Near-infrared Light Triggers Targeted Drug Release Without Harming Tissue

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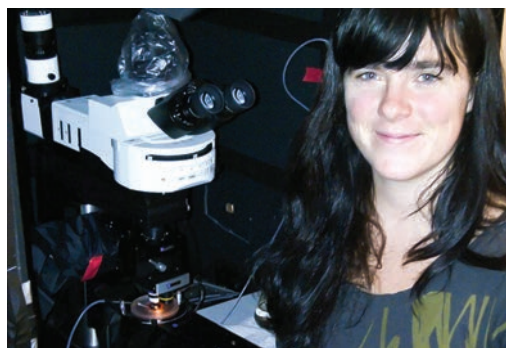

Kim Doré and the two-photon microscope

The precisely controlled release of contents from a capsule to allow the application of a substance to a specific place at a specific time is of potential interest in a number of areas including self-healing materials, nutrient preservation, fragrance release and drug delivery.

A number of methods have been developed to trigger the release of cargo from polymeric particles based on magnetic, electrical, biological, chemical, thermal and photo stimuli.

Light-triggered activation is particularly attractive in biological research because it allows for remote release with highly spatiotemporal resolution. Due to the depth of penetration and low attenuation of near-infrared light (NIR) in biological tissues, wavelengths between 750 and 1300 nm are appealing as potential triggers.

Techniques using NIR in biological systems have already been developed but because of the heat generated, power required and use of toxic compounds these are often detrimental to cells and the surrounding tissue. Other methods require the use of designer polymers that are not widely available. In order to advance the use of NIR-induced payload release, a universal and biologically viable mechanism is required.

Researchers from the University of California, San Diego led by PI Adah Almutairi* have shown that hydrated non-light sensitive polymer particles can release their encapsulated compounds when irradiated with NIR laser light resonant with the vibrational overtone of water at 980 nm. This leads to the conversion of optical energy to heat in the confined water domains without a significant temperature increase to the whole system.

NIR-induced Particle Release

In order to discover how this process works, a number of experiments were carried out using poly(lactic-co-glycolic acid) (PLGA) particles holding model compounds of varying hydrophobicity (fluorescein, Nile blue, Nile red and IR780). PLGA was chosen as the main matrix polymer because it is inherently non-light-sensitive, FDA approved, extensively studied as a drug carrier and widely used in biomaterial applications.

To determine whether this process requires absorption of NIR by water, fluorescein-loaded PLGA particles were irradiated with wavelengths between 780 and 1030 nm at 50 nm increments for 15 minutes; only irradiation at 980 nm induced release, observed as a large increase in fluorescence intensity. (Fluorescein emits more light upon release into the surrounding substrate because it fluoresces more strongly in the polar environment and it is self-quenched at the high concentrations found inside the particles.) Further investigations found that the amount of released material was proportional to the amount of energy provided to the system as tested by different amounts of irradiation time.

This release is not dependent on short, focused, high-energy light pulses but varies depending on the average photon energy, as tested with differing laser excitation powers from (170 to 350 mW). Therefore, lower power continuous wave laser light, less damaging to biological tissues, can be used with this technique.

Scanning electron microscope (SEM) images of the PLGA particles were taken using an Agilent 8500 FE-SEM system. They showed no change in morphology before and after irradiation, although particles did appear to aggregate.

This suggests that the particles may be becoming more rubbery as they are heated above their glass transition temperature (T_g), but the process is not degrading them. Further evidence for the lack of degradation is provided by gel permeation chromatography of irradiated and non-irradiated particles. These display no change in retention time and no small molecular peaks.

The more control that can be exercised over a triggering system, the more useful it becomes. In order to test whether the NIR-induced release from PLGA particles was permanent after the irradiation stopped, the NIR laser was activated for 5-minute periods at 15-minute intervals. Under these conditions, fluorescein release occurred briskly when the laser was on but returned to its initial rate when off. This is further evidence that the particles are not degraded during irradiation and also demonstrates powerful control over the release of cargo.

980 nm NIR also induced release of other dyes of varying hydrophobicity (Nile red, Nile blue and IR780) from PLGA particles, although more polar dyes were released faster.

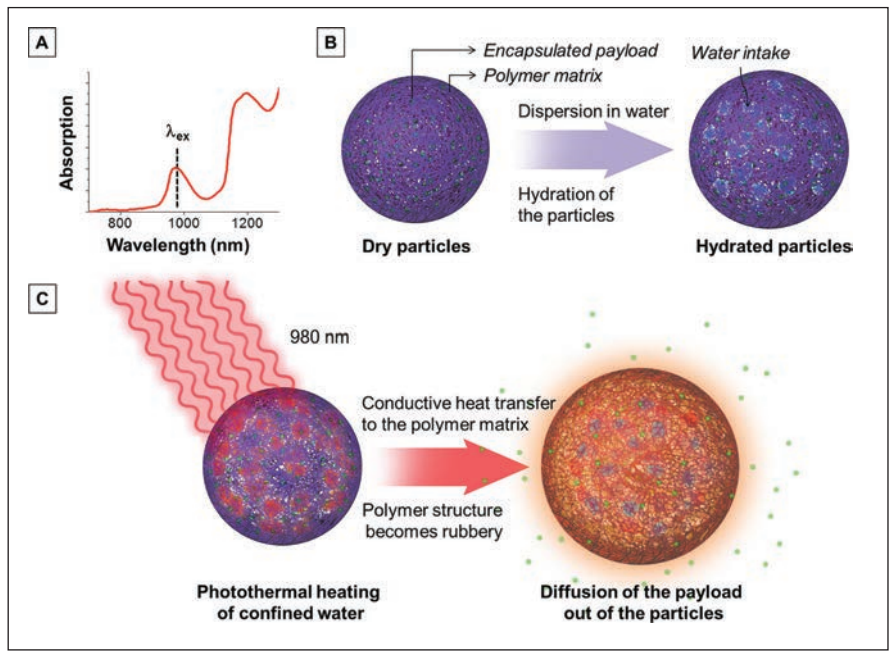

Figure 1. Schematic diagram of NIR-Induced release mechanism

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Confined Water Heating and Payload Release

To ensure that the release of fluorescein is due to the heating of encapsulated water, the release from PLGA particles hydrated with H_2O and deuterated water (D_2O) were compared. D_2O does not absorb NIR-light at 980 nm and therefore fluorescein should not be released when irradiated at this wavelength if the proposed mechanism is correct. Fluorescein particles containing D_2O released a similar amount of cargo when irradiated as the non-irradiated H_2O particles, agreeing with the hypothesis.

This mechanism is not dependent directly on the polymer, which means it should work in other polyester systems without inherent light sensitivity at 980 nm. Linear polyesters like PLGA are biodegradable and biocompatible and can be made with many different chemical and mechanical properties.

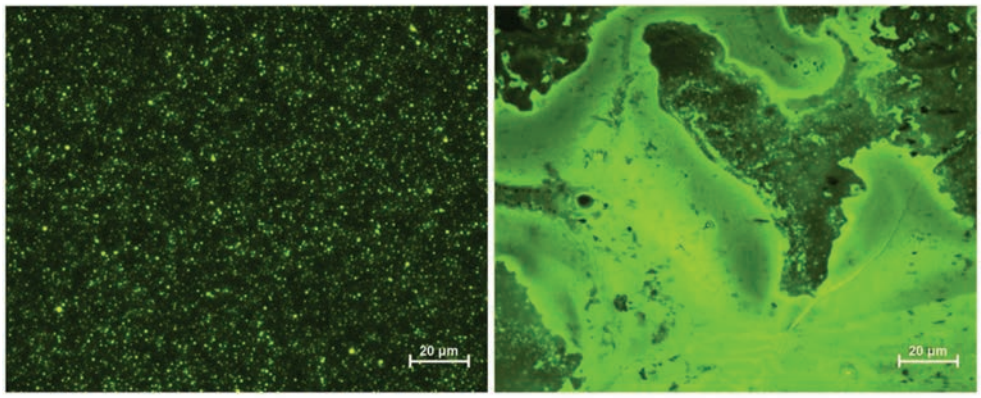

Figure 2. Fluorescence microscopy images of fluorescein loaded particles before (left) and after (right) irradiation with 980 nm light

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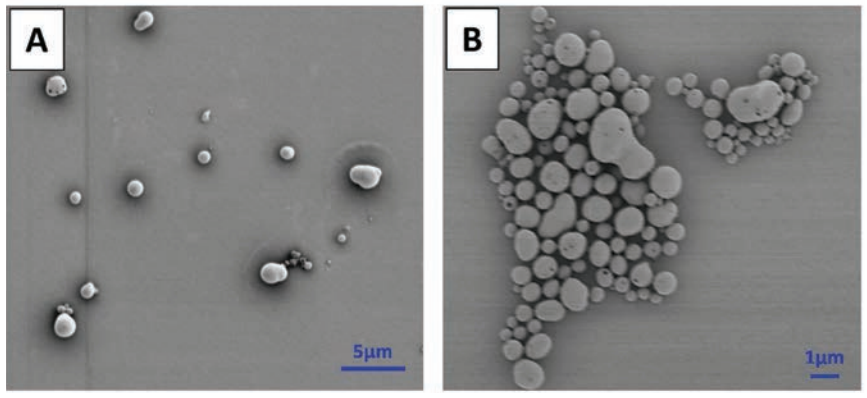

Figure 3. SEM images of fluorescein-loaded PLGA particles (A) before and (B) after irradiation

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The team of researchers created two such polyester particles loaded with Nile Red dye. NIR exposure led to the release of the dye much like PLGA while no release was observed from non-irradiated particles. It is therefore likely that these polyesters use the same mechanism as PLGA. Further investigation showed that this mechanism also works in particles made of certain polysaccharides.

Fluorescence lifetime imaging microscopy (FLIM) on a Scientifica SliceScope two-photon microscope was used to indirectly measure the intra-particle temperatures during irradiation compared to a standard curve of fluorescein lifetime when heated directly. The change in lifetime inside the particles reflected an increase in temperature to 34, 45 and 54 °C following 5, 10 and 15 minutes of irradiation respectively. However, the lifetime of the surrounding free fluorescein did not decrease upon irradiation. Therefore, polymeric particles are selectively heated; the temperature of the aqueous environment does not increase.

Intracellular Release

The next task for the researchers was to examine whether this would work inside cells. To do this they created a polyester particle with a T_g close to 37 °C. They loaded these particles with fluorescein diacetate (FDA), a non-fluorescent compound that becomes highly fluorescent inside cells when it is hydrolysed by esterases. The particles were then incubated with macrophages for 3 hours. When irradiated for 15 and 30 minutes the cells showed a 28- and 71-fold increase in cell-associated fluorescence compared with a 14-fold increase in non-irradiated cells. This indicates more of the cleaved FDA in the intracellular space outside of the particles where esterases can more easily catalyse the reaction and therefore confirms release from the particles.

In order to check that the irradiation at 980 nm was not harmful to the cells their cell count and viability were measured. No change in cell viability and only a small decrease in cell density were observed.

This new method of using NIR to trigger the release of a payload from polymer particles has overcome a number of issues that have plagued other methods that have recently been developed.

Notably this technique uses lower powers, has a greater number of potential uses and eliminates the need for heavy metals or other toxic elements. This allows the technique to work well within cells without compromising the viability of those cells. Other benefits of this system include the ability to apply multiple doses from the same particles and to control the rate of release by changing the average photon energy.

Possible applications include self-healing capsules, on demand delivery of cues for cell proliferation, differentiation and migration and light-triggered drug delivery in *in vitro* and *in vivo* tissues. This may have particular use in animals where light has direct access.

Further work on using different absorption wavelengths of water and advances in creating better polymer particles should help increase the efficiency and efficacy of this technique.

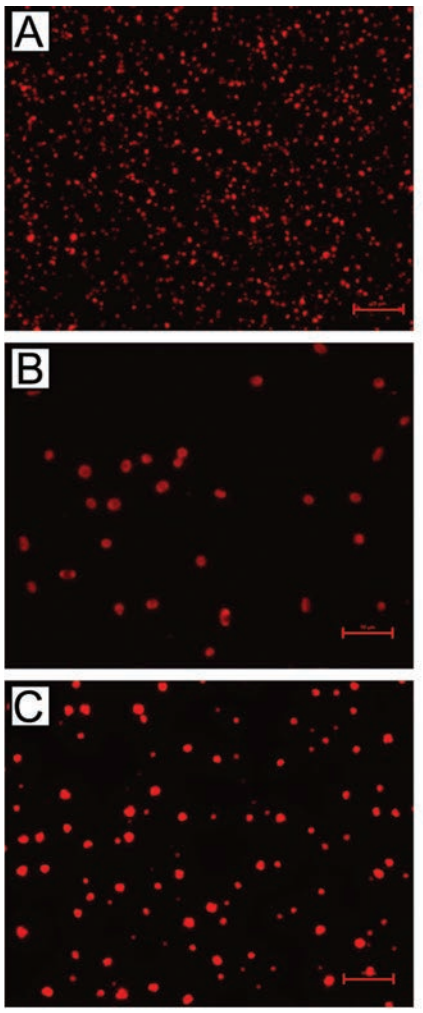

Figure 4. Fluorescence microscope images of Nile-red-loaded: (A) polyester 1 particles; (B) polyester 2 particles; and (C) acelated dextran particles

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References

*"Near-Infrared-Induced Heating of Confined Water in Polymeric Particles for Efficient Payload", Mathieu L. Viger †, Wangzhong Sheng ‡, Kim Doré §, Ali H. Alhasan † , Carl-Johan Carling †, Jacques Lux †, Caroline de Gracia Lux †, Madeleine Grossman , Roberto Malinow §, and Adah Almutairi † **

†Skaggs School of Pharmacy and Pharmaceutical Sciences, ‡Department of Mechanical and Aerospace Engineering, Materials Science and Engineering Program, §Center for Neural Circuits and Behavior, Division of Biology, Department of Neuroscience and Section of Neurobiology, Department of Chemistry and Biochemistry, and KACST–UCSD Center of Excellence in Nanomedicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0600, United States. In ACS Nano, 2014, 8 (5), pp 4815–4826*