On-Demand Drug Delivery System Using Micro-organogels with Gold Nanorods

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ABSTRACT: In this study, we designed a biocompatible drug carrier: micro-organogels prepared by emulsification using vegetable oils and self-assembled gelator fibers. Flurbiprofen was chosen as a hydrophobic model drug and is classified as a nonsteroidal anti-inflammatory drug. In the absence of NIR light, flurbiprofen encapsulated in micro-organogels with gold nanorods (GNRs) was released slowly, while release was accelerated in the presence of NIR light due to the increase in the temperature surrounding the GNRs that transforms the gels into liquid. These results suggest that our system can be efficiently used as a versatile scaffold for on-demand drug delivery systems.

KEYWORDS: Micro-organogels, on-demand drug delivery, gold nanorods, near-infrared, flurbiprofen

Interest in developing responsive biomaterials with flexible physical and chemical properties has continuously increased in the fields of optoelectronics, sensor devices, surface coating, and drug delivery.^{1–3} In particular, reversible phase changes in response to environmental stimuli and physiochemical factors, such as pH, temperature, enzymes, and external forces involving visible light, near-infrared, ultrasound, and electric and magnetic fields, enable the use of these materials as drug-carrier scaffolds for the efficient delivery of therapeutics.^{4–6} Since most drugs require frequent and continuous dosing over a long duration of time, and their concentrations must remain in a specific effective range to avoid overdosing or underdosing, new versatile systems that are able to be externally manipulated and spatially programmable in an easy manner should be developed and enhanced.^{7,8}

Light-activated materials have several benefits, including ease of control, locally selective exposure, and nontoxicity to body tissues, and thus are particularly promising drug delivery systems.^{9–11} In contrast to gold nanoparticles (GNPs), which have strong absorption in the range of 520–600 nm that is harmful to human body, gold nanorods (GNRs), containing light-activated scaffolds with strong absorption of near-infrared (NIR) light in the range of 650–900 nm, are not harmful and particularly useful as they exhibit a strong photothermal effect by plasmon resonance.^{12–14} That is, the localized heat induced by NIR light, which is able to penetrate body tissues at a depth of up to 10 cm without significant damage to surrounding tissue, can be utilized in various therapies and various medical

fields.¹⁵ Organogels are semisolid systems that are thermally reversible and capable of encapsulating hydrophobic therapeutics; thus, they can be employed as responsive scaffolds in on-demand drug delivery systems by combining the ability of the particles to circulate for long durations of time and to illuminate the target object.^{16–19}

Wound healing is an orderly and timely reparative process that replaces devitalized cellular structures and tissue layers following injury and requires a dynamic balance between scar formation and tissue regeneration, and the processes differ in various tissues.^{20,21} Generally, wound healing is a self-perpetuating process, though various therapeutic treatments including antihemorrhagics, antibiotics, anti-inflammatory drugs, and growth-factors can be administered at desired time periods for facilitation.²² Thus, effective cutaneous drug delivery systems should be studied to enhance efficacy of therapeutic wound treatment.²³ Due to the difficulty of directly supplying drugs for tissue regeneration after scar formation, stimuli-responsive drug delivery systems in wound treatment could be a versatile option for supplying additional doses via external stimulation.^{24,25}

In our study, micro-organogels were prepared by emulsification using vegetable oils immobilized within the spaces of the

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Figure 1. Schematic illustration for formation of GNR contained micro-organogels and their drug release upon NIR irradiation.

3D-networked structure formed by the physical interaction between self-assembled gelator fibers. Flurbiprofen, which is a member of the phenylalkanoic acid derivative family of nonsteroidal anti-inflammatory drugs (NSAIDs) that is used to control inflammation and reduce pain,²⁶ was used as the small hydrophobic model drug. In the absence of NIR light, flurbiprofen encapsulated in the micro-organogels with GNRs was released slowly, while the release was accelerated in the presence of NIR light due to an increase in temperature that transformed the gels into liquid. These results suggest that our system could be efficiently used as a versatile scaffold for drug delivery.

Supplying additional doses via external stimulation after the initial therapeutic treatment in wound healing enables to open a new way for effective cutaneous drug delivery systems. In our system, after introduction with other therapeutic reagents for wound healing, the micro-organogels might be stayed in the diseased region even after scar formation. Therefore, the drugs will be slowly released upon NIR irradiation from the micro-organogels stayed in the diseased region. Micro-organogels exhibiting thermo-reversible properties and a high capacity for hydrophobic drugs were prepared via emulsification by ultrasonication with vegetable oils (Figure 1). 12-HSA was used as gelators that are one of representative organogelators, which are relatively nontoxic.²⁸ The oil mixture, which contains flurbiprofen, ODT-GNRs, and HSA gelators (1–5 wt % in oils), was emulsified in an aqueous BSA solution by ultrasonication with high intensity for a fine control. After purification via repeated centrifugation and resuspension, the formation of the prepared micro-organogels was confirmed using optical microscopy, fluorescence microscopy, scanning electron microscopy, confocal laser scanning microscopy, and UV-vis spectroscopy.

Figure 2 shows TEM images and the optical properties of GNRs during the formation of micro-organogels. Initially, CTAB-GNRs exhibiting a 3.5 ± 1.2 aspect ratio and strong absorption in the range of 650–800 nm were successfully prepared via a seed-mediated growing method (Figure 2a,d).

Figure 2. TEM images for (a) CTAB-GNRs, (b) ODT-GNRs, and (c) ODT-GNRs in oils and (d) UV-vis spectra for CTAB-GNRs, ODT-GNRs, and ODT-GNRs in oils and ODT-GNRs in a microparticle. All scale bars are 50 μm in a–c.

No significant change was observed in shape or optical properties of the GNRs even after ligand-exchanging to ODT, though the small red-shift was seen after dissolving in oils, retaining the peak shape (Figure 2b–d). However, the broad absorption peak observed after microparticle formation was thought by environmental change surrounding gold

nanorods in micro-organogels. Initially, the phase change of ODT-GNRs to hydrophobic and viscous oils causes the small red-shift, while the big red-shift is due to their confinement in tiny micro-organogels, forcing GNRs to be close together.^{29,30} The most important property of the micro-organogels is that they possess broad and strong absorption in the range of 750–1100 nm. This phenomenon enables them to respond to light stimuli with high sensitivity and in a stable manner.

To confirm the formation of microparticles encapsulating hydrophobic drugs, coumarin 6 was first dissolved in an oil solution instead of flurbiprofen. As shown in Figure 3a, the

Figure 3. (a) Optical microscopy image with a SEM image inset and (b) fluorescent microscopy image of micro-organogels. (c) Confocal microscopy image of a single micro-organogel and (d) a graph of its profile. (e) Graph of their thermal transition traces that are dependent on the concentration of HSA gelators in oils.

optical and SEM images indicate robust formation of micro-organogels with an average diameter of 3.2 μm. Additionally, encapsulation of hydrophobic coumarin 6 was confirmed using fluorometer microscopy (Figure 3b). To further investigate the successful formation of micro-organogels stabilized with BSA, 10% of fluorescein isothiocyanate-labeled BSA (FITC-BSA) was used with BSA in an aqueous solution. During

emulsification, the hydrophobic moiety of BSA allows embedding it to the hydrophobic microgels, while the hydrophilic moiety of BSA helps to disperse in aqueous environment. The successful adsorption of FITC-BSA onto the surface of the microparticles was confirmed using confocal laser scanning microscopy. In the confocal image, the green fluorescent corona indicates the homogeneous formation of FITC-BSA onto a microparticle that stabilizes the hydrophobic micro-organogels in an aqueous environment (Figure 3c). As shown in the Figure 3d, the fluorescence section profile graph of the corona shows only two strong peaks with no additional fluorescence that indicates the FITC-BSA was selectively embedded onto micro-organogels not to the core.

The transition temperature of the prepared micro-organogels from gels into liquid was achieved by analyzing the change in transmittance via UV–vis. As the amount of HSA gelators in the oils increases, the transition temperature increases, which is consistent with previous research³¹ (Figure 3e). However, an earlier transition was observed in our micro-organogels due to the presence of GNRs. Temperature traces of solutions containing thermoresponsive microparticles (2 wt % of HSA and 52.6 °C of transition temperature) upon NIR irradiation (0.36 W/cm²) were first recorded using an infrared camera. As shown in the Figure 4a–c, the following temperatures were

Figure 4. Temperature traces of Eppendorf tubes containing micro-organogels measured using an infrared camera (a) with no light and with GNRs, (b) with NIR light and no GNRs, and (c) with both NIR light and GNRs. (d) Light-triggered drug release profiles with and without light. Total flurbiprofen amount contained in 1 mL of solution in each tube is 80 ng, and its concentration is 80 μM.

observed: 25.9 °C (with no light and with GNRs), 29.3 °C (with NIR light and with no GNRs), and 61.4 °C (with both NIR light and GNRs). These results indicate that the micro-organogels can efficiently respond after NIR irradiation causes a thermal increase in the surrounding GNRs. Cumulative flurbiprofen release from the micro-organogels was further

tested in a PBS solution using a mini dialysis device. One Eppendorf tube was irradiated by NIR light, and the other was not stimulated. PBS in two tubes was frequently exchanged at the proper time ranges. As shown in Figure 4d, the release profile of flurbiprofen was slow in the absence of light, but rapidly increased in the presence of NIR light. That is, NIR irradiation caused an increase in temperature that transformed the gels into liquid and accelerated drug release. These results demonstrate the potential of our micro-organogels for use as versatile scaffolds in the delivery of various therapeutics over a range of intended time periods.

In the present study, we designed biocompatible drug carriers: micro-organogels prepared by emulsification using vegetable oils and self-assembled gelator fibers. Flurbiprofen was chosen as the hydrophobic model drug and is classified as a nonsteroidal anti-inflammatory drug. The formation of the prepared micro-organogels was confirmed using optical and fluorescence microscopy, scanning electron microscopy, and confocal laser scanning microscopy, and their thermal responsiveness and drug release was recorded using an infrared camera and LC–MS/MS, respectively. In the absence of NIR light, flurbiprofen encapsulated in the micro-organogels with GNRs was released slowly, while release was accelerated in the presence of NIR light due to an increase in temperature that transforms the gels into liquid. These results suggest that our system could be efficiently used as a versatile scaffold for on-demand drug delivery systems. In addition, our externally controlled system has the potential to open new pathways for efficient wound therapy. Though a traditional anti-inflammatory drug was initially used for a model drug in our study, our system will be further extended with other therapeutics involving various growth factors in the future.

■ EXPERIMENTAL PROCEDURES

Materials and Instruments. Fluorescein isothiocyanate bovine serum albumin (FITC-BSA), phosphate buffer solution (PBS), soy bean vegetable oil, 12-hydroxystearic acid (HSA) gelator, 1-octadecanethiol (ODT), gold(III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), cetyltrimethylammonium bromide (CTAB), sodium borohydride (NaBH_4), silver nitrate (AgNO_3), ascorbic acid (AA), and flurbiprofen were purchased from Sigma-Aldrich. All chemicals were used without further purification. Slide-A-Lyzer mini dialysis device (10K MWCO 0.1 and 0.5 mL) was purchased from Thermo Scientific. A diode laser system with a wavelength of 852 nm, continuous wave operating mode, and >2000 mW output power was used as a light stimulus. Temperature traces were recorded using a Ti95 infrared camera (Fluke, Washington, USA). Transmission electron microscopy and scanning electron microscopy images were acquired on a JEOL 1000CX operating at 80 keV and JSM-6510, respectively (JEOL, Japan). The concentration of the drugs that were transferred using a mini dialysis device was measured using an API 4000 QTrap instrument (AB Sciex, CA, USA).

Preparation of ODT-GNRs. CTAB-coated GNRs were prepared according to a known procedure, the seed-mediated growing method.²⁷ Briefly, a brown-yellow seed solution was prepared by adding an aqueous NaBH_4 solution (0.6 mL, 10 mM) into an aqueous mixture of HAuCl_4 (0.25 mL, 10 mM) and CTAB (7.5 mL, 10 mM). Then, an AA solution (0.192 mL, 100 mM) was slowly added into another precursor mixture of AgNO_3 (0.18 mL, 10 mM), HAuCl_4 (1.2 mL, 10 mM), and CTAB (28.5 mL, 100 mM) with mild stirring until the mixture became colorless. After adding 0.06 mL of the seed solution, the reaction mixture was gently mixed for 10 s and left undisturbed for at least 8 h in a water bath (at 25–29 °C). The GNR solution was purified by centrifugation repeatedly, and the CTAB-GNRs was further modified in the presence of an excess of 1-octadecanethiol (ODT) ligands (50 mg, 30 mL) in toluene with

stirring under N_2 for 48 h. The prepared CTAB-GNRs and ODT-GNRs were verified through UV–vis and TEM analysis.

Preparation of Micro-organogels Containing Flurbiprofen and ODT-GNRs. Flurbiprofen was first dissolved in vegetable oils (2 mg/mL) under vigorous stirring. Hydrophobic ODT-GNRs were dissolved in hexane (100 nM), as shown in the Figure 1. Both solutions were mixed together at the same volume ratio for 1 h, and a little aggregation was removed using syringe filters when necessary. Subsequently, the resulting mixture was evaporated by rotary evaporator and vacuum to completely remove the hexane. A varied wt % portion of HSA gelator was dissolved in the oil mixture at a temperature range of 40–60 °C. The flurbiprofen and ODT-GNRs with micro-organogels were prepared by ultrasound. Initially, the oil/water emulsion was formed by mixing 5 mL of 2 wt % aqueous FITC-labeled BSA solution and 1 mL of vegetable oils containing HSA gelator, flurbiprofen, and GNRs. Fine control for emulsion formation was performed using high intensity ultrasound (ultrasonic processor KSS-650D, Korea Process Technology Co., Ltd. South Korea) for 2 min. Subsequently, the solution was cooled down to room temperature, showing a white milky suspension (Figure 1). The micro-organogels were separated from the excessively used reaction mixture by centrifugation and rinsed with water by repeating resuspension and centrifugation. The formation of the prepared micro-organogels was confirmed using optical microscopy (HR-3RF, Huvitz, Korea), fluorescence microscopy (BX53, Olympus, Japan), scanning electron microscopy (SEM) (JSM-6510, JEOL, Japan), and confocal laser scanning microscopy (Leica TCS SP8, Germany).

NIR Irradiation for Drug Release. A 0.5 mL micro-organogel solution was added to a mini dialysis device, and the device was placed into an Eppendorf tube containing 1 mL of PBS. With continuous mild agitation, each tube was collected for an intended period, and a new tube was replaced with a fresh PBS. For the light stimulus, the device was continuously irradiated by a diode laser (850 nm) at an energy level of 0.36 W/cm² for 25 min. Positive ion electrospray-ionization liquid chromatography with triple quadrupole mass spectroscopy (LC–MS/MS) was used for the pharmacokinetic study of the collected flurbiprofen using an API 4000 QTrap instrument (AB Sciex, CA, USA). The LC–MS/MS was calibrated using ketoprofen, with 3 μL injection volume and 2 min run time. In flurbiprofen, precursor and product ions were set at m/z 245 and 199, respectively. Samples were chromatographed on a Scherzo SM-C18 column (3 μ , 2 $\times$ 75 mm; Imtakt Corporation, Kyoto, Japan) that was eluted with a isocratic flow (eluent A, 0.1% formic acid (v/v) in H_2O ; eluent B, 0.1% formic acid (v/v) in acetonitrile; 70% of B in 2 min) at a flow rate of 0.3 mL/min at 40 °C.

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Notes

The authors declare no competing financial interest.

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