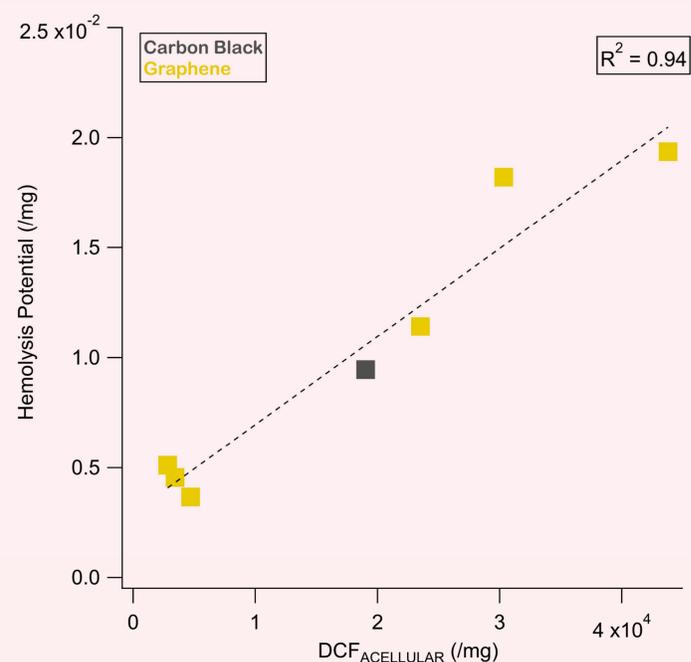


Figure 2. Hemolysis Potential versus $DCF_{ACELLULAR}$ ROS production and linear regression slope (dashed line).

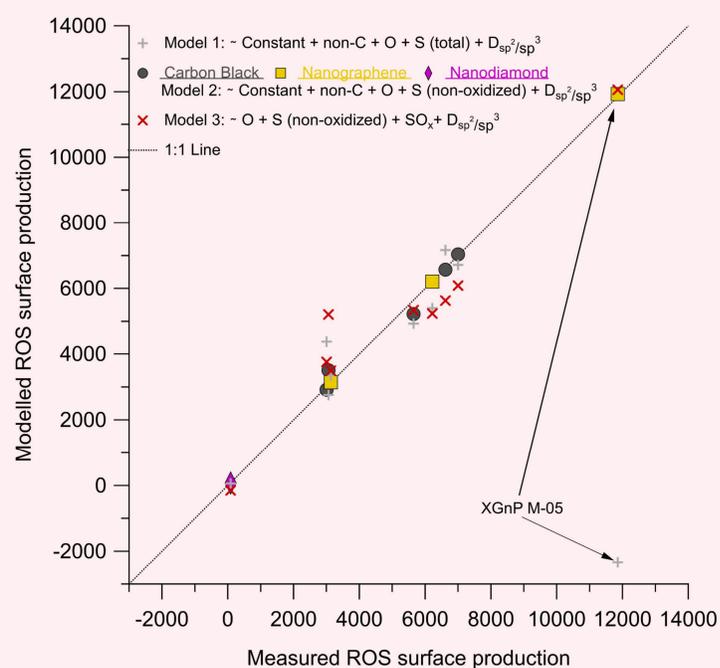


Figure 3. Linear regression models to predict ROS surface production based on the XPS analysis of nanomaterial surface composition ($R^2 = 0.99$, $p < 10^{-4}$).

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