

Gold nanoparticles for one step DNA extraction and real-time PCR of pathogens in a single chamber†

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The optothermal properties of nanoparticles are of interest for biosensors and highly sensitive biochip applications. In this respect, the longitudinal resonance of Au nanorods was used to transform near infrared energy into thermal energy in a microfluidic chip. The resulting heat generated effectively caused pathogen lysis. Consequently the DNA was extracted out of the cell body and transferred to a PCR system. This resulted in the successful demonstration of a one step real-time PCR system for pathogen detection without removal or changing of reagents.

Emerging viruses and re-emerging diseases by adapted pathogens are global threats that are placing new demands on timely and accurate diagnosis.¹ To confront this threat the creation of portable point-of-care medical diagnostic systems, and the miniaturization and integration of complex devices based on microfluidic principles have slowly matured into what is commonly known as 'lab-on-a-chip'. These devices are capable of purifying, isolating, and characterizing samples in one neat portable package.² For example, the optothermal properties of nanoparticles, such as gold (Au), are of interest for biological sensing and pathogen detection utilizing such sensitive 'lab-on-a-chip' devices. Gold nanorods have two kinds of surface resonance effects with external electromagnetic energy along the longitudinal and transversal axis, respectively. In this paper, the longitudinal resonance was used to transform near infrared energy into thermal energy in a microfluidic chip resulting in the generated heat effectively causing *E. coli* pathogen lysis. By using the unique optothermal properties of Au nanorods one-step pathogen detection is realized without removing or changing reagents. This is important as removal of reagents can ultimately effect the overall working efficiency of the microfluidic device. We previously reported on a rapid and practical pathogen detection system using efficient DNA extraction and subsequent real-time PCR in a single microchip with the assistance of laser irradiation and magnetic beads.³ Micron-sized magnetic beads were used to deliver thermal and mechanical energy to the

pathogens during lysis; however, the magnetic beads had to be removed after lysis due to their optical hindrance during PCR detection. In addition, during the removal step, almost all of the SYBR[®] Green dye was undesirably removed, along with the beads, due to the surface charges and properties of the magnetic beads³ resulting in the inability to detect DNA amplification in real-time. A potential solution is to use nanoparticles instead of micro-particles due to their high surface to volume ratio, and sedimentation free movement during the analysis process.⁴⁻⁶ Of late, metal and semiconducting nanoparticles have been applied in the emerging field of nanomedicine (*i.e.* the application of nanotechnology for prevention, diagnosis, and treatment of diseases).⁷⁻⁹ Among these are Au nanoparticles.¹⁰⁻¹³ These types of nanoparticles have high absorption cross sections in the visible and near infrared (NIR) range, and have the ability to scatter visible and NIR light *via* surface plasmon resonance. In this work they have been used as an optothermal biosensor marker for performing real-time PCR within a microfluidic environment. Rod-shaped, high aspect ratio (AR), Au nanoparticles possess larger absorption cross sections when compared to spherical ones and may serve as better optothermal conversion sources with a higher signal to noise ratio than can be obtained in NIR based biosensor systems.¹⁴⁻¹⁹ Herein we describe a novel approach for pathogen cell lysis, subsequent DNA extraction, and a real-time PCR using Au nanoparticles (see Fig. 1). We first hypothesized that nanoparticles, especially Au nanoparticles, are capable of generating sufficient heat to extract DNA from pathogens by heating sample solutions *via* laser irradiation. To test this, we have synthesized various Au nanoparticles (varying aspect ratio) along with silica-coated Fe₂O₃ magnetic nanoparticles and studied the optothermal properties of these various nanoparticles (Fig. 2a). The Au and silica-coated Fe₂O₃ magnetic nanoparticles were prepared as described previously (Fig. 2b-e).^{18,20}

To observe the correlation between the nanoparticle type and temperature increase, the particles were dispersed in deionized (DI) water at concentrations resulting in the same absorption intensity at 450 nm (see Fig. S1†), after which the temperature of each heated solution was measured. An infrared laser having a wavelength of 808 nm (1 W) was used as the excitation source.

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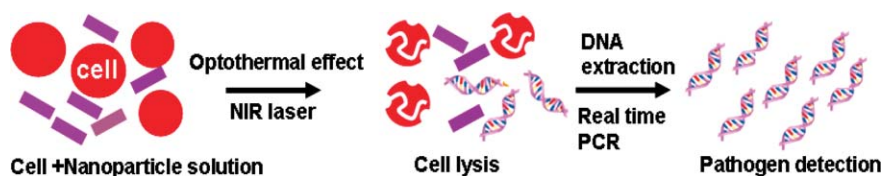


Fig. 1 A schematic of the experimental procedures. One step DNA extraction and real-time PCR detection of pathogens in a single chamber using nanoparticles as an optothermal medium. We have found that Au rods have higher optothermal efficiency as compared to spherical beads and silica coated magnetic nanoparticles. The DNA was extracted out of the cell body and transferred to the PCR system to effectively probe the sample for pathogens.

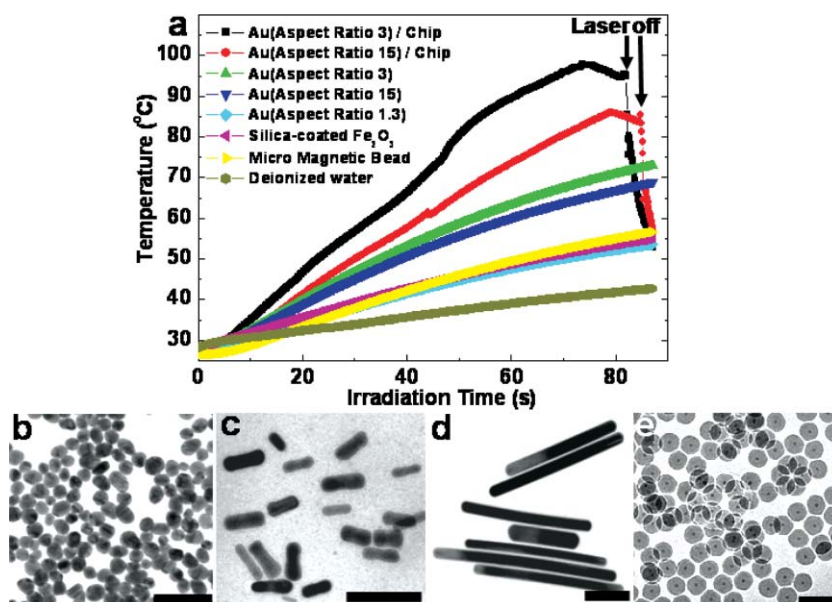


Fig. 2 Optothermal effects of Au and magnetic nanoparticles. (a) Au nanorods (black and red lines) confined in a single microfluidic chamber showed a higher rate of temperature increase as compared to the nanorods in the open glass vials. TEM micrographs of the Au nanoparticles; (b) AR 1.3 (diameter *ca.* 20 nm), (c) AR 3 (diameter *ca.* 20 nm), (d) AR 15 (diameter *ca.* 18 nm), and (e) silica coated Fe₂O₃, diameter \sim 45 nm. Scale bar, 100 nm.

To measure the temperature increase of the solutions in glass vials, a thermocouple (K type, Omega) interfaced with a data acquisition system (34970, Agilent, CA, USA) was used. As expected from the absorption spectra, the gold nanoparticles and nanorods showed different efficiencies on the temperature of the solutions (see Fig. S2[†]), denoted as black and red lines, and in vials, denotes by other colored lines, (Fig. 2, S1). The short Au rods (AR 3) were more efficient at absorbing 808 nm laser radiation in both the microchips and glass vials when compared to the long rods (AR 15). The efficiency on the temperature increase of each nanorod was proportionally dependent on the laser power (see Fig. S3[†]). When using these nanoparticles for real-time pathogen PCR detection, we used the same conditions for laser irradiation so that the efficiency can be compared with respect to temperature increase, cell lysis, and real-time PCR. The amount of DNA produced is quantified by threshold cycle (C_t) values, the fractional cycle number at which the fluorescence passes the fixed threshold. Besides the inefficient temperature increase (Fig. 2) and optical problem when put into a microchip when compared to Au nanoparticles, micro magnetic beads inhibited the fluorescence detection of SYBR[®] Green I completely (Fig. 3a). The PCR reaction itself is not hindered by the presence of micro magnetic

beads, as the amplified PCR products can still be detected by gel electrophoresis (data not shown).

On the other hand, silica-coated magnetic nanoparticles²⁰ could simply be the nano-size counterpart of micro magnetic beads, having a similar negatively-charged surface. Because of their weak absorbance of the 808 nm laser (Fig. S1[†]), even at higher concentrations (2 \times), silica-coated Fe₂O₃ nanoparticles showed less efficient heat absorption compared to the gold particles (Fig. 2). In addition, we found a significant inhibitory effect of silica-coated magnetic nanoparticles on PCR (Fig. 3a) at this concentration. The solution must be diluted more than 8 times to remove the inhibitory effect on PCR (data not shown).

Corresponding to the weak absorbance spectrum at 808 nm (Fig. S1[†]), spherical Au nanoparticles (AR 1.3) showed weaker temperature-increase capability after laser irradiation than gold nanorods (Fig. 2). When spherical gold nanoparticles are used in a quantitative real-time PCR with SYBR[®] Green I dyes (Fig. 3a), we observed no effect on C_t , suggesting that spherical gold nanoparticles are compatible and can be used for DNA quantification by real-time PCR. However, possibly due to the surfactant charge effect, we observed a reduced R_n (normalized reporter value, the fluorescence emission intensity of the reporter dye) (Fig. 3b). These results suggest that spherical

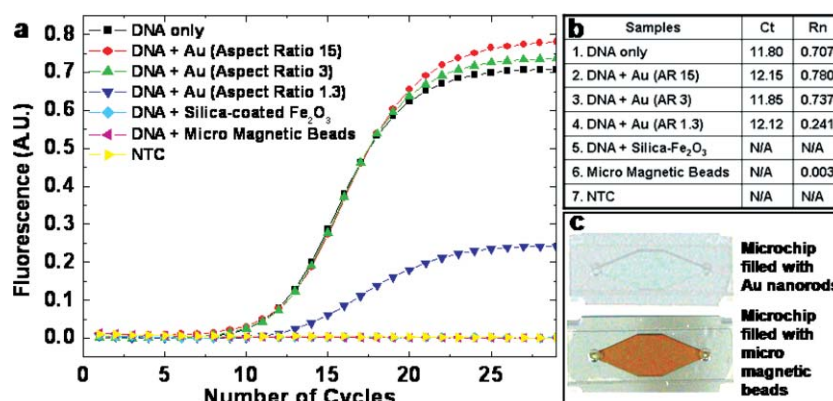


Fig. 3 Effects of Au and magnetic particles on real-time PCR with purified DNAs. AR 3 and AR 15 showed no inhibitory effects on real-time detection of amplified DNAs using SYBR Green I. (a) Real-time PCR results containing various nanoparticles and purified genomic DNA of *E. coli* in a single microfluidic chamber is plotted by the GeneSpector[®] Micro PCR (SAIT, Korea). (b) Real-time PCR results of (a) in C_t and R_n values. AR 3 and AR 15 did not change the C_t and R_n values while other particles significantly affected real-time PCR detection of *E. coli* genomic DNAs. (c) The optical difference between Au nanoparticles and microbeads in the microchip used for real-time PCR.

Au nanoparticles are not optimal for one step DNA extraction and real-time PCR detection of pathogens.

Two different Au rods (AR 3, 15) were also synthesized and their optothermal effects were compared (Fig. 2). The short rods (AR 3) had better absorbance values (Fig. S1[†]) and temperature-increasing ability (Fig. 2) using an 808 nm laser. However, the lysis efficiency of both rods with *E. coli* cells in the microchip showed little differences (Fig. 4a, red and green), suggesting that the energy absorbance of long Au nanorods are efficient enough to achieve similar results when compared to the short ones. When the low volume (8 μ l) microchips were used, the temperature increase was efficient for both sets of nanorods (Fig. 2a, black and red). Given that the temperature increase is much more efficient in the microchip,³ the lysis efficiency reached saturation at a threshold concentration implying these nanoparticles can be used for cell lysis. It is also evident that Au nanorods are optically superior to micro magnetic beads when mixed with samples and loaded in a microchip (Fig. 3c). Furthermore Fig. 3 shows that

Au nanorods (AR 3 and AR 15) have no inhibitory effects on PCR detection of DNA using SYBR Green I fluorescent dyes. Contrary to Li *et al.*, we demonstrate that Au nanorods have an additional advantage on PCR efficiency and optical detection because of heat transfer properties.²¹ However, a significant enhancement on the PCR reaction itself was not seen. In our microchip PCR system, we use a silicon microchip for heat transfer, which greatly enhances the sensitivity of PCR. Another advantage is that the optical detection signal is intensified by the reflective surface of the particles.

Finally, we tested whether Au nanorods enhance the lysis efficiency of pathogens and checked the possibility of real-time pathogen detection in an 8 μ l chamber without exchanging the buffers and reagents for real-time PCR after laser irradiation (Fig. 4). *E. coli* BL21 (10000 cells μ l⁻¹) was added in premixed PCR reagents with LightCycler[®] FastStart Enzyme (Roche Diagnostics) containing Au nanorods (AR 3 or AR 15). The solution was irradiated for 30 s using a portable sample

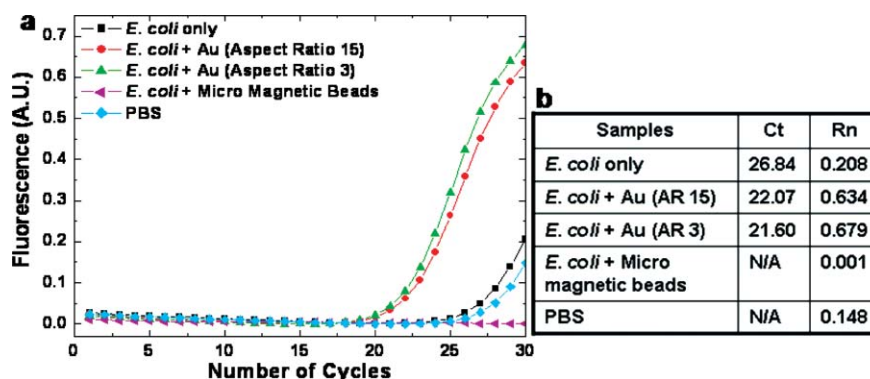


Fig. 4 *E. coli* BL21 cells were added to the premixed PCR solutions containing AR 3 or AR 15. The total process of pathogen detection from the cell lysis to the real-time polymerase chain reaction (PCR) was performed successfully in one chamber of the microchip with premixed PCR solution and without removal of the Au nanorods. (a) Real-time PCR results plotted by the GeneSpector[®] Micro PCR (SAIT, Korea). The *E. coli* cells were not efficiently detected without Au nanorods and showed only nonspecific detection of DNA as no template negative control (NTC) was found in PBS buffer (Black square). A melting curve analysis and gel running by the Bioanalyzer 2100 (Agilent Technologies) confirmed that no amplified band was detected (data not shown). Magnetic microbeads completely blocked the optical detection of real-time PCR (Purple Arrow Heads). Both short and long Au rods (AR 3 and AR 15) efficiently break *E. coli* cells after laser irradiation, which enables pathogen detection by real-time PCR without any optical hindrance. (b) Real-time PCR results of (a) in C_t and R_n values.

preparation device ($58.3 \times 57.9 \times 37.0$ mm, 148 g) with a 1 W laser as previously described.³ Real-time PCR (GeneSpector®, Micro PCR SAIT, Korea) was then performed without removing the Au nanorods (Fig. 4).

The cycle differences (C_i value) 3.5 between the samples indicate a DNA concentration difference of about 100-fold. Since a C_i value larger than 26 is regarded as a non-specific amplification (data not shown), it is evident that without Au nanorods, *E. coli* genomic DNA is not detected (black line). As expected, micro magnetic beads completely inhibited the optical detection of amplified DNA (purple line). In contrast, both AR 3 and AR 15 (green and red lines respectively) showed efficient lysis of *E. coli* cells and quick pathogen detection by real-time PCR using SYBR Green I without any optical hindrance (C_i values, 21.60 and 22.07 respectively). When a different Taq polymerase was used (Solgent, Korea) without hot start, we obtained even better C_i values (17.31 and 18.67 for AR 3 and 15 respectively) with the Au nanorods, suggesting that Au nanorods are also compatible with different Taq polymerases. The lysis mechanism by Au nanorods is similar to our previous LIMBS (Laser-Irradiated Magnetic Beads System) except we further developed a new system using the unique optothermal effects of Au nanorods after laser irradiation. In conclusion, we successfully applied Au nanorods for one step DNA extraction and real-time PCR detection of pathogens, which we expect will hold great promise for point of care diagnostic applications.

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