

## Short Communication

# Laser-induced emission from dye-doped nanoparticle aggregates of poly (*DL*-lactide-co-glycolide)

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**Stable nanoparticles of poly (*DL*-lactide-co-glycolide) (PLGA) have been prepared, which is photosensitized with near-infrared chromophores doped into the particle. Laser-induced white-light emission is observed from aggregates of the dye-doped nanoparticles, which shows spatial interference of nonlinear optical processes as the underlying mechanism. The accompanied break-up of the aggregates is promising as a possible mechanism for laser-triggered release of drugs.**

**Key words:** Nanoparticles, poly(*DL*-lactide-co-glycolide), aggregates, near-infrared laser, multiple scattering

## INTRODUCTION

Poly (*DL*-lactide-co-glycolide) (PLGA) is a biocompatible and biodegradable polymer that is widely studied for biomedical applications (Murakami et al., 1999). PLGA nanoparticles are promising drug carriers, and also considered to be useful in pharmaceutical coating and surface modification. In drug delivery applications, remote-triggering capability is often an advantage. One of such possibilities is to use laser pulses to control drug released from photosensitized carriers. Therefore, it is of interest to formulate dye-doped PLGA nanoparticles and study their responses to laser-pulse irradiation. A recent study has established a new preparation technique called the modified spontaneous emulsification solvent diffusion (MSESD) method, by which monodisperse, stable PLGA nanoparticles can easily be prepared without using chlorinated solvents or surfactants (Murakami et al., 1999). An appropriate choice of organic solvents in this method may facilitate the process of dye-doping into nanoparticles.

Nanoparticle aggregation is known to have a profound effect on laser-particle interactions. Interference between multiply scattered laser fields often results in strong localization of light in the vicinity of rough surfaces of the aggregates, enhancing nonlinear optical phenomena such as surface-enhanced Raman scattering (SERS) and laser-induced breakdown (LIB) (Suzuki et al., 2006). Such enhancement is also potentially useful in therapeutic applications, where a less power is usually required for the incident laser beam to minimize side effects.

In this study, we have successfully prepared dye-doped

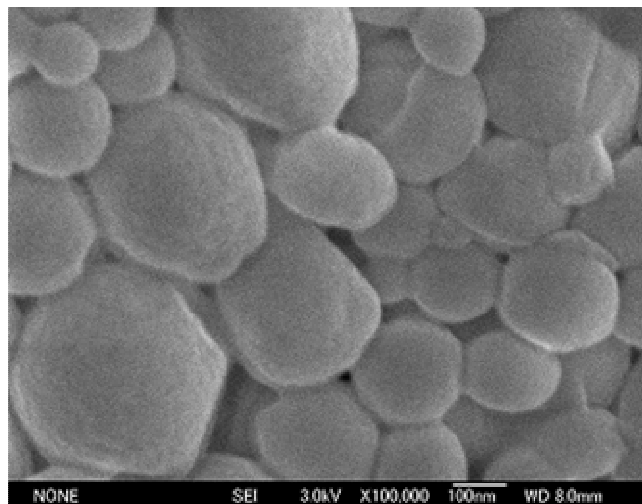
PLGA nanoparticles by the MSESD method, and examined interactions between laser pulses and aggregated nanoparticles via optical microscopy. As a photosensitizer, we have chosen a near-infrared (NIR) dye, *N,N,N',N'*-tetrakis(*p*-diethylaminophenyl)-*p*-benzoquinone-bis-imonium perchlorate (IRG-023), in view of the advantage of NIR light having a deep penetration depth into biological tissue. The dye is a hydrophobic cation and strong non-fluorescent absorber at 1064 nm; it converts the absorbed photon energy into heat via vibronic relaxation, that is, it is a photothermal sensitizer (termed 'molecular heater') (Chen et al., 1992).

## MATERIALS AND METHODS

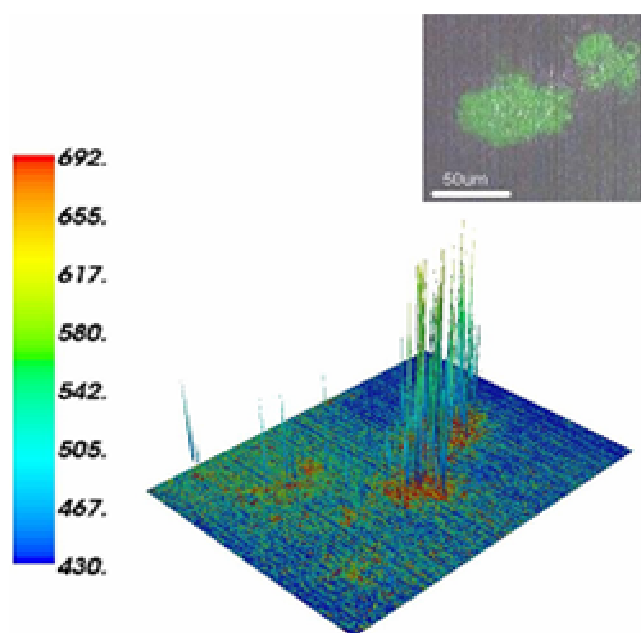
PLGA with a molecular weight of 40,000 - 75,000 and a copolymer ratio of lactide:glycolide = 50:50 (Sigma), poly(vinyl alcohol) (PVA) with a molecular weight of 13,000 - 23,000 and an 87 - 89% hydrolyzation degree (Aldrich), and IRG-023 (a trade name; Nippon Kayaku) were used as received. All other chemicals used were of reagent grade. Solutions were prepared with highly deionized water from a reverse-osmosis-electrodeionization water purifier (Millipore Elix-3). PLGA nanoparticles were prepared by the MSESD method as follows. PLGA (130 mg) and IRG-023 (8.3 mg) were dissolved in acetonitrile (2.7 ml) under sonication, followed by an addition of methanol (1.3 ml). The solution was then added to a 4% (w/w) aqueous PVA solution (20 ml; filtrated through a 0.20  $\mu$ m membrane filter) at the rate of 2.0 ml/min, and the mixture was stirred (700 rpm) for another 10 min. The resulting nanoparticle dispersion was isolated by centrifugation (5600 G, 10,000 rpm, 1.5 h), which was further purified by repeated centrifugation (20 min, 3 times) and finally by freeze-drying in a Schrenk tube. The morphology of Pt-sputtered samples of nanoparticle aggregates was observed with a field-emission scanning electron microscope (JEOL JSM-6700F).

Our excitation source is a collimated, Q-switched Nd: yttrium-alu-

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**Figure 1.** Typical scanning electron micrograph of PLGA nanoparticle aggregates.



**Figure 2.** Spatial profile of laser-induced emission from PLGA nanoparticle aggregates. The emission intensity (relative scale) at each pixel is three-dimensionally plotted over the CCD pixel plane. Inset: image of white speckles of emission captured by optical microscopy.

minium-garnet (YAG) laser ( $\lambda = 1064$  nm) with a pulse duration of 5 ns, a spot diameter of 2.6 mm, and an attenuated pulse energy of 200 mJ (Continuum Surelite I - 10). A wet sample of nanoparticle aggregates is irradiated with single-shot pulses under a home built optical microscope, which is equipped with an objective and a matched condenser having ultra-long working distances that allow enough space for the laser beam to hit the sample at an incident angle of  $\sim 30^\circ$ . Laser-induced emission is captured with a CCD camera (Philips PCVC840K).

## RESULTS AND DISCUSSION

The dye-doped PLGA nanoparticles are spherical with smooth surfaces, having diameters ranging between 100 and 300 nm (Figure 1). The spherical shape is essentially intact in the aggregates, i.e., the nanoparticles are not fused, which implies that the surface of the nanoparticles is stabilized with the adsorbed PVA layers against fusion and coagulation. Incorporation of the dye chromophores into the nanoparticles has been verified by the presence of a broad NIR absorption band centered at 800 nm. It is, however, significantly blue-shifted from that in acetonitrile (1060 nm), possibly reflecting a lower ground-state energy due to hydrophobic adsorption or stacking inside the nanoparticle.

The proposed mechanism of the MSED method (Murakami et al., 1999) is based on the solubility lowering of polymers by two water-miscible organic solvents: one (alcohol) is relatively hydrophilic and thus a poor solvent for PLGA, whereas the other (e.g., acetone or acetonitrile) is rather hydrophobic and a poor solvent for PVA. When the non-aqueous PLGA solution is mixed with the aqueous PVA solution, microscopic droplets of the former solution form via quasi-emulsification; the alcohol and then the other solvent diffuse away from the droplets, which cause coacervation of PVA around them and solidification of PLGA, and thus results in the formation of the surface-protected spherical nanoparticles. In our case, the dye is insoluble in methanol or water, so that it is expected to stay inside the PLGA droplets, until the solidification is complete, throughout the process. Therefore, it is likely that the dye chromophores are embedded inside the body of the PLGA nanoparticles rather than being exposed to the PVA layers or the aqueous phase. It is consistent with the fact that the dye-doping has no effect on the size and morphology of the nanoparticles.

Upon the laser-pulse irradiation, the aggregates emit white light (Figure 2). The spatial profile of the emission is not uniform but composed of speckles, each size being close to the optical resolution limit of our system. The speckle pattern also shows a trace of interference, appearing as curved segments (as opposed to white dots or spots). The emission is strongest from the larger aggregates ( $\sim 50$   $\mu\text{m}$ ), whereas it is practically absent in the smaller ones ( $< 5$   $\mu\text{m}$ ). It suggests that multiple scattering by a collection of nanoparticles is essential in generating the emission.

We have also observed cavitation (bubble formation) and break-up of the aggregate structure during the laser irradiation, which are among the common features in laser-induced breakdown (LIB). A notable difference is, however, the presence of interference in the emission. In the case of LIB in silver-silica nanoshell aggregates in water (Suzuki et al., 2006), the white light is essentially the incoherent blackbody emission that comes from hot spots generated by the localized optical heating; thus, no interference is observed in the spatial emission profile. In contrast, the interference observed here indicates that the underlying mechanism of emission is (at least partial-

ly) a coherent optical process. Accordingly, it is more likely that nonlinear optical processes in the embedded dye chromophore are responsible, such as combination of second-harmonic, stimulated Raman scattering, and supercontinuum generation (Alfano, 2005; Boyd, 2002). These processes may be triggered by the strong local optical field that is created via the extensive multiple scattering. On the other hand, the photoacoustic effect and possibly the shock-wave formation due to optical heating is a possible cause of the cavitation and the aggregate break-up. The laser-induced break-up is especially promising for optically triggered drug release.

In conclusion, we have demonstrated our objective of establishing a simple procedure for preparation of dye-doped PLGA nanoparticles that have excellent stability and feasibility for potential biomedical applications. The significance of this study is that the observed laser-induced emission from the aggregates has a distinctive feature from that of the blackbody emission in its coherent character and underlying mechanism, implying the importance of multiple scattering and nonlinear optical processes.

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#### REFERENCES

- Alfano RR (2005). *The Supercontinuum Laser Source: Fundamentals with Updated References*. Springer.
- Boyd RW (2002). *Nonlinear Optics, Second Edition*. Academic Press.
- Chen S, Lee IYS, Tolbert WA, Wen X, Dlott DD (1992). Applications of ultrafast temperature jump spectroscopy to condensed phase molecular dynamics. *J. Phys. Chem.* 96: 7178-7186.
- Murakami H, Kobayashi M, Takeuchi H, Kawashima Y (1999). Preparation of poly(DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. *Int. J. Pharmaceut.* 187: 143-152.
- Suzuki H, Koike T, Suzuki I, Kawabata T, Lee IYS (2006). Localization of laser-induced breakdown in aggregates of silver nanoshells. *Sci. Technol. Adv. Mater.* 7: 290-295.