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Attachment and Growth of PC12 Neural Cells in Presence of Multifunctional Nanocarriers for Early Detection of Radiation Induced Damages

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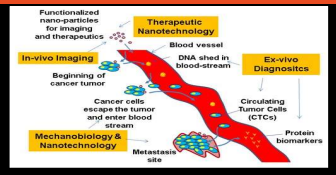
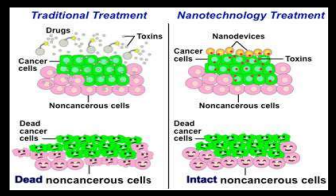
Overview

- Chronic low-dose galactic cosmic ray (GCR) radiation can aggravate of disease states like cardiovascular, cerebrovascular, microvascular, and radiation induced central nervous system damages.
- These can result in acute and/or late changes in cognition, memory, reaction time, and behavior.
- Unavailability of point-of-care minimally-invasive rapid tests that are able to determine the extent of personal sustained tissue-specific damages is a significant problem.
- In our research we are trying to design nano substrates that can facilitate neural growth and attachment.
- Nanotube substrates are an excellent and unique candidate in that regard.
- Therefore, in our research we are utilizing Boron Nitride Nanotubes (BNNT) as the growth substrate which is biocompatible unlike Carbon Nanotubes(CNT) which show a high level of toxicity.

Why Biomedical Nanotechnology is the "Way to GO"?



Novel applications in the area of cellular detection, targeting, and drug delivery.



Transportation and release of therapeutics to the specific target only

- Diseases are initially expressed at molecular and cellular scales and the study of biological molecules and diseased cells require "theranostic" devices at same size scales.
- Thus, Nano-bioengineering applications hold the potential to achieve improved treatment efficacy, decreased therapy time, and use of smaller quantity of the therapeutic agents.

Introduction

- Boron nitride nanotubes (BNNT) have a characteristically high degree of chemical and thermal stability, are less cytotoxic than carbon nanotubes (CNTs) and possess excellent piezoelectric properties; making them an extremely attractive candidate for potential biomedical applications.
- However, several issues (e.g., poor dispersibility, and agglomeration in the intracellular environment) limit the use of BNNT-based systems.
- In this work, we have evaluated the attachment and growth pattern from E.coli cells and PC12 cells in the presence of this novel nanoscale system.
- E.coli cells and PC12 cells were cultured and exposed to 100 µg/ml, 300 µg/ml, 400 µg/ml, and 1200 µg/ml of BNNT concentration levels to observe the cell density and growth.
- We interpret this data to indicate that the suspended BNNTs up to a moderately high concentration of 300 µg/ml in the culture media do not deleteriously affect cellular growth, or adversely affect the cellular health of the E.coli/ PC12 model neural cells.

Multifunctional Nano-Scale Bio-Marker Design

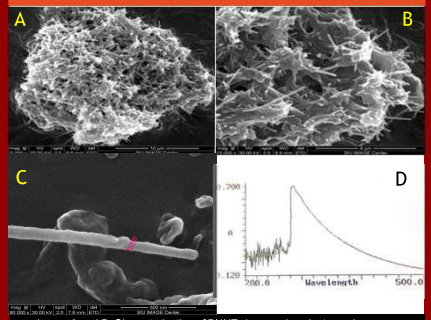
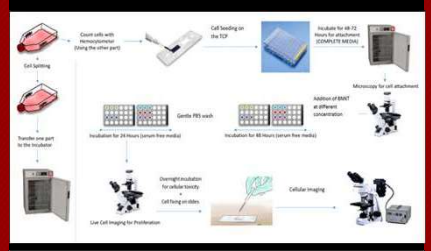


Image A and B: Characterization of BNNT via scanning electron microscopy
Image C: BNNT- 84nm in diameter
Image D: UV-Vis Spectrum of synthesized BNNT

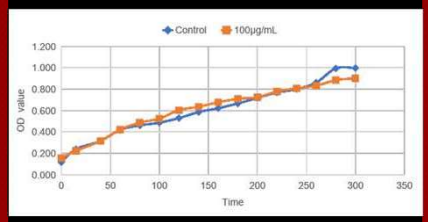
Experimental Methods and Design for *E.coli* Cells

- Dissolve BNNT in water and sonicate for 15 minutes at medium strength.
- Check for homogeneity, if not sonicate for another 15 minutes.
- Add 3-5 drops of SDS (1 mg/ml from stock) until the dispersion can be seen.
- Due to hydrophobicity of BNNT metal surface, Sodium Dodecyl Sulfate (1 mg/ml) has been used for dispersion.
- SDS renders the hydrophobic-hydrophobic interaction with metallic surface and conducive to disperse the BNNT in water by probe sonication.
- E. coli (K 12) was grown aerobically at 37 °C in an incubator at 100 rpm overnight to stationary phase.
- Luria-Bertani (LB) medium (10 gm/L of Tryptone, 5 gm/L yeast extract and 10 gm/L sodium chloride) was used for the bacterial culture.
- The cultures were incubated at 37 °C, shaking at 100 rpm and the optical density was measured using A600nm at selected times to determine growth rates.
- 600 nm is selected, because to measure bacterial growth by light scattering it is best to pick a wavelength where absorption is at a minimum and for most bacterial cultures wavelengths around 600 nm are a good choice.

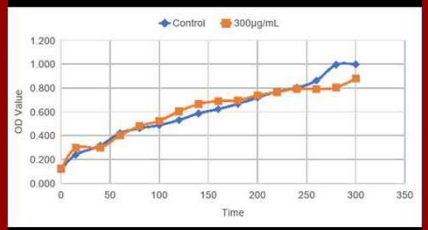
Experimental Methods and Design for PC12 Neural Cell Model



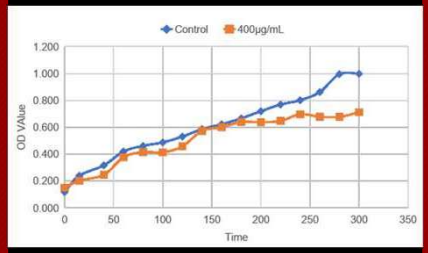
BNNT Toxicity Data



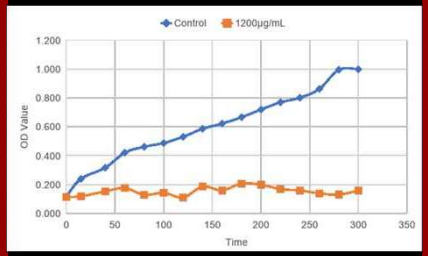
Cellular toxicity (Optical Density) variation over time at 100 µg/ml



Cellular toxicity (Optical Density) variation over time at 300 µg/ml



Cellular toxicity (Optical Density) variation over time at 400 µg/ml



Cellular toxicity (Optical Density) variation over time at 1200 µg/ml

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Morphology and growth patterns of PC12 neural modal cells.

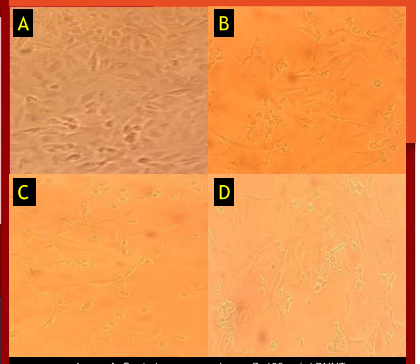

Image A: Control
Image B: 100 µg/ml BNNT
Image C: 200 µg/ml
Image D: 400 µg/ml

Ongoing work and current work in progress

- Toxicity assessments with PC12 model neuronal cells
- Cell and neurite outgrowth and cells density assessment.

Conclusion

- The synthesized Boron Nitride Nanotubes show biocompatibility at moderately low concentrations showing its potential to be used as a growth substrate to further extract neurite growth.
- Excellent cell growth shown in PC12 cells in the presence of the BNNTs is indicative of strong substrate surface attachment, which is also considered an important parameter for efficient early detection of biomolecules.
- Minimal toxicity and excellent intracellular uptake demonstrated the potential for novel therapeutic applications.
- Provide the basis for designing a highly effective nano-patterned substrate for facilitating biomolecule detection and radiation induced damages at the cellular level.

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