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Polyethylenimine-Enhanced Alumina Nanoscale Adjuvant for Cervical Cancer Vaccine

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ABSTRACT

Aluminum oxide nanoparticles (Al2O3 NPs) have been shown to increase the efficiency of cell-mediated immune response. Specifically, CD8 and CD4 immune response is required for T cell activation by dendritic cells. These nanoparticles, when functionalized with peptides and other molecules, can be used as vaccine in cancer treatment. HPV-induced cervical cancer expresses E6/E7 antigens. E6/E7 proteins were attached using surface modification of the Al2O3 NPs; different types of molecules were tested to see which adhered the highest amount of protein and produced the strongest cell response. Protein measurements were done using bicinchoninic acid assay (BCA assay) and spectrophotometry. CD8 and CD4 immune response was measured in vivo using flow cytometry. In vitro measurements of immune response were done using the TC1 tumor line. When coated on the nanoparticles and mixed with E6/E7 protein, the polymer polyethylenimine (PEI) proved to be most effective at strengthening the immune response in vaccinated mice.

Our findings in this study demonstrate the growing importance of applied physics in the fields of medicine and biology. Fabrication and characterization of nano-materials are important for improving vaccine delivery and ensuring effectiveness.

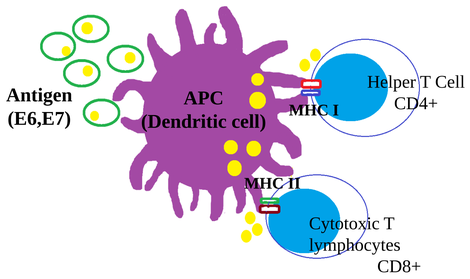
METHODS & MATERIALS

Synthesis and characterization: Al2O3 nanoparticles (40-80 nm) were functionalized via ultra-sonication. Imaging of the PEI-coated Al2O3 NPs was performed on the transmission electron microscope. The specimen was dried in a vacuum oven on a lacy carbon grid. The FEI Tecnai F-20 200kV field emission high resolution TEM was used for imaging. E6/E7 protein attachment to the NPs was carried out overnight. measurement of protein attachment was done using the spectrometer Nanodrop's protein assay and BCA assay.

T cell activation: Antigen was injected into C57BL/6 mice by intramuscular injection. One week post vaccination, the spleens were harvested and re-stimulated with E6/E7, OVA, and anti-CD3. After surface staining with PE CD8, FITC CD4, and intracullelar staining with APC IFNgamma, T cell response was measured by flow cytometry.

We also measured the capability of the dendritic cells pulsed by PEI enhanced Alumina NP carrying antigen to activate CFSE (carboxyfluorescein diacetate succinimidyl ester)-stained naïve OT-1 T cells when incubated with cancer cell line that responds to E6/E7.

T CELL IMMUNE PROCESS

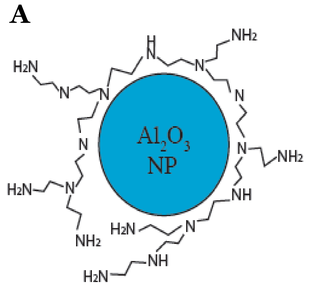


Antigen presenting cells (APCs) such as dendritic cells (DCs) process and present antigen to naïve T cells via the Major Histocompatability Complex (MHC). MHCII activates CD4+ helper T cells which activate other immune cells against the antigen . MHCI activate CD8+ T cells which kill antigen-positive cancer cells.

RESULTS

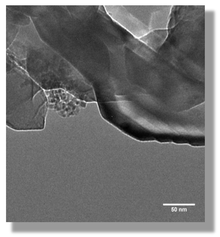
Modification of Alumina NPs with Polyethylenimine

A

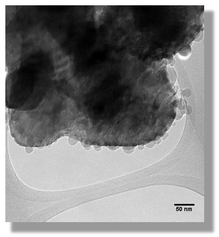


Surface modification of Al2O3 NPs with PEI was accomplished via ultra-sonication. Diagram A is an illustration of the functionalization. Figure 1a is a transmission electron microscope (TEM) image of naked alumina. Figures 1b and 1c show that PEI is coating the Al2O3 NP. The electron beam from the microscope cause the polymer to form bubbles. Figure 1c is a high magnification of the sample, showing the lattice fringes of the polycrystalline Al2O3.

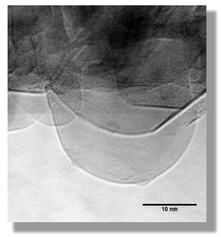
1a



1b

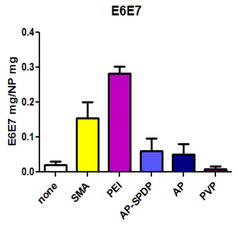


1c

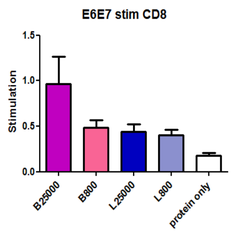


PEI efficiently enhance alumina nanoscale adjuvancy

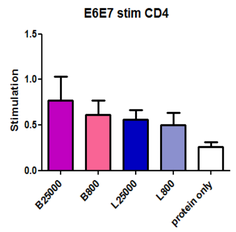
2a



2b

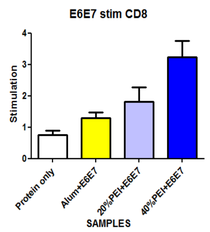


2c

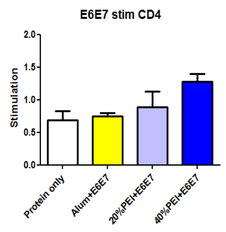


Different molecules were analyzed for their efficiency at protein attachment (Figure 2a). Al2O3 NPs were coated with the polymer styrene maleic anhydride (SMA), PEI, the chemical aminophenol (AP), aminophenol cross-linked with SPDP (SPDP), and polyvinylpyrrolidone (PVP). After determining that PEI was the best candidate for improving antigen delivery, different forms and weights of PEI were tested in vivo. Mice were injected with five different samples: branched PEI at 25k Daltons ("B25000") and 800 Da ("B800"), linear PEI at 25k Da ("L25000") and 800 Da ("L800"), and E6/E7 (protein only). Flow cytometry determined that the branched PEI at 25kDa stimulated the most CD8 and CD4 immune response when re-stimulated with E6/E7 protein (figures 2b and 2c).

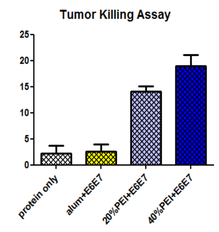
3a



3b



3c



Different titrations of PEI-coated Al2O3 NPs were tested in vivo to compare CD4 and CD8 immune response to re-stimulation (figures 3a and 3b). They were also tested to see the effectiveness of killing TC1 tumor cells (figure 3c). The NPs were compared with Alum, which is an FDA approved adjuvant.

CONCLUSION

Using transmission electron microscopy, the presence of branched polyethylenimine (PEI) around the alumina NPs could be detected and imaged.

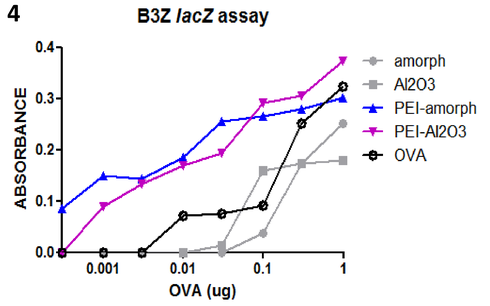
PEI-modified Al2O3 NPs most efficiently loaded proteins. Thus, it demonstrated to be highly effective in improving antigen delivery by Al2O3 NPs.

Out of the different structures and weights of PEI, the branched polymer (25kDa) produced the most CD4 and CD8 response. Higher titrations of the NPs also produced higher T cell signals.

FUTURE WORK

Improve dispersion- the PEI-enhanced alumina NPs do not stay dispersed in solution and settles to the bottom. This makes accurate injection dosage difficult to determine.

4



Amorphous Al_2O_3 NPs stay well-dispersed when coated with PEI. We have done a simple *in vitro* assay using frozen DC2.4 and B3Z T cells. These T cells respond to ovalbumin (OVA), so we attached OVA to the NPs. CPRG reagent staining and spectrometer absorbancy reading show the T cell response to DCs pulsed with different NPs at different titrations of antigen (figure 4). The size of the amorphous NPs are too small, 15nm, so we need to find an optimal size so the PEI coating does not overwhelm the capabilities of the alumina.

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ACKNOWLEDGEMENT

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