

Fluorescence Enhancement from Nitro-Compound-Sensitive Bacteria within Spherical Hydrogel Scaffolds

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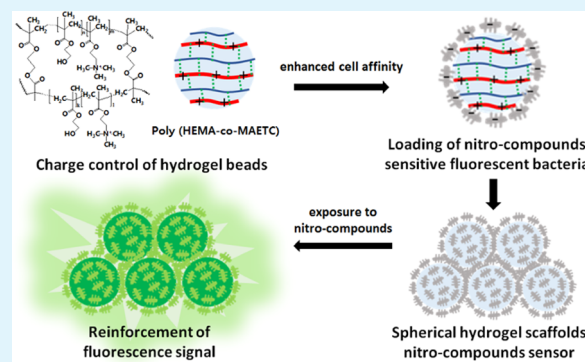
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S Supporting Information

ABSTRACT: For the safety of both production and life, it is a very significant issue to detect explosive nitro compounds in a remote way or over a long distance. Here, we report that nitro compounds were detected by the bacterial sensor based on hydrogel microbeads as a platform. Green fluorescent protein-producing *Escherichia coli*, which was genetically engineered to be sensitive to nitro compounds, was loaded within poly(2-hydroxyethyl methacrylate) [poly(HEMA)]-based hydrogel beads, in which fluorescent signals from bacteria were concentrated and strong enough to be easily detected. For efficient loading of negatively charged bacteria, the surface charge of poly(HEMA)-based beads was controlled by copolymerization with 2-(methacryloyloxy)-ethyltrimethylammonium chloride (MAETC) as a cationic monomer. With the addition of MAETC, the cell affinity was nine times enhanced by the interaction between the positively charged poly(HEMA-co-MAETC) beads and negatively charged bacteria. The increased cell affinity resulted in an enhancement of a sensing signal. After exposure to 2,4,6-trinitrotoluene, a typical explosive nitro compound, the fluorescence intensity of bacterial sensors using poly(HEMA-co-MAETC) beads having 80 wt % MAETC was five times increased compared to those based on poly(HEMA) beads. This amplification of the fluorescent signal enables easier detection of explosives efficiently by a remote detection, even over a long distance.

KEYWORDS: poly(HEMA-co-MAETC) beads, surface-charge-controlled hydrogel beads, electrospraying method, photopolymerization, biosensor for TNT sensing



INTRODUCTION

Due to increased dangers by chemical terrorism or abundant use of industrial chemicals, it is a crucial issue to detect explosives effectively for the safety of both production and life. Especially, nitro compounds such as 2,4,6-trinitrotoluene (TNT), 1,3-dinitrobenzene, and 2,4-dinitrotoluene are explosive organic components containing one of the most common explosives, which have been used in landmines, bombs, and so on. To detect them, metal detectors by humans or odor detection by well-trained animals has been mainly used. Recent research studies have reported TNT sensors with highly sensitive detection of sub-ppm TNT by monitoring a change of electrical properties after TNT exposure in a field-effect-transistor-based system.¹ However, these close detection methods have several limitations. For example, metal detection is restricted in the case of a plastic-bodied landmine^{2,3} and an unexpected explosion sometimes hurts human or animals during detection, which demand remote or long-distance

detection methods.⁴ As one of the various strategies to overcome these problems, fluorescence-based bacterial bio-reporters have been considered as a promising method. These are genetically engineered microbial strains for detecting nitro compounds from the vapor of explosives with advantages of low cost, environmental safety, and remote detection.⁵ However, the weakness and short lifetime of fluorescent signals are still considered as disadvantages of the nitro compound detection using fluorescence-based bacterial bio-reporters.^{5,6}

In this regard, a hydrogel bead can be a useful carrier for genetically engineered bacteria to collect and concentrate fluorescent signals by encapsulating bacteria within a bead, resulting in amplification of the fluorescent signal. It enables

Received: February 3, 2019

Accepted: March 26, 2019

Published: March 26, 2019

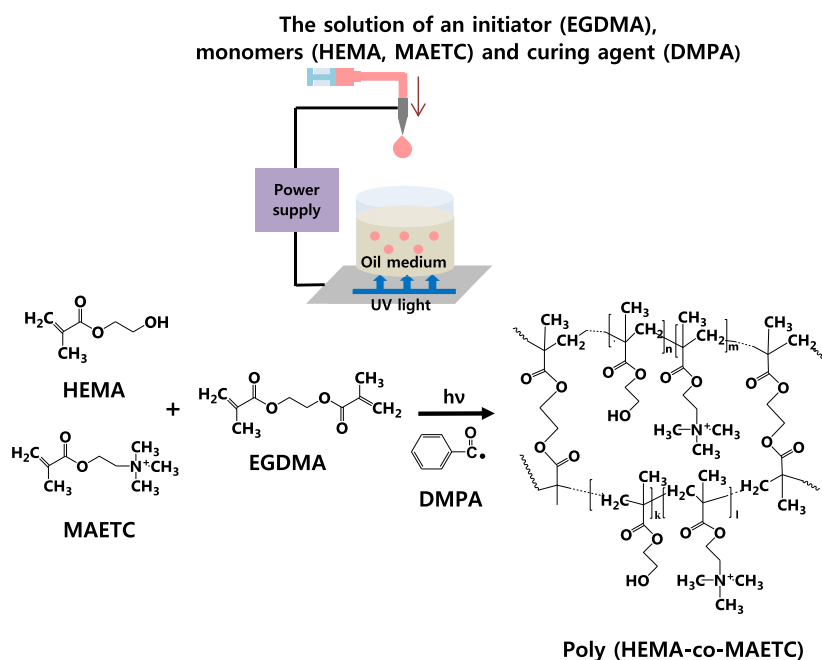


Figure 1. Schematic diagram of the fabrication method for synthesis of poly(HEMA-co-MAETC) hydrogel microbeads.

easy detection of explosives efficiently by remote detection, even over a long distance with a specific optical scanning system.⁷ Hydrogels are physically or chemically cross-linked polymers having hydrophilic polymer chains.^{8–12} They can absorb waters up to thousands of times their dry weight, which provides good environments for the nutrition of bacteria. Hydrogels obtained from 2-hydroxyl methacrylate (HEMA) have been studied in diverse applications because of its biocompatibility, good mechanical properties, environmental safety, easiness of synthesis, etc.¹³ To apply hydrogel beads as useful carriers for nitro-compound-sensitive bacteria, the cell affinity for bacterial attachment is required to be enhanced for high loading amount of bacteria and their mechanical properties are investigated for sustainable detection when hydrogel beads are spread around the suspected area.

Here, we report that nitro compounds were detected by the bacterial sensors fabricated within hydrogel beads. Poly-(HEMA)-based hydrogel beads were successfully fabricated via electrospray using a photopolymerization method, which was used as a platform for bacteria-based biosensors for detection of nitro compounds. Electrospraying has been reported as a promising method for fabrication of beads due to advantages of the lack of an organic solvent, encapsulation with a wide range of materials, and the easy control of bead size.^{14–17} The size of the microbeads was controlled by the voltage applied in the electrospray device or the size of nozzles ranging from 400 μm to 2.5 mm in diameter. The swelling behavior and mechanical properties of hydrogel microbeads were controlled by copolymerization with 2-(methacryloyloxy) ethyl trimethylammonium chloride (MAETC) as a cationic comonomer at different ratios of monomers between HEMA and MAETC.^{18,19} Copolymerization of a cationic monomer with HEMA allows the control of the surface charge of hydrogel beads, which results in an enhancement of cell affinity by electrical interaction between positively charged beads and negatively charged bacteria, compared to that of nonionic poly(HEMA) beads. Nitro compound sensors based on poly(HEMA-co-MAETC) hydrogel beads were prepared by

loading green fluorescent protein (GFP)-producing *Escherichia coli*, which was genetically engineered to respond to degraded products of nitro compounds by degradation through bacterial metabolism, and their performance was studied by varying MAETC composition (0–80 wt %) in hydrogel beads. In this study, the nitro compound sensing behavior of microbead-typed sensors was investigated by exposure to TNT, which is the main explosive component in a landmine.²⁰

RESULTS AND DISCUSSION

Poly(HEMA-co-MAETC) microbeads were obtained via an electrospraying method with free radical photopolymerization (Figure 1). When the droplets of the precursor solution containing HEMA and MAETC as co-monomers, ethylene glycol dimethacrylate (EGDMA) as a cross-linking agent, and 2,2-dimethoxy-2-phenylacetophenone (DMPA) as a photo-initiator generated via electrospraying were dropped in oil medium, their spherical shape was maintained due to water/oil (W/O) phase separation. Upon UV exposure, the radicals generated from DMPA initiated the copolymerization of HEMA, MAETC, and EGDMA, resulting in the gelation of droplets.^{21,22}

The size of the fabricated beads was controlled by varying the applied voltages and the diameter of the nozzle through which the solution was extruded (Figure S1). First, increasing the voltage resulted in relatively small beads, with diameters of 600–300 μm at 5 and 20 kV with a nozzle diameter of 32 G. Hartman et al. previously reported the effect of the applied voltage on bead size.^{23,24} Droplet diameter is proportional to the liquid flow rate and inversely proportional to the applied voltage. Additionally, the diameter of the nozzle also affected the size of beads. Increasing the nozzle diameter led to increased bead sizes, from 400 to 300 μm for 27 and 32 G at 20 kV because the diameter of the nozzle is proportional to the diameter of the pendant droplet neck at the end of the nozzle.²⁵

The effect of EGDMA as a cross-linking agent for HEMA in the poly(HEMA) beads on the swelling behavior and

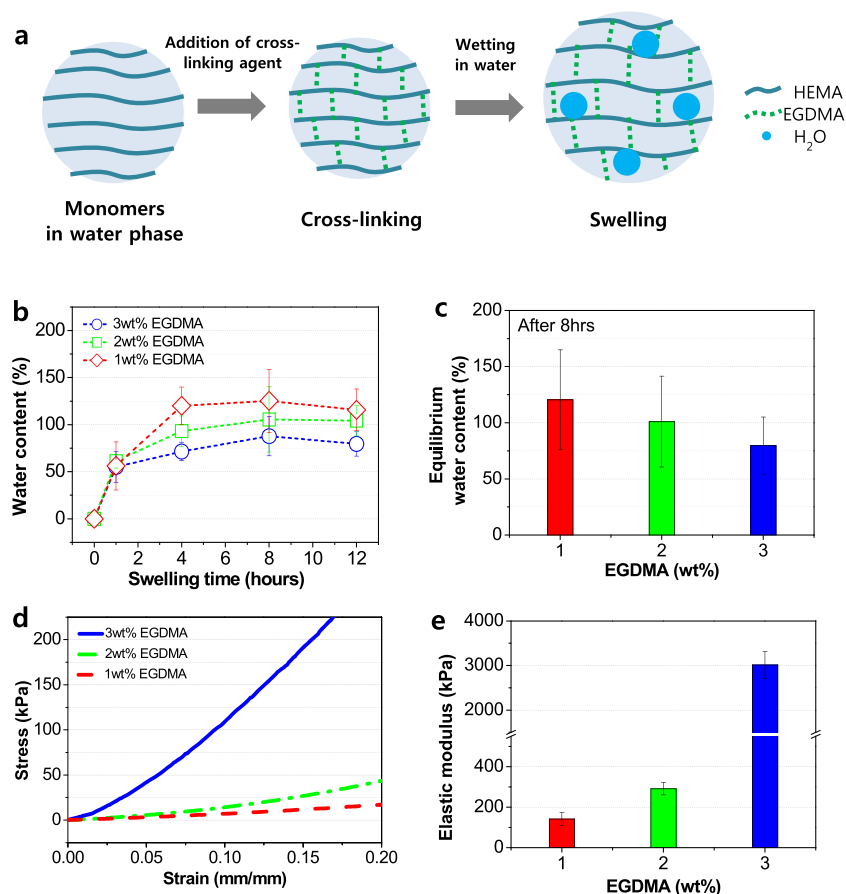


Figure 2. Effect of the cross-linking degree on the swelling behavior and mechanical properties of poly(HEMA) with respect to the concentration of EGDMA; (a) is a schematic diagram of swelling behavior, (b) and (c) are water content with increased swelling time and equilibrium water content, respectively, and (d) and (e) are the stress–strain curve and calculated elastic modulus of poly(HEMA) microbeads, respectively.

mechanical properties was analyzed (Figure 2). Increasing the content of EGDMA to HEMA from 1 to 3 wt % resulted in decreased swelling behavior. Dried beads were swollen in water and saturated after 4 h, indicating equilibrium water contents of 120, 100, and 80% at 1, 2, and 3 wt % EGDMA, respectively (Figure 2a–c). Conversely, a higher EGDMA monomer concentration resulted in an increased elastic modulus, from 100 to 3000 kPa at 1–3 wt %, respectively (Figures 2d,e and S2). These results clearly demonstrated that the cross-linking density of the beads could be controlled by the concentration of EGDMA as a cross-linking agent.²²

Copolymerization of MAETC as a cationic monomer contributed to the fabrication of positively charged beads. Figure S3 shows the Fourier transform infrared (FT-IR) spectra of poly(HEMA-co-MAETC) (80 wt % MAETC). The C=O stretching vibration peak by the methacrylate group appears at 1735 cm⁻¹. Also, the C–H peaks by –CH₃ (2883 cm⁻¹) and CH₂ (2952 cm⁻¹) stretching and –OH peaks (3426 cm⁻¹) were observed in poly(HEMA-co-MAETC). Specifically, the characteristic –C–N stretching (1243 cm⁻¹) peak appears due to trimethylammonium (–N⁺(CH₃)₃) groups giving a positive charge.¹⁸ Analysis of the surface charge using ζ -potential values indicated that the fabricated poly(HEMA-co-MAETC) beads had a positive charge from 50 wt % MAETC to HEMA (Figure 3f) because of the presence of –N⁺(CH₃)₃. The addition of the cationic monomer affected the properties of the beads. In Figure 3b, the water contents of all fabricated beads were saturated within 4 h of incubation in distilled water.

The equilibrium water contents of poly(HEMA-co-MAETC) drastically increased from 120 to 2500% at 0–80 wt % MAETC, respectively, and poly(MAETC) indicated 3000% equilibrium water content (Figure 3c). When water diffuses into hydrogels during a swelling process, there is an osmotic swelling pressure generated between the polymeric network and the surroundings. The total osmotic swelling pressure (π_{total}) is determined by mixing of the polymer chain with a solvent (π_{mix}), the elastic response of the polymer network (π_{elast}), mixing of ions from solution (π_{ion}), and the electrostatic interaction of ionized groups upon swelling (π_{elect}).¹⁹ Therefore, using MAETC as a cationic monomer likely had led to enhanced π_{total} with increased π_{elect} by electrostatic interaction between water molecules and ionic functional groups in the poly(HEMA-co-MAETC) beads, resulting in higher water content than that of poly(HEMA) beads (Figure 3a). Additionally, the mechanical properties of the poly(HEMA-co-MAETC) beads were also significantly influenced by the presence of cationic MAETC, as compared with those of poly(HEMA) beads. The elastic modulus of the beads indicated two opposite effects of charged groups with increasing cationic monomer. In this ionic hydrogel, elastic modulus was increased from 130 to 630 kPa at 0–40 wt % MAETC with an increased charge density of the beads by additional cross-linking in the hydrogel. However, a continuous increase of ionic groups resulted in a decreased elastic modulus from 630 to 150 kPa at 40–100 wt % MAETC composition. The increased charge density led to greater

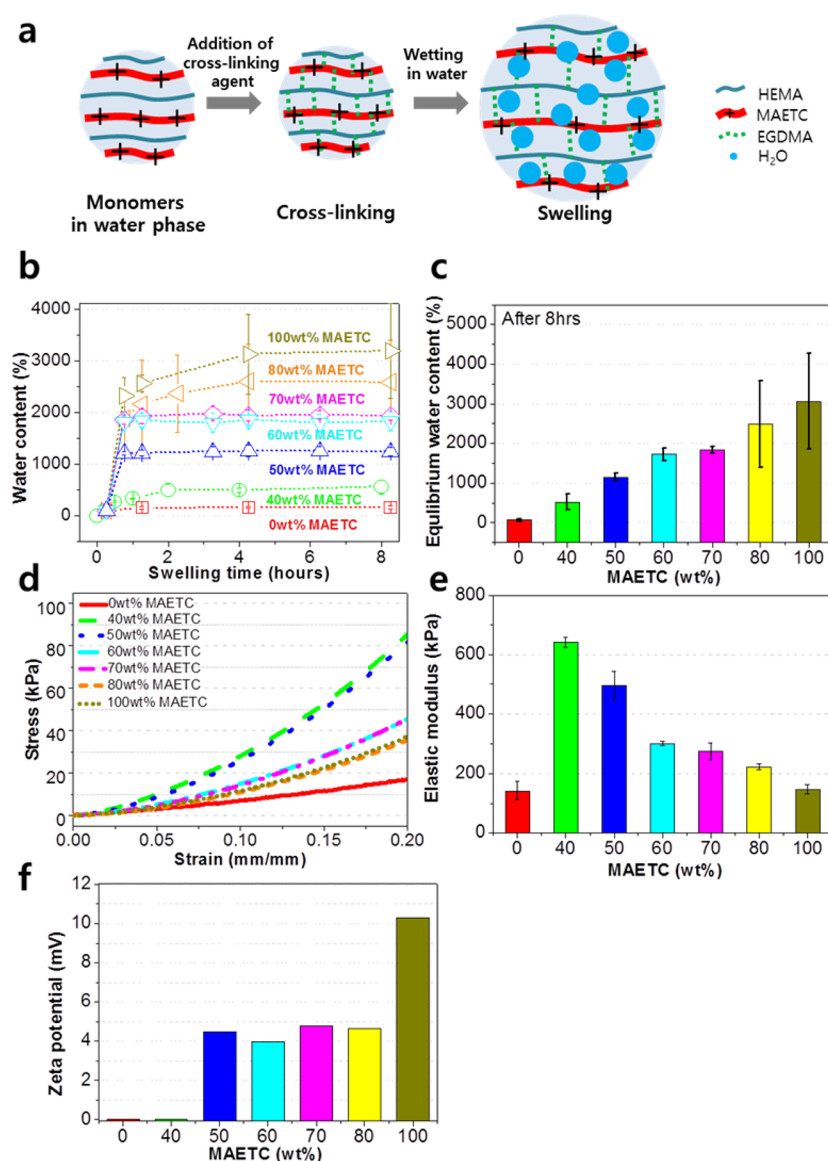


Figure 3. Variation of the swelling behavior and mechanical properties of poly(HEMA-co-MAETC) by charge control with increased MAETC concentration; (a) is the schematic diagram of swelling behavior, (b) and (c) are water content with swelling time and equilibrium water content, respectively, and (d) and (e) are the stress–strain curve in the compression test and elastic modulus, respectively, and (f) is the ζ -potential of poly(HEMA-co-MAETC) beads [1 wt % EGDMA to monomer (HEMA + MAETC)].

electrostatic repulsion between polymer chains, leading to decreased mechanical strength (Figures 3d,e and S4).^{26,27}

The cell attachment to the surface of fabricated beads was analyzed after incubation of the beads into bacterial solution. The cell attachment was analyzed by observation of the fluorescence signal of genetically engineered *E. coli* BL21-(DE3) with pBbE7K-*gfp+* loaded onto the surface of fabricated beads with 0–80 wt % MAETC composition (Figures 4 and S5). As a result, the cell affinity was increased by positively charging poly(HEMA-co-MAETC) beads with increased MAETC composition from 0 to 80 wt % (Figure 4a–f). Generally, the microorganism has a negative charge because the cell membrane contains carboxyl and phosphate groups.⁶ Therefore, the surface potential of beads as a substrate had an important effect on cell attachment. In Figure 4g, it can be seen that the normalized fluorescence intensity of poly(HEMA-co-MAETC) beads increased from 1.2 to 9.0 times in comparison with that of poly(HEMA) beads with 40–

80 wt % MAETC to HEMA because of their positive trimethylammonium functional groups. As a result, a maximum nine times higher fluorescence intensity value was obtained in poly(HEMA-co-MAETC) beads with 80 wt % MAETC, having a surface potential of +10.3 mV, compared with that of poly(HEMA) beads, having a surface potential of approximately 0 mV (Figure 3f).

To demonstrate the utilization of hydrogel beads as biosensors for detection of nitro compounds, genetically engineered GFP-producing *E. coli* [*E. coli* (MG1655 pPROBE-*P_{yqjFmut}-gfp+*)] in response to nitro compounds was attached within poly(HEMA-co-MAETC) beads. One of the nitro compounds, TNT, which is a typical explosive component, was used to analyze the sensing performance. It has been reported that the *yqjF* promoter (*P_{yqjF}*) in genetically engineered *E. coli* is activated when some sort of nitro compounds are treated. When *P_{yqjF}* is activated by nitro compound derivatives, it leads to the initiation of GFP

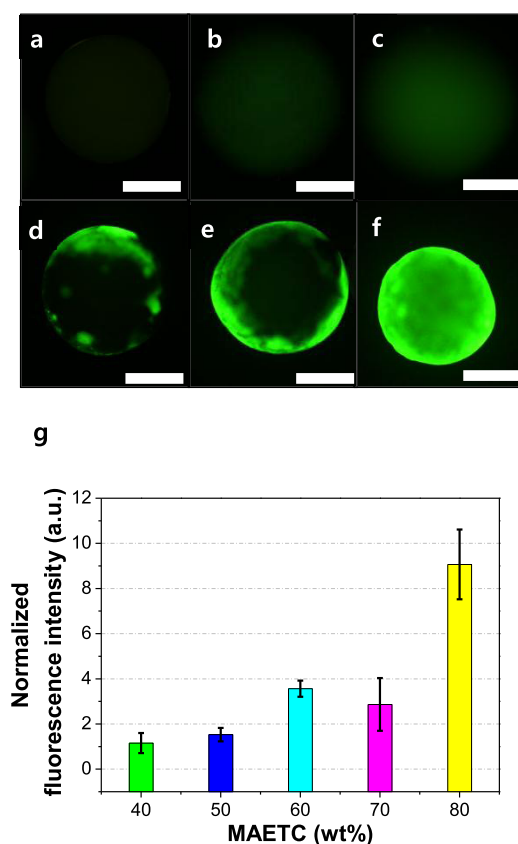


Figure 4. Cell affinity of poly(HEMA-co-MAETC); fluorescence microscopy images of beads with the sensor *E. coli* [BL21(DE3) pBbE7K-egfp+] as a function of MAETC composition [(a) 0, (b) 40, (c) 50, (d) 60, (e) 70, and (f) 80 wt % MAETC to HEMA] (scale bar: 500 μ m). (g) is the normalized average fluorescence intensity of poly(HEMA-co-MAETC) beads to that of poly(HEMA) beads (count: 10 ea. for each condition).

expression.²⁸ Fluorescence signals of fabricated bead-type sensors were measured by exposure to TNT. (Figure 5). When TNT was exposed to the beads that were immersed in the prepared 100 ppm TNT solution, green fluorescence was detected from the beads as expected. The MAETC composition in the beads had an effect on the fluorescence intensity after exposure to TNT. When the fluorescence signal of the reactive bacteria on the poly(HEMA-co-MAETC) beads from 0 to 80 wt % MAETC composition was observed (Figure 5b–d), the strongest signal was exhibited on the bead with 80 wt % MAETC. The fluorescence intensity with exposure to TNT, which was normalized to that of the signal without TNT, was about five times increased at the 80 wt % MAETC compared to that of poly(HEMA) (Figures 5e and S6). The enhanced cell affinity of poly(HEMA-co-MAETC) beads by a positively charged control with increased MAETC composition resulted in a stronger fluorescent signal as compared to those with lower MAETC composition (Figure 5a). Their sensing behavior was analyzed as a function of TNT concentration and exposure time (Figure 6). Hydrogel beads with bacteria were exposed to TNT moieties by dipping them in a TNT solution for a fixed duration. When biosensors were dipped in 100 ppm TNT solution for a shorter time less than 48 h, fluorescence intensity was linearly increased to five times compared with that before exposure to TNT. This increase of fluorescence intensity indicates that bacteria embedded within

hydrogel beads were proliferating even in a TNT solution using nitro substituents as a nutritional nitrogen source, i.e., degradation products of TNT.^{28,29} Then, P_{yqjF} in more bacteria was activated and an enhancement of the fluorescence signal was produced. However, after more than 48 h, the fluorescence intensity was saturated, which means that the capacity of hydrogel beads for bacteria reached a limit due to the lack of space within hydrogel beads for more proliferation of bacteria and TNT was degraded by bacterial metabolism. When these biosensors were dipped in a lower concentration (10 ppm) of TNT, the growth of bacteria and the activation of P_{yqjF} were limited due to the lack of nitro compounds. Therefore, the fluorescence intensity was saturated earlier ($\sim$ 24 h) and the increasing ratio of fluorescence intensity was smaller than that in 100 ppm TNT solution. These results demonstrated that by tuning the chemical composition of the beads the biological function of the engineered bacteria cultured on the beads could be significantly enhanced, which could be utilized as a highly sensitive cell-based nitro compound sensor.

CONCLUSIONS

Poly(HEMA-co-MAETC) microbeads were obtained via the electrospraying method to generate droplets of gel precursor solution, followed by photoinitiated radical polymerization. The size of fabricated beads was controlled by the applied voltage and the size of the nozzle used for electrospraying. An increased voltage induced higher energy in the solution to overcome its surface tension, resulting in smaller droplets, whereas an increased nozzle size formed an increased pendant droplet neck at a given voltage, resulting in larger droplets. The swelling behavior and mechanical properties of the microbeads could be modified by changing the ratio of HEMA and MAETC. With the addition of cationic MAETC to HEMA, enhanced swelling behavior with an increased water content of a maximum 2500% at 80 wt % MAETC and enhanced mechanical properties having an approximately 4.8 times increased elastic modulus (630 kPa at 40 wt % MAETC) compared with those of poly(HEMA) (130 kPa) were obtained. In addition, the cell affinity of the positively charged poly(HEMA-co-MAETC) beads increased to a maximum nine times compared to that of the poly(HEMA) beads due to the electrostatic interaction between bacteria and the surface of beads. These biocompatible charge-controlled beads were applied as the microbial-based sensor for the detection of nitro compounds such as TNT, which is an explosive component. For the investigation of the sensing performance, GFP-producing *E. coli* cells responding to derivatives of nitro compounds, which were formed by degradation through bacterial metabolism, were loaded within the beads having various MAETC compositions from 0 to 80 wt %. After exposure of TNT to the hydrogel bead with bacteria, the fluorescence signal was enhanced in poly(HEMA-co-MAETC) beads with 80 wt % MAETC, indicating five times increased value compared to that of poly(HEMA). This result signified that the positively charged poly(HEMA-co-MAETC) beads by copolymerization with MAETC as a cationic monomer contributed to the enhanced sensing performance for nitro compound detection by increasing the cell affinity. These results suggested promising applications of hydrogel beads for detection by degradation of the explosive component from munition including landmines as an efficient and safe method in a remote way or over the long distance.

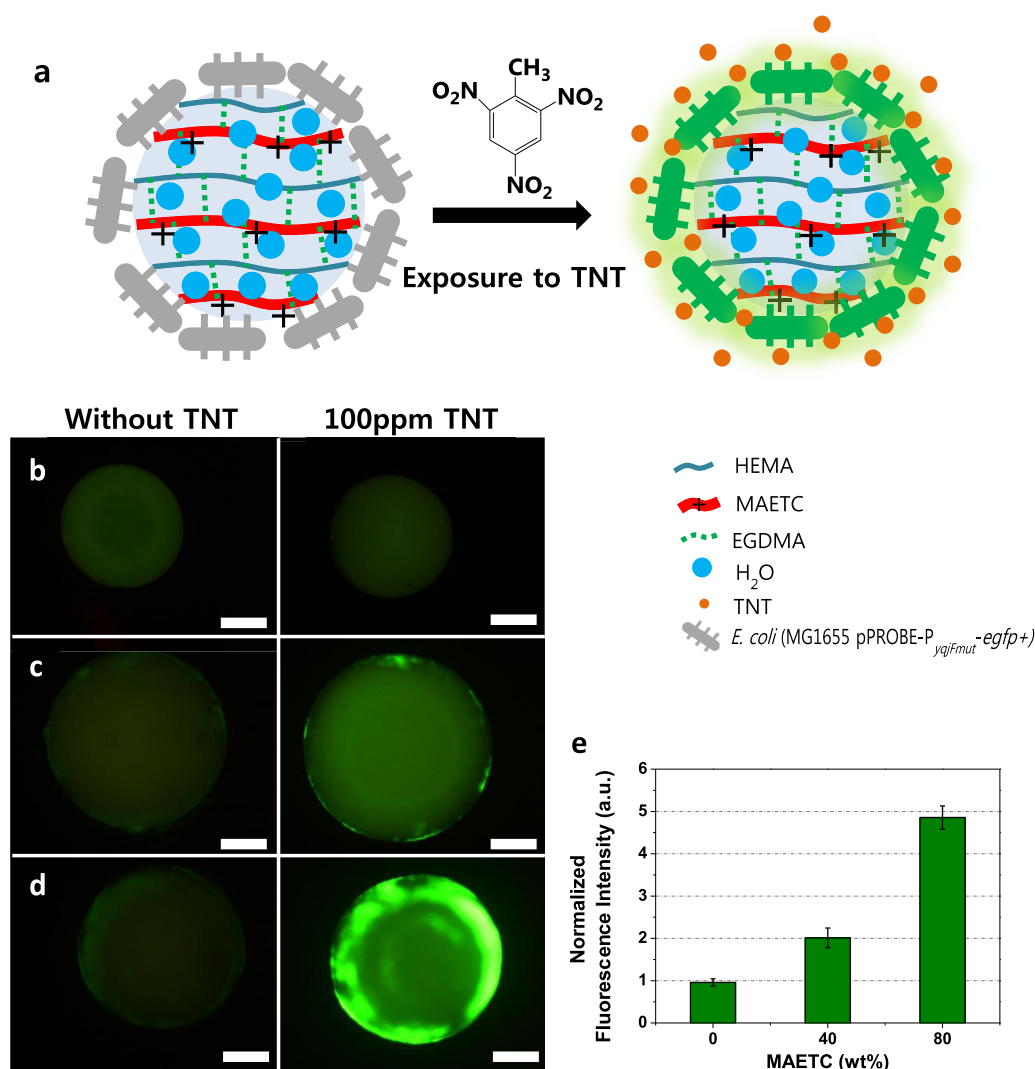


Figure 5. Response of poly(HEMA-co-MAETC) beads with *E. coli* (MG1655 pPROBE-P_{yqjFmut}-egfp+) exposed to TNT; (a) is the schematic diagram of GFP expression of the sensor by exposure to TNT. (b), (c), and (d) are fluorescence microscopy images of poly(HEMA-co-MAETC) beads with 0, 40, and 80 wt % MAETC without TNT and in the presence of 100 ppm TNT for 3 days, respectively (scale bar: 500 μm). (e) is the normalized fluorescence intensity of the beads with MAETC composition after exposure to 100 ppm TNT for 3 days to that of beads in the absence of TNT.

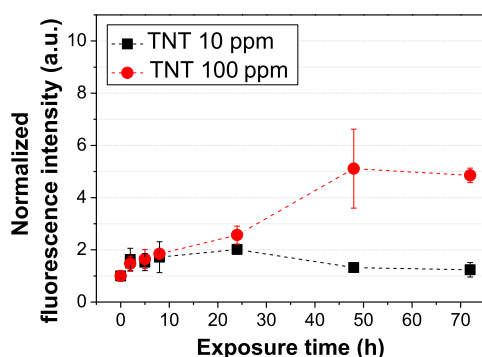


Figure 6. Sensing behavior as a function of TNT concentration and exposure time using poly(HEMA-co-MAETC) microbeads having 80 wt % MAETC.

MATERIALS AND METHODS

2-Hydroxyethyl methacrylate (HEMA, 97%, JUNSEI Chemical Co., Ltd.) and [2-(methacryloyloxy)ethyl]trimethylammonium chloride (MAETC, 80 wt % in H₂O, Sigma-Aldrich) were used as monomers.

Ethylene glycol dimethacrylate (EGDMA, 98%, Sigma-Aldrich) was used as a cross-linking agent. As a photoinitiator, 2,2-dimethoxy-2-phenyl acetophenone initiator (DMPA, trade name-IRGACURE 651, BASF) was used. For fabrication of beads in the water/oil (W/O) phase during the electrospraying procedure, mineral oil (heavy, DAEJUNG) was used. Luria-Bertani (LB) broth (LB broth) and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. 2,4,6-Trinitrotoluene (TNT) was received from the Agency for Defense Development of the Republic of Korea with approval for the research purpose.

Poly(HEMA-co-MAETC) hydrogels were prepared via a novel electrospraying method by photopolymerization. The monomer solutions with various MAETC compositions ranging from 0 to 100 wt % MAETC to HEMA were mixed with EGDMA as a cross-linking agent at room temperature. The EGDMA weight fraction to (HEMA and MAETC) monomer was varied from 1 to 3 wt %. DMPA as a photoinitiator was added to the monomer solution [(HEMA and MAETC):DMPA = 50:1 (w/w)]. In all of the monomer solutions, the monomer to water ratio was 4:1 [(HEMA and MAETC):H₂O, w/w]. The prepared solution was transferred into the nozzle of an electrospray device by control of the flow flux using a syringe pump (KD-scientific). A droplet of solution was dropped into a container filled with mineral oil under various electric fields, with the applied

voltage ranging from 0 to 20 kV. In the droplet, due to W/O phase separation, the monomers underwent photopolymerization with UV light exposure (365 nm, VL-4.LC, MARNE-LA-VALLEE CEDEX, France). After polymerization, the hydrogel beads were collected and washed with ethanol and deionized water separately in a centrifuge.

To analyze the surface charge, the ζ -potential values of fabricated beads were measured (380 dls, Nicomp). Images of the beads were observed using an optical microscope (S16C, MIC). The chemical structure of polymerized poly(HEMA-co-MAETC) was analyzed by Fourier transform infrared (FT-IR) spectra (Spectrum 100, Perkin Elmer). The swelling behavior was analyzed by calculating water content for each bead, where W_s and W_d are the weights of swollen and dried beads, respectively (eq 1). The mechanical properties were measured through compression testing of beads (model 3343, Instron, 0.1 N load cell), and using Hertz theory, the elastic modulus (E) was calculated in the strain region from 0.05 to 0.15 (mm/mm), where F is the measured force, ν is Poisson's ratio (0.5), R is the initial radius of beads, and h is the displacement (eq 2).^{30,31}

$$\text{water content(\%)} = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

$$E = \frac{3}{4} \frac{F(1 - \nu^2)}{R^{1/2} h^{3/2}} \quad (2)$$

To analyze the cell affinity of charge-controlled beads, we used *E. coli* BL21(DE3) carrying a pBbe7K-*egfp+* plasmid that expresses an improved green fluorescent protein (GFP) under the control of the isopropyl β -D-1-thiogalactopyranoside-inducible T7 promoter, which was constructed by transforming *E. coli* strain BL21(DE3) with the GFP plasmid (Figure S7).³² pBbe7K-*egfp+* is a plasmid containing the GFP-encoding gene. The plasmid was constructed by replacing the RFP-encoding gene of the biobrick plasmid pBbe7K-*rfp* with *egfp+*.^{33,34} The GFP-expressing *E. coli* in LB broth medium was cultured in a shaking incubator at 37 °C and 200 rpm. The fabricated charge-controlled beads were dipped into the prepared bacteria solution (OD₆₀₀ = 0.5) for 12 h and were washed with PBS. The optical signal of charge-controlled beads with GFP-producing *E. coli* by cell attachment was analyzed using a fluorescent microscope (Nikon, Eclipse Ti). The fluorescence intensity was obtained using "ROI statistics" tool in imaging software (Nikon, NIS elements). In the fluorescent microscopic image, the mean fluorescence intensity was averaged over intensities in pixels of a defined area as a common pixel measurement. The obtained fluorescence intensity of poly(HEMA-co-MAETC) beads in each condition was normalized to that of poly(HEMA) beads (count: 10 ea. for each condition).

The charge-controlled poly(HEMA-co-MAETC) beads were used as substrates for biosensors with genetically engineered fluorescent bacteria sensitive to nitro compounds. The fabricated beads were coated with GFP-producing *E. coli* MG1655 with pPROBE-*P_{yqjFmut}-egfp+* [*E. coli* (MG1655 pPROBE-*P_{yqjFmut}-egfp+*)]. The plasmid encoding the GFP+ under the control of the nitro-compound-inducible *yqjF* mutant promoter was constructed.^{20,28,35} The plasmid with the *yqjF* promoter and the *egfp+* sequence at the downstream of the promoter was transformed. The *yqjF* promoter (*P_{yqjF}*) is activated when some sort of nitro compounds are treated.²⁸ When *P_{yqjF}* is activated by nitro compounds, it leads to the initiation of GFP expression. Additionally, 12 mutations were introduced into *P_{yqjF}* (*P_{yqjFmut}*). These mutations have been reported to efficiently increase the activity of *P_{yqjF}* (Figure S8).²⁰ The GFP-expressing *E. coli* in LB broth medium was cultured in a shaking incubator at 37 °C and 200 rpm. The beads were dipped into bacterial solution (OD₆₀₀ = 1) for 12 h and rinsed with PBS. The nitro compound sensing performance was analyzed using TNT as one of the nitro compounds. TNT was dissolved in acetone and diluted in LB broth. To observe the reactivity of the bead-type nitro compound sensor, poly(HEMA-co-MAETC) beads with the sensor *E. coli* MG1655 cells were dipped into prepared TNT solution for 72 h and washed with PBS. The fluorescence intensity of poly(HEMA-co-MAETC) beads by GFP-producing *E. coli* after exposure to TNT detection was normalized to

that of poly(HEMA-co-MAETC) beads in the absence of TNT (count: 10 ea. for each condition).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.9b02262.

Variations in the size of poly(HEMA) beads with the nozzle size and applied voltage in electrospraying method (Figure S1); stress-strain curves of poly(HEMA) beads with respect to the content of EGDMA (Figure S2); FT-IR spectra of poly(HEMA-co-MAETC) with 80 wt % MAETC (Figure S3); stress-strain curves of poly(HEMA-co-MAETC) beads with the content of MAETC (1 wt % EGDMA) (Figure S4); (a) optical and (b) fluorescence microscopy images of 40 wt % MAETC to HEMA in the poly(HEMA-co-MAETC) beads with GFP-producing *E. coli* [BL21(DE3) pBbe7K-*gfp+*] (Figure S5); effect of MAETC composition on the fluorescence intensity of a nitro compound sensor with the *E. coli* (MG1655 pPROBE-*P_{yqjFmut}-egfp+*) on the poly(HEMA-co-MAETC) without and with 100 ppm TNT (Figure S6); construction of *E. coli* [BL21(DE3) pBbe7K-*egfp+*] (Figure S7); construction of *E. coli* (MG1655 pPROBE-*P_{yqjFmut}-egfp+*) for detection of nitro compounds (Figure S8) (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

H.L. acknowledges the financial support for this work by the National Research Foundation of Korea Grant funded by the Korean Government [NRF-2015R1A5A7037615 and 2017R1A2B2010552] and the Civil Military Technology Cooperation Center [15-CM-SS-03].

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