Probing Morphology and Cellular Processing of Peptide Amphiphile Micelle Vaccines Utilizing Electron Microscopy

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Peptide amphiphiles (PAs) are a class of diblock biomaterials comprised of hydrophilic peptide head(s) conjugated with hydrophobic lipid tail(s) which self-assemble into peptide amphiphile micelles (PAMs) in water. PAMs play a significant role in biomedical research, having shown promise as cancer therapies, prophylactic vaccines, and regenerative solutions. Our preliminary studies demonstrate that a PAM vaccine is more effective than a traditional subunit vaccine (Fig. 1). However, the immunoinductive mechanism of PAM vaccines is still poorly understood. For instance, it is not well known how PAM vaccine physical properties, such as size, shape, and surface charge, affect its cell uptake ability. In this study, we investigated ways to control PAM morphology by tweaking materials chemistry. Additionally, we studied several staining types and procedures as well as optimized imaging quality to allow for better exploration of PAM morphology. We also utilized fluorescent microscopy and studied PAM trafficking within the cells. Further, we have ongoing experiments utilizing high pressure freezing to track peptide amphiphile trafficking within cell compartments using transmission electron microscopy.

Morphology characterization of PAMs was achieved by negative staining utilizing transmission electron microscopy. Different stains, including nano-tungsten, uranyl formate, and uranyl acetate, were applied to achieve optimal image quality. A macrophage like monocyte cell line, RAW 264.7, was incubated with PAMs over different time courses either on a TEM grid for high pressure freezing or on a coverslip for fluorescent microscopy.

PAM shape and size can be controlled by introducing a zwitterion-like polypeptide region, (Lys-Glu-Lys-Glu-Lys-Glu-Lys-Glu) (Fig. 2). Additionally, nano tungsten staining gave the best imaging quality for PAM samples. PAMs were not internalized into endosomes based on confocal fluorescent microscopy studies.
Figure 1. PAM vaccines have better performance than traditional subunit vaccines in promoting dendritic cell activation in vitro (left panel, red peaks represent PAM vaccine formulations and blue peaks represent conventional subunit vaccine formulations) and in vivo (right panel, higher antibody titers for micelle vaccines).

Figure 2. PAs with two different peptide sequences, OVA\textsubscript{BT} (upper panel) or OVA\textsubscript{cytoT}, yield similar structure micelles. (Palm = palmitic acid)