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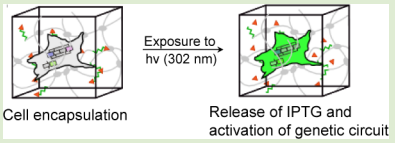
Photomodulation of Cellular Gene Expression in Hydrogels

Anirudha Singh,[†] Tara L. Deans,[†] and Jennifer H. Elisseeff*

Translational Tissue Engineering Center, Wilmer Eye Institute, and Department of Biomedical Engineering, Johns Hopkins University, 400 North Broadway, Baltimore, Maryland 21231, United States

Supporting Information

ABSTRACT: Biomaterials are designed to mimic aspects of various extracellular matrix environments, through chemical modifications to input biological or chemical signals. However, the dynamic nature and timing of gene expression during cellular events is much more difficult to mimic and control in these synthetic environments. Here, we utilized concepts of photochemistry combined with click chemistry for synthetic biology applications to modulate cellular gene expression in poly(ethylene glycol) (PEG) hydrogels. Specifically, a genetic inducer, isopropyl β-D-1-thiogalactopyranoside (IPTG), is covalently linked to PEG via a biocompatible and easy to synthesize 2-(2-azido-6-nitrophenyl)ethoxycarbonyl (ANPEOC) photocleavable moiety that, on a short exposure to UV light, effectively releases IPTG and activates gene expression of enhanced green fluorescence protein (EGFP). We anticipate that combining concepts of material chemistry with synthetic biology will further enable the construction of highly defined engineered niches that are capable of controlling both intrinsic and extrinsic cellular events.



Materials chemistry plays an important role in designing biomaterials for instructing cell behavior in many biomedical applications including cellular homeostasis,[1] tissue regeneration,[2−4] wound healing,[5,6] and targeted drug release.[7] These complex biological processes involve multiple signals that originate from both the extracellular matrix (ECM), and from the intrinsic cellular control of gene products.[6] Polymeric scaffolds can be designed to mimic aspects of various ECM environments, through chemical modifications to input biological or chemical signals.[8] However, the dynamic nature and timing of gene expression during cellular events is much more difficult to mimic and control in these synthetic environments. Approaches in synthetic biology aim to build genetic circuits that instruct cells to perform in predictable ways.[9−14] Coupling approaches in biomaterials and synthetic biology should improve our ability to recreate aspects of the cellular niche by endowing materials with the ability to regulate genetic circuits and, therefore, dynamically control gene expression patterns.[15] Hydrogels are a unique class of biomaterials that are hydrophilic, biocompatible, and amenable to modifications for conferring functional or biological responsiveness within the hydrogel.[16−25] We have previously employed these characteristics to modify poly(ethylene glycol) (PEG) hydrogels with the attachment of the genetic inducer, isopropyl β-D-1-thiogalactopyranoside (IPTG) via an ester bond. Upon hydrolysis of this ester bond, IPTG is released and becomes available to activate genetic circuits within the encapsulated cells.[15] Combining biomaterials and genetic circuits enables construction of three-dimensional (3D) microenvironments that are capable of controlling both intrinsic and extrinsic cellular events in highly defined engineered niches. Moreover, the ability to manipulate aspects of material properties with external stimuli in order to control switching on and off of specific genes is an important advance to spatially and temporally controlling gene expression for basic research and biomedical applications. In contrast to our earlier published method that is environmentally dictated,[15] we developed an externally controlled method that successfully demonstrated both spatial and temporal control. In this study, we utilized concepts of photochemistry combined with click chemistry for synthetic biology applications to modulate cellular gene expression in PEG hydrogels. Photocleavable bonds are attractive options to control the release of genetic inducers from biomaterials because photoactivation is easily controlled in space and time,[26−32] and applying this technology to induce genetic circuits in 3D materials would allow the spatial and temporal activation of specific genes. Moreover, by incorporating a novel and easy to synthesize photocleavable linker to PEG hydrogels, we demonstrate that these light-responsive moieties in biomaterials enable the controlled release of IPTG and subsequent gene expression (Figure 1A). The method we developed to temporally and spatially control the activation of gene expression in hydrogels involves the attachment of IPTG to PEG chains via a biocompatible photolabile moiety, 2-(2-azido-6-nitrophenyl)ethoxycarbonyl (ANPEOC; Figure 1A).

We utilized an NPEOC moiety to link IPTG to the PEG chains, making PEG-IPTG hydrogels for the temporal control of gene expression in PEG hydrogels. This functionalized PEG is capable of forming hydrogels in the absence and presence of cells, and when exposed to 302 nm UV light, the photolabile moiety degrades and releases IPTG in its original chemical state without any residual functional groups. We attached λIPTG using click chemistry (Scheme 1), and the controlled release of IPTG then becomes available to activate genetic circuits within the encapsulated cells (Figure 1B). First, the commercially available product, 2-(2-bromo-6-nitrophenyl)ethanol (**1**) was

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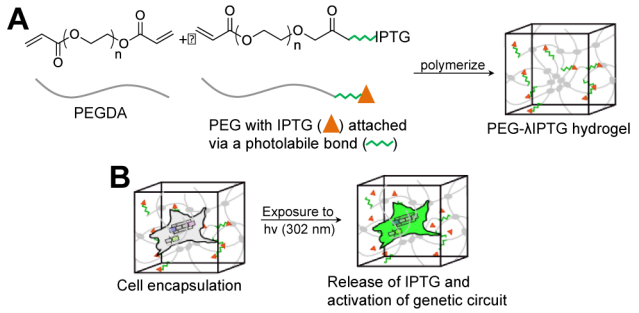
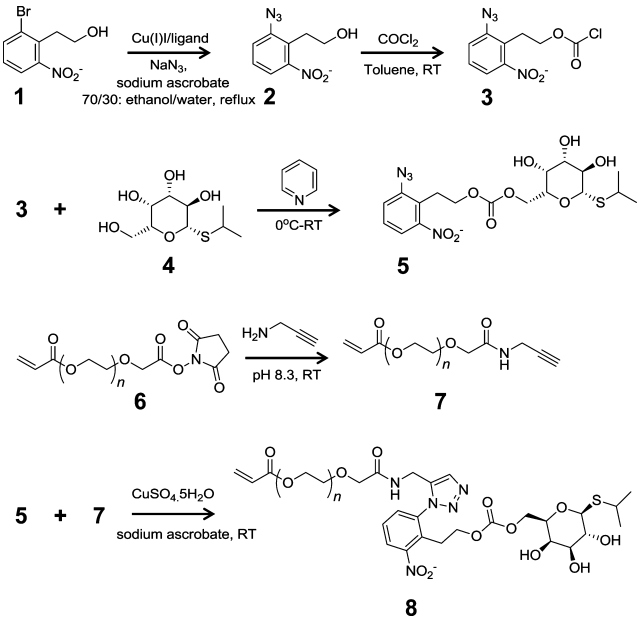


Figure 1. PEG-λIPTG hydrogels. (A) IPTG (orange triangle) was chemically attached to PEG chains via a photolabile bond (green line) using click chemistry. (B) CHO cells stably transfected with the LTRi_EGFP genetic circuit encapsulated in PEG-λIPTG hydrogels. Upon exposure to 302 nm UV light, the photolabile bond breaks, releasing IPTG from the PEG chains and activating EGFP gene expression within the cells.

Scheme 1. Synthesis of PEG-λIPTG (8)[a]



[a]The azide-functionalized photolabile NPEOC (**2**) was synthesized from (**1**) by copper-assisted nucleophilic substitution reaction in the presence of copper(I) iodide/*trans*-*N*,*N*′-dimethylcyclohexane-1,2-diamine, sodium azide, and sodium ascorbate at 110 °C for 18 h. Product (**3**) was synthesized by treating (**2**) with phosgene in toluene overnight at room temperature. Upon chemical reaction of IPTG (**4**) with the photolabile moiety (**3**) in pyridine, the photolabile IPTG (**5**) was obtained. Acrylate-PEG-NHS (**6**) was treated with propargyl amine in $NaHCO_3$ aqueous solution (pH 8.3) to yield acrylate-PEG-propargylate (**7**), which upon reaction with (**5**) under copper-based click chemistry condition yields PEG-λIPTG (**8**).

converted into an azide functionalized nitrophenyl moiety (**2**) by copper-assisted nucleophilic substitution reaction,[33−37] which was further converted into its carbonyl chloride derivative (**3**) and conjugated to IPTG (**4**) to yield λIPTG (**5**).

Next, we utilized acrylate-PEG-NHS (**5**) and propargyl amine to synthesize PEG chains with a terminal alkyne group (**7**), which reacts with azide-functionalized nitrophenyl IPTG (**5**) under the click chemistry reaction conditions[38] to yield PEG-λIPTG (**8**). ^{1}H (Figure 2) and ^{13}C NMR and FTIR-ATR spectroscopies (Figures S1−S2) confirmed the structures of the

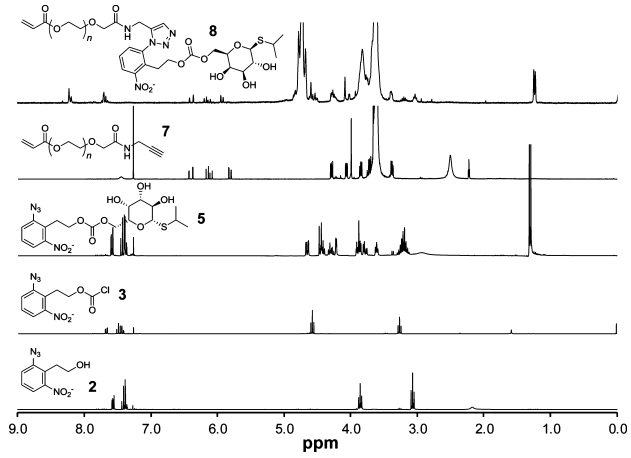


Figure 2. ^{1}H NMR spectrum of PEG-λIPTG (**8**) and its comparison to that of the starting materials.

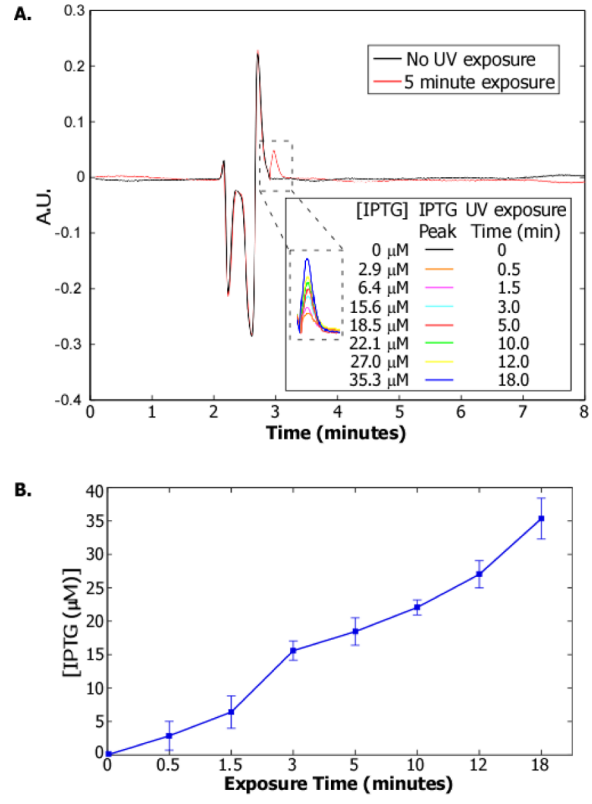


Figure 3. Release of IPTG from PEG-λIPTG gels. (A) HPLC of IPTG in solution (0.05 mg/mL) after exposure to 302 nm UV light. Inset is an overlay of peaks corresponding to UV exposure time. (B) Temporal control of IPTG release from PEG-λIPTG hydrogels.

products at each chemical modification step, including the final product, PEG-λIPTG.

Given the confirmed attachment of IPTG to PEG via the NPEOC photolabile moiety, we investigated the light-triggered release of IPTG. We exposed aqueous solutions of PEG-λIPTG (0.3 mg/mL) to ultraviolet light (UV, wavelength 302 nm) for various amounts of time. HPLC analysis confirmed that the UV light cleaved the photolabile moiety, separating the IPTG from the PEG chain[15] (Figure 3A). The amount of IPTG released was directly related to the length of exposure to UV light (Figure 3B).

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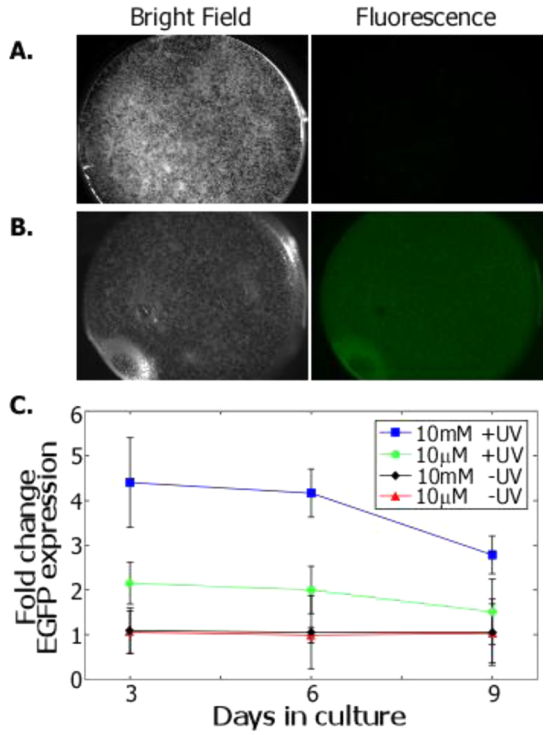

Figure 4. Temporal control of gene activation in PEG-λIPTG hydrogels. CHO cells stably transfected with LTRi_EGFP were encapsulated in PEG-λIPTG hydrogels and either (A) not exposed to 302 nm UV light or (B) exposed to 302 nm UV light for 5 min. (C) Quantitative RT-PCR over a 6 day time course. Cells were exposed to UV light at t = 0.

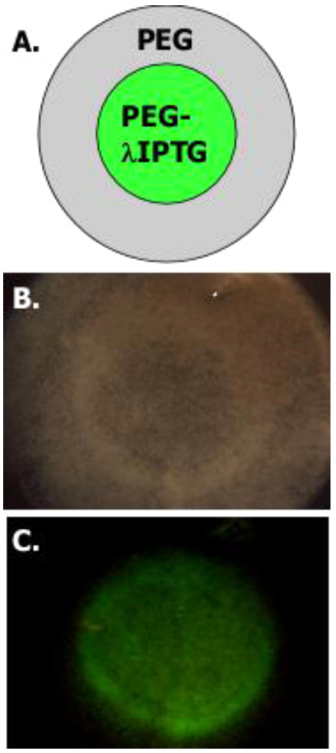

Figure 5. Spatial control of gene expression. (A) Schematic of pattern made. (B) Bright field image of cells encapsulated in PEG alone (outer ring) and PEG-λIPTG (inner area). Cells were exposed to UV light after polymerization. Images were taken 3 days after UV exposure: (B) bright field image; (C) fluorescent image.

Ultimately, this chemistry enables the controlled release of inducer molecules from PEG hydrogels to be used for the temporal control of gene expression in the encapsulated cells within the material. To demonstrate that the means of purifying the PEG-λIPTG, the UV wavelength, and length of exposure required to activate gene expression are cytocompatible, 1×10^6 Chinese Hamster Ovarian (CHO) cells stably transfected with LTRi_EGFP[9] were encapsulated into the PEG-λIPTG hydrogels. LTRi_EGFP is a synthetic genetic switch that is capable of regulating enhanced green fluorescence protein (EGFP) with the addition of IPTG.[9] We kept the UV exposure within 5 min to minimize the phototoxicity.[39] Because 18.5 μM IPTG released with a 5 min exposure is sufficient to activate gene expression in the LTRi_EGFP circuit,[9] stably transfected CHO cells encapsulated in PEG-λIPTG hydrogels were exposed to a 5 min pulse of 302 nm UV light. Cells were encapsulated 24 h prior to photoinduction, and three days after UV exposure, genetic circuit activation was assessed based on EGFP fluorescence (Figure 4). Control cells that were encapsulated yet did not receive UV exposure did not express EGFP, demonstrating that IPTG is stably maintained in the PEG hydrogel by the photolabile bond. In contrast, EGFP fluorescence is readily detected in encapsulated cells exposed to 302 nm UV light, indicating that the LTRi_EGFP genetic circuit is activated in the CHO cells (Figure 4).

Furthermore, the chemistry presented enables spatial control of gene expression. To demonstrate this, PEG and PEG-λIPTG (500 μM IPTG) both containing stably transfected CHO cells with LTRi_EGFP were patterned (Figure 5). After polymerization, the gels were exposed to 302 nm of UV light. Three days after UV exposure, a distinct pattern of gene expression was observed using fluorescent microscopy (Figure 5c).

In summary, we developed a biomaterial that has the genetic inducer, IPTG, covalently linked to PEG via a 2-(*o*-nitrophenyl)ethoxycarbonyl photocleavable bond. IPTG is effectively released by a short, cytocompatible pulse of 302 nm UV light upon which IPTG enters the cells encapsulated within the material to activate gene expression. These results demonstrate that concepts of material chemistry can be used to engineer 3D microenvironments for spatiotemporal photoregulation of gene expression. These 3D biomaterials can be used to engineer cellular niches to control intrinsic and extrinsic cellular events.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jhe@jhu.edu. Tel.: +1-410-614-6834.

Author Contributions

†These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

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