

Co-delivery of targeted hypoallergens and resiquimod powders using silk fibroin microneedles for effective allergen-specific immunotherapy

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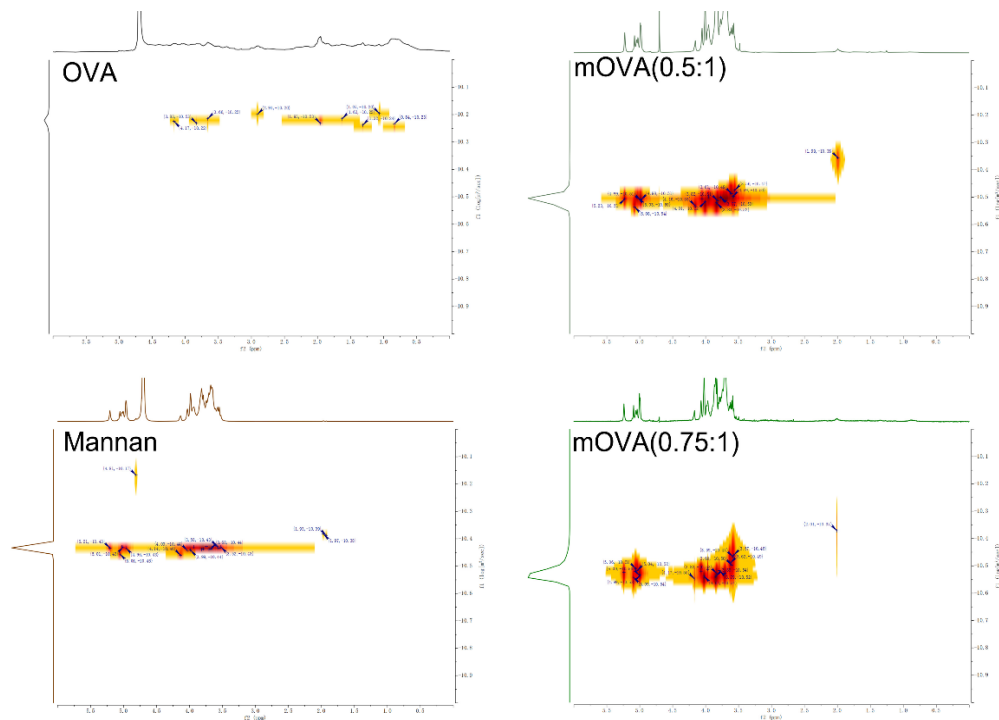


Figure S1. 2D-DOSY spectra for OVA, mannan and mOVA. OVA (black); Mannan (brown); mOVA (green): at ratio of 0.5: 1 and 0.75: 1.

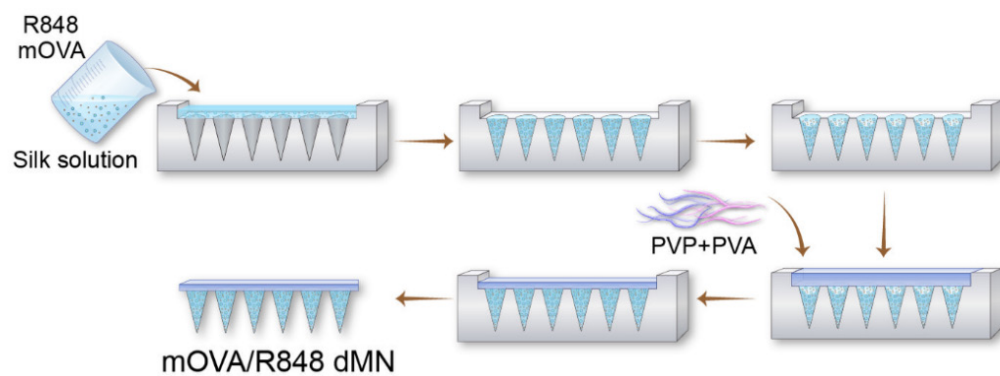


Figure S2. Fabrication of mOVA/R848 dMN arrays.

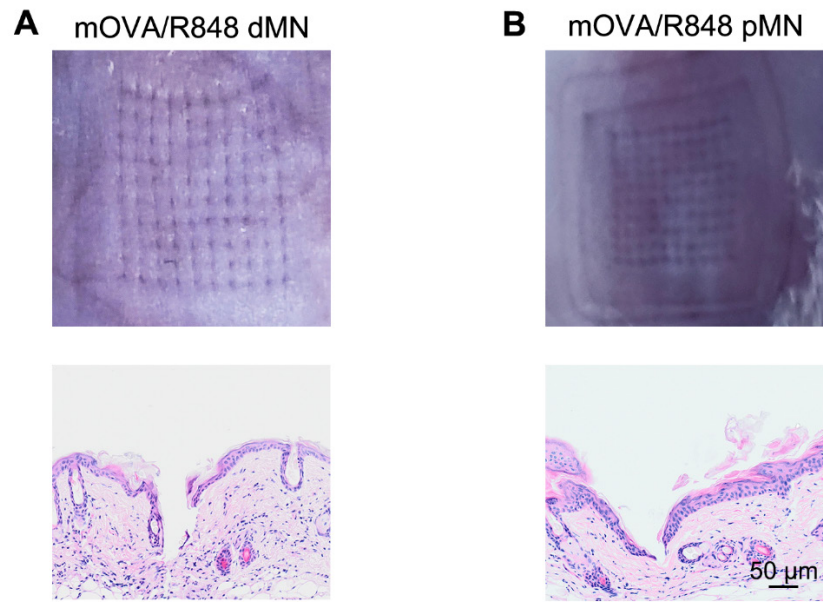


Figure S3. The holes created by mOVA/R848 dMN and mOVA/R848 pMN arrays on skin samples and their corresponding H&E staining results. Scale bar = 50 μm .

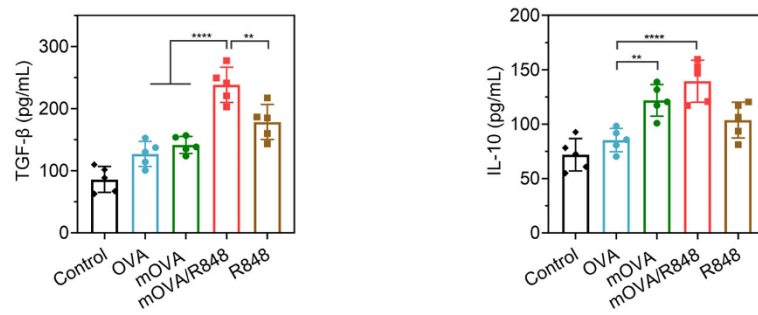


Figure S4. TGF- β and IL-10 from T cells activated by pretreated BMDCs. The data are presented as means \pm SDs ($n = 5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

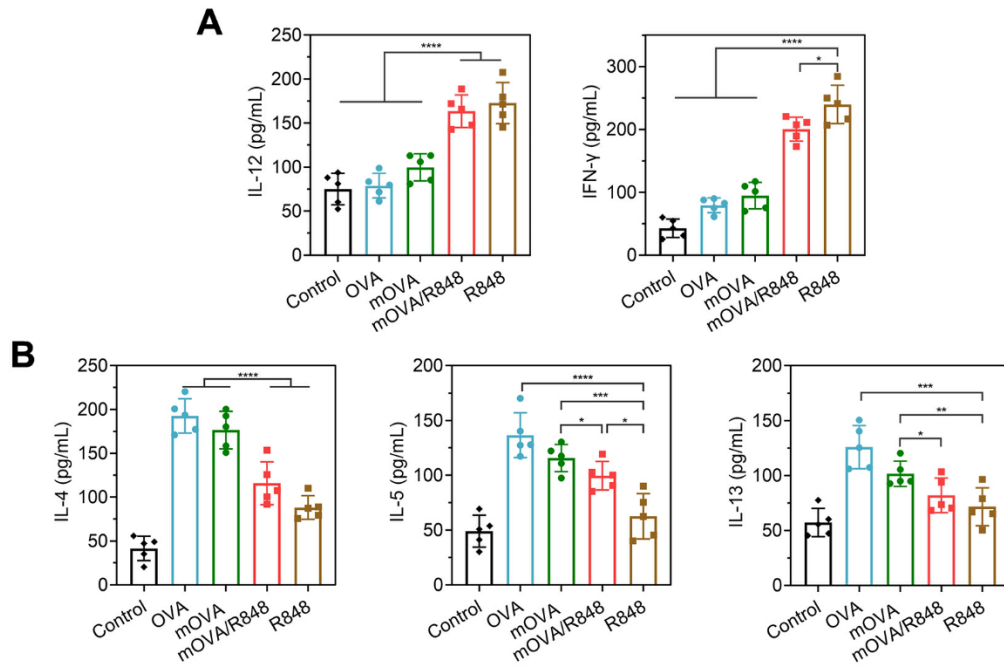


Figure S5. Secretion of (A) IL-12, IFN- γ and (B) IL-4, IL-5, IL-13 from T cells activated by pretreated BMDCs. The data are presented as means \pm SDs ($n = 5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

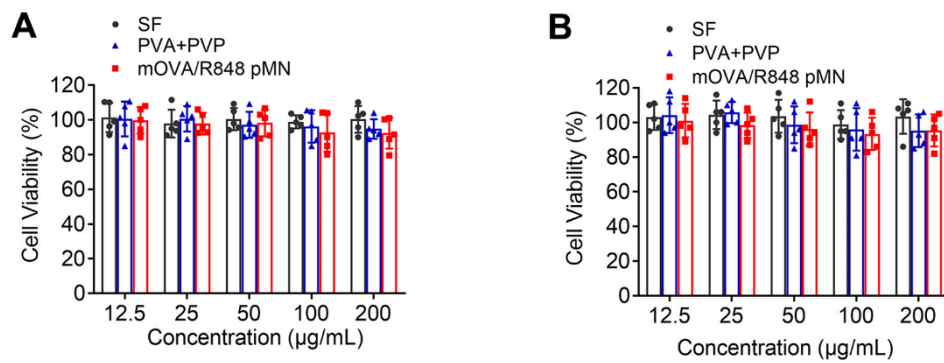


Figure S6. Cell proliferation with different extracting solutions. The data are presented as means \pm SDs ($n = 5$).

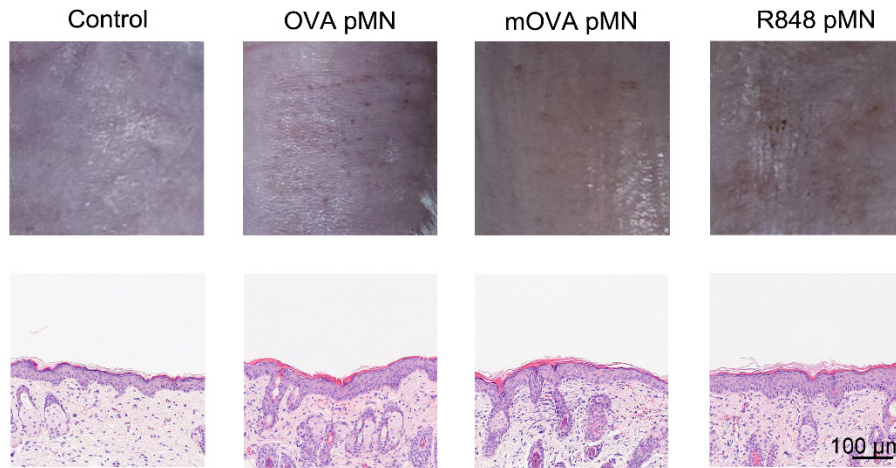


Figure S7. Representative images of skins at the treated site of mice and their corresponding H&E staining results. Scale bar = 100 μm .

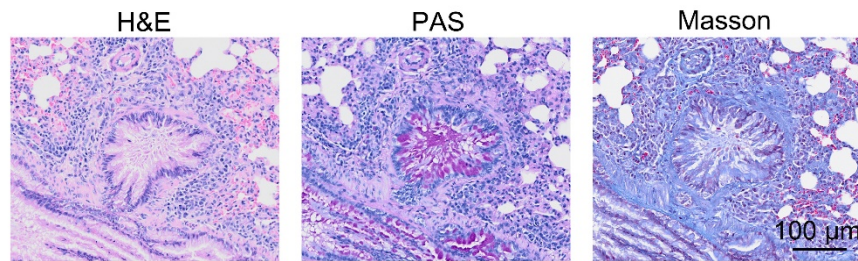


Figure S8. Representative H&E, PAS and Masson staining of lung sections for allergic asthma mice. Scale bar = 100 μm .

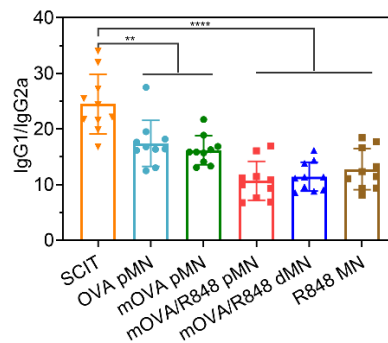


Figure S9. The ratio of OVA-specific IgG1 to OVA-specific IgG2a in serum. The data are presented as means \pm SDs ($n = 10$). ** $P < 0.01$, **** $P < 0.0001$.

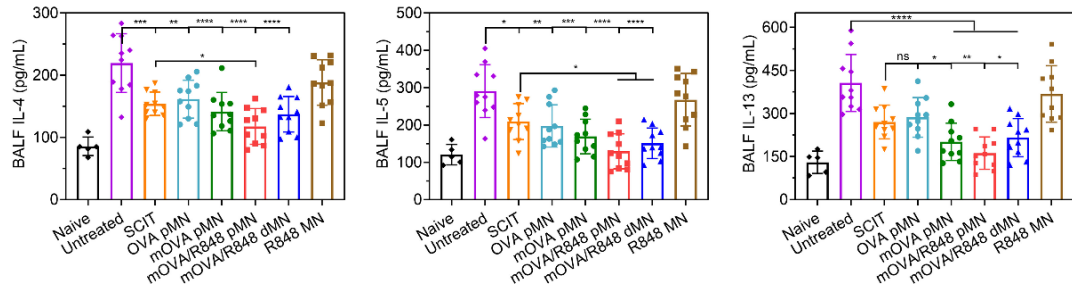


Figure S10. The levels of IL-4, IL-5, IL-13 in BALFs. The data are presented as means \pm SDs (naive: n = 5; other: n = 10). * P < 0.05, ** P < 0.01, *** P < 0.001, and **** P < 0.0001.

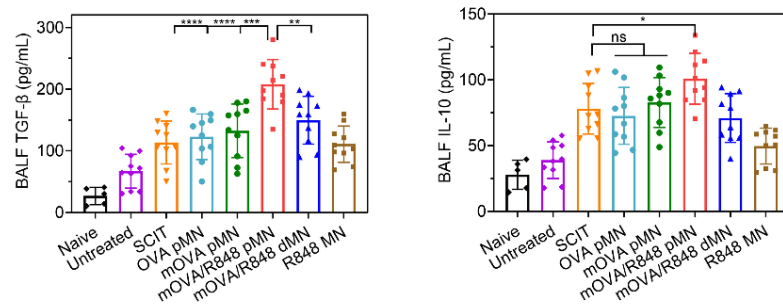


Figure S11. The levels of TGF- β and IL-10 in BALFs. The data are presented as means \pm SDs (naive: n = 5; other: n = 10). * P < 0.05, ** P < 0.01, *** P < 0.001, and **** P < 0.0001.

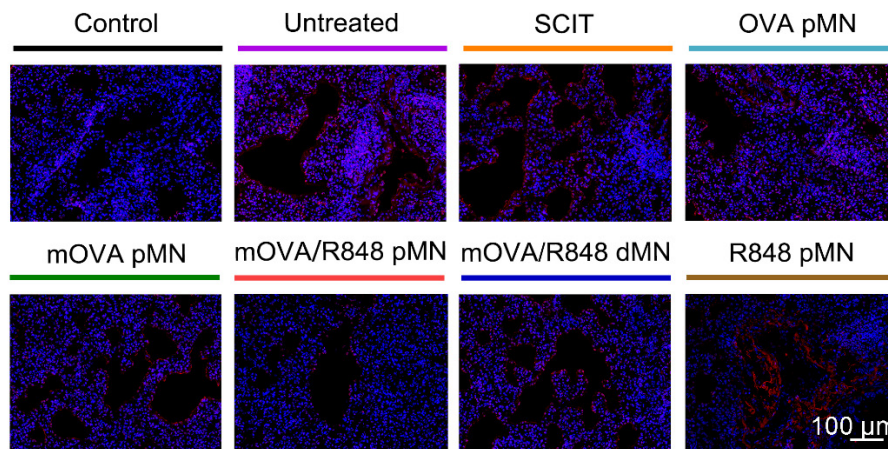


Figure S12. ROS in lung tissue was observed by DHE staining. Scale bar = 100 μ m.

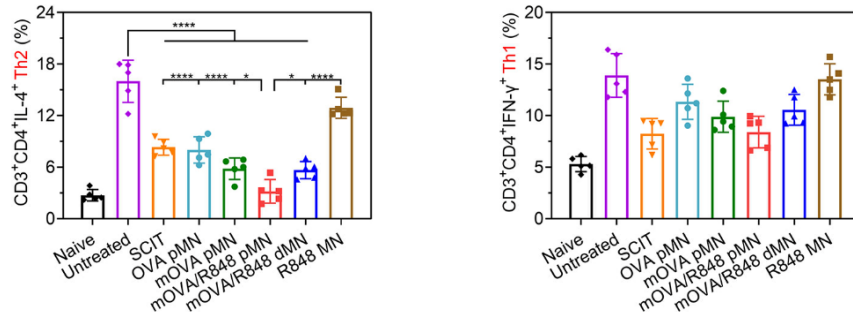


Figure S13. The proportion of Th2 and Th1 cells in splenocytes. The data are presented as means \pm SDs ($n = 5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

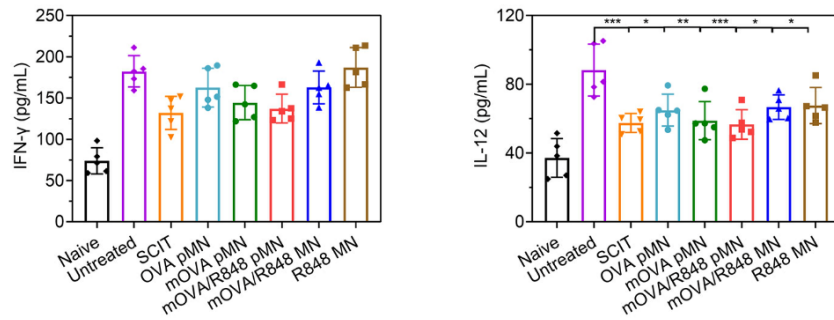


Figure S14. Secretion of IFN-γ and IL-12 from splenocytes. The data are presented as means \pm SDs ($n = 5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

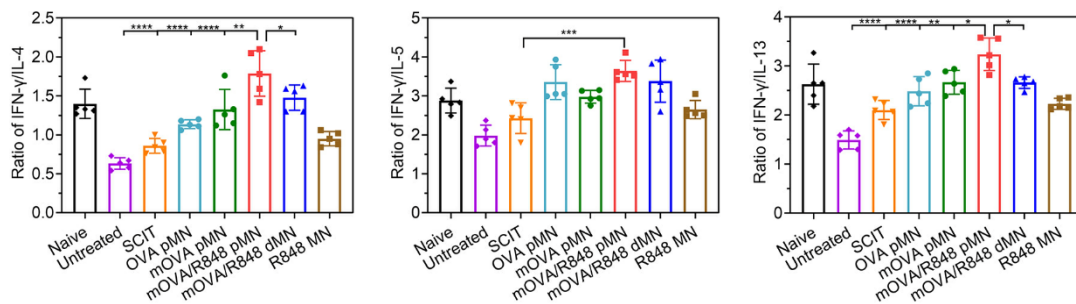


Figure S15. The ratios of IFN-γ/IL-4, IFN-γ/IL-5, IFN-γ/IL-13 in splenocytes. The data are presented as means \pm SDs ($n = 5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

Table S1. OVA and Mannan quantification in mOVA conjugates with different ratios.The data are presented as means \pm SDs (n = 3).

Mannan (mg/mL)	OVA (mg/mL)	Feed Man/OVA ratio	Incorporated Man/OVA Ratio
5	10	0.5: 1	0.54: 1
7.5	10	0.75: 1	0.72: 1
10	10	1: 1	0.70: 1

Table S2. Loading capacity in MN arrays. The data are presented as means \pm SDs (n = 3).

	OVA loading (μ g)	R848 loading (μ g)
OVA pMN	27.1 \pm 1.2	
mOVA pMN	25.7 \pm 2.8	
mOVA/R848 pMN	25.4 \pm 3.0	2.3 \pm 0.5
mOVA/R848 dMN	25.3 \pm 2.5	2.1 \pm 0.3
R848 pMN		2.8 \pm 0.2

Table S3. Antibodies used in the study.

Antibody	Company	Clone	Catalog No.
CD11c (PE)	BioLegend	N418	177307
CCR7 (Alexa Fluor® 647)	BD Pharmingen	4B12	560766
MHC Class II (FITC)	eBioscience	M5/114.15.2	11-5321-81
CD40 (PerCP-eFluor™ 710)	eBioscience	1C10	46-0401-80
CD86 (PE-Cy7)	eBioscience	GL1	25-0862-80
CD3 (FITC)	eBioscience	145-2C11	11-0031-81
CD4 (PE)	BioLegend	GK1.5	100407
CD25 (PE-Cy7)	eBioscience	PC61.5	25-0251-81
FOXP3 (Alexa Fluor® 647)	BD Pharmingen	MF23	560401
IL-4 (PE-Cy7)	eBioscience	11B11	25-7041-80
IFN- γ (APC)	eBioscience	XMG1.2	17-7311-81
Fixable Viability Dye eFluor™ 780	eBioscience		65-0865-14

Table S4. ELISA kits used in the study.

ELISA kits	Company	Catalog No.
OVA-sIgE	Yifeixue Bio Tech	YFXEM00075
OVA-sIgG1	Yifeixue Bio Tech	YFXEM00656
OVA-sIgG2a	Yifeixue Bio Tech	YFXEM00657
IL-4	Yifeixue Bio Tech	YFXEM00057
IL-5	Yifeixue Bio Tech	YFXEM00012
IL-13	Yifeixue Bio Tech	YFXEM00013
IL-10	Yifeixue Bio Tech	YFXEM00065
TGF- β	Yifeixue Bio Tech	YFXEM00561
IFN- γ	Yifeixue Bio Tech	YFXEM00039
IL-12	Yifeixue Bio Tech	YFXEM00599