Small Extracellular Vesicles (sEV) from Precision Porous Templated Scaffold Resident Cells Modulate T Cell Inflammatory Signaling via TLR4

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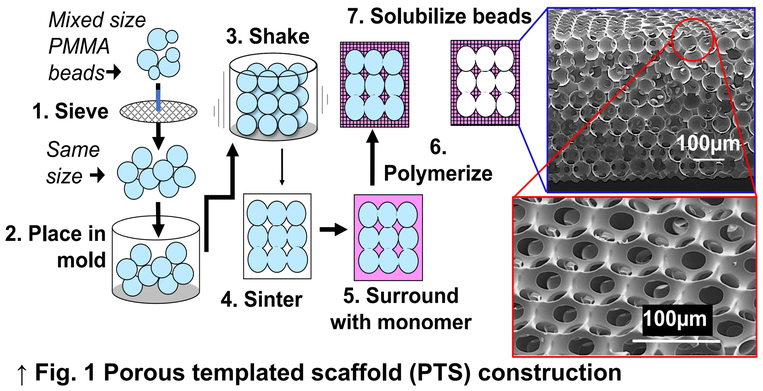
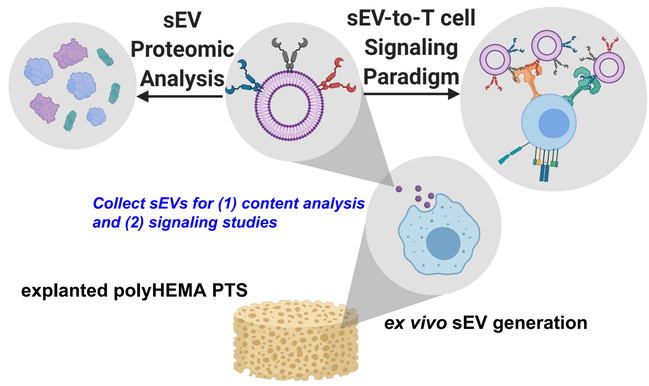
1. Background



↑ **Fig. 1 Porous templated scaffold (PTS) construction**

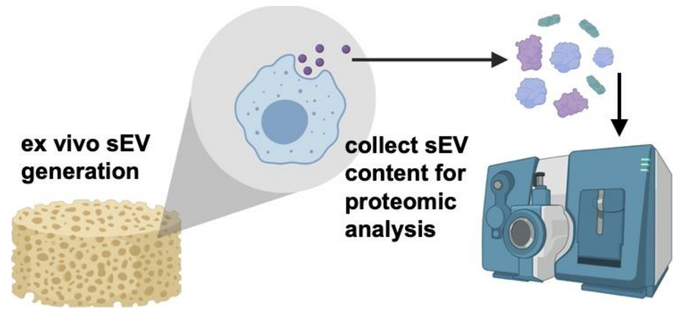
- PTS with 40µm diameter pores induce pro-healing effects
- PTS-derived sEVs stimulate T cells and adjust T_{reg} and TH1 populations
- Working to quantify the mechanism of sEV-to-T cell signaling

2. Objective

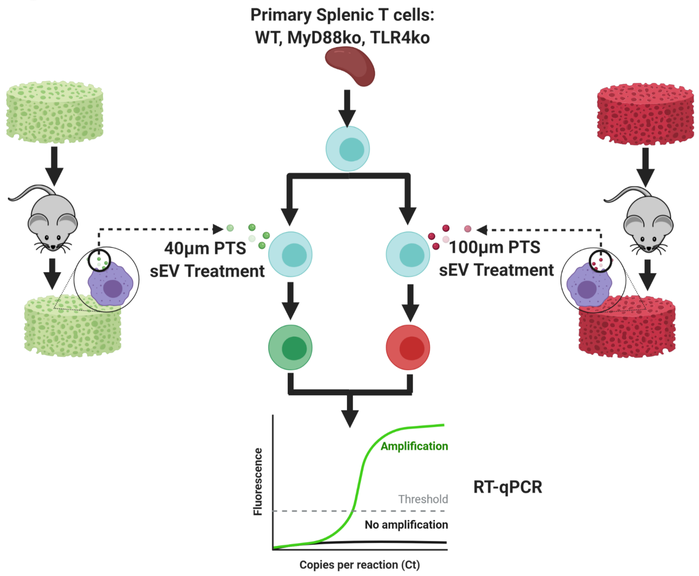


↑ **Fig. 2 Study Objective: We seek to quantify the mechanism of small extracellular vesicle (sEV) signaling that affects the phenotype of CD4⁺ T cells within the PTS.**

3. Methods



↑ **Fig. 3. Proteomic analysis of PTS resident cell-derived sEVs.**



↑ **Fig. 4. qPCR to assess TLR4 effector signaling of splenic T cells from WT, TLR4ko, and MyD88ko mice treated with PTS-derived sEVs**

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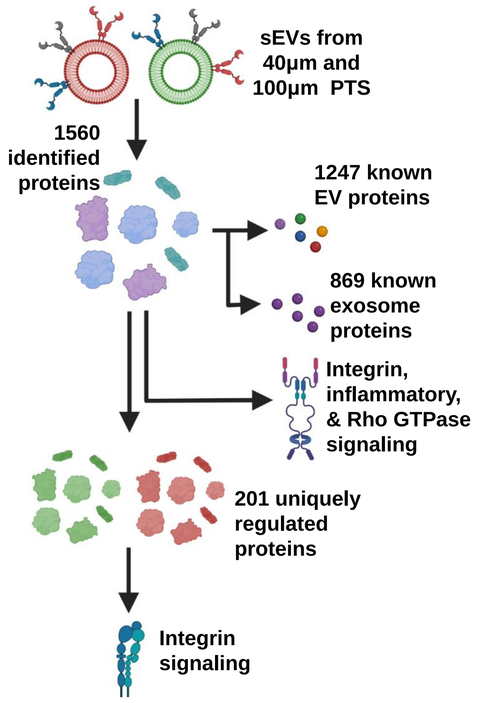
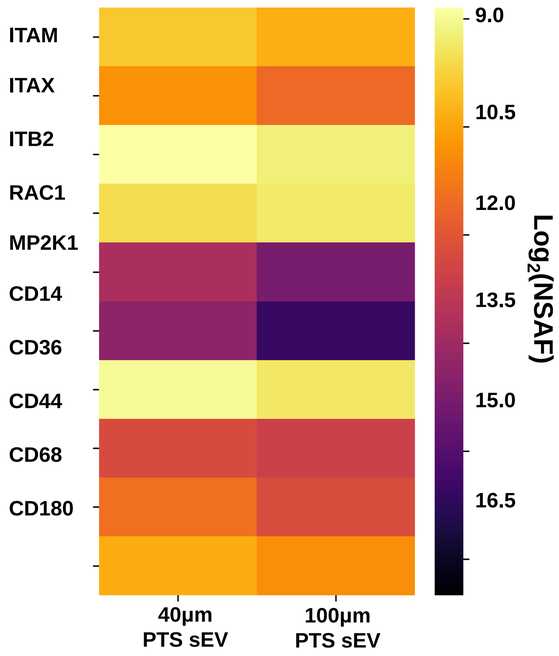
Figures 2 - 5 created with BioRender™ Software.

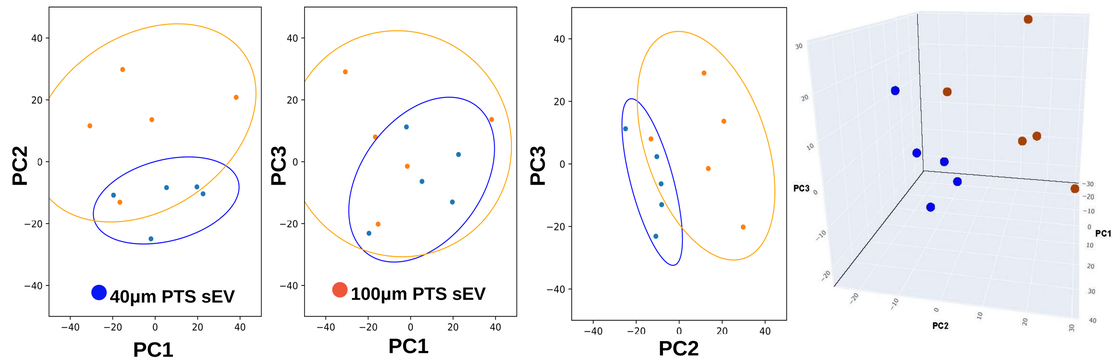
4. Results



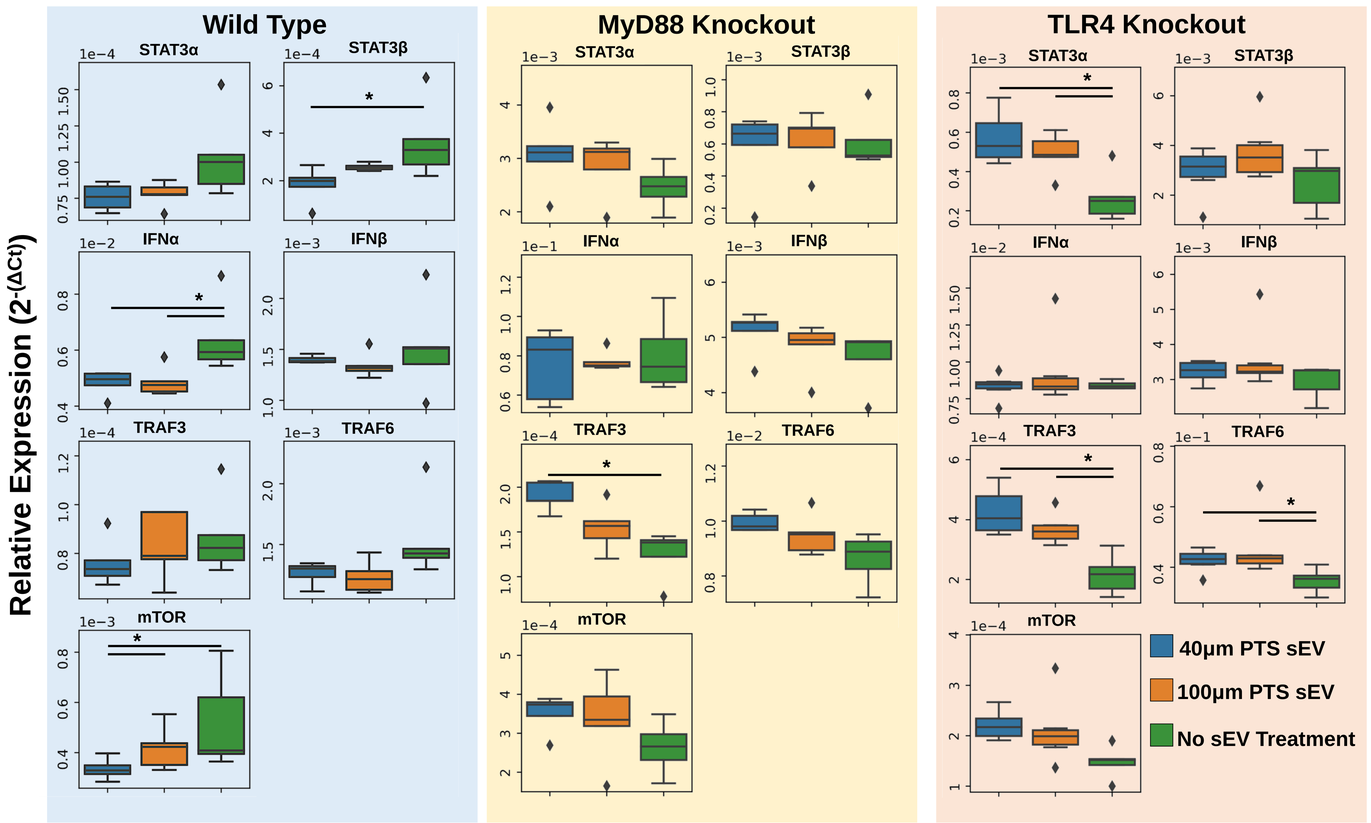
←**Fig. 5 PTS sEVs contain immunomodulatory EV and exosome associated proteins, and are differentially regulated as a result of PTS pore size**



← **Fig. 6 Key immunomodulatory proteins within sEVs are regulated by PTS pore size; macrophages and monocytes may influence PTS sEV signaling.**



←**Fig. 7 3D PCA of protein expression in 40µm and 100 µm PTS sEVs (PC1: 27.11%, PC2: 17.91%, PC3: 15.25%). Circles represent 95% confidence intervals. PC1 and PC3 show similarities in protein expression between the two sEV populations, while PC2 shows differential expression.**



← **Fig. 8 TLR4 effector RT-qPCR panel of PTS resident cell-derived sEV treated splenic T cells collected from WT, MyD88KO, and TLR4KO mice. MyD88 dependent downregulation of IFNα occurred as a result of sEV treatment, while mTOR and STAT3β were downregulated only in T cells treated with sEVs from 40µm PTS. TLR4 deficiency caused sEV treatment to upregulate TRAF3, TRAF6, and STAT3α, indicating a suppressive role for MyD88 independent TLR4 regulation of sEV-to-T cell signaling.**

5. Conclusions

- **The proteomic content of sEVs from 40µm and 100µm PTS are similar but possess key immunomodulatory differences**
- **MyD88-dependent TLR4 activation induces anti-inflammatory sEV-to-T cell signaling, particularly from 40µm PTS sEVs**
- **MyD88-independent TLR4 activation regulates activation of other pathways (e.g. TCR, TLR2) by PTS sEVs**

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