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Facile synthesis of polylactide coarse microspheres as artificial antigen-presenting cells

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Experimental section

1. Materials

Poly(L-lactide) (PLLA) (Mw. 50000, 10000, 5000, 50:50) was purchased from Jinan Daigang Biomaterial Co., Ltd (Jinan, P. R. China). PVA was kindly supplied by Sinopec Sichuan Vinylon Works (Chongqing, P. R. China). Poly(allylaminehydrochloride) (PAH, M_W~17,500) was obtained from Sigma-Aldrich. BSA (M_W~66,000) was purchased from Solarbio TechnologyCo., Ltd (P. R. China).The silk fibroin was purchased fromHuzhouXintiansi bio-tech Company (P. R. China) and purified before use. C57BL/6 mouse were provided by Beijing HFK bioscience (Beijing, P. R. China) and maintained according to National Cancer Center Institutional Review Board. Fetal bovine serum (FBS), RPMI1640 culture medium and penicillin-streptomycin were purchased from HyClone (Los Angeles, USA).

Murine IL2 was purchased from PeproTech Inc. (Rocky Hill, USA). Avidin(Mw. 66,000, isoelectric point = 10.5) and Micro BCA Protein Assay Kit were provided by Thermo Fisher Scientific (Waltham, USA). MojoSort™ Mouse CD3 T Cell Isolation Kitwas obtained from Biolegend (San Diego, USA). CCK-8 kit was bought from Dojindo, (Rockville, USA). CBA Mouse IFN-γ Flex Set kit, biotinylated anti-CD3ε(145-2C11) and anti-CD28 (37.51) antibodies were purchased from BD biosciences (San Jose, USA). Biotin (5-fluorescein) conjugate was supplied by Sigma-Aldrich, (St. Louis. USA). Iron Oxide Nanoparticles (15nm) were supplied by Ocean Nanotech (San Diego, USA). All other chemical reagents were of analytical grade and obtained from commercial sources. Ultrapure water used in all experiments was produced by Milli-Q Synthesis System, Millipore Corp (Billerica, USA).

2. Synthesis of aAPCs

2.1. Synthesis of plain and magnetic PLLA smooth microspheres

For the smooth microspheres(sMS) synthesis, using a single emulsion-solvent evaporation technique, 225 mg of PLLA (Mw. 50000) was dissolved in 15 mL of dichloromethane to obtain a 15mg/mL solution. The resultant polymer solution was injected dropwise into 100 mL of PVA aqueous solution (1%, w/v) under constant mechanical agitation with the aid of a high-speed homogenizer (Ultra-Turrax T-18 basic, Ika, Germany) for 5min to emulsify. The resulting microparticle emulsification was followed by slow magnetic stirring overnight at room temperature to evaporate organic solvent from the bulk completely. When the sMS were hardening, washed the sMS three times with ultrapure water through centrifugation. To yield magnetic PLLA sMS, Fe_3O_4 nanoparticles were added to the dichloromethane solvent. Other parameters were the same as the above plain sMS synthesis, except 3600 rpm instead of 7200 rpm in the process of high-speed homogenization.

All of polymeric microspheres in this study were made from PLLA with Mw. 50000 unless otherwise noted.

2.2. Synthesis of polyelectrolyte-coated PLLA microspheres

The coating of polyelectrolytes on as-synthesized PLLA microspheres was carried out by layer-by-layer technique. Typically, 2 mg/mL of PAH or BSA was incubated with PLLA microspheres (~10^8 particles/mL)alternately until 3 layers were formed in 0.5 M of NaCl solution,followed by three washings with 0.5 M of NaCl.

Then, 1 mL of 2% of glutaraldehyde aqueoussolution was added to cross-link the layers. After centrifugation,30 mM of sodium borohydride was used to stop the cross-linkingreaction for 30 min and was followed by washing with waterthree times.

PAH/silk fibroin-coated microspheres were prepared using a similar protocol.

2.3. Synthesis of PLLA-based coarse microspheres

PLLA microspheres, PAH/BSA- or PAH/silk fibroin-coated PLLA microspheres and PLLA microspheres incorporating iron nanoparticles were incubated with Tetrahydrofuran (THF) for 30min thoroughly washed with ultrapure water to remove organic solvents. Repeated the etching step 3 times, then collected cMS by centrifugation.

2.4. Solubility of PLLA with different molecular weight

Desired amount of PLLA raw materials with different molecular weight (Mw. 50000, 10000, 5000) was incubated with THF for 30 minutes to investigate the solubility, respectively.

2.5. Ligand coupling to the MS surface

3×10^6 MS wereresuspended in 1mL of PBS buffer containing 1mg of avidin and non-specific adsorption was carried out at 4℃ by rotating overnight. The binding efficiency was calculated by Micro BCA protein assay kit. Washed the MS 2 times by centrifugation and resuspended them in 1mL of PBS, biotinylated anti-mouse CD3ε and anti-CD28 mAb were added at a concentration of 0.24 μg/mL to 6 μg/mL and incubated for 30min. PE Goat anti-Armenian Hamster IgG from e-Bioscience (San Diego, USA) was used to detect anti-CD3 antibody, FITC Goat anti-Syrian Hamster IgG from e-Bioscience(San Diego, CA, USA) was used to detect anti-CD28 antibody. Mean fluorescence intensity (MFI) of second antibody was detected by flow cytometry LSRII, BD Biosciences (San Jose, USA).

3. Primary T cells isolation and culture condition

After the mice sacrificed, the spleen was dissected out and homogenized through a cell strainer. Primary T cells were isolated from C57BL/6 mouse splenocytes by negative selection using the MojoSort™ Mouse CD3 T Cell Isolation Kit (Biolegend; San Diego, CA). T cells were incubated in RPMI 1640 supplemented

with L-glutamine, non-essential amino acids, 10 % FBS, 1 % penicillin-strptomycin and 100 U / mL murine IL2, with a final concentration of 1 × 10^6 cells / mL.

4. Characterizations

4.1 Scanning electron microscopy (SEM)

SEM (ZEISS, SUPRA 55VP) was employed to observe the topology of PLLA microspheres. Polymeric microspheres were deposited on a silicon wafer and air-dried prior to observation. The samples were gold-coated in an ion coater (EIKO，IB-3) and measured at an operation voltage of 1.0 kV.

4.2 Zeta-potential measurements

The Zeta-potential values of sMS and cMSwith or without avidin adsoption were measured using dynamic light scattering (DLS, Zetasizer Nano-ZS, Malvern).

4.3 Confocal laser scanning microscopy (CLSM)

CLSM was used to evaluate aAPC-T cell conjugate formation. T cells were isolated using a CD3-negative isolation kit from Biolegend (San Diego, USA) and labeled with Cell Tracker Green from Thermo Fisher (Waltham, USA). 1×10^6T cells were incubated with 5×10^5sMS-aAPCs or cMS-aAPCs for 60 min at 37℃ in a φ 20mm glass bottom dish. Cell-aAPCs interactions were visualized during a 1-hour-incubation by confocal imaging. Images were acquired on a Perkin Elmer UltraviewVox(PerkinElmer, USA) laser scanning confocal at 60× magnification.

4.4 Critical point drying

1×10^6 T cells were seeded on poly-l-lysine-coated glass slid from Solarbio (Beijing, P. R. China) and incubated with 5×10^5sMS-aAPCs or cMS-aAPCs for 60 min at 4℃.Then T cells were fixed with 2.5% electron microscopy-grade glutaraldehyde from Leagene(Beijing, P. R. China), followed with dehydration in a serial dilutions of water and ethanol, dried under vacuum using critical point drier (Quorum k850), cells were gold-coated in an ion coater (EIKO，IB-3) and imaged with the SUPRA 55VP (Carl Zeiss, Germany) at 3.0 kV.

4.5 Polyclonal expansion and cytokine secretion detection

Avidin coated sMS or cMS were co-cultured with T cells at a ratio of 1:2 respectively in RPMI1640 complete medium supplemented with 100 U/mL murine

IL2 for 72 hours at 37℃. Cell viability was assessed by the CCK-8 Cell Viability assay kit, and no stimulatory or inhibitory effect was observed relative to control populations. In order to examine the effect of surface topology on T cell expansion, 1×10^5 sMS-aAPCs or cMS-aAPCs were incubated with T cells at a ratio of 1:2 at an antibody concentration from 40 ng/mL to 1000 ng/mL at 37℃ for 5 days. Polyclonal expansion of T cells was analyzed by CCK-8 kit and the secretion of IFN-γ was measured by CBA kit using flow cytometry.

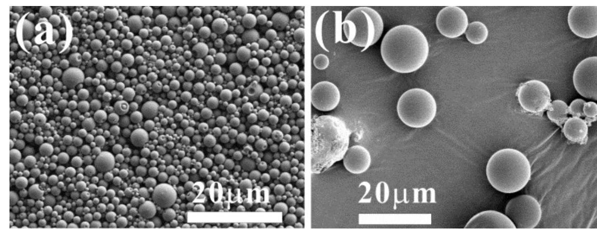

Fig. S1 SEM images of PLLA microspheres (a) and PLLA microspheres incorporating iron nanoparticles (b) fabricated by single emulsion-solvent evaporation method.

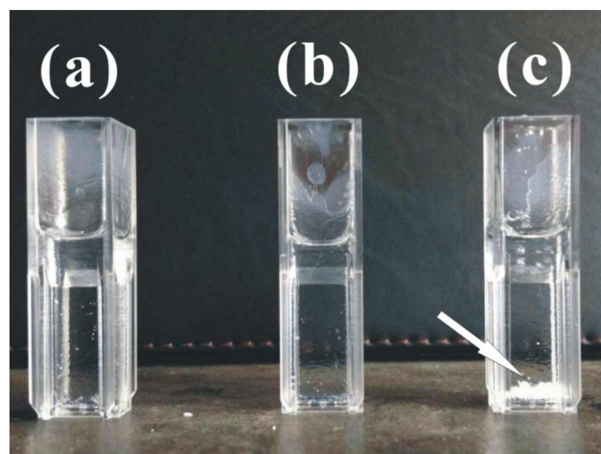

Fig. S2 Digital picture of PLLA raw materials with different molecular weight after 30 minutes of incubation with THF.(a), 5000; (b), 10000; (c), 50000.PLLA sample was soluble in (a) and (b). The arrow in (c) indicates the precipitation of PLLA in THF.

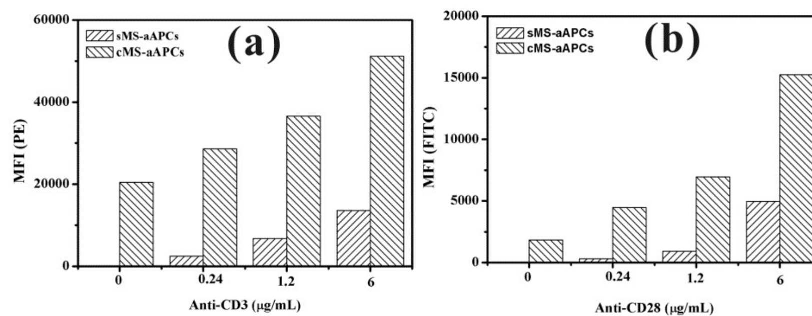

Fig. S3 Antibodies immobilization on sMS and cMS.(a), anti-CD3; (b), anti-CD28.

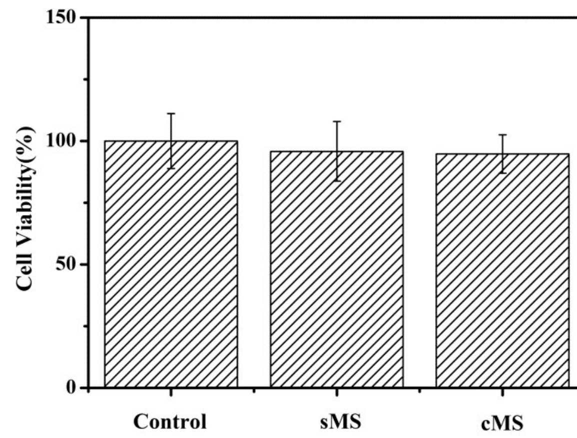

Fig. S4 Cell viability of sMS and cMS.

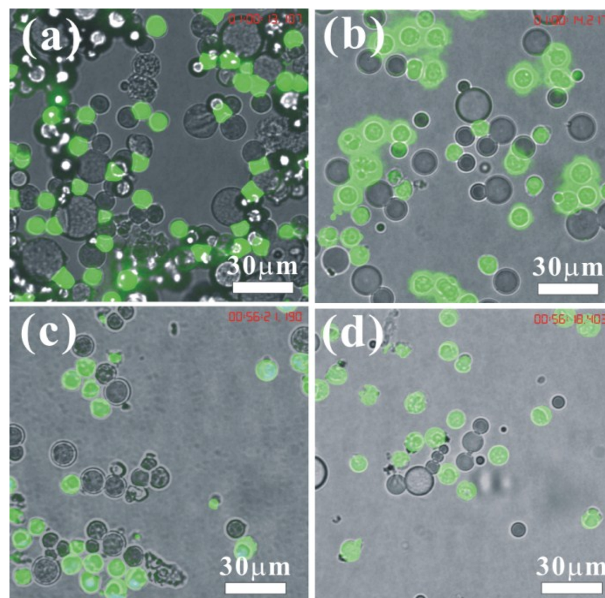

Fig. S5 CLSM images of T cell-aAPC conjugates. (a), cMS-Abs; (b), sMS-Abs; (c), cMS; (d),sMS.

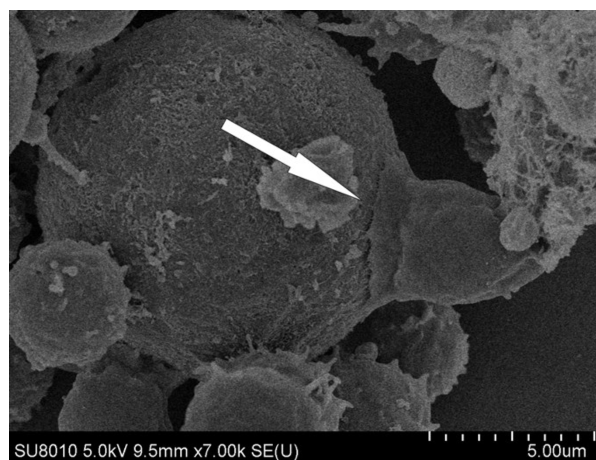

Fig. S6 SEM image of T cell-aAPC conjugates formation after critical point drying. aAPCs are PLLA cMS-Abs. The arrow shows the tight conjugation which indicates the immunological synapses formation.

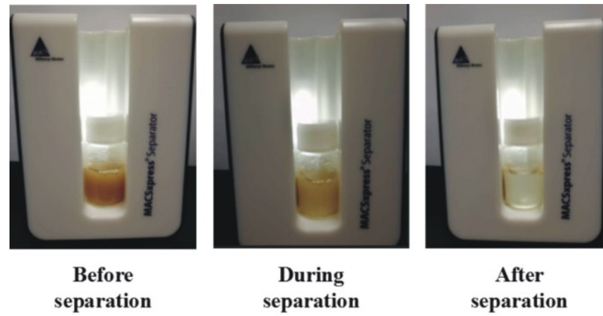

Fig. S7 Digital pictures of magnetic separation process between T cells and cMS-aAPCs using magnetic separator.