Generating Dendritic Cell-Mimetic Artificial Antigen Presenting Cell for Optimized T Cell Activation

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Primary CNF Tools Used: Hitachi TM3000 SEM, Malvern Nano ZS Zetasizer

Abstract:

This project aims to develop dendritic cell-mimetic artificial antigen-presenting cells using wrinkled polystyrene particles and chemically treated sunflower pollens to enhance T cell activation. Polystyrene particles are fabricated via solvent evaporation-induced interfacial instability, while pollen shells are prepared through acid-base treatment. Both are coated with polydopamine and conjugated with activation antibodies. Particle size and morphology are characterized using CNF tools: the Zetasizer and Hitachi TM3000 SEM. Future work will focus on improving surface morphology and antibody accessibility to enhance T cell stimulation.

Summary of Research:

Mechanical cues such as substrate stiffness and surface topography are important in regulating T cell activation [1]. This project aims to generate dendritic cell-mimetic artificial antigen presenting cells for optimized T cell activation using synthetic polystyrene particles and natural sunflower pollens respectively. The resulting protruding morphology facilitates the formation of an interaction area between naïve T cells and artificial T cells that mimics the natural immune synapse, which enhances T cell activation and proliferation.

The polystyrene particles are generated using interfacial instability of emulsion droplets during solvent evaporation using the method similar to the method described by Liu et al [2]. Polystyrene emulsion droplets are via homogenization, where the organic phase has polystyrene and 1- hexadecanol dissolved in chloroform, while the continuous phase has sodium dodecyl sulfate and glycerol dissolved in deionized water. The droplet size is optimized by tuning the polymer concentration and homogenization speed. After homogenization, the droplets are solidified under controlled solvent evaporation condition to trigger interfacial instability and form wrinkled surfaces. The solidified wrinkled polystyrene particles are incubated with deionized water and ethanol respectively to remove residual chloroform, sodium dodecyl sulfate, glycerol, and 1-hexadecanol. Clean particles are coated with polydopamine and conjugated with activation antibodies for T cell activation.

Sunflower pollens are first defatted and then incubated with acid and base respectively to obtain clean, hollow pollen shell for biomedical applications combining the protocols in previous literatures [3], [4]. For the defatting, sunflower pollens are washed subsequently with deionized water, acetone, and cyclohexane. Defatted sunflower pollens are incubated with phosphoric acid and then potassium hydroxide to remove the internal cytoplasmic contents. Clean pollen shells are also coated with polydopamine and then conjugated with activation antibodies for T cell activation.

Both dendritic cell-mimetic artificial antigen presenting cells are co-cultured with native CD4 T cells extracted from mice for three days. After the three-day activation, T cells are stained and examined under flow cytometry to check for activation markers.

Two CNF tools are used to characterize the polystyrene particles and sunflower pollens: Malvern Nano ZS Zetasizer is used for dynamic light scattering measurements to measure the diameter of polystyrene particles generated under different homogenization conditions. Hitachi TM3000 SEM is used to characterize the morphology of polystyrene particles and sunflower pollens.

Conclusions and Future Steps:

For the polystyrene artificial antigen presenting cells, the effect of homogenization speed on particle diameter is shown in Figure 1. The optimal particle diameter is 4-5 microns, which is similar to that of naïve T cells. A lower homogenization speed of 2.8k rpm generates

particles with a diameter of 1.14 µm, which is larger compared to those generated at a homogenization speed of 5.2k rpm. To further increase the particle diameter, polymer concentration is further increased. The resulting particles are characterized with instruments outside of CNF and the results are not shown here. The largest polystyrene particles have a diameter of approximately 4-5 um.

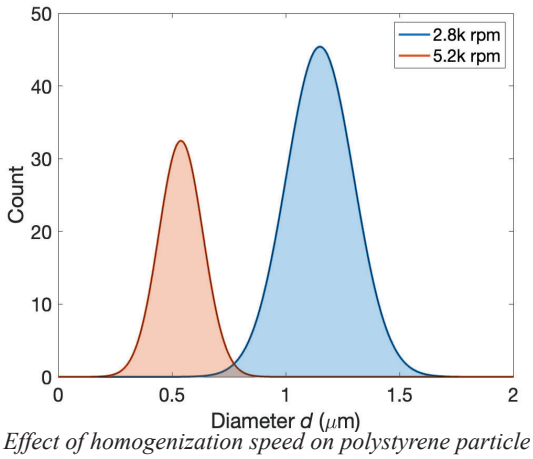

Figure 1: Effect of homogenization speed on polystyrene particle diameter obtained by dynamic light scattering.

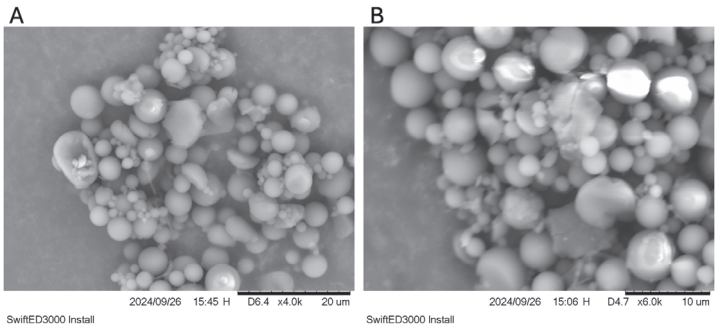

Figure 2: Effect of dialysis against ethanol on polystyrene particle morphology. After (A) 4 days, (B) 6 days dialysis.

The wrinkling is first assumed to happen during the ethanol wash when 1-hexadecanol leaves the droplet, and the ethanol wash is first done via dialysis instead of direct incubation. Figure 2 shows the morphology of polystyrene particles sampled after 4 days and 6 days of dialysis against ethanol respectively. Particles with a diameter of 4-5 um is difficult to image clearly using the tabletop SEM. There's no significant difference in morphology between particles sampled after 4 days and 6 days of dialysis against ethanol, suggesting that wrinkling is not due to 1-hexadecanol leaving the structure. Alternatively, we assume that wrinkling happens during solidification instead of the ethanol wash, which is later confirmed by Gemini SEM results. For easier imaging, we later switched to Gemini SEM, and the results are not shown here.

The morphology of untreated pollens, acid treated pollens and acid and base treated pollens are shown in Figure 3. Pollens remain intact after acid treatment and base treatment and the spiky features are preserved. Acid treatment opens the apertures on pollen surface, suggesting removal of internal components.

Future work will focus on optimizing the accessibility of conjugated antibodies on pollen shells for more effective activation and increasing the percentage of wrinkled polystyrene particles.

References:

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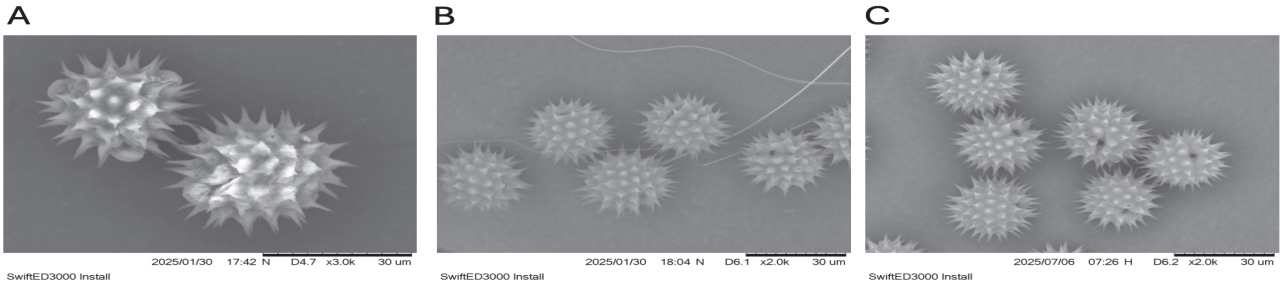

Figure 3: Effect of chemical treatment on pollen morphology. (A) Untreated pollens, (B) Acid treated pollens, (C) Acid and base treated pollens.