Supplementary Information (SI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2024

Supplementary Information

Formulation and evaluation of PVA-based composite hydrogels: physicochemical, leachables, and in vitro immunogenicity studies

Achmad Himawan, Anna Korelidou, Ana M. Pérez-Moreno, Juan L Paris, Juan Dominguez-Robles, Lalitkumar K Vora, Andi Dian Permana, Eneko Larraneta, Robert Graham, Christopher J Scott, Ryan F Donnelly

Preparation of the polymeric stock solutions

Poly(vinyl alcohol) (PVA): A 25% w/w PVA solution was created by mixing PVA granules with deionized water and heating the mixture at 80°C overnight with intermittent stirring.

Poly(vinylpyrrolidone) (PVP): A 40% w/w PVP solution was prepared by dissolving PVP powder in deionized water, allowing the mixture to hydrate overnight, and then stirring until completely dissolved.

Chitosan: A 5% w/w chitosan solution was made by combining chitosan powder with 10% v/v acetic acid. The mixture was allowed to hydrate overnight with a rotator mixer set to 12 rpm.

Freeze-drying cycles

Step	Temp (°C)	Pressure (torr)	Time (min)	Step
1	5	760	10	Hold
2	-40	760	60	Ramp
3	-35	190	180	Hold
4	-30	190	60	Ramp
5	-30	190	180	Hold
6	-25	190	60	Ramo
7	-25	190	180	Hold
8	25	190	120	Ramp
9	25	190	360	Hold
10	25	50	360	Hold

Cell culture condition

DC2.4 Cell Culture and Maintenance:

This study utilized the DC2.4 mouse dendritic cell line. These cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, nonessential amino acids, L-glutamine, and β-mercaptoethanol.

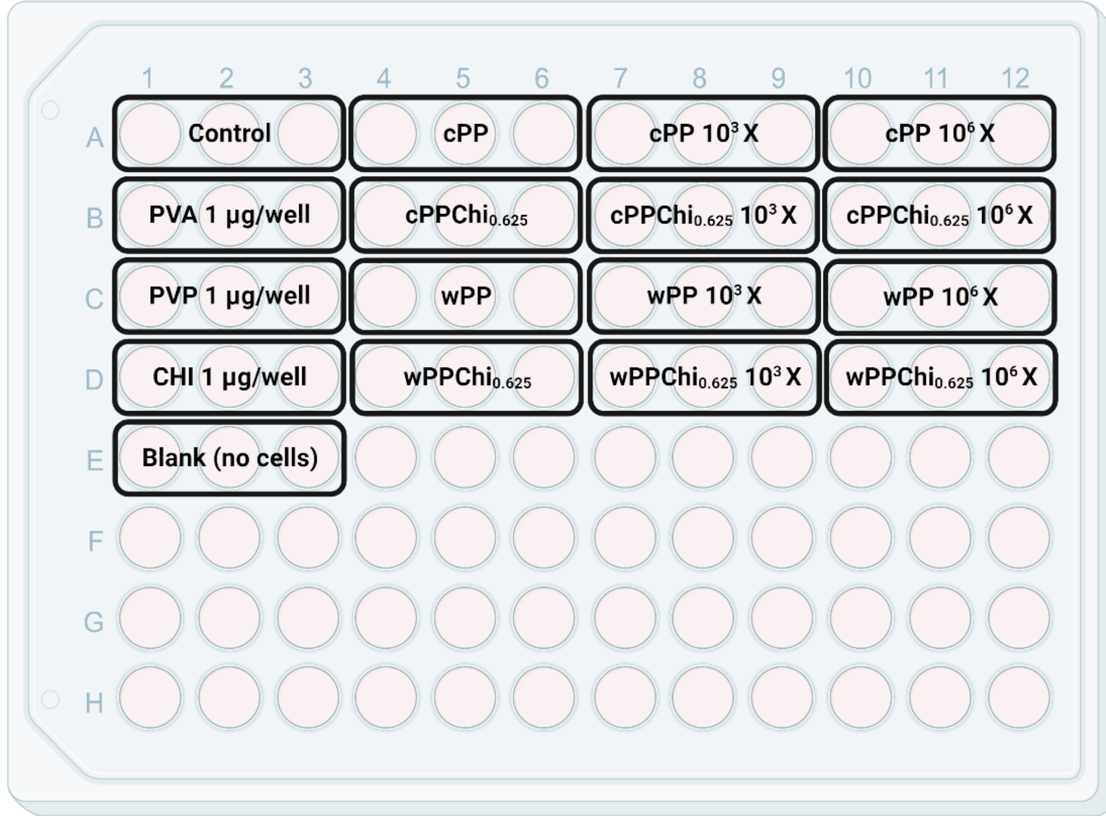
Cell Thawing and Expansion:

Upon thawing, DC2.4 cells were transferred to a sterile tube, diluted with 9 mL of RPMI-1640 medium, centrifuged at 300 rcf for 2-3 minutes, and resuspended in 15 mL of fresh medium. The cells were then transferred to a T75 tissue culture flask and incubated at 37°C with 5% CO2. The medium was changed every two or three days until the cells reached confluence.

Cell Passage and Seeding:

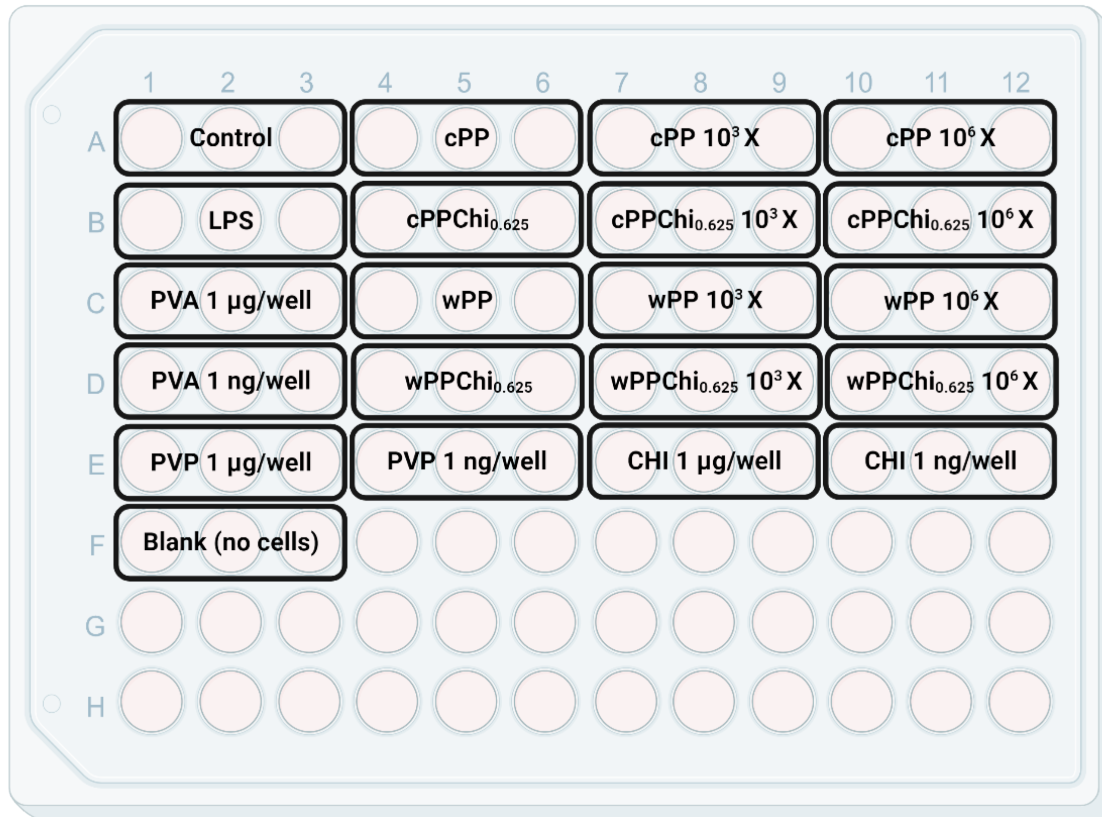
For cell passage, the culture medium was removed from the flask, and the cells were rinsed twice with 1× PBS (pH 7.4; 10 mM). Trypsin-EDTA solution (0.25% trypsin, 1 mM EDTA) was added to detach the cells, and the mixture was incubated for 3-5 minutes at 37°C. The cells were then resuspended in 8 mL of RPMI-1640 medium, transferred to a 15 mL conical tube, and centrifuged at 300 rcf for 3-5 minutes. The supernatant was removed, and the cells were resuspended in 2 mL of RPMI-1640 medium. A 15 µL aliquot of the cell suspension was taken and placed on a cell counting slide. The cells were counted manually with a confocal microscope at 10X magnification. A dilution was made to seed the cells into a 96-well culture plate with a cell density of 500 cells/µL. 100 µL of the diluted cell suspension was added to each well, and the remaining cells were passaged. The cells in the well plate were incubated in a humidified incubator at 37°C with 5% CO2.

Schematic diagram for cell treatment in the cytotoxicity study



Control = 100 µL of media; Polymer (PVA/PVP/CHI) 1 µg/well = 100 µL of 10 µg/mL polymer working solution; Formulation (cPP/cPPChi0.625/wPP/wPPChi0.625) = 100 µL of undiluted hydrogel extract; Formulation (cPP/cPPChi0.625/wPP/wPPChi0.625) 103 × = 100 µL of 1:1.000 dilution of hydrogel extract; Formulation (cPP/cPPChi0.625/wPP/wPPChi0.625) 106 × = 100 µL of 1:1.000.000 dilution of hydrogel extract; Blank (no cells) = 100 µL of media.

Schematic diagram for cell treatment in the DC activation study



Control = 100 µL of media; LPS = 100 µL of 100 ng/mL LPS solution in supplemented RPMI-1640; Polymer (PVA/PVP/CHI) = 1 µg/well = 100 µL of 10 µg/mL polymer working solution; Polymer (PVA/PVP/CHI) = 100 µL of 10 ng/mL polymer working solution; Formulation (cPP/cPPChi0.625/wPP/wPPChi0.625) = 100 µL of undiluted hydrogel extract; Formulation (cPP/cPPChi0.625/wPP/wPPChi0.625) 103 X = 100 µL of 1:1.000 dilution of hydrogel extract; Formulation (cPP/cPPChi0.625/wPP/wPPChi0.625) 106 X = 100 µL of 1:000.000 dilution of hydrogel extract; Blank (no cells) = 100 µL of media.

Preparation of the treatment solution.

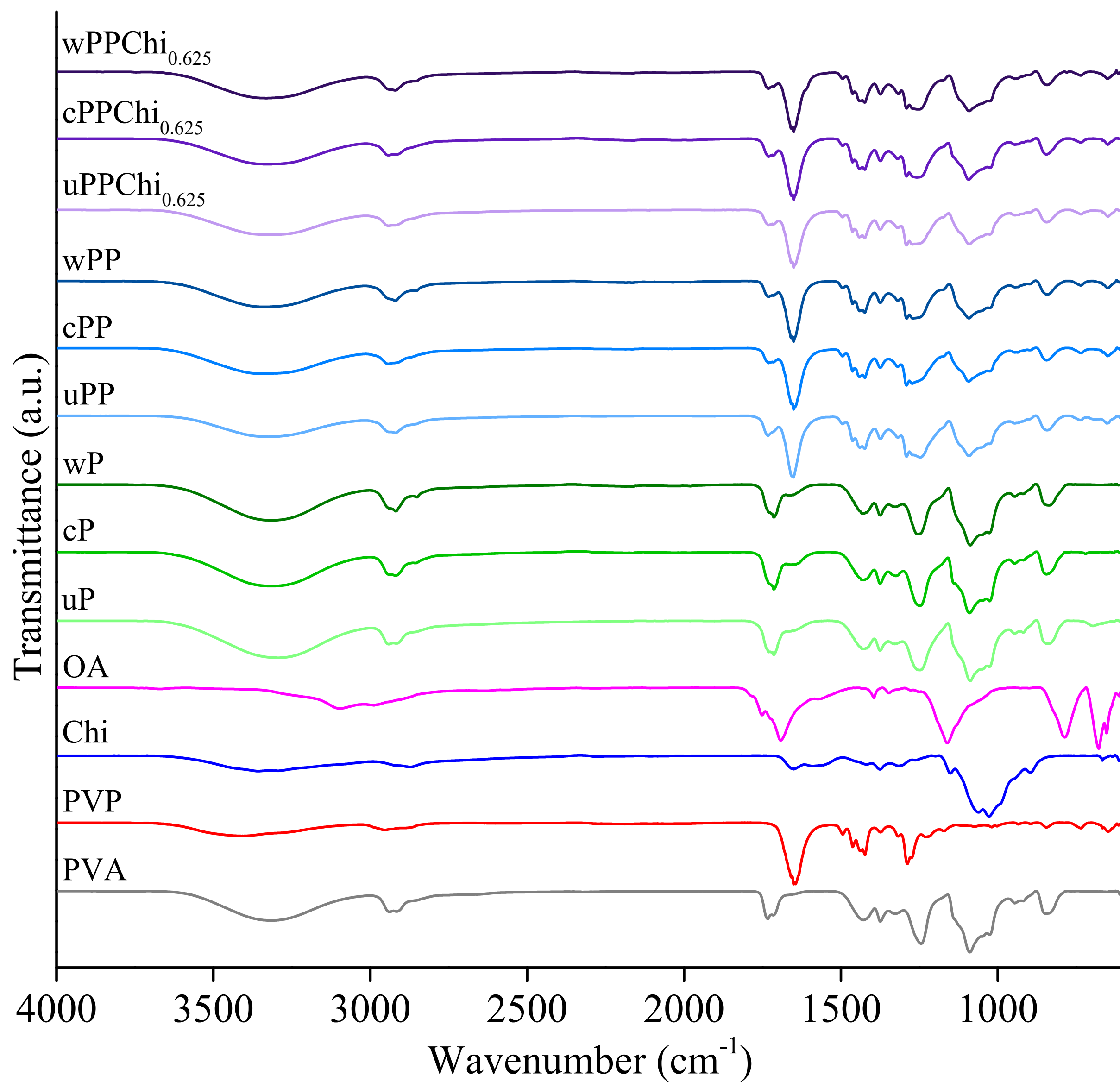
Preparation of hydrogel extracts in cell expansion medium

Approximately 60 mg of each hydrogel formulation (cPP, $cPPChi_{0.625}$, wPP, $wPPChi_{0.625}$) was placed in a 6-well plate and sterilized under UV light. The hydrogels were then immersed in 1.2 mL of supplemented RPMI-1640 medium for 24 hours. After discarding the swollen hydrogels, the remaining media, referred to as undiluted hydrogel extracts, were collected. These extracts were further diluted 10^3- and 10^6-fold with fresh supplemented RPMI-1640 medium to obtain diluted hydrogel extracts.

Preparation of polymer solutions in cell expansion medium

Aqueous solutions of PVA and PVP were prepared by dissolving 20 mg of each polymer in 10 mL of Milli-Q water. The chitosan solution was prepared in an acidic environment using 1% acetic acid and was subsequently neutralized with sodium hydroxide. The final concentration of each polymer solution was 2 mg/mL. These stock solutions were then diluted with supplemented RPMI-1640 medium to create working solutions of 10 µg/mL and 10 ng/mL.

The complete IR spectra from wavenumbers 4000 cm-1 to 750 cm-1 of the hydrogels and their individual constituent



The list of peaks of interest from all constituent materials

Chemical group vibrational mode	Wavenumber (cm^{-1})			
	PVA	**PVP**	**Chi**	**OA**
v O-H water (moisture)	-	3425	-	-
v O-H alcohol[*]/carbohydrate[†]	3328	-	3360	-
v N-H amine		-	3360, 3294	-
v O-H acid	-	-	-	3101
v C-H asymmetric, ring	-	2954	2921	-
v C-H asymmetric, chain	2939	2982	-	-
v C-H symmetric, chain	2917	2925	-	-
v C-H symmetric, ring	-	2897	2875	-
v C=O ester, saturated	1733	-	-	-
v C=O ester, unsaturated	1716	-	-	-
v C=O carboxylic acid	-	-	-	1693
v C=O amide (amide I)	-	1651	1651	-
δ N-H amide (amide II)	-	-	1593	-
v C-N	-	1288	-	-
v C-O (crystallinity)	1141	-	-	-
v C-O-C (glycosidic linkage)	-	-	1151	-
v C-O-C (carbohydrate ring)	-	-	1028	-
v C-O (saccharide vibration)	-	-	665	