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α-Al_2O_3 Nanowire Delivery of Soluble Antigen for Cross- Priming of Cytotoxic T-cells

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One of the most effective methods for detection and destruction of tumor cells is therapeutic cancer vaccination. The induction of tumor-specific T-cell immunity relies on efficient cross presentation of tumor associated antigens. We previously showed that α-Al_2O_3 nanoparticles (NPs), as antigen carriers, are phagocytosed favorably by dendritic cells (DCs) and efficiently enhance cross-presentation of ovalbumin (OVA) to Thy1.1$^+$ OT-I CD8$^+$ T cells.[1] The morphological differences between the nanowires and nanoparticles may affect the antigen delivery and the efficacy for antigen cross-presentation.

To test this hypothesis, we synthesized α-Al_2O_3 NWs via chemical vapor deposition of Al sheet (98.5% purity) and TiO_2 nanoparticles (97.5% purity) in an alumina boat. Similar to the method reported by Peng et al.,[2, 3] the chemical vapor deposition reactor was heated to 1050 – 1150 ^oC with a ramp rate of 25^{o}C/min under flow of 100 sccm of argon. After reactions for 60 min and cooling with argon flow, the resulting fine white-grey powder was collected and characterized by a scanning electron microscope (SEM) and a transmission electron microscope (TEM). FIG 1a shows that high yield branched NWs were grown. Energy dispersive X-ray spectroscopy (EDX) analyses reveals that these NWs are Al_2O_3 (FIG 1b). The branched Al_2O_3 NW consisted of a thick trunk and many thin needle-like NWs, well-aligned, on the surface of the trunk (FIG 1c). HRTEM imaging of a partial Al_2O_3 NW indicates that these NW are single crystalline and the lattice spacing in two directions of 2.379Å, 3.500 Å match (110) and (012) planes of α-Al_2O_3. As-synthesized α-Al_2O_3 NWs were ultrasonicated and purified using centrifuge forming the suspension of well-dispersed α-Al_2O_3 NWs. Using the same protocol as conjugating OVA to α-Al_2O_3 NPs, we conjugated OVA to α-Al_2O_3 NWs. To compare the efficiency of cross-presenting α-Al_2O_3 NP-OVA or α-Al_2O_3 NW-OVA, we used these conjugates to pulse 0.2 million DCs. Six hours later, the pulsed DCs were washed and employed to prime 1 million CFSE-labeled Thy1.1$^+$ OT-I CD8$^+$ T cells. After a 66 hour incubation, cells were collected and treated with marked antibodies of CD8-PE and Thy1.1-APC. The percentage of proliferated Thy1.1$^+$ OT-I CD8$^+$ T cells was measured using BD FACS Calibur flow cytometry. α-Al_2O_3 NW-OVA successfully cross-primed antigen-specific T cells, however the efficiency was 15 % lower in comparison to the α-Al_2O_3 NP-OVA. To clarify the shape-induced efficiency difference of cross-presentation, tracking the antigen carriers of α-Al_2O_3 NW and α-Al_2O_3 NP in sub-cellular compartments will be conducted.

[1] H. Li et al., has been submitted to *Nature Nanotechnology* (2010).
[2] X. S. Peng et al., *J. Phys. Chem. B* 106 (2002) 11163.
[3] X. S. Fang et al., *J. Phys.: Condens. Matter* 16 (2004) 4157.
[4] This research was supported by Oregon Nanoscience and Microtechnologies Institute and National Science Foundation.

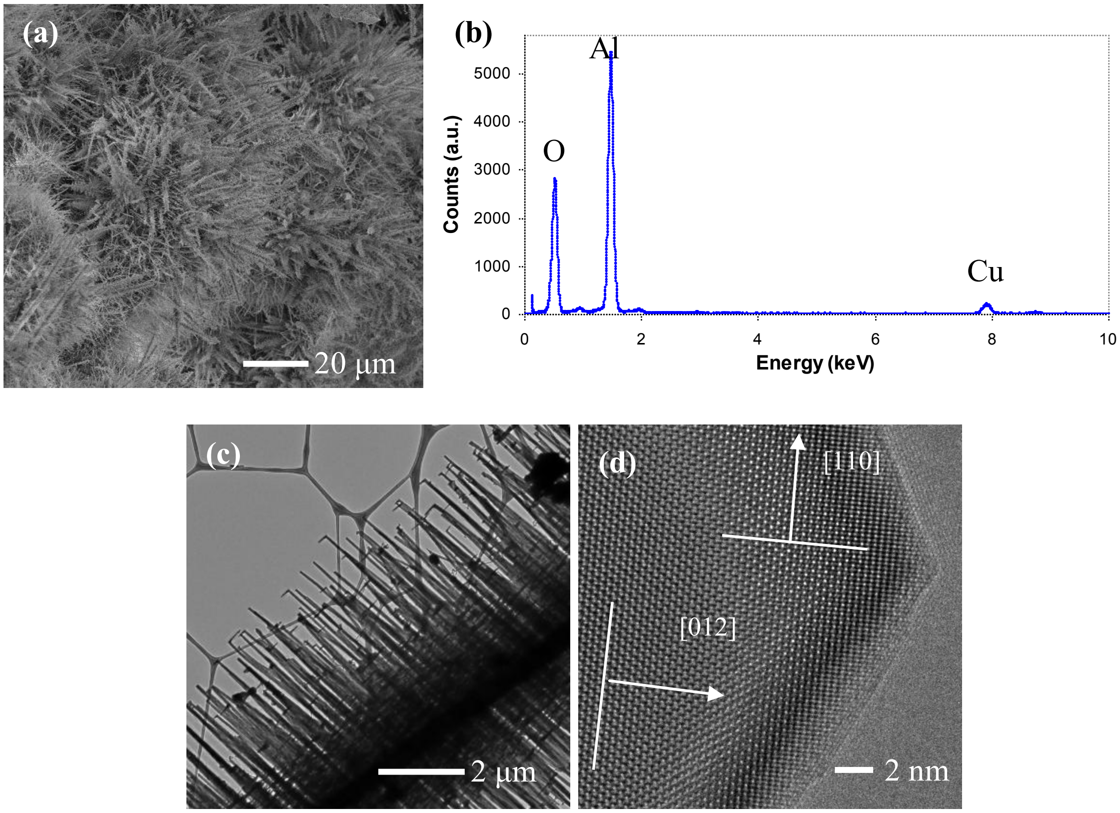

FIG. 1. (a) SEM image of Al_2O_3 NWs grown at 1100°C for 60 min. (b) EDX spectrum of as-synthesized Al_2O_3 NWs. (c) and (d) low- and high-resultion TEM images of as-synthesized Al_2O_3 NW.

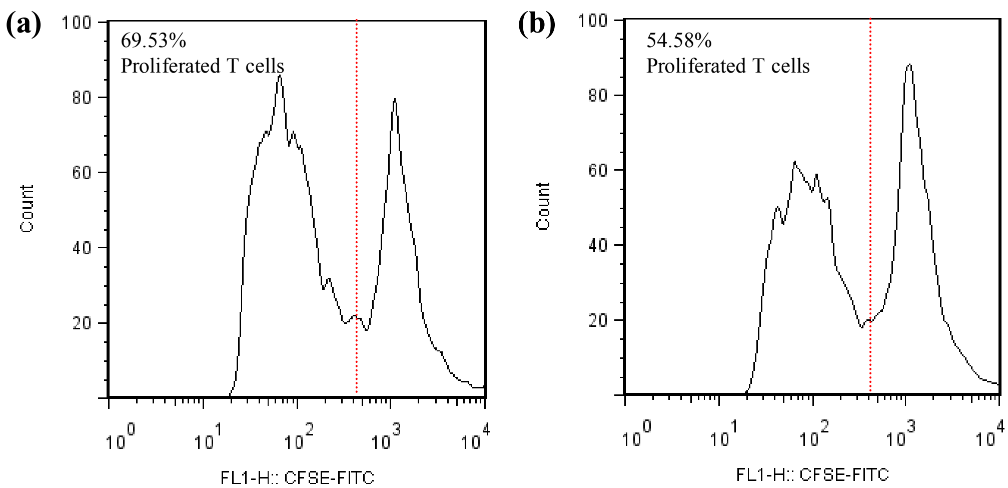

FIG. 2. The percentages of proliferated Thy1.1$^+$ OT-I CD8$^+$ T cells cross-primed by DCs pulsed with (a) 0.01μg/mL of α-Al_2O_3 NP-OVA and (b) 0.01μg/mL of α-Al_2O_3 NW-OVA.

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