Artificial Antigen Presenting Cells for Tumor Immunotherapy: Shape and Size Matter

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Statement of Purpose: Biodegradable polymers can be used to synthesize microparticles and nanoparticles, and through surface modifications, these particles can mimic biological antigen presenting cells. These biomimetic artificial antigen presenting cells (aAPCs) are a promising platform to modulate the immune system. We fabricate an automated multi-dimensional thin film stretching device and then use this device for the generation of anisotropic polymeric microparticles and nanoparticles (**Fig 1A**). We next utilize these anisotropic particles to construct aAPCs of various sizes and shapes and evaluate their efficacy at stimulating cytotoxic T-Cells *in vitro* and *in vivo*. We demonstrate that both particle size and particle shape are critical parameters for immunomodulation and that these anisotropic aAPCs are capable of generating enhanced T-Cell proliferation *in vivo*.

Methods: The automated stretcher consists of two sets of mechanical grips that are mounted onto aluminum guide rails and can precisely control the stretch of PVA/glycerol films. A 2D stretcher was constructed and automated by computer control of stepper motors. This enables us to have control over the strain rates applied to the visco-elastic films. PLGA microparticles and nanoparticles were made by single-emulsion and then embedded into the films, which were stretched in an oven by the film stretcher. Anisotropic particles were removed from the film by cooling, film dissolution, and then particle purification. aAPCs were constructed by covalently coupling an antigen specific signal ("signal 1," a peptide-in-MHC-IgG dimer) and an activation signal ("signal 2," anti-CD28 mAb) to the surfaces of the particles. All imaging was conducted with a Leo FESEM scanning electron microscope or a Hitachi 7600 TEM. To evaluate aAPC immune activation *in vitro,* a T-Cell stimulation assay was utilized. Primary splenocytes were isolated from fresh PMEL mice and CD8+ T-Cells were isolated. The CD8+ cells were then stained with carboxyfluorescein succinyl ester and labeled CD8+ T-Cells were mixed with various aAPCs to evaluate T-Cell proliferation by flow cytometry and through manual cell counts. To evaluate *in vivo* proliferation, one day prior to adoptive transfer, transient lymphopenia was induced in C57/BL6 mice. The following day, CD8+ T-cells were isolated from Thy1.1+ PMEL mouse spleens. CD8+ T-cells were co-incubated with spherical, non-spherical, or no particles (2 mg per 10^6 cells) for 1 hour at 4 ºC. Recipient Thy1.2+ C57/BL6 mice tail veins were intravenously injected with 1 x 10^6 PMEL T-cells with or without particles.

Results: We have successfully fabricated an automated thin film stretcher for control over biomaterials via thin film stretching in 1D and 2D (**Fig 1A**). This allowed us to generate anisotropic polymeric particles of various sizes and shapes, including those that mimic red blood cells (**Fig 1B**). These micro and nanoparticles can be used to construct micron-scale aAPCs and nano aAPCs (naAPCs). While at high particle doses *in vitro*, different particle shapes can lead to high levels of T-Cell activation, at mid-range particle dose (0.1 mg / 100,000 cells), shape dependency on T-cell activation is the strongest. As an example, the 2-fold stretched naAPC induce a 15-fold expansion of the T-Cells compared to the spherical particles, which induce a 3-fold expansion. *In vivo*, similar trends are seen with ellipsoidal naAPC leading to increased proliferation compared to spherical naAPC or adoptive transfer without particles (**Fig 1C**).

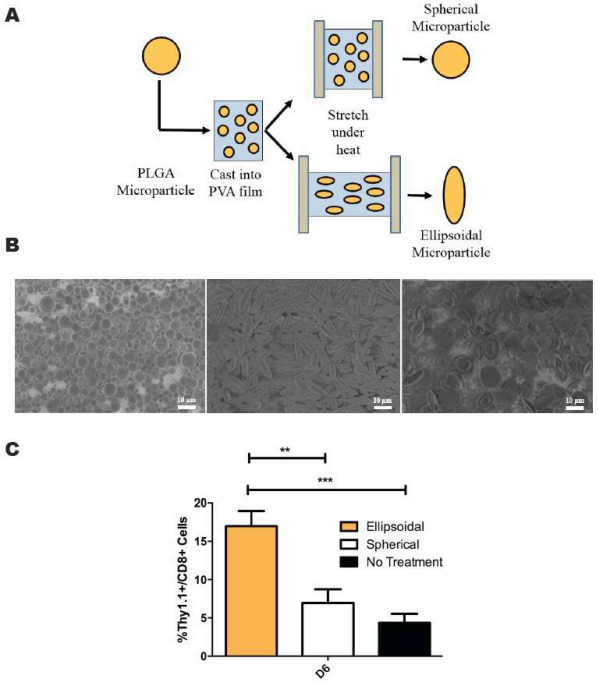

Fig. 1: (**A**) Scheme to control particle shape. (**B**) Particles constructed of various sizes and shapes. (**C**) Ellipsoidal nano aAPCs lead to superior *in vivo* T-cell proliferation compared to spherical nano aAPC.

Conclusions: A 2D automated stretching device was successfully constructed to fabricate anisotropic particles. Particle shape and size were both determined to be important parameters for the activity of polymer-based aAPCs *in vitro* and *in vivo*.

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