Electronic Supplementary Material (ESI) for Lab on a Chip.

Microfluidic On-demand Engineering of Exosomes towards Cancer Immunotherapy

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Supplemental Information

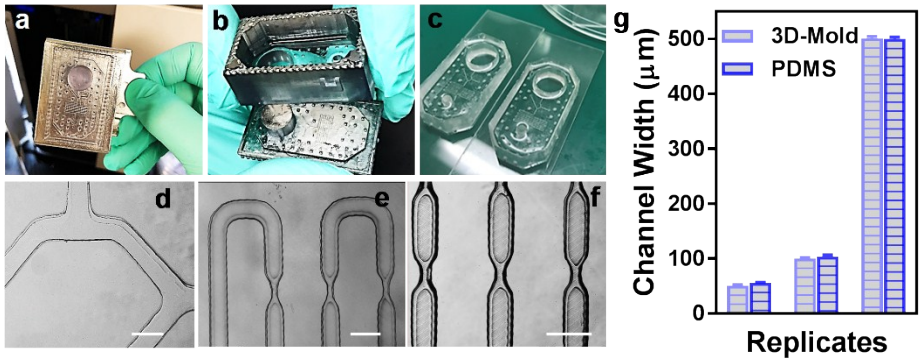


Figure s1. Illustration of 3D printing approach for one-step producing 3D mold and replicating PDMS microfluidic device integrated with cell culture and downstream exosome isolation, surface engineering, and on-demand photo release.

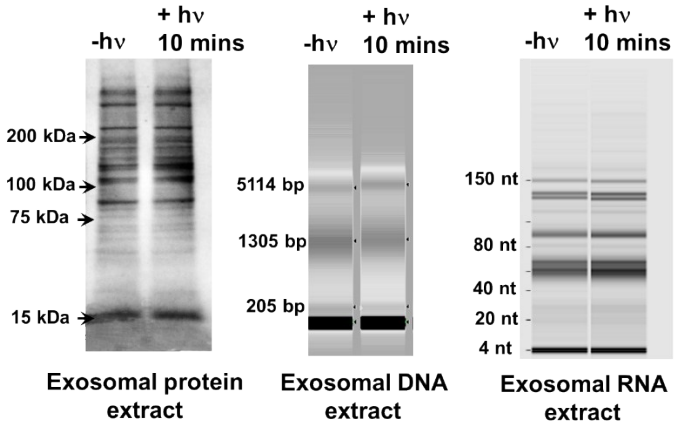


Figure s2. Investigation of the side-effect of UV exposure on exosome molecular contents in terms of proteins, DNAs and RNAs.

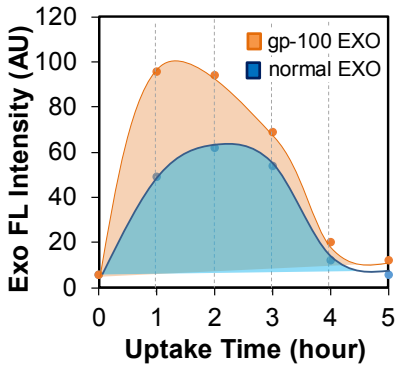


Figure S3. The fluorescence intensity analysis for showing the cellular uptake rate of gp-100 engineered exosomes and native exosomes.

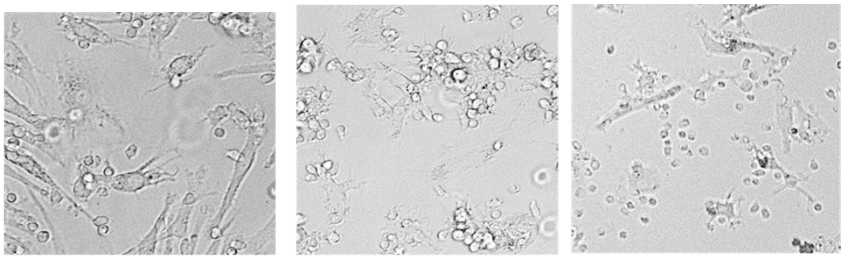


Figure s4. Human leukocytes culture under different stimulation conditions: 1) negative control is the leukocytes without any stimulation; 2) PWM protein stimulation as the positive control; 3) The gp-100 engineered exosome stimulation.

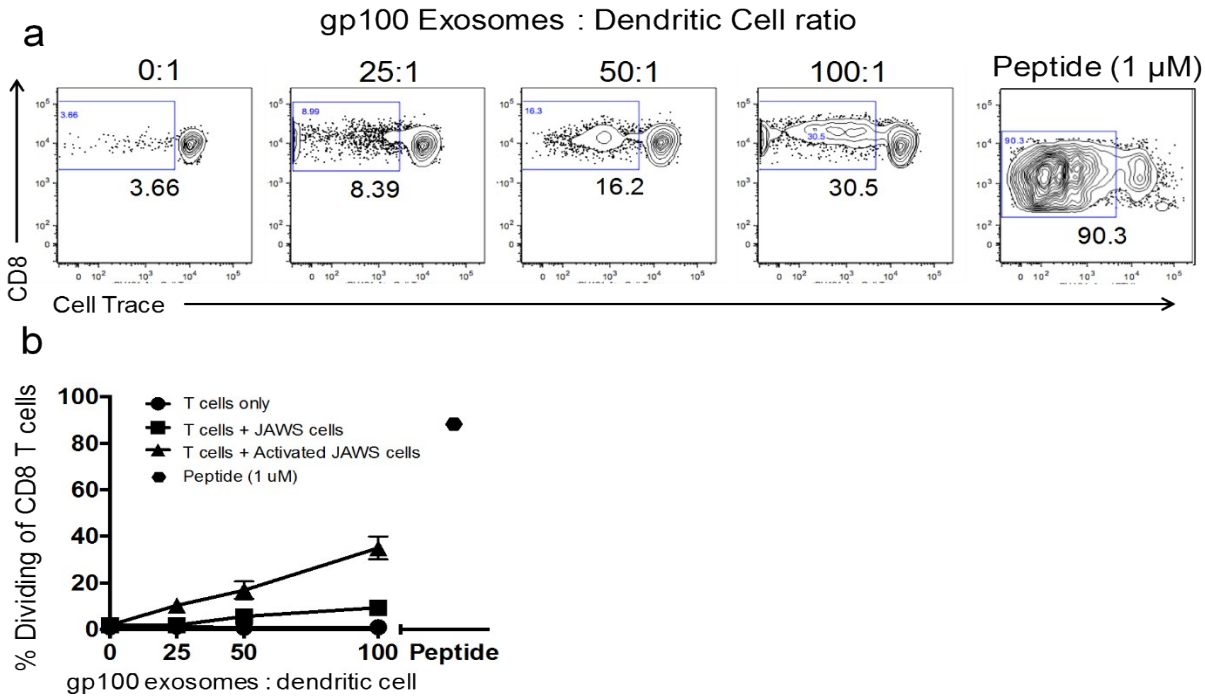
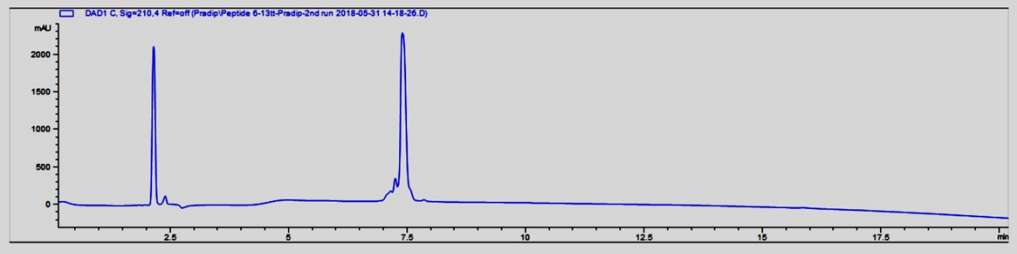


Figure s5. ex vivo testing of surface-engineered exosomes for activating transgenic mice spleen-derived CD8+ T cells. a) depicts representative flow plots from wells containing T cells + Activated JAWS cells with increasing concentrations of the gp100-engineered exosomes. The spiking gp-100 in μM serves as positive control. b) depicts the cumulative data from all four culture conditions showing the CD8+ T cell dividing rate under stimulation. The results are representative of 3 independent experiments with three duplicate wells for each culture condition (RSD < ~ 5%).

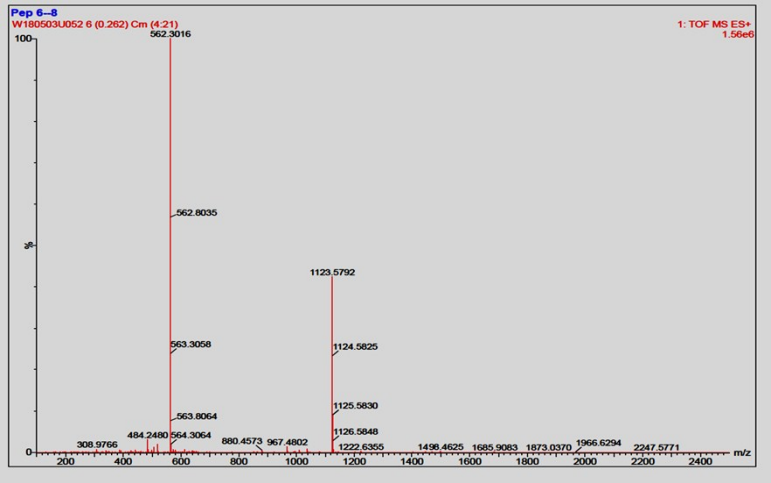
Tumor peptide synthesis and characterization

The protocols follow standard Fmoc chemistry. The peptides where cleaved using a solution of 92.5:2.5:2.5:2.5 TFA:TIPS:H2O:DODt and the crude peptides where purified using preparative HPLC (gradients of water/ acetonitrile (90:10 to 0:100 containing 0.1% TFA over 40 min) and lyophilized to obtain white powder. Analytical HPLC traces were acquired using an Agilent 1100 quaternary pump and a Hamilton PRP-1 (polystyrene-divinylbenzene) reverse phase analytical column (7 μm particle size, 4 mm x 25 cm) with UV detection at 210 nm. The eluents were heated to 45 °C to reduce separation of rotational isomers, and elution was achieved with gradients of water/ acetonitrile (90:10 to 0:100 containing 0.1% TFA) over 20 min. Low-resolution mass spectra (LRMS) were obtained using a Waters Micromass ZQ 4000 instrument with ESI+ ionization.

Peptide gp-100 Sequence: RLMKQDFSV
Chemical Formula: C49H82N14O14S1
Molecular Weight: 1123.33

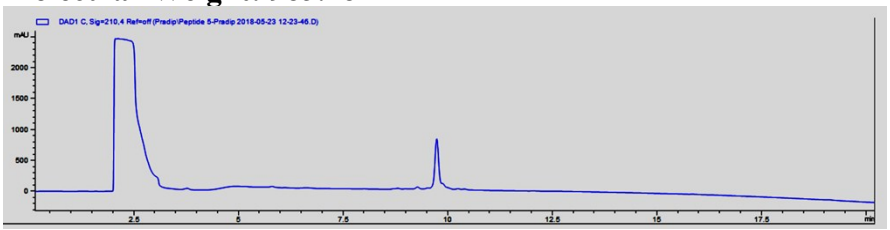


Analytical HPLC profile of sythesized peptide gp-100. Retention time = 7.51min (monitored at 210 nm). Purity > 90% by HPLC.

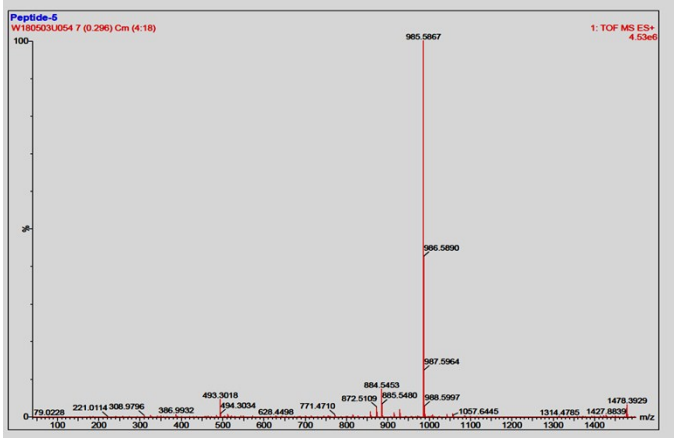


Low-resolution mass spectrum of peptide sythesized gp-100

Peptide MART-1 Sequence: ELAGIGILTV
Chemical Formula: C45H80N10O14
Molecular Weight: 985.18



Analytical HPLC profile peptide MART-1. Retention time = 9.80 min (monitored at 210 nm). Purity > 90% by HPLC



Low-resolution mass spectrum of peptide MART-1