Electronic Supplementary Material (ESI) for Biomaterials Science.

Comparative study of α–helical and β-sheet self-assembled peptide nanofiber vaccine platforms: Influence of integrated T-cell epitopes

Supplementary Information

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Supporting Methods:

***In vitro* DOBW presentation assay**

To investigate nanofiber antigen presentation efficiency, bone marrow derived dentritic cells (BMDCs) were seeded in a 96-well plates (0.5 million cells/mL, 100μL) and incubated with either pOVA-Q11 or pOVA-Coil29 at indicated concentrations in RPMI medium for 12 hr. After removal of peptide solution, DOBW reporter T cell hybridomas were added (100μL of 0.5 million/mL cells in complete DMEM media) and cocultured with BMDCs in a humidified CO_2 incubator at 37°C for 12 hr. When DOBW cells received presentation of pOVA (OVA323−339) complexed within the appropriate MHCclass II (I-Ab) by BMDC, they will secret IL-2 and the concentration of IL2 in culture media was measured via IL-2 ELISA (BD, Cat#55148).

Antibodies and flow cytometry

To analyze cell recruitment in the peritoneal cavity, cells isolated from i.p. lavage fluid were stained as previously described.[1] Briefly, cells were treated with 2.4G2 antibodies (2.4G2, BD), followed by staining with anti-mouse IA/IE-FITC (M5/114.15.2, Biolegend), Ly6G-PE (IA8, Biolegend), F4/80-PerCP-Cy5.5 (BM8, Biolegend), CD11c-PE-Cy7 (N418, Biolegend), B220-APC (RA3-6B2, BD), CD11b-APC-Cy7 (M1/70, BD), Ly6C–bV605 (AL-21, BD) and DAPI. Cell subtypes were defined as: Inflammatory monocytes (F4/80 int CD11b+ Ly6C+Ly6G-), conventional DCs (MHCII+CD11c+F4/80-Ly6C-), plasmacytoid DCs (B220+Ly6G+CD11cintF4/80-), macrophages (F4/80+CD11b+), neutrophils (CD11b+ Ly6C+Ly6G+F4/80-), or eosinophils (CD11b+ Ly6CintLy6Gint F4/80int). To determine the antigen-specific memory B cell frequency, single-cell suspensions were prepared from spleens of immunized mice after treatment with ACK lysis buffer. Splenocytes were first incubated with biotinylated antigen peptide (20 μg/mL) on ice for 1 h. After wash with staining buffer 3 times, cells were stained with streptavidin-APC-Cy7 for 30 min at 4°C, followed by treatment with 2.4G2 antibodies and staining with IgD-FITC (11-26c.2a, Biolegend), CD138-PE (281-2, BD), CD3e-PerCP-Cy5.5 (145-2C11, BD), CD95-PE-Cy7 (Jo2, BD), CD38-APC (90/CD38, BD), and B220-v605 (RA3-6B2, eBioscience) for 30 min at 4°C, with antigen-specific memory B cells defined as antigen$^+$B220$^+$CD3e$^-$IgD$^-$CD138$^-$CD95$^-$CD38$^+$.

To analyze B cell phenotypes in lymph nodes after vaccinations, single-cell suspensions were isolated from lymph nodes at designated time points after immunizations, and were stained with GL7-FITC (GL7, BD), CD138-PE (281-2, BD), CD95-PE-Cy7 (Jo2, BD), B220-APC IRA3-6B2, BD), IA/IE-APC-Cy7 (M5/114.15.2, Biolegend), CD86-V605 (GL1, BD), and CD3e-PerCP-Cy5.5 (145-2C11, BD), following 2.4G2 antibody treatment.

To examine the Tfh cell frequency in lymph nodes, after Fc receptor blocking with 2.4G2 antibodies, cells from draining lymph nodes were first stained with biotin-CXCR5 (2G8, BD) for 1 hour at 4 °C, followed by streptavidin-APC (BD), CD4-FITC (GK1.5, Biolegend), CD44-PE (IM7, BD), PD-1-PE-Cy7 (RMP1-30, Biolegend), CD3e-PerCP-Cy5.5 (145-2C11, BD). Tfh cell populations were defined as (CD3e$^+$CD4$^+$CD44$^+$CXCR5$^+$PD-1$^+$).

Antigen-specific Tfh cell identification

Single cells were isolated from draining lymph nodes 10 days after the final immunizations. Cells were cultured in 24-well TC-treated culture plates at a density of 2×10^6 cells/mL for 30 hours in the presence of either pOVA peptide, Coil29 (5×10^{-3} mM), or PBS. The cells were subsequently stained with biotin-CXCR5 (2G8, BD) for 1 hour at 4 °C, followed by streptavidin-APC (BD), CD4-FITC (GK1.5, Biolegend), CD44-BV711 (IM7, BD), PD-1-PerCP-Cy5.5 (RMP1-30, Biolegend), CD19-APC-Cy7 (6D5, Biolegend), CD25-BV786 (PC61, BD), and OX40-PE-Cy7 (OX-86, eBioscience). Antigen-specific Tfh cell population is defined as (CD19$^-$CD4$^+$CD44$^+$CXCR5$^+$PD-1$^+$CD25$^+$OX40$^+$). Flow cytometry was performed using a BD Fortessa instrument.

Supporting Data

Table S1 Peptides used in this paper. All peptides were purified by HPLC, and their masses were confirmed by MALDI

pOVA-Coil29 NH_2-ISQAVHAAHAEINEAGR SGSG QARILEADAEILRAYARILEAHAEILRAQ-Amide	MW= 5348 g/mol
Coil29 Ac-QARILEADAEILRAYARILEAHAEILRAQ-Amide	MW= 3345 g/mol
pOVA-Q11 NH_2- ISQAVHAAHAEINEAGR SGSG QQKFQFQFEQQ-Amide	MW= 3529 g/mol
Q11 Ac-QQKFQFQFEQQ-Amide	MW= 1527 g/mol
pOVA Ac-ISQAVHAAHAEINEAGR-Amide	MW= 1773 g/mol
Coil29a Ac-QARILEADAEILRAY-Amide	MW= 1773 g/mol
Coil29b Ac-ILEADAEILRAYARI-Amide	MW= 1758 g/mol
Coil29c Ac-ADAEILRAYARILEA-Amide	MW= 1716 g/mol
Coil29d Ac-EILRAYARILEAHAE-Amide	MW= 1796 g/mol
Coil29e Ac-YARILEAHAEILRAQ-Amide	MW=1795 g/mol
E214-Coil29 Ac-KFEGTEDAVETIIQAIEA-SGSG-QARILEADAEILRAYAEILEAHAEILRAQ-Amide	MW= 5553 g/mol
E214-Q11 Ac-KFEGTEDAVETIIQAIEA-SGSG-QQKFQFQFEQQ-Amide	MW= 3761 g/mol
PADRE-Q11 NH_2-aKXVAAWTLKAa SGSG QQKFQFQFEQQ-Amide X: cyclohexylalanine; a: D-alanine	MW= 3038 g/mol
E214 Ac-KFEGTEDAVETIIQAIEA-Amide	MW= 1963 g/mol

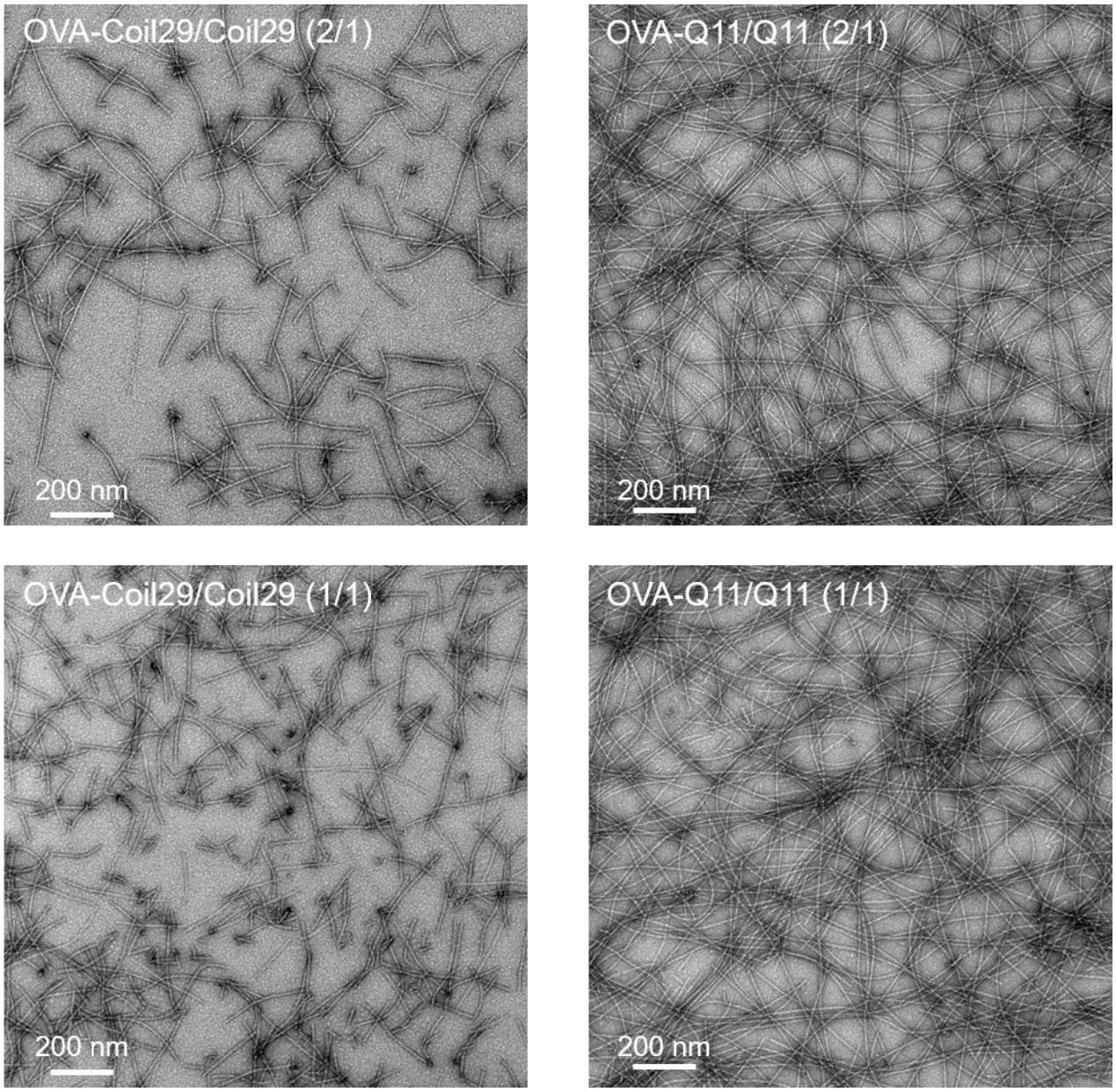

Figure S1. Self-assembled pOVA-CoiI29 at OVA-CoiI29/CoiI29 ratios of 2:1 and 1:1 exhibited reduced fiber lengths, but the morphologies of pOVA-Q11 nanofibers were consistent at these different formulation ratios.

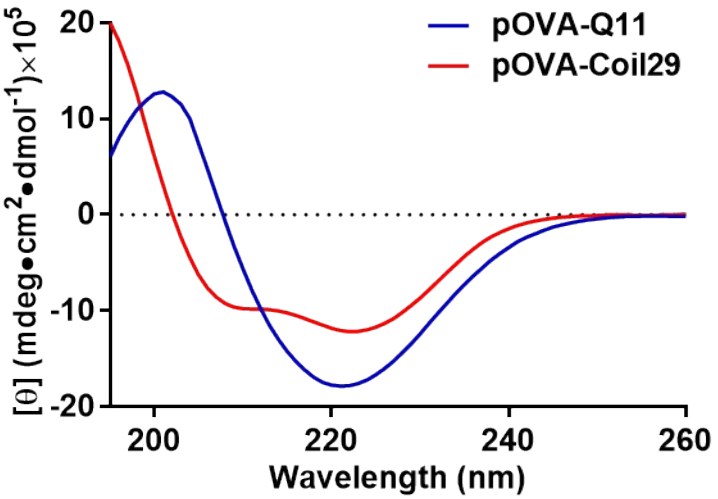

Figure S2. Self-assembled pOVA-Q11 exhibited β-sheet secondary structure, while pOVA-Coil29 adopted α-helical secondary structure by circular dichroism spectroscopy in 1×PBS.

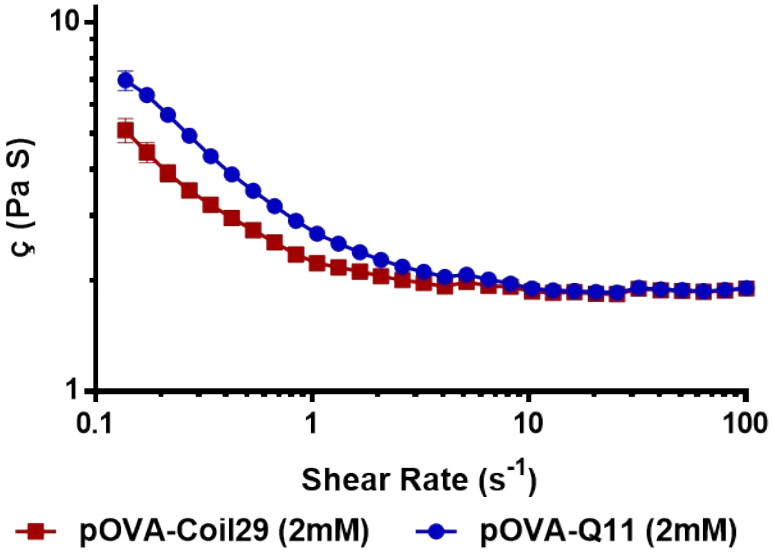

Figure S3. Viscosity versus shear rate for pOVA-Coil29 and Q11 nanofibers. Similar shear-thinning behavior was observed in both nanofiber samples at a concentration of 2 mM in 1×PBS.

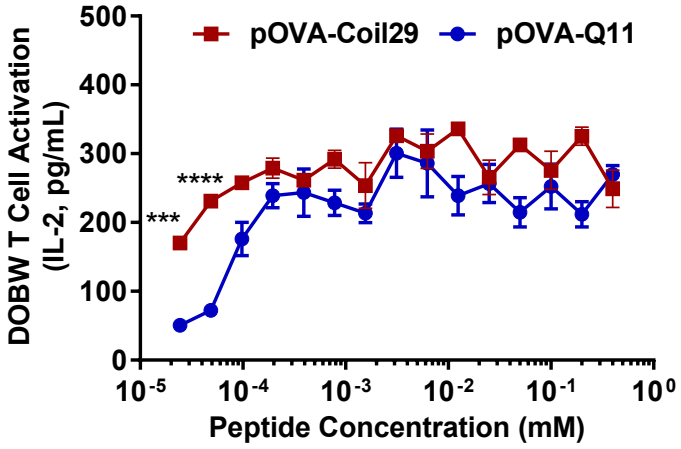

Figure S4. BMDCs acquired, processed, and presented the OVA epitope similarly when cultured with pOVA-Coil29 and pOVA-Q11 nanofibers. Apporpriately processed and presented OVA peptide was detected using DOBW T cells, which produce IL-2 when they encounter OVA presented in class-II MHC molecules. (*** P<0.001, **** P<0.0001 were calculated with multiple t test using Holm-Sidak method for multiple comparison correction.)

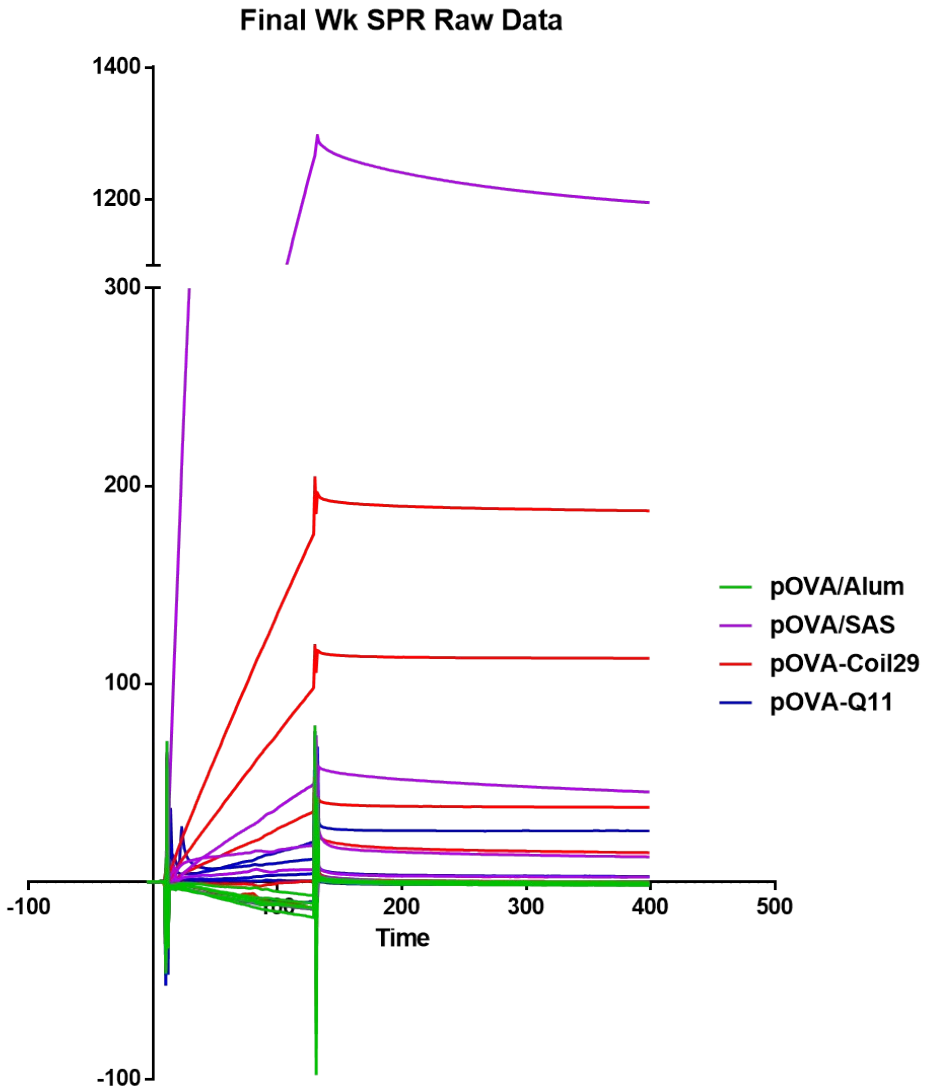

Figure S5. SPR curves for all serum samples after booster immunizations.

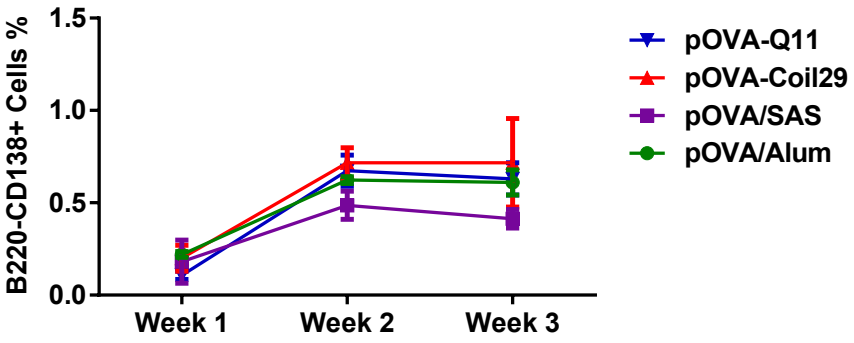

Figure S6. All four groups showed similar abilities to stimulate plasma cell differentiation. Cells are gated on DAPI$^-$CD3e$^-$ cells.

References

1. J. Chen, R. R. Pompano, F. W. Santiago, L. Maillat, R. Sciammas, T. Sun, H. Han, D. J. Topham, A. S. Chong and J. H. Collier, *Biomaterials*, 2013, **34**, 8776-8785.