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Quantitative Comparison of a Nanoengineered Alumina Coated CNT Arrays to SiO2 Coated CNTs and Solution Based Delivery System

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Investigating Ovalbumin Delivery by Amorphous Alumina-coated Nanowire Arrays

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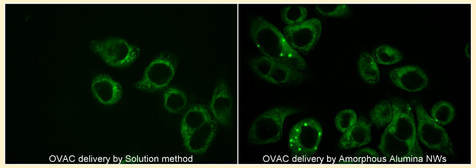
Introduction

There has been a growing need for nanoengineered biocompatible materials to serve as drug delivery platforms for cancer. In this research, vertically aligned alumina nanowires (NWs) were fabricated by high yielding, tightly controlled atomic layer deposition[1] to serve as a platform for delivering biomolecules[2] which act as cancer therapeutics.



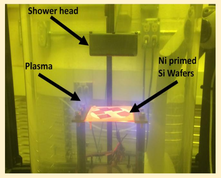
Preliminary results indicate that compared to a solution delivery, NWs are successful and more effective at delivering OVA to HeLa cells and with dendritic cells (DCs). Results confirm that there is an increased amount of dye spots noted in the NW delivery method, suggesting that more dye is delivered per cell. It is hypothesized that this is due to a changed in concentration gradient induced by the NWs.

Aim

To further understand the interaction of cells with the NW arrays, in this work, the mechanism of antigen – ovalbumin (OVA) delivery was investigated. Here in, we aim to investigate the interaction of whether the NWs use macropinocytosis (MP) and receptor meditated endocytosis (RME) mechanisms to digest OVA or if one method is predominantly used. Solution based ovalbumin delivery *(in-vitro)* was used to compare results. To assess mechanistic results, delivery was assessed by confocal microscopy.

Methods

3.5 nm nickel was sputter coated onto silicon wafers by physical vapor deposition (PVD). Carbon nanowires arrays (CNTs) were grown on plasma enhanced chemical vapor deposition systems. Acetylene was used (50 sccm) as the carbon source along with ammonia (200 sccm) and nitrogen gases chemical vapor deposition (PECVD) at 800 ºC, 630 V for 30 minutes.



The CNTs were then coated with alumina by atomic layer deposition (ALD) completed at Oregon State University by Dr. John Conley Jr.'s team using a patented process.

Ovalbumin Delivery to DCs

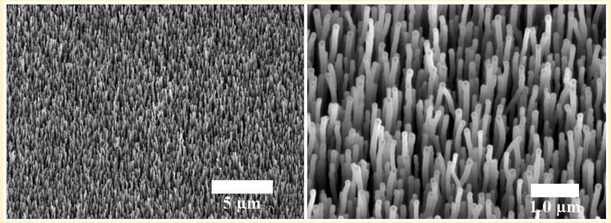
Dendritic cells (murine, immortalized bone marrow derived) were cultured with Gibco RPMI, supplemented with 10 % fetal bovine serum and 1 % Penicillin Streptomycin and incubated at 5 % CO_2 and 37 ºC.

To investigate RME mechanism and to provide evidence towards protein delivery through the NW arrays, DQ OVA was chosen as the antigen. DQ OVA was attached to the NWs using a chemical linker 3-aminopropyl-trimethoxysilane (APTMS). DCs were then subcultured (added) on the NWs and incubated for 8 hours. Cells were then removed from the NWs and added to new petri dishes for imaging purposes. Samples were fixed with a 4% paraformaldehyde solution, BSA and washed with PBS at 19.5 hours. They were further treated with BSA (bovine serum albumin).

To investigate the MP mechanism OVA alone was delivered by NWs and Lucifer Yellow (LY) dye was used. Since LY specifically reacts to fluid phase changes, cells were imaged live on the NW arrays after 4 hours.

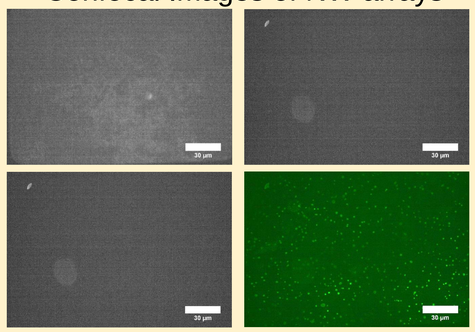
Samples were imaged using a Leica Ni8, Yokogawa Nipkow spinning disk confocal system. A water immersion, dipping lens (60x) was used to image live cells as well as fixed samples. Both DQ OVA (BODIPY dye) and LY dye were imaged with using λ_{ex} at 488 nm.

NW Characterization with SEM



Representative SEM images of amorphous alumina coated NWs were taken using a 30 tilt to show features of the NW arrays. The tips of the NWs are darker indicating Ni.

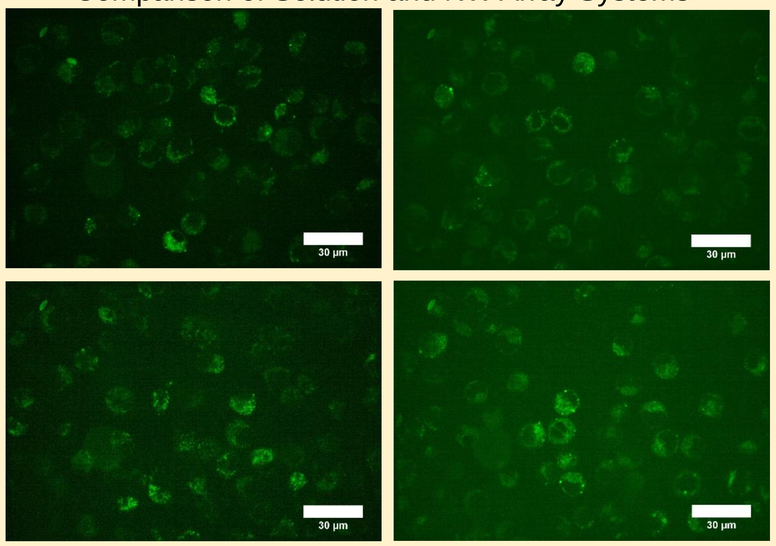
Confocal Images of NW arrays



Top L: NW arrays with linker. **Top R**: NW arrays with OVA
Bottom L: NW arrays with linker, OVA, LY dye. **Bottom R:** NW arrays with linker, OVA, LY dye, cells.
Results show LY dye only fluoresce if OVA + cells are added

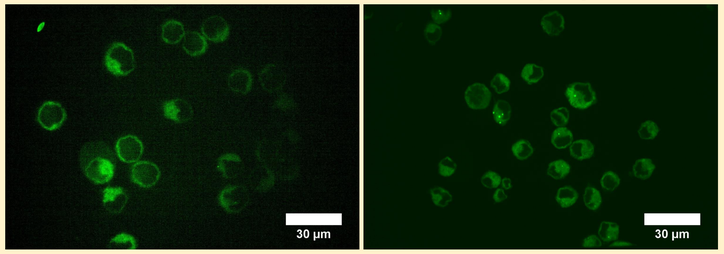
Results

Ovalbumin Delivery through Macropinocytosis - a Comparison of Solution and NW Array Systems



Top + Bottom L: OVA delivery by NW arrays
Top + Bottom R: OVA delivery by solution based system
Results show that both solution and NW array showed no significant difference in the use of macropinocytosis pathway. Ovalbumin protein 250 µL [0.1 mg/mL] and Lucifer Yellow 250 µL [10 µg/mL] were delivered by NWs and by solution delivery.

Ovalbumin Delivery through Receptor Mediated Endocytosis – Results of the NW Array System



Results show conclusively that DQ OVA is digested and processed by DCs when delivered through the NW arrays. DQ OVA protein is fully quenched outside the cell, upon lysosomal proteolysis, peptide sequences fluoresce at 505 nm. Results imaged after 19.5 hours of DQ OVA digestion
L: DQ OVA 250 µL [10 µg/mL] delivery Wafer 1
R: DQ OVA 250 µL [10 µg/mL] delivery Wafer 2

Conclusions and Next Steps

Previous results confirm that there is an increased amount of dye spots noted in the NW delivery method, suggesting that more dye is delivered per cell. It is hypothesized that this is due to a changed in concentration gradient induced by the NWs. Preliminary results and observations in this work does not show any significant difference in macropinocytosis mechanism. DQ OVA comparison with solution based delivery system and quantitative results with flow cytometry should be completed. Additionally, the amorphous alumina coating should be further investigated and compared with a flat alumina base to isolate the effects of the 3D growth medium typical of NW arrays. In the future, semiconductors and biocompatible materials such as SiO_2 should be also be investigated.

Acknowledgments

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