Interaction of Single Walled Carbon Nanotubes (SWCNTs) with Hydrogels: Toxicological Implications

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1: Motivation & Objectives

➢Since their discovery, Single-Wall Carbon Nanotubes (SWCNTs) have attracted great attention due to their unique electrical and mechanical properties.

➢With anticipated increases in production over time, SWCNTs would likely enter waste streams, reach natural terminal sinks such as rivers and lakes, where they would interact with aquatic organisms and impact ecological functions.[1]

➢However, conflicting findings have been reported, in that for the same model organism, both toxic and non-toxic effects are detected.[2-4] These variations are likely due to the heterogeneous nature of SWCNT suspensions, i.e., the presence of both Metallic (***m***-SWCNTs) and Semiconducting species (***s***-SWCNTs) in as produced batches (**Figure 1**); and lack of standardized experimental conditions.

➢A common method for separation of SWCNT suspensions is column-based using an agarose gel stationary phase.[5,6]

➢This method has potential for scale up to produce large scale separations of the two fractions for use in eco-toxicology studies.

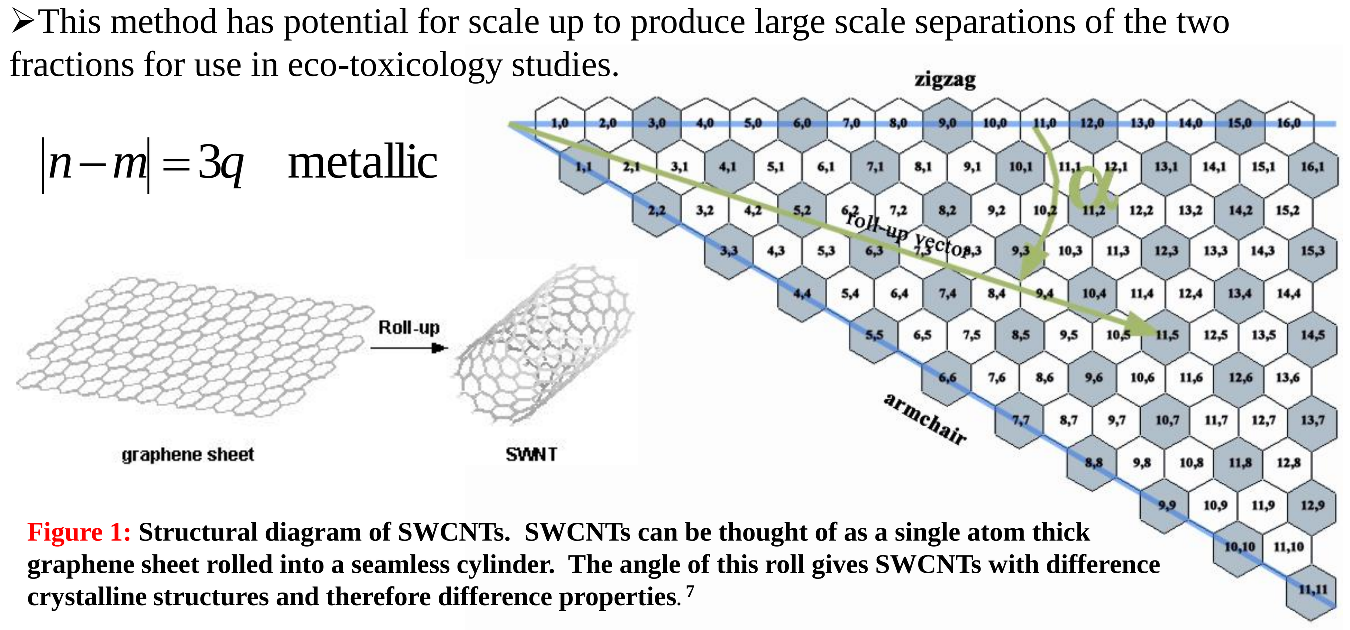

Figure 1: Structural diagram of SWCNTs. SWCNTs can be thought of as a single atom thick graphene sheet rolled into a seamless cylinder. The angle of this roll gives SWCNTs with difference crystalline structures and therefore difference properties.[7]

OBJECTIVES & GOALS

➢Achieve a large scale separations of SWCNT types (***m- and s-***) using agarose gels.

➢Characterize and assess the biological impacts of each of the fractionated SWCNT types on the growth of *Pseudokirchneriella subcapitata* when suspended in non-toxic surfactants.

2: Selective Adsorption

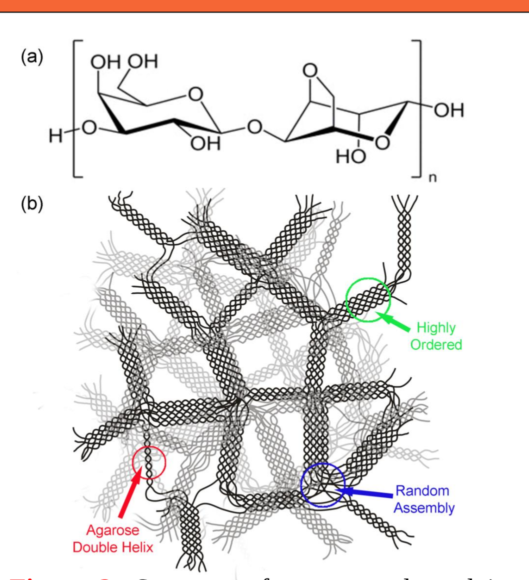

Figure 2: Structure of agarose gel used in selective adsorption.(a) Monomeric unit of agarose. (b) Porous network of agarose gel

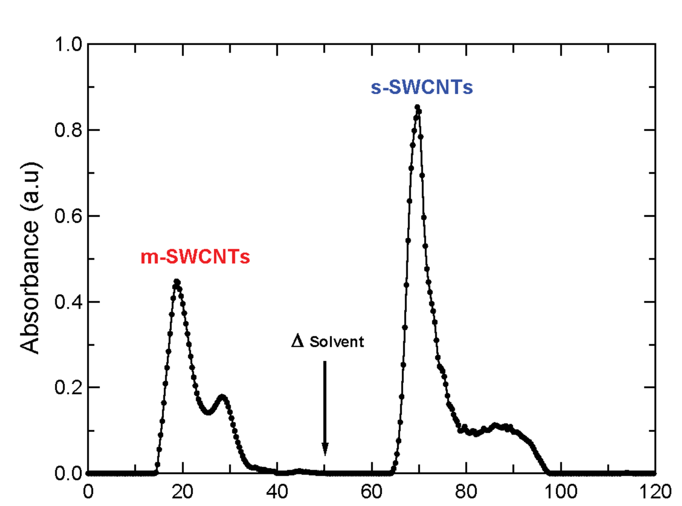

Figure 3: Separation of SDS-SWCNTs by Selective Adsorption. Elution curve of separation; all absorbance data collected at 626nm.

➢Agarose Gels are used as stationary phase due to their intricate porous structure (**Figure 2**). Column volume (CV) for these separations is 40mL.

➢***m***-SWNCTs are eluted with 1 CV of 1% Sodium Dodecyle Sulfate (SDS) solution, while ***s***-SWCNTs are retained on the gel.

➢***s***-SWCNTs are eluted only after a change in eluent. 2 CV of 2% Sodium Cholate (SC) are used in this case resulting in the separation illustrated in **Figure 3**.

3: Results & Discussion

3.1 Separation Mechanism

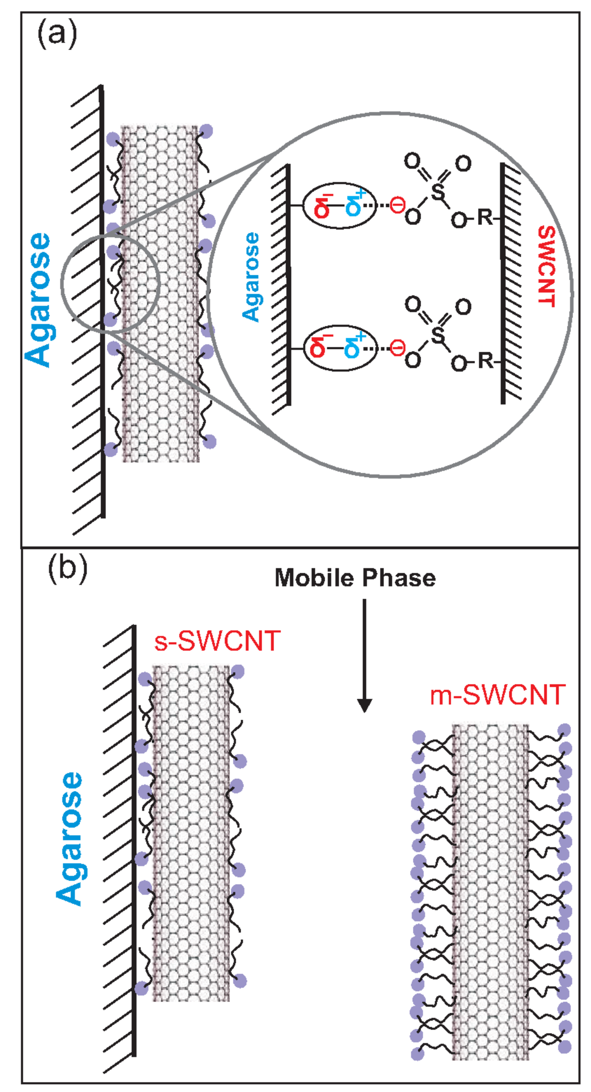

Figure 4: Mechanism of interaction and selectivity during agarose gel-based separations of SDS SWCNTs.[8]

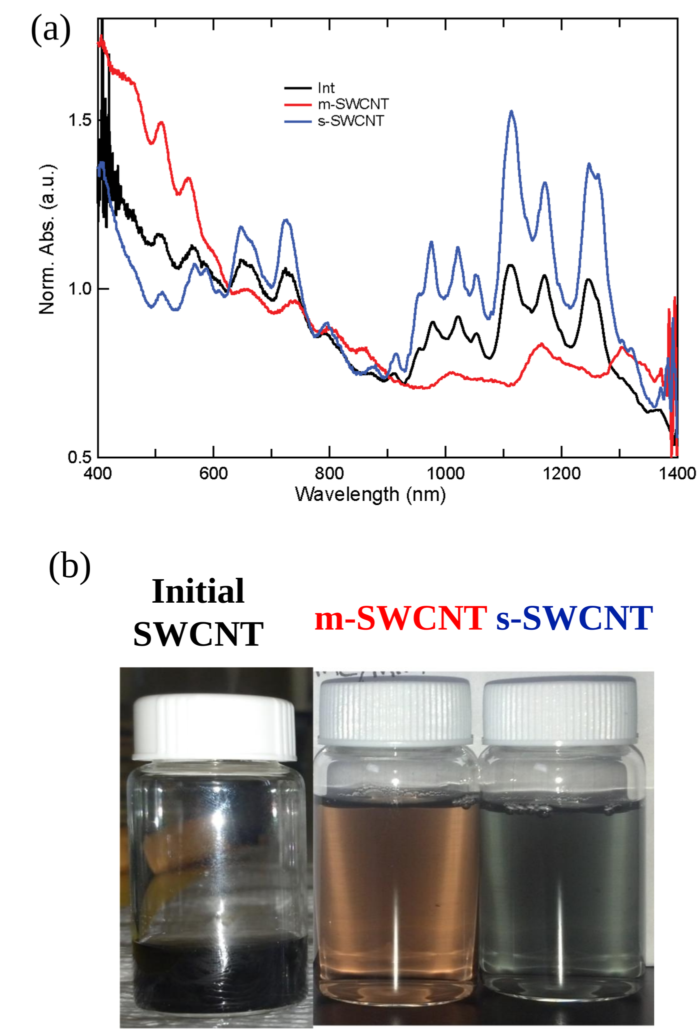

Figure 5: Separated Fractions of SWCNTs. (a) UV-NIR Absorbance of fractions. Data has been normalized at 626nm. (b) Representative vials of collected fractions

➢Ion-Dipole Interactions between anionic head groups of SDS and dipoles of agarose are the dominate force driving separations.[8]

➢Image charges on SWCNTs govern selectivity

➢Polarizability of ***m***-SWCNTs > ***s***-SWCNTs resulting in increased magnitude image charge on ***m***-SWCNTs

➢Larger image charge on ***m***-SWNCTs limits interaction with agarose gel:

a) Direct repulsion between image charge and dipole

b) Increase in local charge screening alters surfactant aggregation number on ***m***-SWCNT surfaces. (**Figure 4**)

3.2. Preliminary Studies of the Biological Response of *P. subcapitata* to SWCNTs

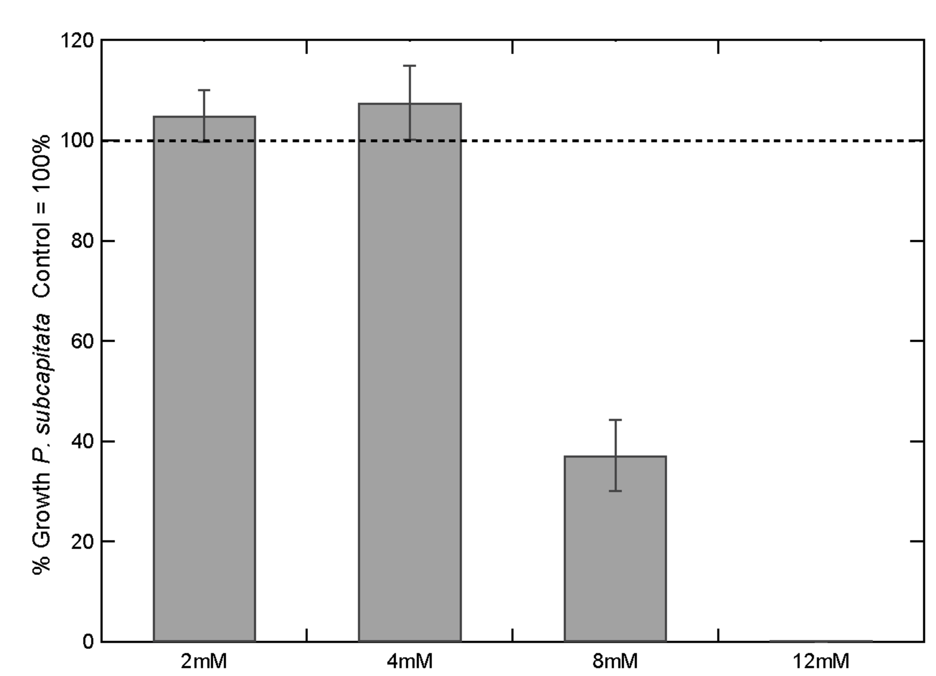

Figure 6: Concentration range finding for Sodium Cholate (SC) on X-axis, to be used as surfactant to suspend SWCNTs. The bars represent the response of *P. Subcapitata* to increasing concentrations of SC. Concentrations ≤4mM are non toxic.

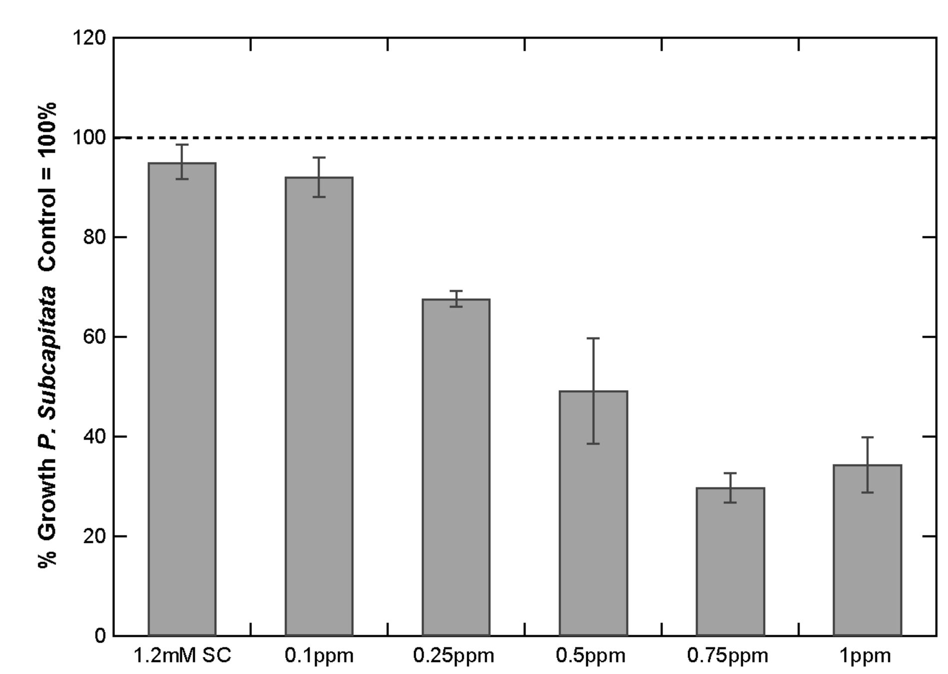

Figure 7: Growth response of *P. Subcapitata* to increasing concentrations of as produced SWCNTs (mixture of *m-* and *s*-SWCNTs) on X-axis. Suspensions have been stabilized with non-toxic concentrations of SC.

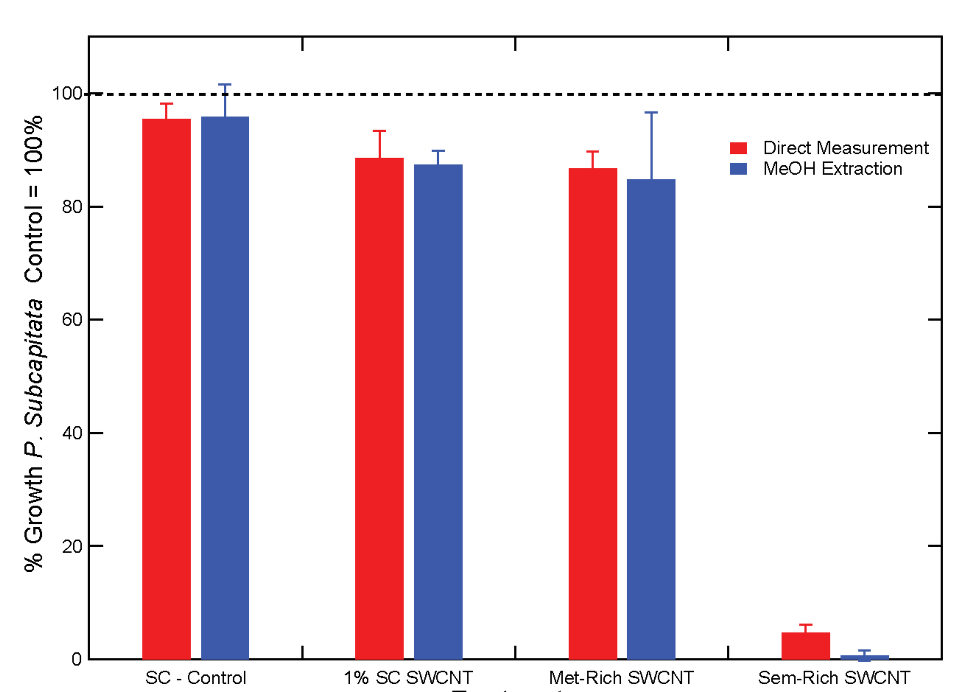

Figure 8: Preliminary response of *P. Subcapitata* exposed to a fixed SWCNT concentration of 0.5ppm using type separated suspensions. SWCNT fractions were washed and re-stabilized with SC to remove any residual toxic surfactant (SDS).

➢Sodium Cholate (SC) does not significantly effect *P. Subcapitata* growth at concentrations ≤4mM. Toxicity studies were therefore conducted under SC concentration below this threshold. (**Figure 6**)

➢Increasing conc. of SWCNTs suspended in non-toxic level of SC results in a significant growth inhibition of *P. Subcapitata* starting at SWCNT concentrations as low as 0.25ppm (**Figure 7**)

➢Dose response of *P. Subcapitata* to as produced SWCNT suspensions indicates 50% inhibition at concentrations of 0.5ppm (**Figure 7**)

➢Preliminary toxicity results obtained using *P. Subcapitata* and identical concentrations of separated SWCNTs indicate that the ***s***-SWCNT fraction is the main driver of the toxicity of the mixture shown in Figure 7 (**Figure 8**)

4: Conclusions

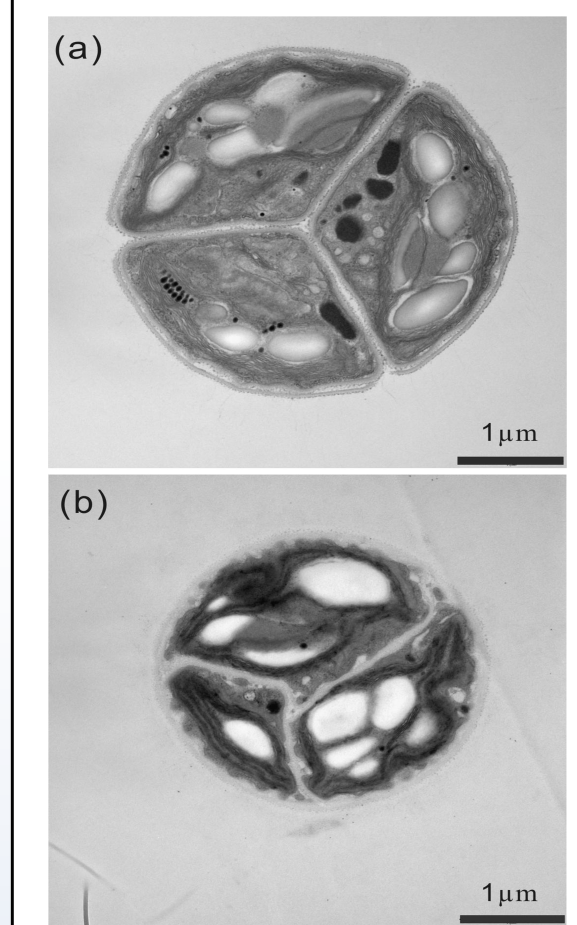

Figure 9: TEM images of *P. subcapitata* from control growth medium (**a**) and from a treated medium containing 0.5 ppm of SWCNTs suspended in a non-toxic surfactant (**b**).

➢Preliminary results demonstrate the agarose gel based separation of SDS suspended SWCNTs is efficient and should be scaled up to produce significant amount of speciated SWCNTs

➢Unlike SDS that is highly toxic to aquatic organisms, SC appears to be ideal for suspending SWCNTs as it minimizes toxicity. However, the downside of this environmental-friendly approach is the extra step of replacing the SDS coating SWCNTs after separation by SC.

➢A significant difference in biological response is observed when the organism used in this study is exposed separately to identical concentrations of ***m***-SWCNTs and ***s***-SWCNTSs suspended in non-toxic levels of SC.

➢Further dose response studies are needed and are ongoing

5: Looking Forward

➢While a clear difference is observed in the biological response expressed by the green algae exposed to either ***m***-SWCNTs or ***s***-SWCNTs, the mechanisms of observed toxicity need to elucidated.

➢We will take advantage of our improved understanding of the selective adsorption that led to the separation of SWCNTs into specific (*n.m*) types to extrapolate on potential SWCNT-cell membrane interactions

➢In addition to solid phase analyses (e.g. TEM), we are currently planning to use HPLC-MS analysis to gain insight into potential changes in the biochemistry of molecules present in the cell membrane.

➢Additionally, mechanisms of toxicity previously reported in the literature for different engineered nanomaterials (**Figure 10**) will be investigated using algal cells harvested from different points along the sigmoid growth curve.

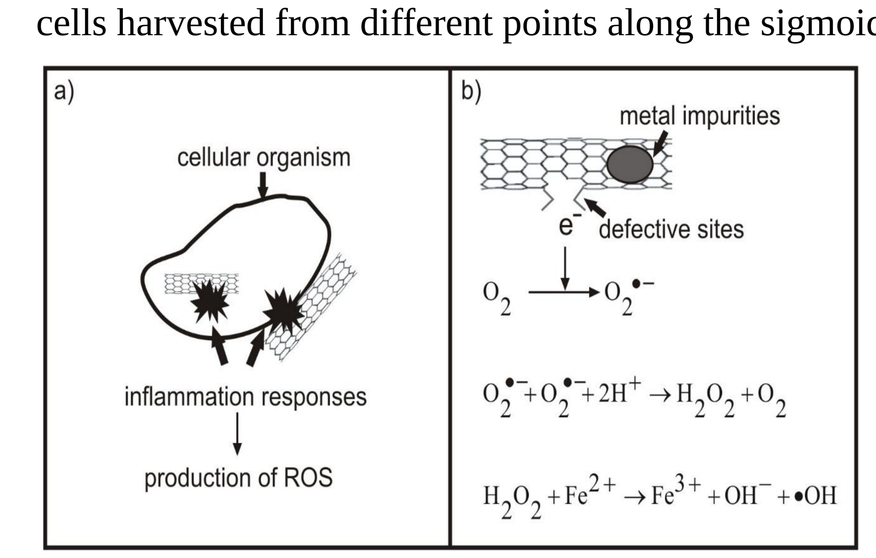

Figure 10: Possible mechanisms of reactive oxygen species production in the presence of SWCNTs. ROS may be produced by (a) inflammation, or (b) electron release from reactive sites and subsequent Fenton reaction of transitions metals.

6: Acknowledgements

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