Electronic Supplementary Material (ESI) for Biomaterials Science.


# Injectable thermosensitive hydrogel to modulate tolerogenic dendritic cells under hyperglycemic condition

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## Physicochemical characterizations of hydrogel

*Mechanical Testing: rheology analysis and compression test.* Rheology analysis was performed using an TA Instruments AR2000 Rheometer (Waters Corporation, United States) with a 40 mm

2° steel cone geometry. The storage modulus (G') and loss modulus (G'') of the hydrogel formulation were measured at a constant shear force (1 Pa) and constant frequency (1 Hz) at 37 °C as a function of time. For the compression test, Instron 4301 (Instron, United States) equipped with 50 N load cell was used. 5 mL of hydrogel solution was poured into a 12-well cell culture plate and incubated at 37 °C overnight. Compression was applied at a constant rate of 1mm per second until 10 % deformation was reached. Young's modulus was calculated as the slope of the linear portion of the stress and strain curve.

*Swelling test.* Swelling ratio was determined using a protocol reported previously [1]. Hydrogels were prepared and immersed in PBS overnight. The equilibrium mass swelling ratio was calculated by the following equation: Swelling ratio = Ws/Wd x 100 %, where Ws is the weight of fully swollen hydrogel and Wd is the weight of dried hydrogel.

*Degradation assay.* Lysozyme solution was prepared at a concentration of 1 mg/mL in PBS [2,3]. 1mL of the hydrogel solution was introduced into a 4 mL centrifugal tube and incubated at 37 °C for 5 minutes. The gelated hydrogels were then immersed in 3 mL of lysozyme solution for the following timepoints: Day 1, 4, 7, 10, and 14 (degradation solution was replaced every two days to ensure proper enzymatic activity). At the aforementioned timepoints, the degradation solution was emptied, and the hydrogel was frozen and lyophilized. Three hydrogel samples were lyophilized without immersion in lysozyme solution and their average weight was used as the standardized initial weight. Percentage remained was calculated by the following equation: final weight/initial weight x 100%.

*Porosity and surface topography.* Hydrogels were frozen overnight at -20 °C and lyophilized at -80 °C and 0.01 mBar (Labconco FreeZone, United States) 2.5 Liter -84 °C benchtop freeze dryer.

Subsequently they were snapped into smaller hydrogel pieces, followed by sputter coating with gold. The cross-section of hydrogel was then visualized under a scanning electron microscope (Hitachi FlexSEM 1000, Japan). Porosity was determined by following a published protocol [4]. Briefly, porosity was calculated from the following equation: ((Ws-Wd)/Ws)) x 100 %, where Ws is the weight of fully swollen hydrogel and Wd is the weight of dried hydrogel.

*Release of GM-CSF and resveratrol.* 1 µg of GM-CSF and 0.12 mg of resveratrol were added into 5 mL of hydrogel solution and stored at 4 °C overnight for maximal adsorption [5,6]. 0.5 mL of GM-CSF and resveratrol loaded hydrogel solution was transferred into a 24-well plate and incubated at 37 °C for 5 mins to allow hydrogel formation. For the release of resveratrol, 2 mL of PBS was added to each well containing the formed hydrogel. The plate was incubated at 37 °C for different time periods (0.167 h, 2 h, 4 h, 6 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h, 240 h, and 336 h). At each time point, 1 mL of the supernatant was collected and replenished by the same volume of fresh PBS. Absorbance of the collected supernatant was measured at 304 nm (Biotek, United States), and the concentration of resveratrol was calculated from the calibration curve. For the release of GM-CSF, 2 mL of PBS with 1 % bovine serum albumin was added to each well containing the formed hydrogel [7]. GM-CSF is not stable on its own and BSA is used as carrier proteins to improve its stability. The plate was incubated at 37 °C for the aforementioned time periods. At each designated time point, 1 mL of the supernatant was collected and replenished by the same volume of fresh PBS with 1 % bovine serum albumin. Supernatants were immediately stored in a -20 °C freezer. The amount of GM-CSF released was quantified with an enzyme-linked immunosorbent assay (ELISA) kit (Cedarlane Laboratories, Canada) for GM-CSF following manufacturer's instructions.

**Primer sequences for qRT-PCR**

CD80 (5’ – 3’)
Forward: CTCTTGGTGCTGGCTGGTCTTT
Reverse: GCCAGTAGATGCGAGTTTGTGC

CD83 (5’ – 3’)
Forward: TACAGAGCGGAGATTGTCCTGC
Reverse: GCTCGTTCCATGCCAGCTTTAG

CD86 (5’ – 3’)
Forward: CCATCAGCTTGTCTGTTTCATTCC
Reverse: GCTGTAATCCAAGGAATGTGGTC

HLA-DRA (5’ – 3’)
Forward: GTCTGGCGGCTTGAAGAATT
Reverse: ACCTTGAGCCTCAAAGCTGG

SOCS3 (5’ – 3’)
Forward: GTCACCCACAGCAAGTTTCC
Reverse: TCACTGCGCTCCAGTAGAAG

FOXP3 (5’ – 3’)
Forward: GGCACAATGTCTCCTCCAGAGA
Reverse: CAGATGAAGCCTTGGTCAGTGC

TGFß (5’ – 3’)
Forward: TACCTGAACCCGTGTTGCTCTC
Reverse: GTTGCTGAGGTATCGCCAGGAA

IL-10 (5’ – 3’)
Forward: TCTCCGAGATGCCTTCAGCAGA
Reverse: TCAGACAAGGCTTGGCAACCCA

IL-17A (5’ – 3’)

Forward: CTTGGAATCTCCACCGCAAT
Reverse: CACGTTCCCATCAGCGTTG

RORγt (5’ – 3’)
Forward: CTTGCCGTAGGGATGTCTCG
Reverse: GAAGTTCCGTCAGCCCGTT

## References


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