Physical characterization and *in vitro* cytotoxicity screening of single-walled carbon nanotubes (SWNTs)

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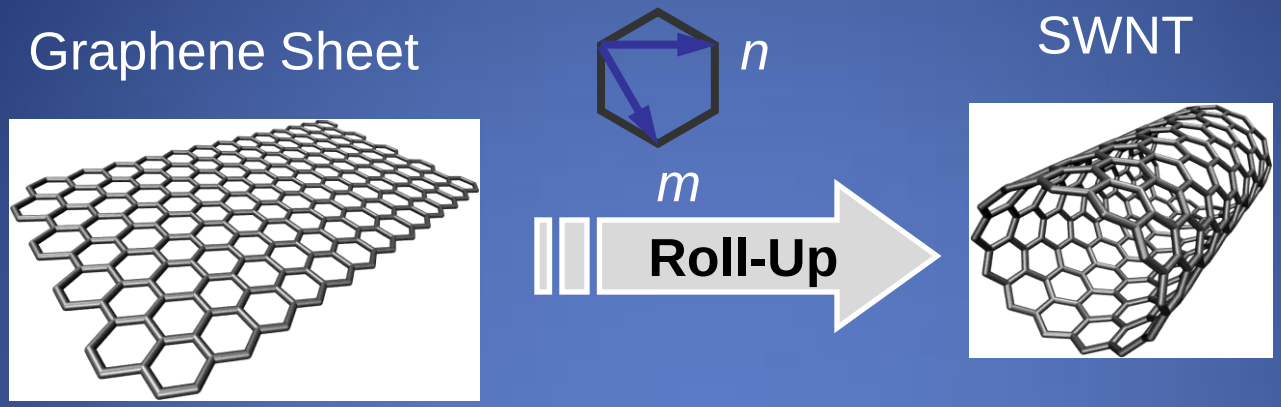
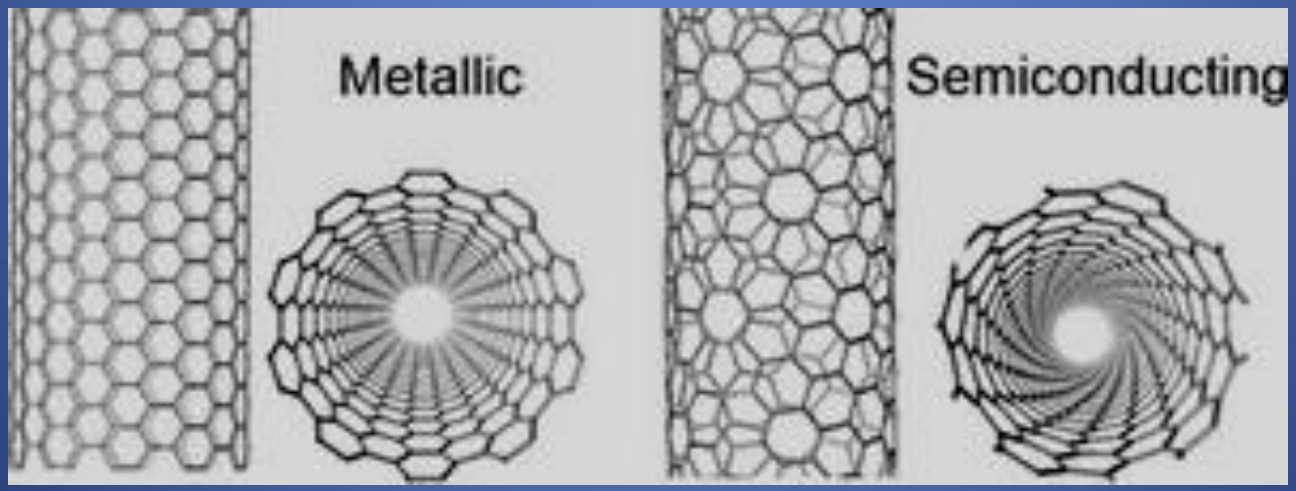
Overview

I. Introduction
II. Physical Characterizations of SWNT Dispersions
III. *In vitro* Cytotoxicity Assays
IV. Removal of Toxic Components
V. Summary
VI. Works in Progress

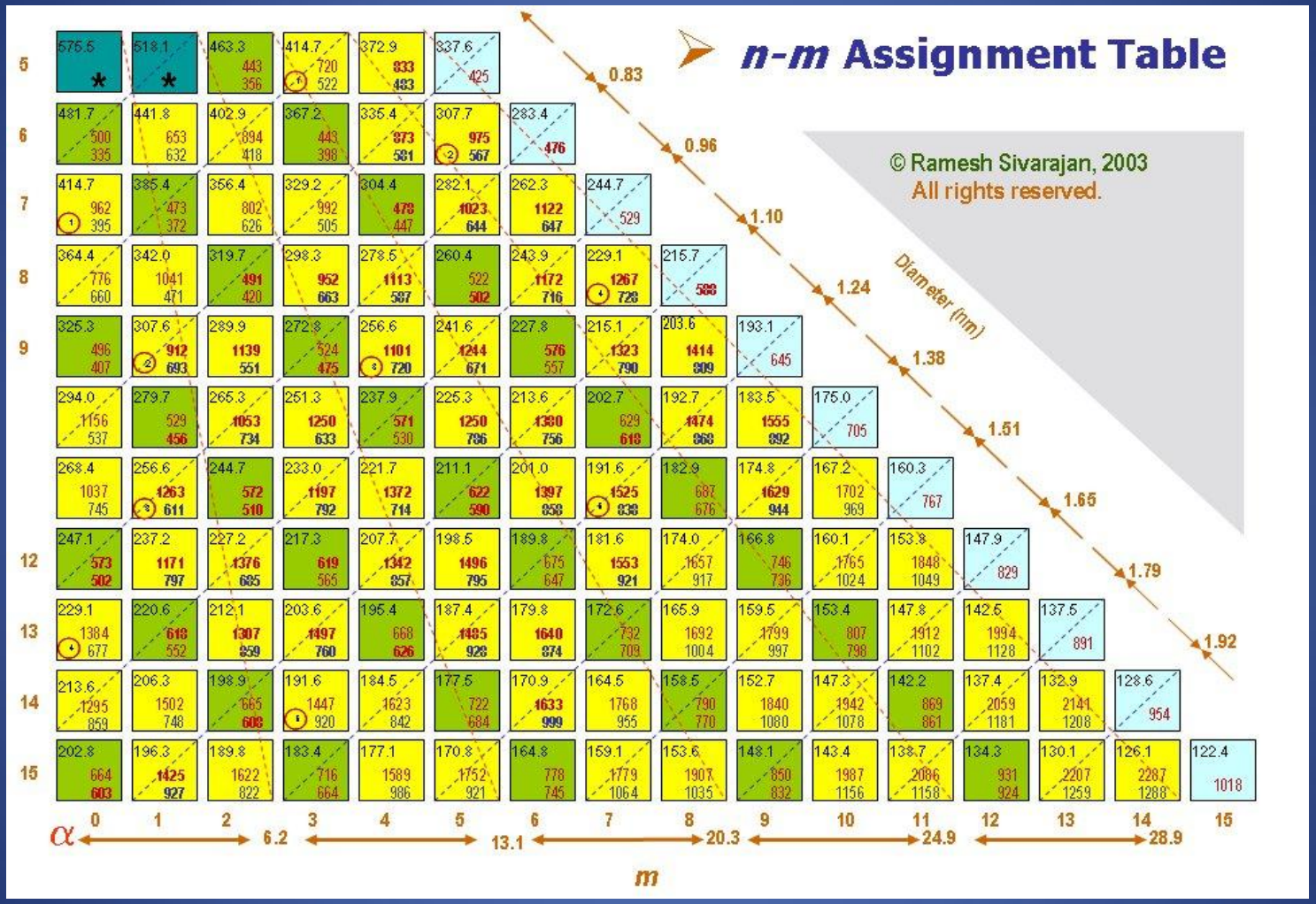
What are single–walled carbon nanotubes (SWNTs)?





Javey, A. , *ACS Nano* (**2008**) *2:* 1329-1335

Variety of SWNT tube types



www.carbonwall.com

Methods used for SWNT synthesis

- Natural, incidental, and controlled flame –
  Nanotubes produced by burning methane, benzene.
- Laser ablation –
  Blast a composite of graphite and metal catalyst with a laser
- Arc discharge –
  Nanotubes produced on graphite electrodes during an arc discharge
- Chemical vapor deposition (CVD) –
  Nanotubes grow on metal catalyst particles as carbon-containing gas break down in a heated reactor.
  - CoMoCAT CVD
  - HiPco CVD
  - Super-growth CVD

Not all SWNT products are created equal.

Most commercially available SWNT products are mixtures

- SWNTs (metallic & semi-conducting)
- Metal catalysts / catalyst support
- Non-tubular carbon (NTC) species
- By-products of purification / modifications

Representative properties of SWNT products

SWNT product	CG100	SG65	SG76	HiPco-R	AP	P2	P3*
Synthesis method	CoMoCAT-CVD			HiPco - CVD	Arc discharge		
Manufacture	Southwest NanoTechnologies (Sigma Aldrich)			Unidym	Carbon Solution Inc. (CSI)		
Carbon (%)	> 90			~ 65	40 – 60	80-90	80-90
Metal(%)	< 10 (Co, Mo)			<35 (Fe)	~ 30 (Ni, Y)	4-7 (Ni, Y)	5-8 (Ni, Y)
SWNT (%)	≥ 65	≥ 77 >50% (6,5)	≥ 77 >50% (7, 6)	NA	NA		
Raman D/G	<0.06			NA	NA		
Length (nm)	450-2300	450-2000	300-2300	100-1000	[1000-5000]	[500–1500]	[500-1500]
Diameter (nm)	0.7-1.3	0.7-0.9	0.7-1.1	0.8-1.2	[2-10]	[4-5]	[4-5]

In-house characterizations of purchased SWNT products

- No mandatory SWNT product specification
- Product specification data provided may not be adequate and/or accurate.
- High quality control is crucial for applications in high-tech industries.
- No ecological information available

Toxicity
no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects
no data available

UTD in-house SWNT analysis:

- ✓ TGA – SWNT and metal contents
- ✓ AFM – individual nanotube length and diameter
- ✓ Raman – nanotube identification and quality assessment
- ✓ ICP-MS – identify metal catalysts and quantification
- ✓ UV-Vis-NIR – identify nanotubes varieties and quality
- ✓ FTIR – identify nanotube surface impurities/modifications

I. Introduction

- > 120 varieties of SWNT tube types
- Commercial SWNTs are synthesized by AD or CVD methods
- SWNT products are mixtures of
  {SWNTs, Metals, NTC, others …}
- Vendor provided data are incomplete/inaccurate
- No ESH data available
- In-house characterization of purchased SWNT products is essential.

The SWNTs produced by different manufacturers are unique.

Know your SWNT product before you use it!

I. Introduction

II. Physical Characterizations of SWNT Dispersions

- Dispersion protocol
- AFM analysis
- Concentration
- Vis-NIR Absorbance Spectroscopy
- Raman analysis

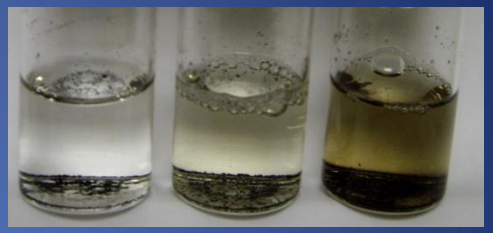
SWNT Dispersion protocol

- SWNT material –

  10 mg purchased SWNT product

- Solutions –

  10 mL HB (10 mM HEPES, 10 mg/mL BSA, pH 7.4)



- Sonication –

  Bath sonication (40K Hz, 120W, cooling coils)

  4 hours in 4^{O}C cold room (bath temperature: 3-12^{o}C)

  Up to 8 samples, 10 mL each, prepared per batch

  ➔ 1 mg/mL SWNT suspensions

- Centrifugation –

  20,000 g, 5 min, collect top 90% supernatant

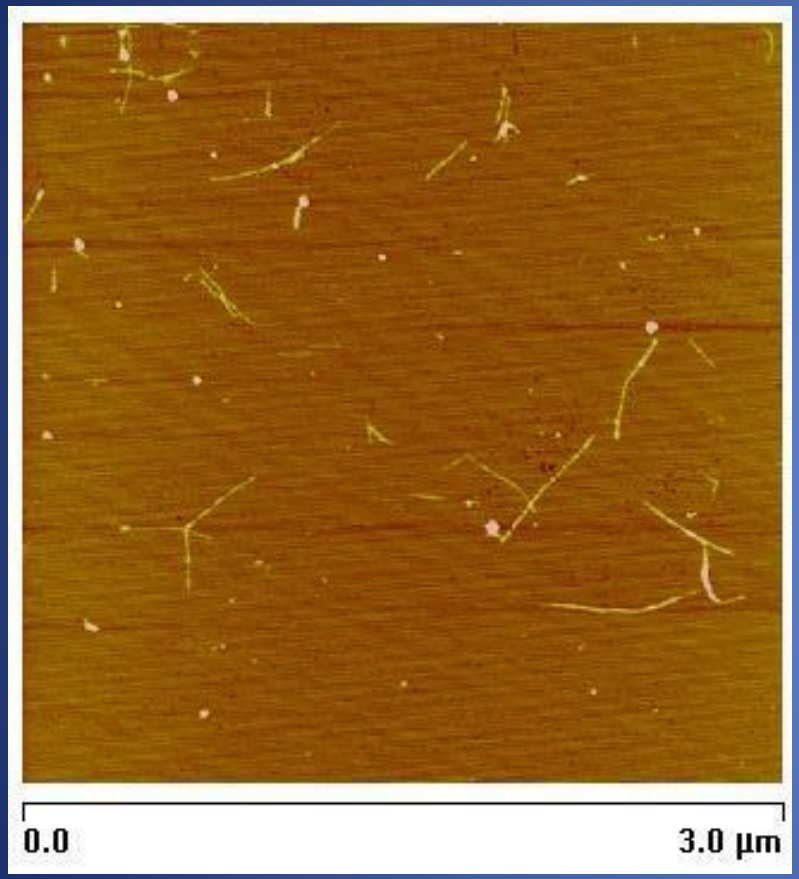
  20,000 g, 30 min, collect top 95% supernatant

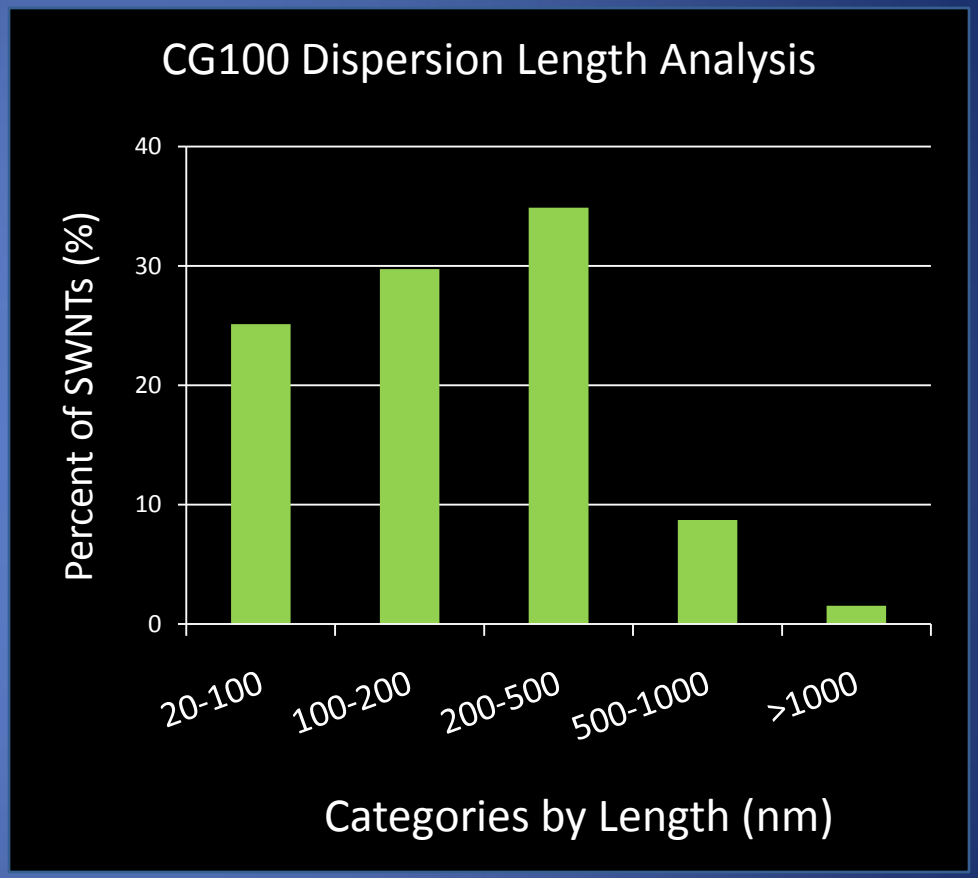
  ➔ SWNT dispersions, TBD mg/mL

- Storage –

  4^{O}C, stable for longer than one month

AFM analysis of SWNT Dispersion





The average SWNT length: 244 nm

Quantify SWNT/NTC concentration in dispersions

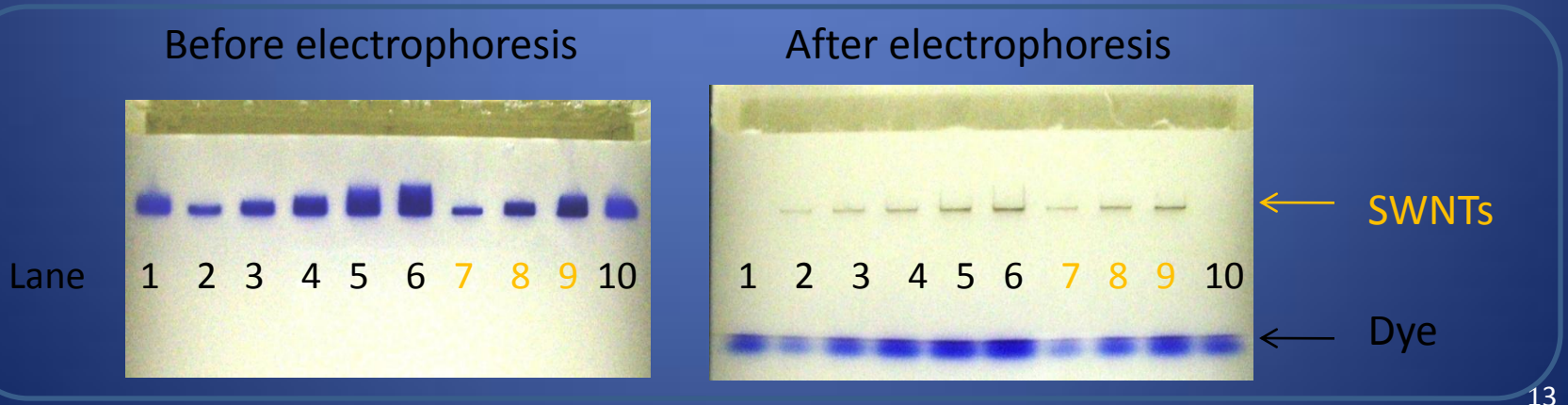
- Samples: a) SWNT suspension (1 mg/mL) b) SWNT dispersion (conc. TBD)
- Lanes 2-6:

  Load 100, 200, 300, 400, and 500 ng of SWNT suspension to construct a pixel intensity vs. SWNT concentration calibration curve
- Lanes 7-9:

  Load 5,10,15 µl of SWNT dispersion
- Apply electrophoresis –

  SWNTs are too big to enter the gel , form a dark band at liquid-gel interface
- Scan the gel and quantify the dark band pixel intensity –

  Band darkness <≈> pixel intensity <≈> SWNT /NTC amount



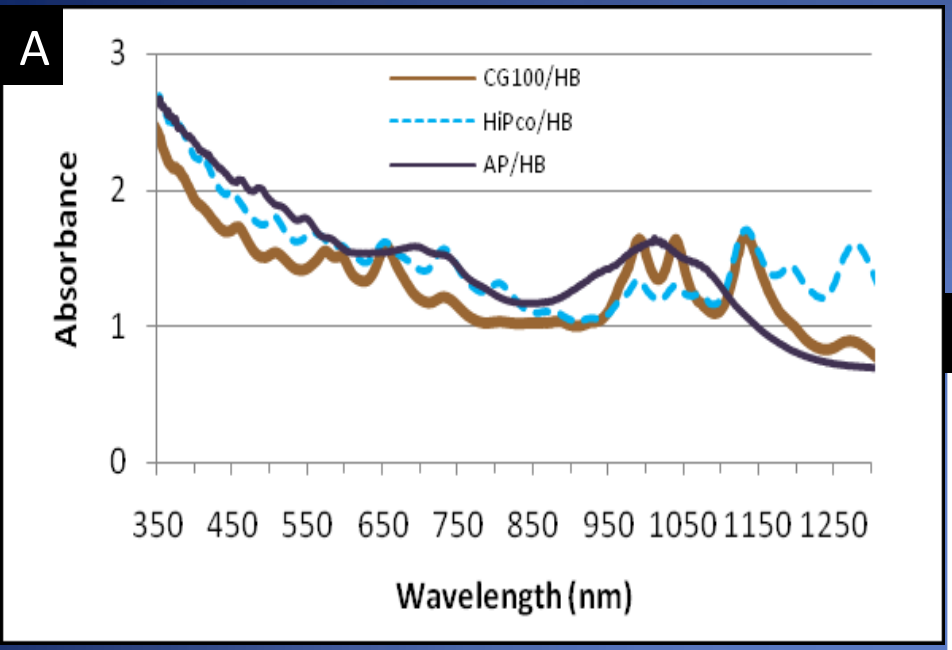
Wang et al., *Anal Chem.* **(2009) 81 (8): 2944-52.**

Physical characterizations of SWNT dispersions

SWNT Dispersion	CG100	SG65	SG76	HiPco-R	AP	P2	P3*
Synthesis method	CoMoCAT			HiPco	Arc discharge		
Conc. (ug/mL)	230 10	301 63	258 31	401 55	241 29	289 41	786 61

Vis-NIR absorbance analysis of SWNT dispersions

A



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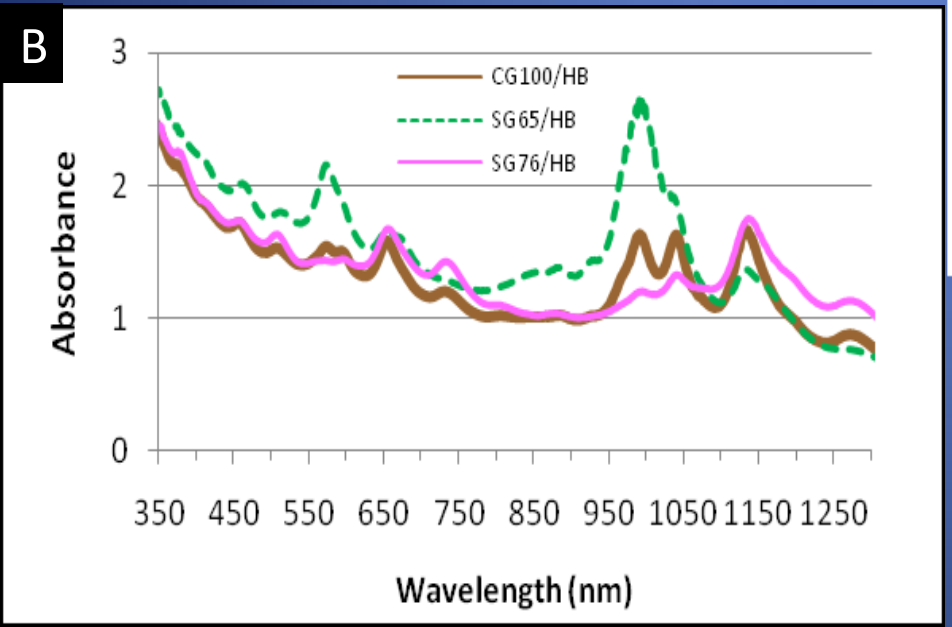
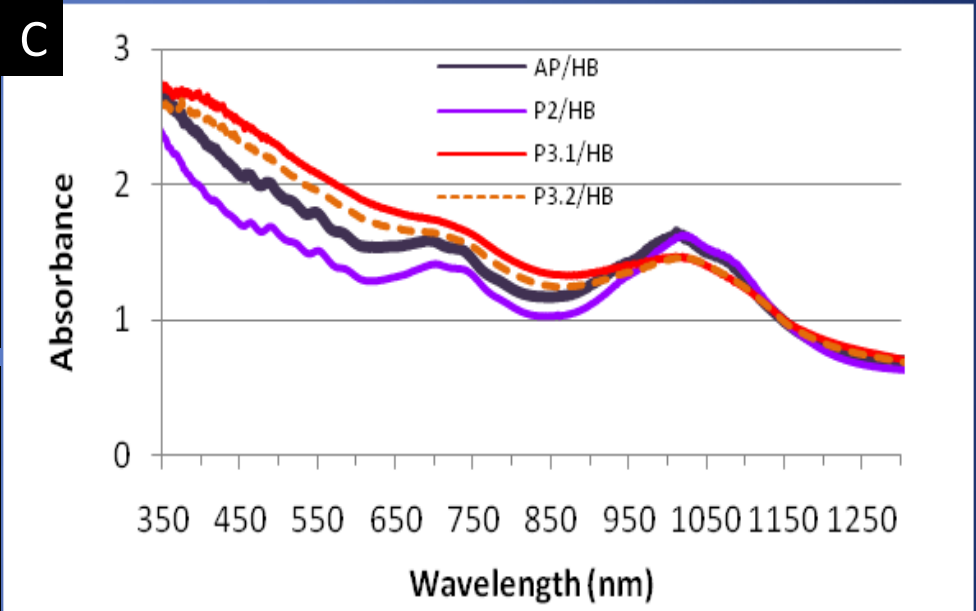
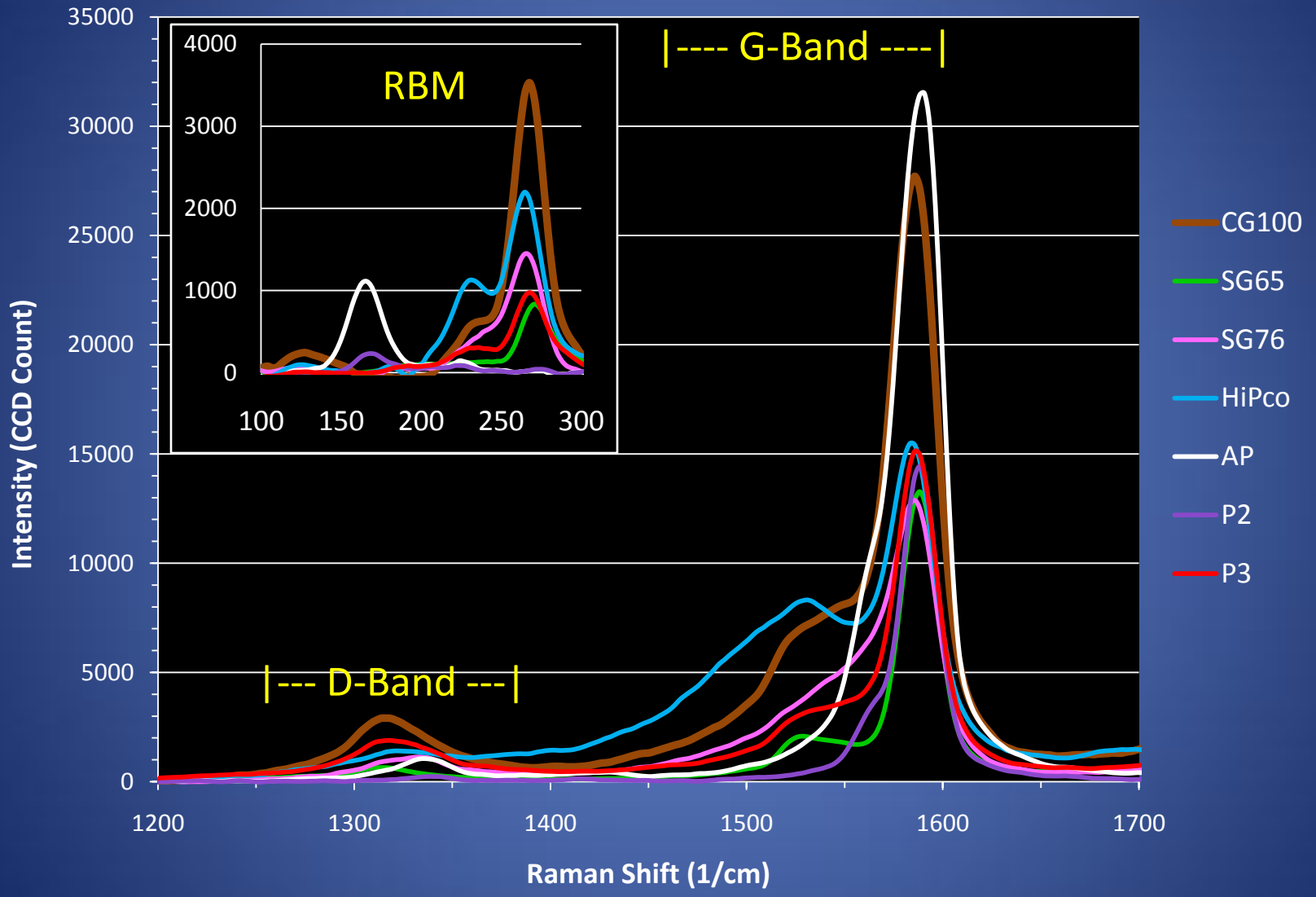


C



Raman analysis of SWNT dispersions



I. Introduction

II. Physical Characterizations of SWNT Dispersions

- Hydrophobic SWNTs can be individually dispersed in biological aqueous solution
- Concentrations of SWNT/NTC in dispersions can be quantified using SDS-PAGE system
- Quality, quantity, and the tube types of SWNTs in dispersions can be measured and analyzed by optical absorption and Raman Scattering.

I. Introduction

II. Physical Characterizations of SWNT Dispersions

III. *In vitro* Cytotoxicity Assays

- Toxicity assessment
- IC_{50}

In vitro SWNT cytotoxicity assay protocol

- Cell culture – Normal Rat Kidney (NRK) cell line
- SWNT dispersions – 100 μg/mL in culture medium
- Incubation –
  - 4,000 cells per well
  - 4 wells per sample per assay
  - 37^{o}C, 3 days
- Assays –
  - Cell Counts

    Count cell number in each well using a Coulter counter
  - Crystal Violet Staining

    Stain cells with Crystal Violet dye which binds DNA

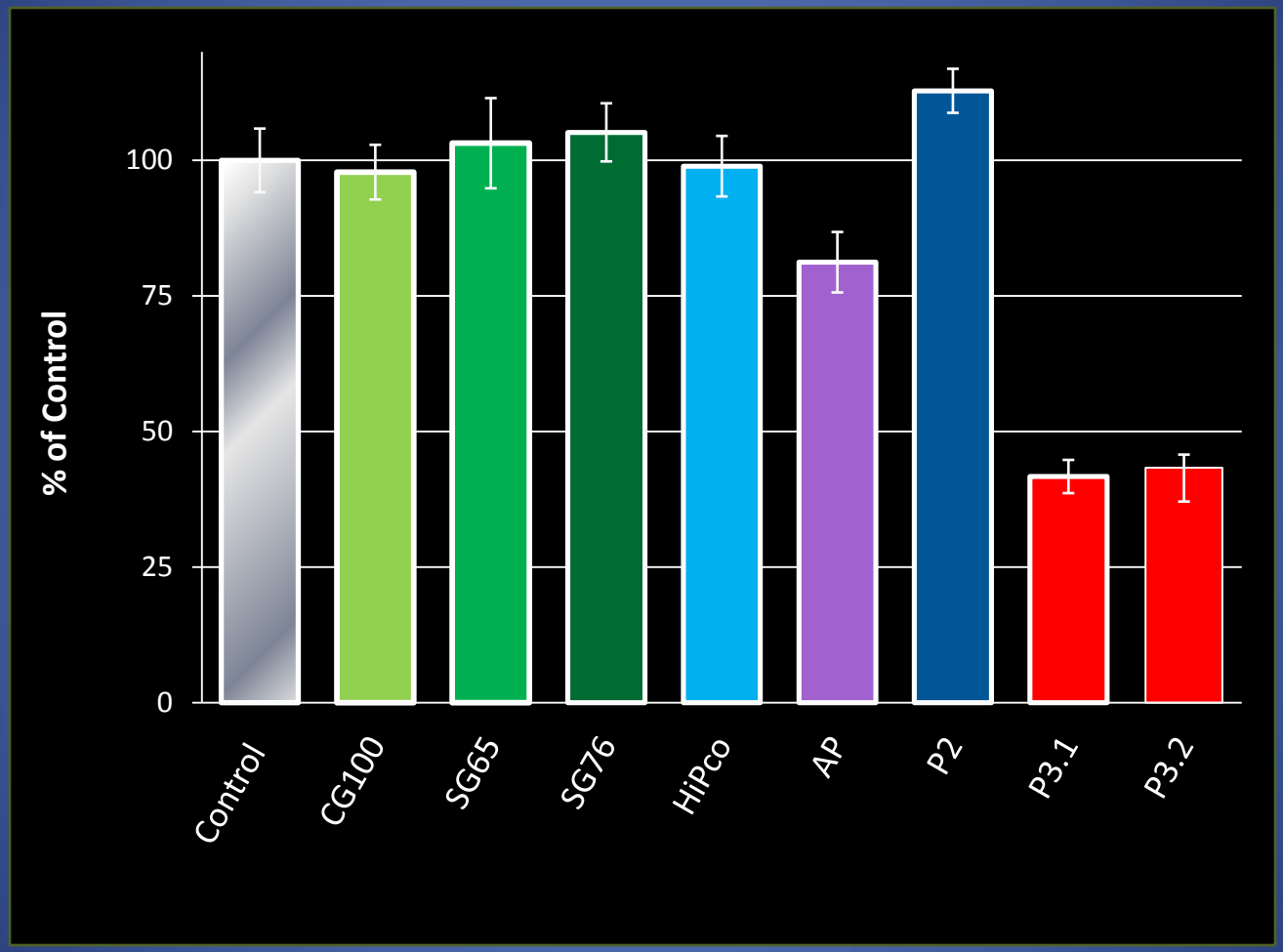
    Elute bound dye with 10% acetic acid

    Transfer the elusion to a 96-well plate

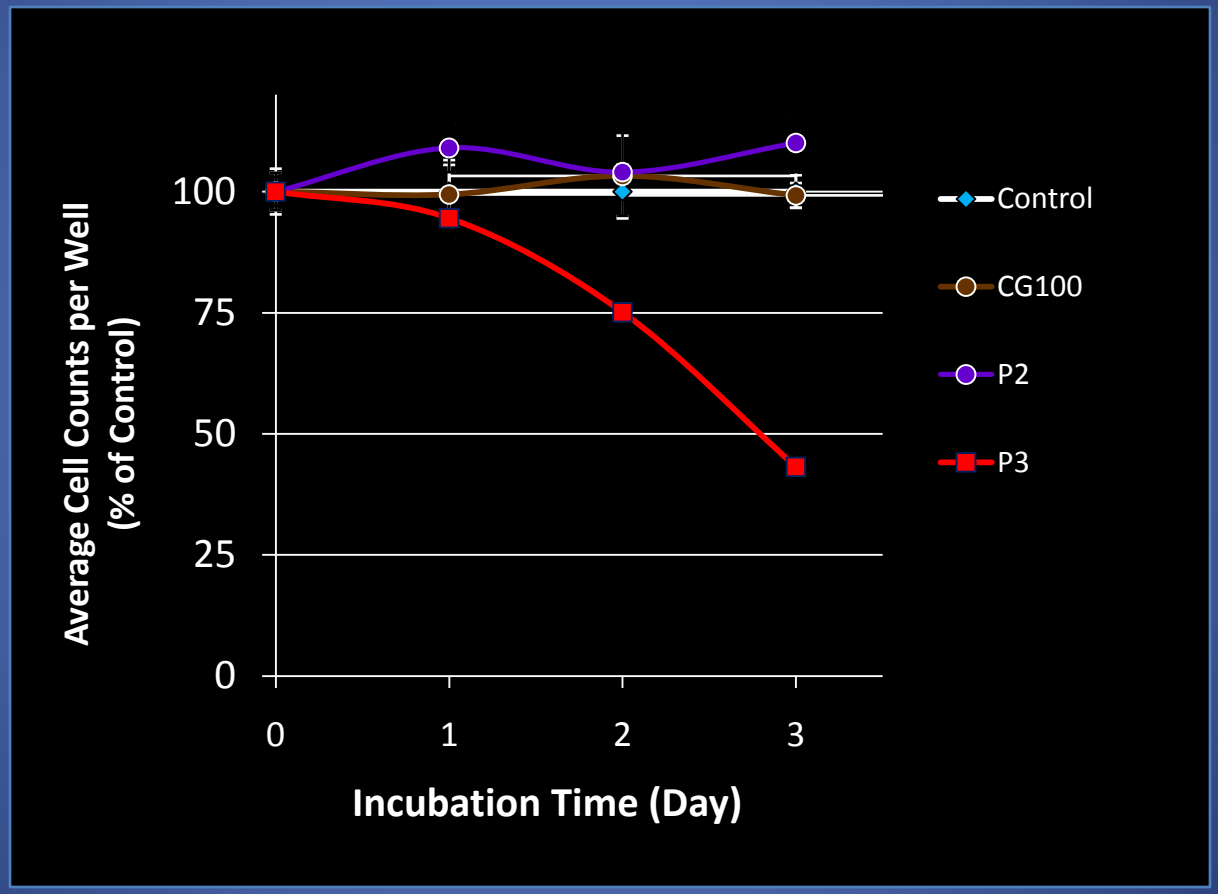
    Measure CV dye intensity at A_{590} using a micro-plate reader

    (A590 <≈> bound CV dye <≈> DNA amount <≈> cell number)

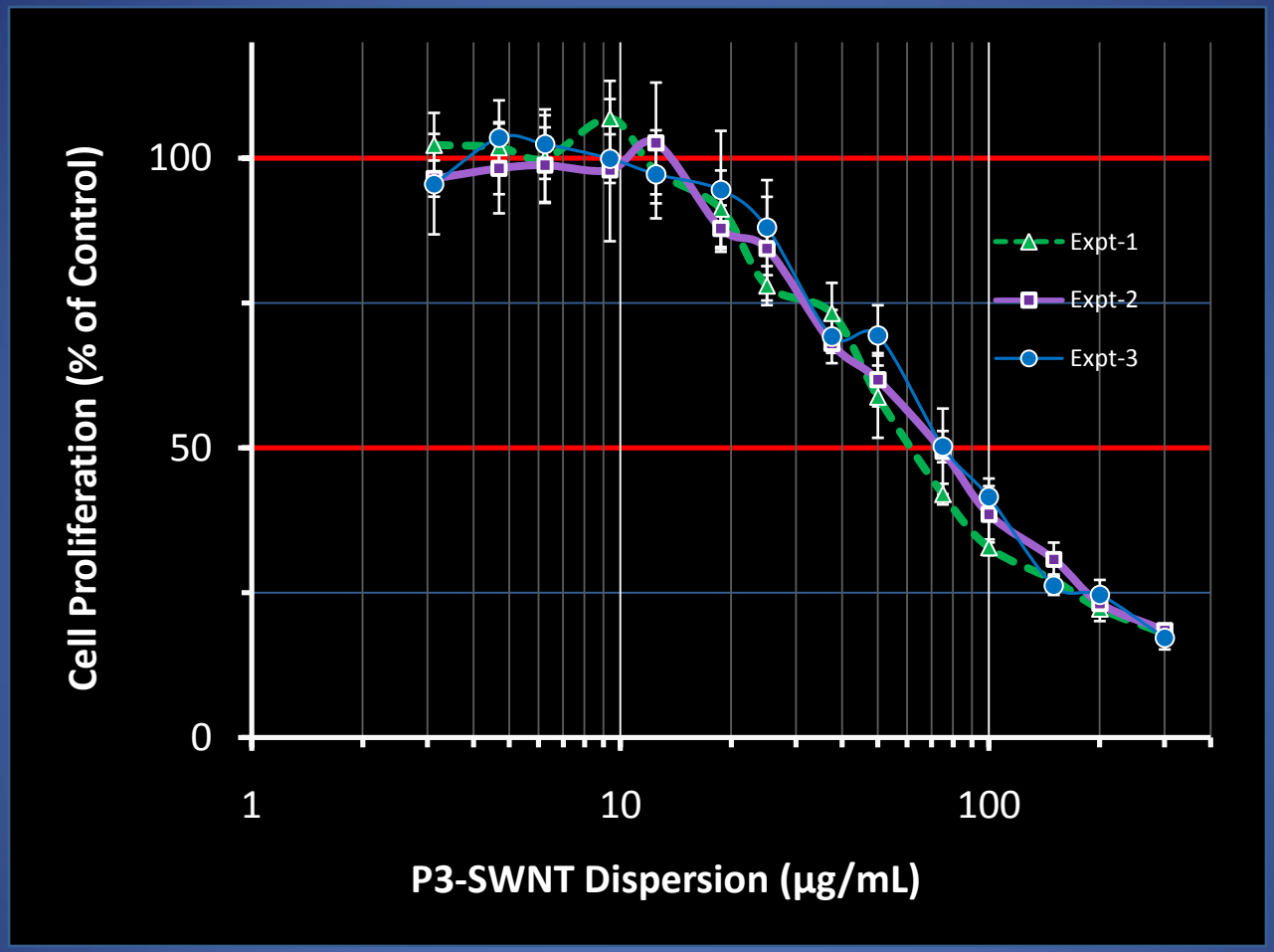
In vitro cytotoxicity assessment of SWNT dispersions



Toxicity of P3-SWNT dispersion as a function of time



Toxicity of P3-SWNT Dispersion as a function of concentration



IC_{50} : the concentration of a drug that is required for 50% inhibition *in vitro*.

P3-SWNT dispersion IC_{50} = 76.5 4.9 µg/mL

I. Introduction

II. Physical Characterizations of SWNT Dispersions

III. *In vitro* Cytotoxicity Assays

- 2 methods were used for toxicity assessment
- 7 purchased SWNT products were tested
- 1 SWNT product is toxic: carboxylated P3-SWNT product
- P3-SWNT toxicity is time dependent
- P3-SWNT toxicity is concentration dependent:
  IC_{50} = 76.5 μg/mL

Only the carboxylated SWNT product contains toxic material.

I. Introduction

II. Physical Characterizations of SWNT Dispersions

III. *In vitro* Cytotoxicity Assays

IV. Removal of toxic components

- Remove toxic material by filtration
- Analysis of filtration materials

Separation of the Toxic Components from P3 Carboxylated SWNTs Product

Step 1:

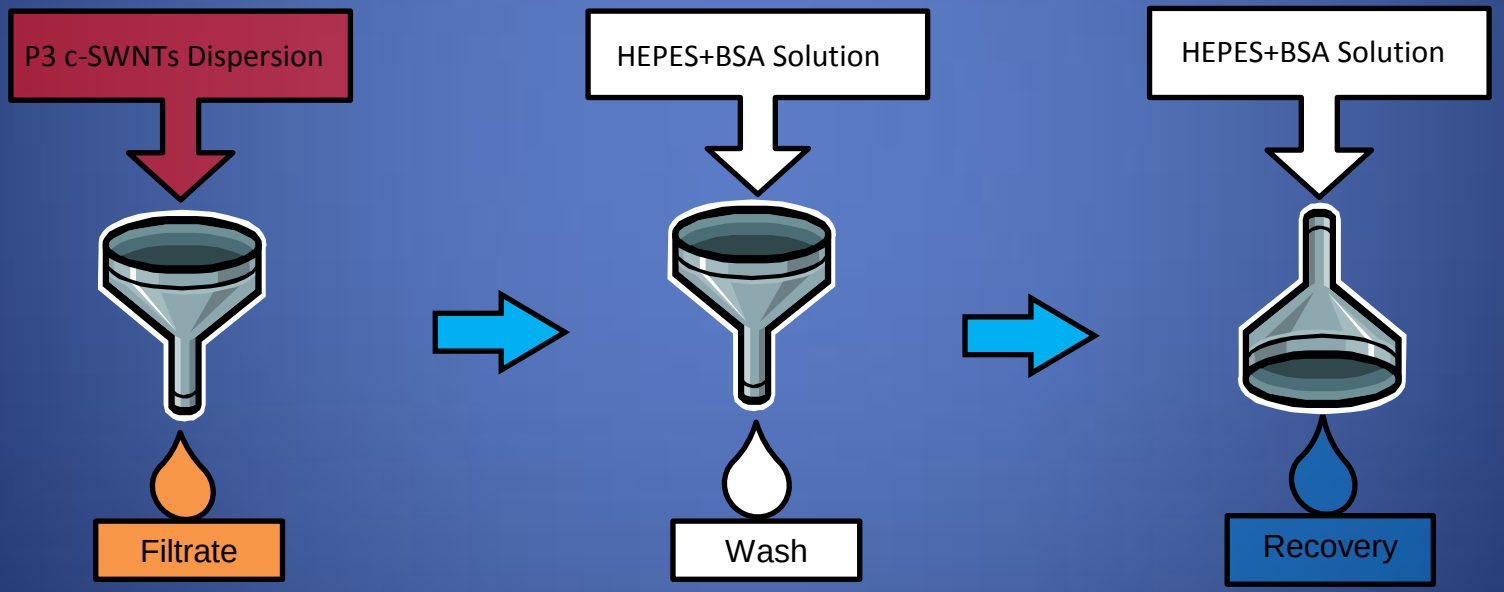
Filter 5 mL diluted P3 dispersion (in HEPES+BSA solution) through a 0.22 µm membrane and collect the Filtrate.

Step 2:

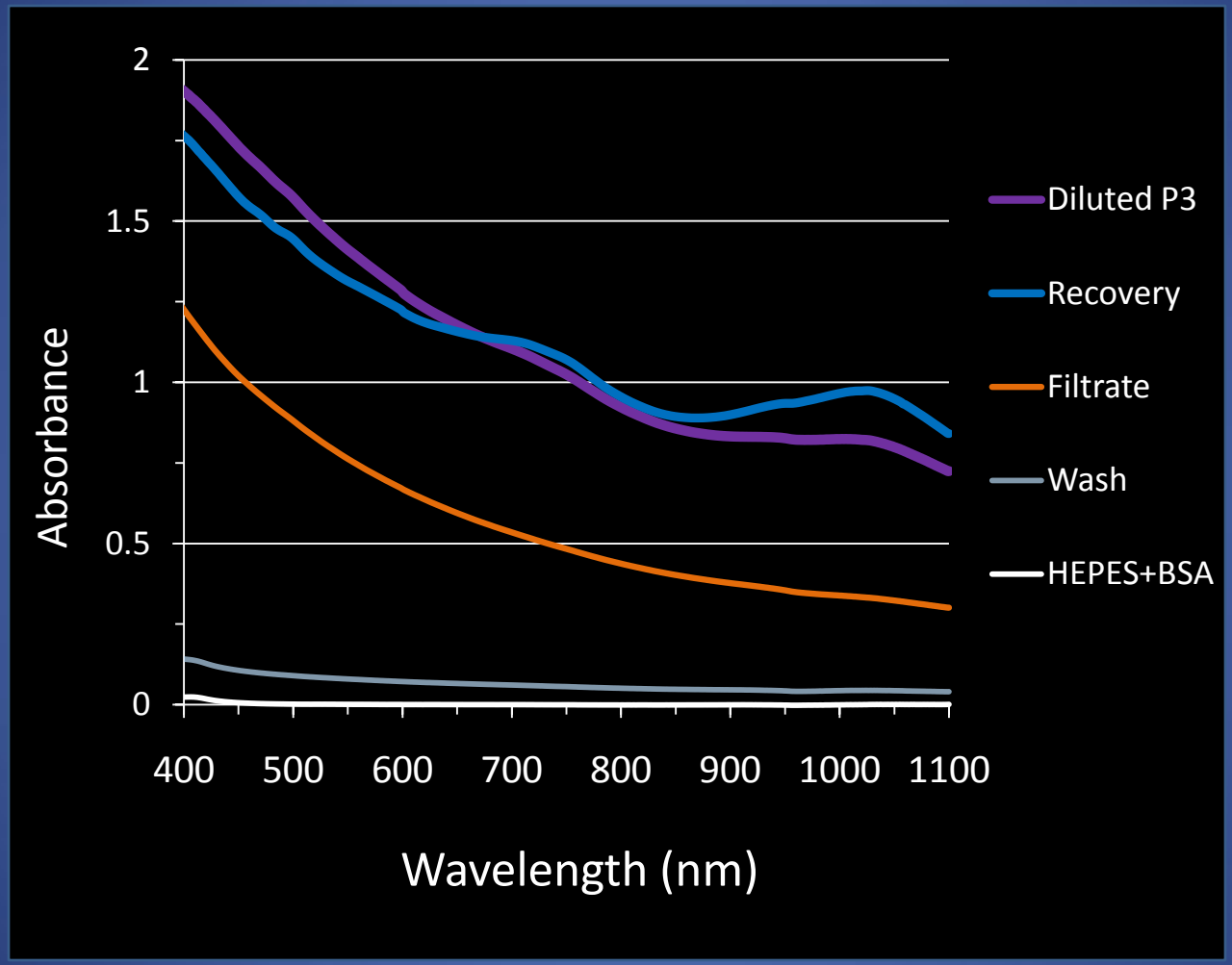
Pass 5 mL HEPES+BSA solution through the same membrane and collect the solution passed through.

Step 3:

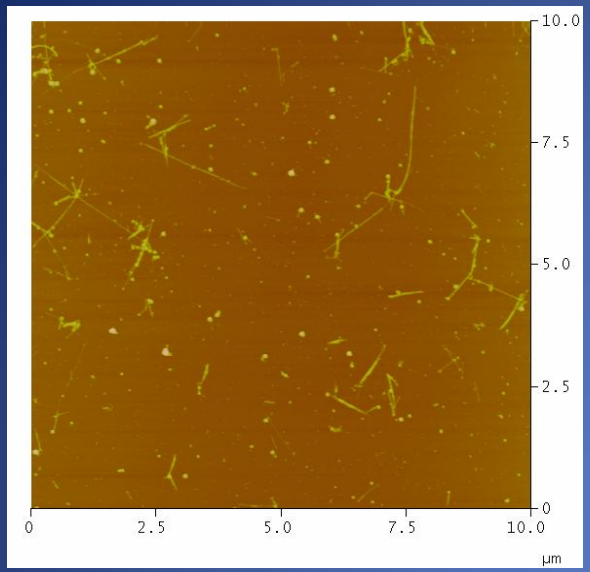
Extract material retained on the membrane with 2 mL HEPES+BSA solution, termed Recovery.



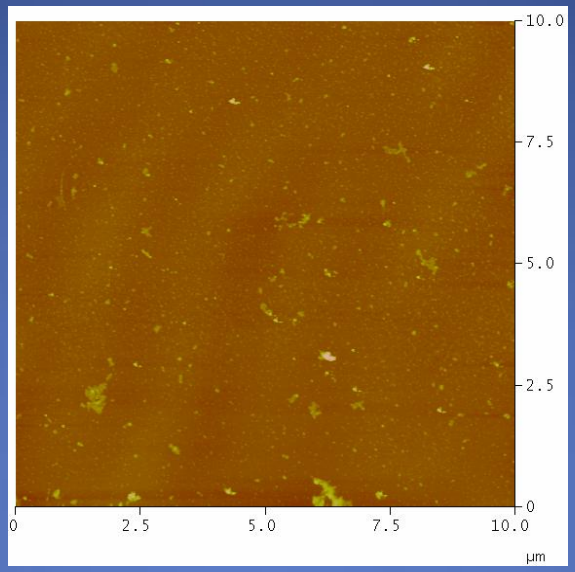
Vis-NIR absorption analysis of P3 filtration materials



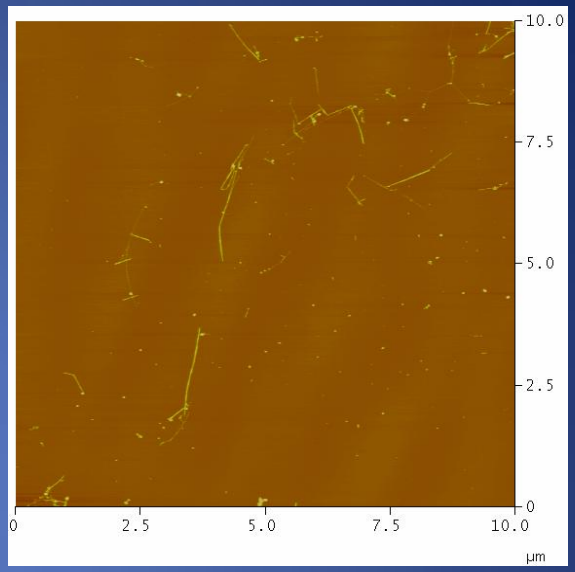
AFM and Raman Analysis of P3 Filtration Materials



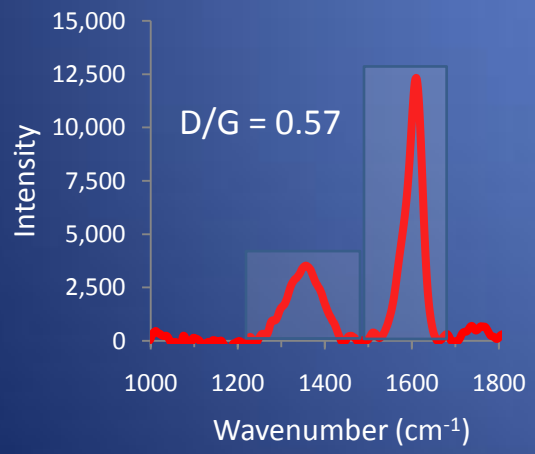
Starting c-SWNTs

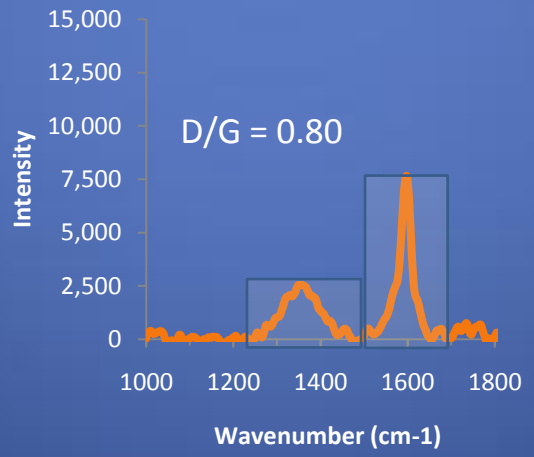


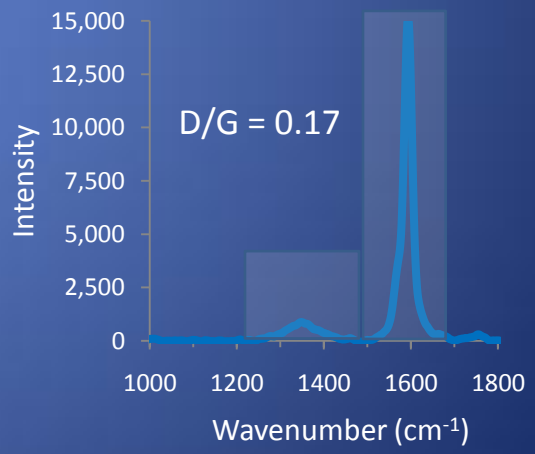
Filtrate



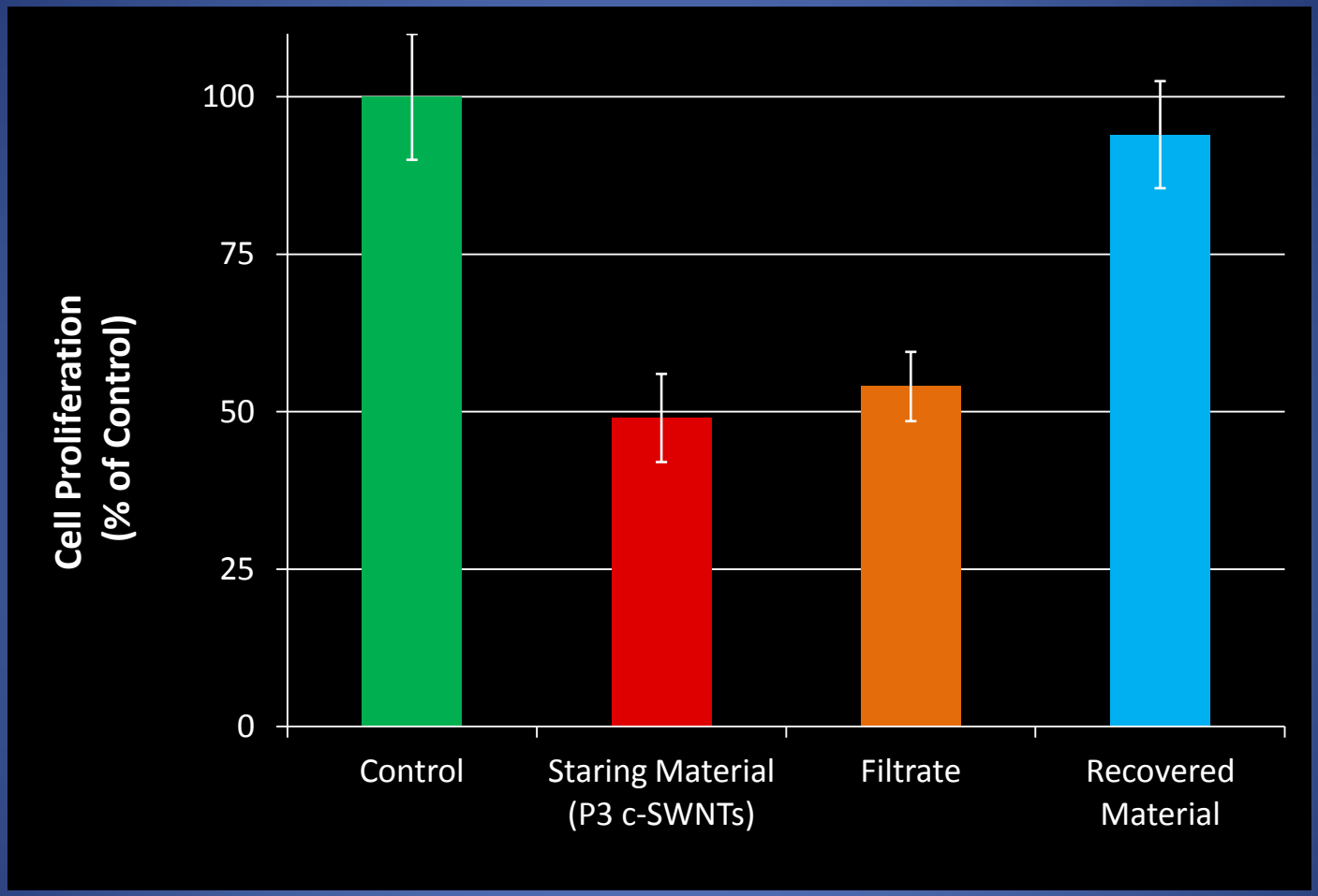
Recovered Material







Filtration Purified Carboxylated SWNTs are Not Cytotoxic



I. Introduction

II. Physical Characterizations of SWNT Dispersions

III. In vitro Cytotoxicity Assays

IV. Removal of toxic components

- The commercially purchased carboxylated P3 SWNT product appear to contain amorphous carbon fragments.
- The toxic components in P3 SWNT product, smaller than 0.22 μm in size, can be removed by filtration.
- The recovered materials, larger than 0.22 μm in size, are not toxic.

V. Summary

- ✓ Commercially obtained SWNT products require physical and chemical characterization to ensure quality, purity, and lack of toxicity.
- ✓ 7 purchased SWNT products were tested for toxicity in a model mammalian cell culture system using 2 different assays.
- ✓ Only one SWNT product tested was toxic as a function of exposure time and concentration.
- ✓ The toxic component in the P3 SWNT product could be removed from the SWNTs by filtration.

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Related research projects

1. SWNT carboxylation <=?=> toxicity
   - carboxylation process creates more amorphous carbon fragments
   - majority of amorphous carbon fragments are removed by filtration

   Are COOH-SWNT products from different sources toxic?

   Does the extent of carboxylation correlate with toxicity?

2. Expand study to other carbon based nano materials
   - double-walled carbon nanotubes (DWNTs)
   - multi-walled carbon nanotubes (MWNTs)
   - graphene oxide

Worsley et al., J Am Chem. Soc. (2009) 131 (508): 18153-8.

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