33. Photocaged Cytokines for Cancer Immunotherapy

(Emory University)

Asset Overview

Product Type	Protein+polymer
Disease Area	Oncology
Indication	Cancer
Current Stage	Lead Optimization
Target	Cytokine IL-2, IL12, IL-15
MoA	The light-sensitive cage enables spatiotemporal activation of these cytokines (IL-2, IL12, IL-15)
Brief Description	• Emory inventors have engineered a new class of cytokines that enable spatiotemporal activation of therapeutic cytokines in response to external light exposure. In nature, some cytokines are expressed in a latent form ("shielded") and remain dormant until a specific condition is triggered. The triggering event leads to activation of these cytokines by "de-shielding" and specific signaling pathways are then activated. • Therapeutically promising cytokines IL-2, IL12, IL-15 are not expressed in a latent form and known to have poor circulation due to their small size and rapid clearance. The light-sensitive cage enables spatiotemporal activation of these cytokines. Signaling pathways can be activated by exposing these light sensitive cytokines to a light source (with specific wavelength – 356 nm (blue) and NIR (730 nm, more clinically relevant light wavelength – pulse oximeter, etc). • "Caged structure" is also just large enough to be above the renal clearance cutoff, leading to prolonged circulation and the observation of less prevalent or severe toxic side effects. These photokines are rapidly triggered, highly color selective, and their activity can be spatially constrained with micrometer-scale resolution.
Intellectual Property	US20220305124A1
Publication	Optical Control of Cytokine Signaling via Bioinspired, Polymer-Induced Latency. Biomacromolecules. (2020)
Inventors	Erik Dreaden, Priscilla Do, Lacey Anne Perdue

Highlights

- The prolonged circulation of scIL-12 may (i) obviate the need for frequent, high dosing and (ii) improve tissue accumulation of the drug in therapeutic settings..
- LED irradiation can de-repress IL-2 latency, promote antigen-specific immunity, and re-activate JAK/STAT pathway signaling.
- Polymer-induced latency may be maintained in vivo over time scales necessary for light-constrained cytokine de-repression.
- Spatiotemporal control of therapeutic cytokine activation leads to controlled local activation and reduces off-target toxicity.
- Prolonged circulation means less frequent dosing is required.
- Biased activity towards CD8+ T-cells and biased immune cell-selectivity.

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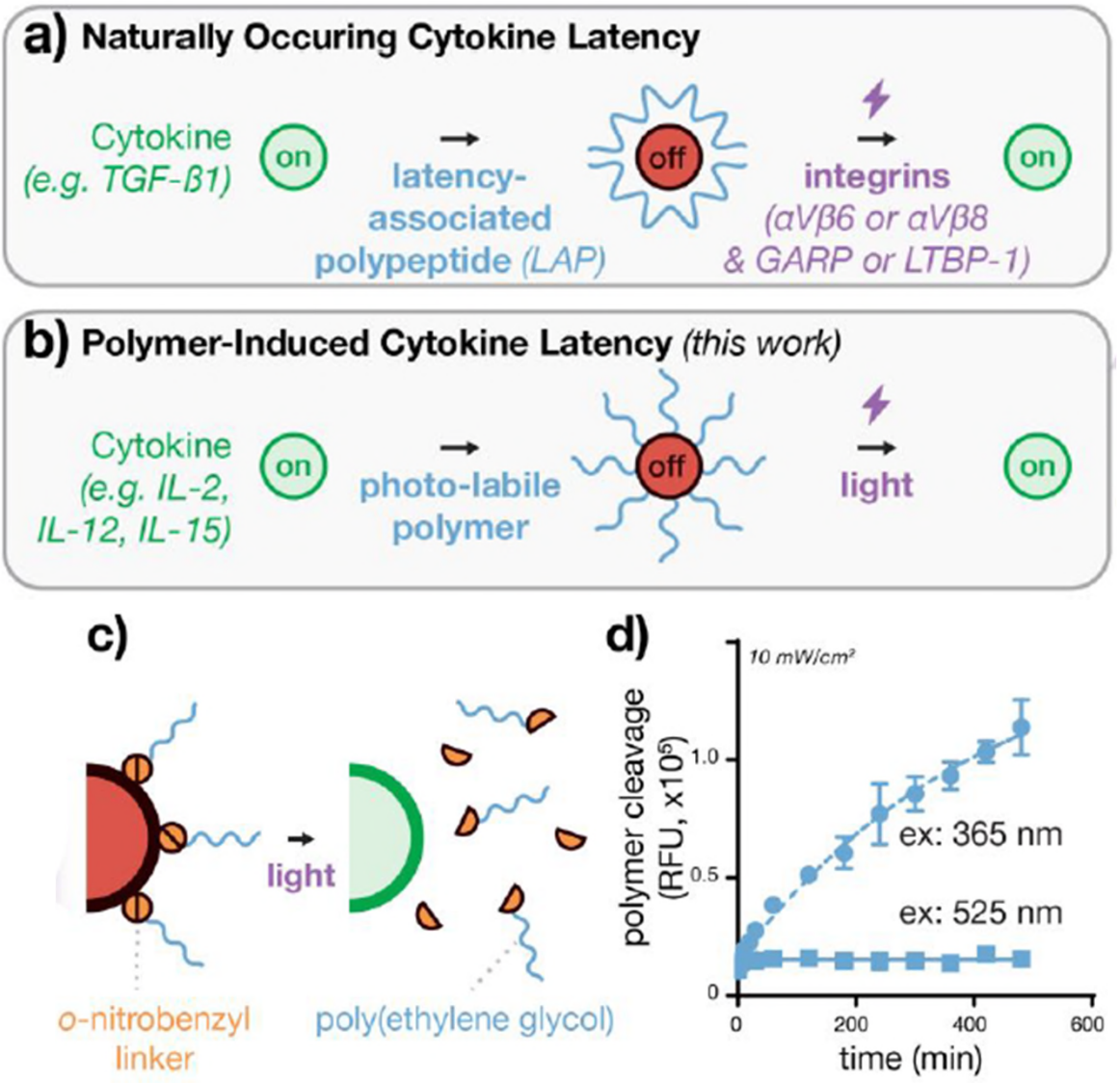
Key Data

Bioinspired cytokine latency via photo-labile polymer modification



a) Transforming growth factor-β1 (TGF-β1) transmits tissue- and cell- specific cytokine signals via association of with latency associated peptide (LAP) which sterically shields and later disassociates from TGF-β1 in response to traction forces caused by the binding of αVβ6 or αVβ8 integrins with either cell membrane-bound GARP or extracellular matrix-bound LTBP-1.
b) Strategy for the induction of reversible latency in cytokines with pleiotropic effects via modificaiton with end-modified, photo-labile polymers.
c) Illustration of the traceless modification strategy used here whereby 20 kDa poly(ethylene glycol) polymer chains are appended to cytokine lysine residues via o-nitrobenzyl groups which (d) are rapidly degraded by blue, but not green, LED light as measured by cleavage-induced fluorescence de-quenching. Data in (c) represent mean±SD of 3 technical replicates.

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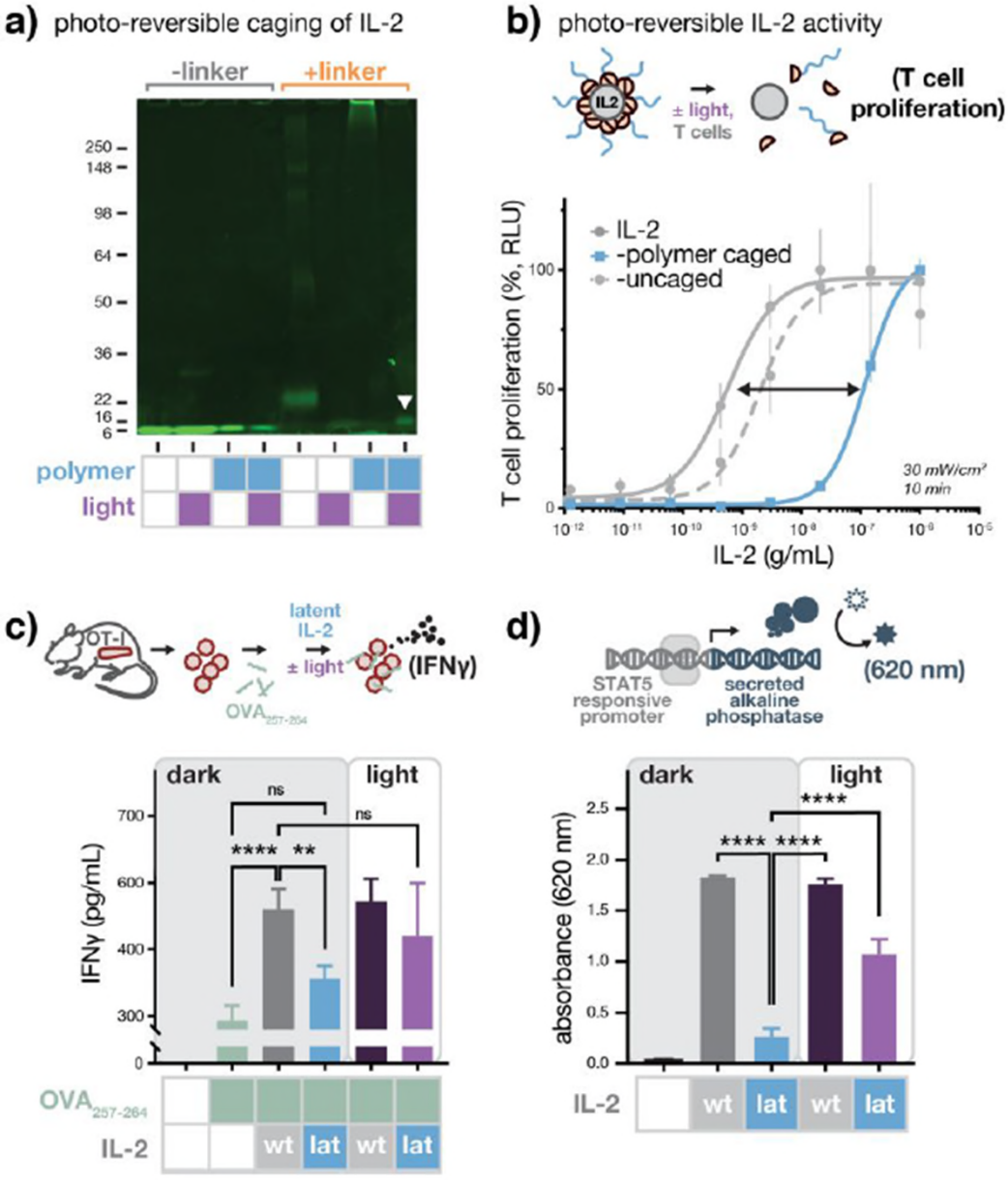
Photo-exposure of latent IL-2 restores protein size and functional activity



a) Electrophoretic mobility shift demonstrating linker- and polymer- dependent modification of IL-2, as well as light-dependent restoration (arrowhead) of wild-type protein mobility as measured by polyacrylamide gel electrophoresis.
b) Polymer-dependent repression and light-induced restoration of IL-2 activity as measured by CTLL-2 T cell proliferation (24 h). Effect of latent IL-2 and uncaged IL-2 (1000 IU/mL molar equivalents) on (c) OVA257–264 antigen-specific T cell activation and (d) JAK/STAT pathway activation as measured ex vivo by ELISA of OT-I splenocyte-secreted interferon gamma (IFN, 24 h) and STAT5 reporter cell secretion of aklakine phosphatase (48 h), respectively. Chromogenic substrate absorption in (d) was monitored at 620 nm. Data represent (b,d) mean±SD of 3 technical replicates and (c) mean±SD of of 6 technical replicates. $p < .01$(**),$p < .0001$(****).

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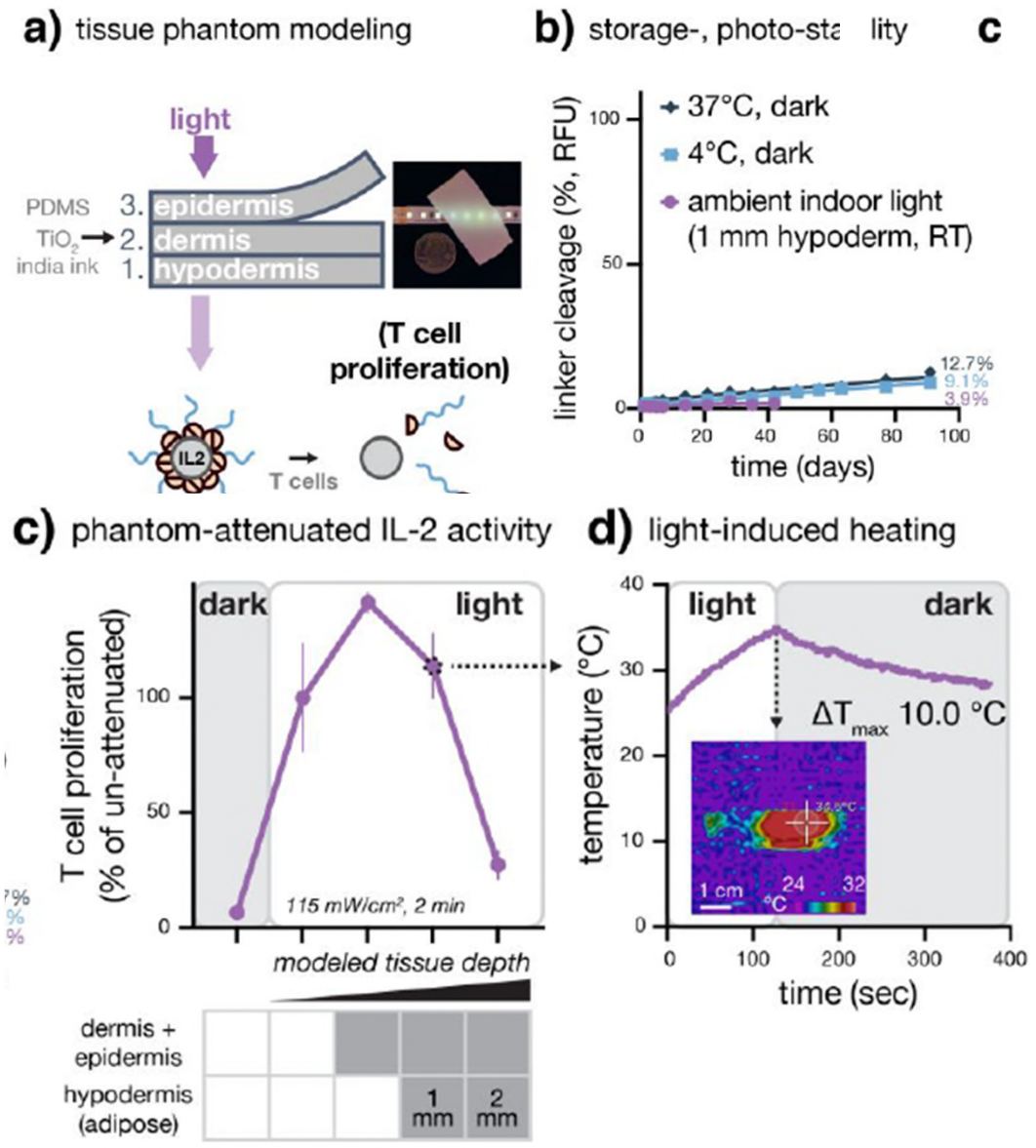

a) Illustration of multilayered tissue phantom construction.
b) Stability of 5 kDa poly(ethylene glycol) polymer photocages under prolonged, aqueous storage and under conditions simulating ambient, indoor light exposure of superficial veins (1 mm depth) as measured by cleavage-induced fluorescence dequenching.
c) Effection of tissue phantom light attention on latent IL-2 activity as measred by CTLL-2 T cell proliferation (24 h).
d) Superficial heating of multilayered tissue phantoms of the indicated thickness as measured by forwardlooking infrared imaging. Data in (c) represent mean±SD of 3 biological replicates. $p < .05$(*), $p < .01$(**), $p < .001$(***), $p < .0001$(****).