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Phototriggered Local Anesthesia

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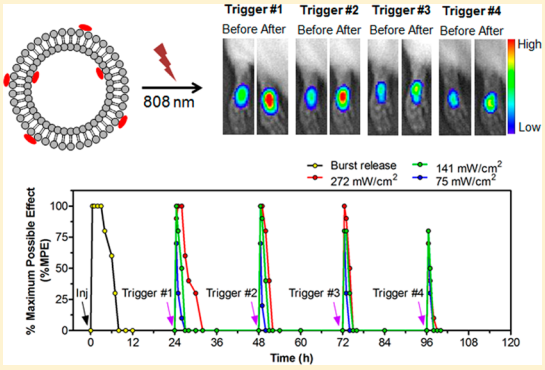
Supporting Information

ABSTRACT: We report a phototriggerable formulation enabling *in vivo* repeated and on-demand anesthesia with minimal toxicity. Gold nanorods (GNRs) that can convert near-infrared (NIR) light into heat were attached to liposomes (Lip-GNRs), enabling light-triggered phase transition of their lipid bilayers with a consequent release of payload. Lip-GNRs containing the site 1 sodium channel blocker tetrodotoxin and the α_2-adrenergic agonist dexmedetomidine (Lip-GNR-TD) were injected subcutaneously in the rat footpad. Irradiation with an 808 nm continuous wave NIR laser produced on-demand and repeated infiltration anesthesia in the rat footpad in proportion to the irradiance, with minimal toxicity. The ability to achieve on-demand and repeated local anesthesia could be very beneficial in the management of pain.



KEYWORDS: *Phototriggering, liposome, gold nanorod, local anesthesia, tetrodotoxin*

The development of an injectable local anesthetic that can be initiated by a single injection then produce repeated on-demand analgesia over extended periods would greatly enhance the quality of life of patients suffering from postoperative or even chronic pain. Such systems would minimize the taking of systemic analgesic medications (e.g., opioids), with all their complications[1] and their clouding of the sensorium. They would not require that patients be tethered to an external device or be maintained as inpatients. Most importantly perhaps, such devices would allow patients to modulate the degree of local analgesia in response to changes in their needs, level of activity, etc.

In recent years there has been increasing interest in remotely triggerable drug delivery systems in which a depot of drug is administered once, then repeatedly actuated via a safe external trigger such as an electromagnetic field[2] or ultrasound.[3] Near-infrared (NIR) light is emerging as a promising method to trigger drug release because of its ability to penetrate relatively deeply into soft tissues.[4,5] NIR-activated drug delivery systems have been widely investigated, particularly those that function by heating a thermosensitive component through coupling to gold nanomaterials.[6,7]

Liposomes are useful for remotely triggered drug delivery because they are injectable, often thermosensitive, and tissue response is generally benign.[8,9] Heating the liposomal lipid bilayer over its phase transition temperature increases its permeability and triggers the release of drugs.[10] Gold nanomaterials have been incorporated within liposomes to actuate drug release by NIR light.[11,12] The gold couples with light from a pulsed or continuous wave source to produce heat, which will induce a phase transition of the lipid bilayer[13,14] or be translated into pressure fluctuations that disrupt the lipid membrane.[15]

In order to maximize the local anesthetic efficacy of each triggered drug release event, we encapsulated tetrodotoxin (TTX) and a second molecule, dexmedetomidine (DMED). TTX is a naturally occurring toxin, whose mechanism of action is unimolecular blockade of site 1 on the extracellular surface of sodium channels on nerves.[16,17] TTX is an extremely potent local anesthetic.[18,19] Unlike commercially available amino-amide and amino-ester local anesthetics, tissue toxicity from site 1 sodium channel blockers (S1SCBs) after injection at peripheral nerves can be minimal,[20] even when delivered for prolonged periods.[21] Coadministration of S1SCBs with adjuvant agents can enhance anesthetic effect. For example, the combination of TTX and the α_2-agonist dexmedetomidine can significantly prolong corneal local anesthesia over that from TTX alone.[22]

Here, to achieve repeated on-demand local anesthesia, gold nanorods (GNRs) that are able to convert NIR light into heat were chemically tethered to liposomes (Lip-GNRs) containing TTX and DMED. The GNRs would raise the temperature of the adjacent liposomal lipid bilayer above its transition temperature, so that it would change from an ordered gel

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phase to a disordered liquid crystalline phase,[23] and release analgesic compounds. The ability to provide repeated sensory blockade triggered by remote NIR irradiation was tested *in vivo*.

Results and Discussion. *Characterization of Lip-GNRs.* Gold nanorods were synthesized (see Methods) and characterized by transmission electron microscopy. They exhibited a maximum absorbance at 808 nm (Figure S1). 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (DPPG) were selected as the liposome lipid components because their transition temperatures (41 °C) are above but close to body temperature.[24] Thiolated PEG-DSPE (HS-PEG-DSPE, ∼2 mol % of the total lipids) was anchored in the lipid bilayer to bind GNRs through gold−thiol interactions.[15]

Liposomes without GNRs were made with no payload (Lip-0), or containing rhodamine 6G (Lip-R6G) or TTX/DMED (Lip-TD). Lip-GNRs were made with no payload (Lip-GNR-0), or containing rhodamine 6G (Lip-GNR-R6G) or TTX/DMED (Lip-GNR-TD) (see Methods). Drugs or a dye were loaded by addition to the hydration buffer, and their concentrations in the purified formulations were measured (Table S1). The GNR content in purified Lip-GNRs as measured by ICP-MS was 0.02 wt %. The purified Lip-GNRs were 3.3 μm in diameter (Figure 1a). Cryo-electron microscopy imaging (cryo-EM, Figure 1b) showed GNRs associated with the liposomes.

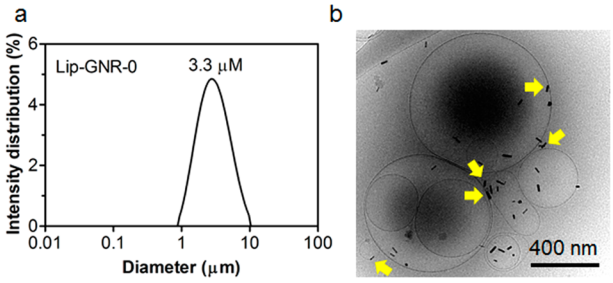

Figure 1. Characterization of blank liposomes conjugated with gold nanorods (Lip-GNR-0). (a) Dynamic light scattering measurement of Lip-GNR-0 demonstrated a mean diameter of 3.3 μm. (b) Cryo-EM image displaying gold nanorods (yellow arrows).

Lip-GNR-TD were not cytotoxic to C2C12 myotubes and PC12 cells over a four-day period (Figure S2). C2C12 and PC12 cells are commonly used in toxicological assays of muscle and nerve, respectively.

Initial studies of the thermosensitivity of Lip-GNRs were done with R6G. R6G was selected as a model dye since at low pH it is positively charged, like TTX. At 37 °C, release of R6G from Lip-GNR-R6G was minimal (Figure S3), similar to the release profile of R6G from Lip-R6G. Less than 20% of dye was released from Lip-GNR-R6G and Lip-R6G after 2 weeks. These data indicated that tethering to GNR did not alter liposome permeability. When incubated at 43 °C, 37% of R6G was released from Lip-GNR-R6G within 8 h, and a cumulative 76.4% of R6G was released by 2 weeks. A similar release profile was observed for R6G release from Lip-R6G at 43 °C. These release profiles verified that Lip-GNRs were sensitive to mild hyperthermia.[25,26]

Photosensitivity of Lip-GNRs. The photosensitivity of Lip-GNRs in PBS was assessed by irradiating Lip-GNR-0 (0.02 wt % of gold and 60−70 mg/mL of lipids) with an 808 nm continuous wave NIR laser for 1−30 min (Figure 2a). The bulk

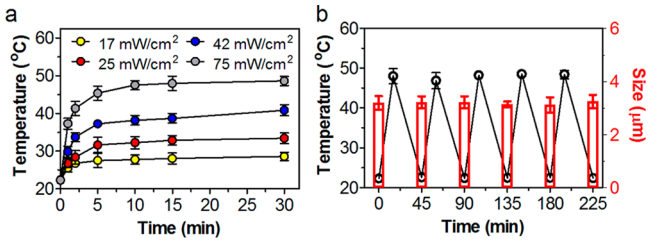

Figure 2. Photosensitivity of blank liposomes conjugated with gold nanorods (Lip-GNR-0). (a) Effect of irradiance and duration of irradiation (808 nm continuous wave NIR laser) on the temperature of a solution of Lip-GNR-0. (b) Effect of five cycles of irradiation with 75 mW/cm^2 NIR laser (15 min each, followed by cooling to RT for 30 min) on the size and photosensitivity of Lip-GNR-0. Lip-GNR-0 were sized (red) at the end of each off-state. The temperature (black) was recorded at the beginning and end of each triggering cycle. Data are means ± SD (n = 4).

temperature of the PBS was measured with an infrared imaging camera. Irradiation caused the temperature of the Lip-GNR-0 solution to increase rapidly during the first 2 min and plateau within 10 min. The plateau temperature increased from 28 to 49 °C as irradiance increased from 17 to 75 mW/cm^2. Irradiation at 75 mW/cm^2 for more than 2 min heated the solution of Lip-GNR-0 above the 41 °C lipid transition temperature.

The stability of phototriggering of Lip-GNR-0 was assessed by repeated NIR laser irradiations at 75 mW/cm^2 for 15 min. The particle size and the photothermal response of Lip-GNR-0 did not change over five cycles of irradiation (Figure 2b). The peak temperature (49 °C) after triggering was higher than the phase transition temperature (41 °C) of the lipids, suggesting that heating the lipid bilayer over the transition temperature did not destroy the liposomes.

To assess the irradiance required for phototriggered release, Lip-GNR-R6G were exposed to various irradiances for 10 min (Figure S4a). The rate of R6G release correlated with irradiance over the range of 0−25 mW/cm^2, then plateaued. Repeated triggering with 10 min cycles at 17 mW/cm^2 caused R6G release after each irradiation (Figure S4b), with the release from the first event being considerably larger than from subsequent ones. Seventeen mW/cm^2 only heated the solution of Lip-GNR-R6G to 28 °C, which is lower than the phase transition temperature of DPPC/DPPG, suggesting that local heating of the liposomes was to a higher temperature than the bulk heating of the medium.

Release of TTX and DMED from Lip-GNR-TD. In the absence of irradiation of Lip-GNR-TD, both TTX and DMED exhibited a small burst release on the first day, followed by slow sustained release (Figure S5). Studies of release from irradiated particles were done in particles where the burst release was first removed by dialysis at 37 °C for 24 h, to reflect the *in vivo* reality that injected particles were only irradiated once the burst release would have resolved (see *in vivo* study below). Release of TTX and DMED was measured under continuous irradiation with an 808 nm NIR laser at 25 mW/cm^2 (Figure 3a). Since triggered release of DMED started to plateau at 10 min, we used that as the duration of irradiation in subsequent studies. Release of both compounds decreased significantly with repeated irradiation (Figure 3b).

Phototriggered On-Demand Infiltration Anesthesia. Ninety microliters of Lip-GNR-TD and 10 μL of Lip-GNR-R6G were coinjected subcutaneously into the plantar aspect of

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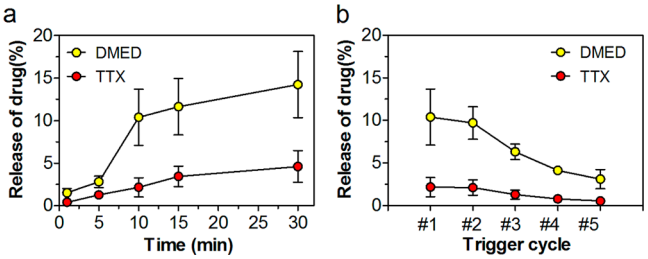

Figure 3. Release of DMED and TTX from liposomes conjugated with gold nanorods (Lip-GNR-TD) under continuous irradiation with an 808 nm continuous wave NIR laser at 25 mW/cm^2. (a) Effect of duration of irradiation on drug release. Drug release was measured independently (separate sample) for each duration of irradiation. (b) Drug release (noncumulative) over repeated 10 min irradiation cycles separated by 30 min. Data are means ± SD (n = 4).

the rat left hindpaw under brief isoflurane general anesthesia; neurobehavioral testing was initiated after the animals had recovered. The local anesthetic effect of Lip-GNR-TD was assessed by noting the vocal or motor (foot withdrawal) response to mechanical stimulation to the rat footpad with Touch Test sensory evaluators, and the duration of local anesthesia was calculated (see Methods). The initial local anesthesia had a median duration of 5.0 h (4.0−7.2 h, interquartile range) (Figure 4a and Table S2). Starting 24 h after injection, after complete resolution of local anesthesia, both footpads were irradiated once a day for 10 min (repeated daily over 4 days). Lip-GNR-TD could be activated *in vitro* at an irradiance of 25 mW/cm^2 (Figure 3). Given that light from an 808 nm NIR laser could be greatly attenuated by rat skin *ex vivo*,[27] we increased the laser irradiance used in the *in vivo* experiments to 75, 141, and 272 mW/cm^2. Each 10 min irradiation event (done under isoflurane anesthesia) triggered local anesthesia in the footpad that had been injected with Lip-GNR-TD (Figure 4a and Table S2) and had no effect of analgesia in the contralateral foot (suggesting a lack of systemic toxicity). Modulation of laser intensity allowed adjustment of the duration and intensity (%MPE) of triggered local anesthesia, as well as the area under the curve (AUC) for those two parameters (Figure 4a−d). The duration of local anesthesia decreased with progressive triggering events, presumably in reflection of the decreasing triggered flux of drugs with each cycle (Figure 3b).

Injection of 100 μL of Lip-TD into the rat footpad induced a similar duration of local anesthesia (median duration of 6.0 h) as from the initial injection of Lip-GNR-TD, which could be due to the burst release of TTX/DMED. Lip-GNR-0 did not produce detectable nerve block. Irradiation (141 mW/cm^2 for

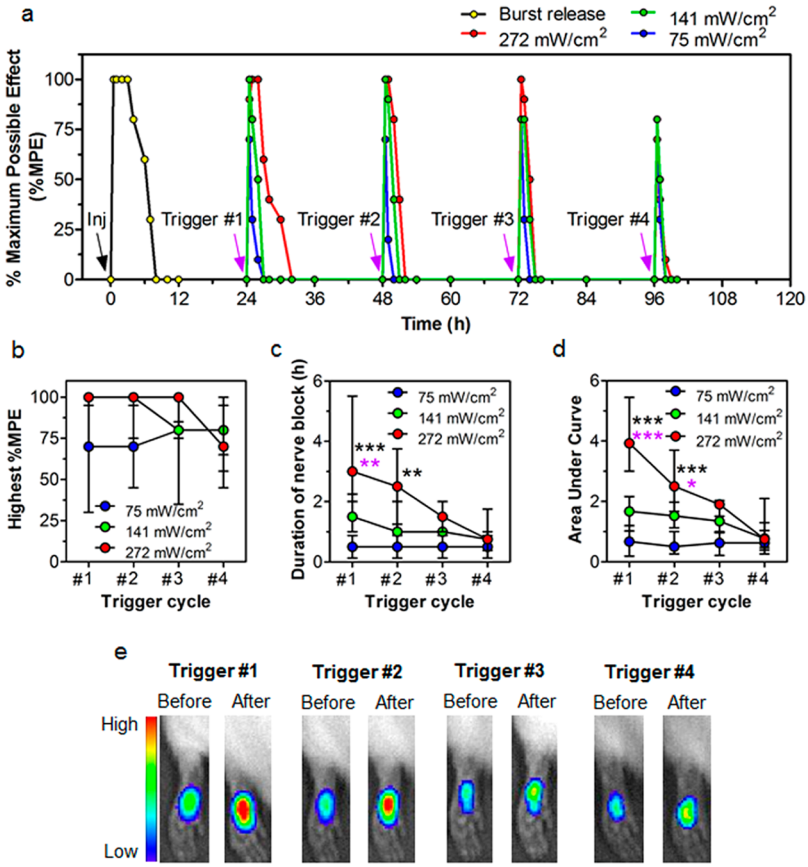

Figure 4. Phototriggered local anesthesia in the rat footpad. (a) Effect on local anesthesia of the footpad of injection (black arrow labeled "Inj") of 100 μL of a mixture of Lip-GNR-TD and Lip-GNR-R6G (volume ratio 9:1) and subsequent irradiation (purple arrows, 808 nm continuous wave NIR laser at 75, 141, and 272 mW/cm^2, for 10 min). Local anesthesia is presented as % maximum possible effect; see Methods. Data are medians (n = 4−6 per group; for the initial local anesthesia, n = 14 for the 3 groups). (b) The highest %MPE and (c) the duration of local anesthesia after each triggering with different laser irradiances. (d) The AUC of the %MPE-time curves for panel a (see Methods). Data are medians with 25th and 75th percentiles in panels b−d. *p < 0.05, **p < 0.01, and ***p < 0.001 (black asterisks, 75 versus 272 mW/cm^2; purple asterisks, 141 versus 272 mW/cm^2). (e) Fluorescence of R6G detected with an *in vivo* imaging system immediately before and after irradiation (purple arrows in panel a).

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10 min) of the injection sites of rats injected with Lip-TD or Lip-GNR-0 24 h after injection produced no detectable local anesthesia (Figure S6). These results indicated that laser irradiation itself did not trigger the release of TTX/DMED from Lip-TD in the absence of GNR and that localized heating by Lip-GNR-0 was not responsible for the local anesthesia.

Phototriggered release of R6G from Lip-GNR-R6G was visualized with an *in vivo* imaging system. The concentration of R6G in Lip-GNR-R6G was 2.33 mg/mL, displaying very low fluorescence because of self-quenching (Figure S7).[28] Rat left footpads were imaged immediately before and after irradiation. Phototriggered release of R6G from Lip-GNR-R6G resulted in brighter imaging since the released R6G was diluted outside of liposomes (Figure 4e).

The temporal arrangement of the triggered local anesthesia could also be altered by changing the timing of irradiation. For example, we could achieve prolonged continuous local anesthesia by irradiating (at 141 mW/cm^2 for 10 min) the footpads of rats receiving Lip-GNR-TD whenever the %MPE dropped below 100%. In this way, the duration of local anesthesia was prolonged from 4.0 h (interquartile range: 4.0−5.5 h) to 13.0 h (interquartile range: 12.0−14.8 h) with four cycles of the triggering (Figure S8).

Tissue Reaction. Rats receiving Lip-0 (no irradiation), Lip-TD (no irradiation), Lip-GNR-TD (no irradiation), and Lip-GNR-TD (4 cycles of laser irradiation at 141 mW/cm^2) were euthanized 8 days after injection, and the footpads were dissected for histological study (Figure S9). There was no injury to the skin and underlying tissues. Inflammation at the injection site, with macrophages and lymphocytes, occurred in all treatment groups, as is commonly seen with injected particles.[21,29,30] Foamy macrophages were observed, likely reflecting uptake of the injected formulations.[29,31] It is unlikely that there was significant neural injury given that we saw little tissue injury or inflammation: those phenomena occur well before there is any nerve injury, as we have documented in other sustained release formulations containing site 1 sodium channel blockers and other compounds.[21,32]

We[21,33−37] and others[38,39] have developed injectable sustained drug release systems that provide prolonged duration local anesthesia lasting days to weeks from one or more injections. However, those formulations have the limitation that once initiated, nerve blockade proceeds relatively monotonically until the drug content is depleted. The development of an injectable local anesthetic that could provide repeated on-demand analgesia after initiation with a single injection could have a significant clinical impact. It would allow patients suffering from postoperative pain or chronic pain to adjust the timing and degree of anesthetic effect. Here, the device could be repeatedly triggered to provide local anesthesia at least four times over a five-day period, after the initial nerve block had worn off. Aside from the initial nerve block, there was no basal anesthetic effect in the absence of irradiation.

NIR irradiation is a promising tool for triggerable *in vivo* applications.[7,27,40−43] However, NIR light may cause burns at high irradiances and/or prolonged irradiation times.[27,41] This concern is particularly relevant in devices that can be triggered repeatedly. Liposome-gold nanomaterial system should be designed so that the irradiance required to fully activate it is minimized. Here, gold nanorods attached to the liposomal membrane were able to mediate local actuation of the release of the payload without heating the bulk medium. (Our Lip-GNRs could be efficiently activated at 17 mW/cm^2, while 75 mW/cm^2 was required to heat the bulk medium over the 41 °C lipid phase transition temperature.)

We have provided proof-of-principle of a system that provides phototriggered release of local anesthetics in a manner that could be adjusted by varying the irradiance and the duration of irradiation. Such adjustment would allow tuning of the duration and intensity of nerve block according to patient needs. Such devices could also be adapted to use in other excitable tissues, e.g., in the brain to prevent or treat seizures.[44]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.5b03440.

Methods section and the results describing the characterization of gold nanorods, *in vitro* cytotoxicity assays, cumulative release of R6G from Lip-GNR-R6G and Lip-R6G, phototriggered release of R6G from Lip-GNR-R6G, release kinetics of TTX and DMED from Lip-GNR-TD, effect of injection of Lip-TD and Lip-GNR-0 on local anesthesia of the footpad, self-quenching effect of R6G, prolonged duration local anesthesia from four laser irradiations after injection Lip-GNR-TD, histological studies, the characterization of formulations, and a tabulation of the durations of local anesthesia in the rat footpad from various treatments (PDF)

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Author Contributions

C.Z. and D.S.K. designed research; C.Z., W.W., J.B.M, S.G., and C.S. performed research; C.Z., W.W., and D.S.K analyzed data; and C.Z., W.W., B.P.T., and D.S.K. wrote the paper.

Notes

The authors declare no competing financial interest.

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