CODE-CHANGEABLE ENCODED MICROPARTICLES FOR MULTI-STEP BEAD-BASED ASSAY

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ABSTRACT

Recording the reaction history of microparticle-based combination assays is important. However, several existing encoding methods cannot change microparticle codes. In this paper, we present a new method of encoding microparticles that uses a photoluminescent material for multiple code writing. 2,2-Dimethoxy-2-phenylacetophenone (DMPA) is a commonly used photoinitiator for free-radical polymerization. DMPA exhibits photoluminescence when irradiated in the ultraviolet region. Photopolymerized microparticles that contain DMPA were generated and then graphically encoded by using the Optofluidic Maskless Lithography system [1]. Our encoding method has advantages such as high coding capacity and long-term durability aside from enabling repeated writing on microparticles. Our encoding method that uses the DMPA photoinitiator can be applicable to multi-step microparticle-based assays.

KEYWORDS

Encoded microparticle, Bead-based assay, Photoinitiator, Encoding, Code.

INTRODUCTION

Graphical encoding methods can generate a large number of codes by using structurally patterned microparticles such as aluminium-patterned SU-8 microbars [2] and hole-patterned hydrogel particles [3]. However, microparticles that are encoded by using these graphical encoding methods cannot be used to record a biochemical reaction history because such microparticles are generated with codes. To be rewritable, these microparticles require surface modification so that they may be tagged. In this context, we introduce a new encoding method for microparticle encoding that uses only ultraviolet (UV) light without surface treatment.

EXPERIMENTAL

Figure 1 shows a schematic diagram of the generation of code-changeable encoded microparticles by using an OFML system. A mixed solution that contains photocurable perfluoropolyether (PFPE) and DMPA photoinitiator is filled between two glass slides. Patterned UV light from a digital micromirror device is projected onto the mixed solution through an objective lens, after which the PFPE monomer is photopolymerized. UV light with a single-circle pattern generates a disk-shaped microparticle, as shown in Figure 1(a). Figures 1(b) and (c) exhibit the code-changeability of encoded microparticles. A code of this microparticle is created by using a star-patterned UV light. By changing the area of UV irradiation, another code is produced on the same particle. As described in Figure 1(d), these codes emit green light when they are excited by blue light because the DMPA initiator becomes photoluminescent after irradiation by UV light, as shown in Figure 1(f). That is, the DMPA in the polymerized network is used as encoding material, as shown in Figure 1(e). For magnetic nanoparticle addition and silica-coated particle generation, we used photopolymerizable resins of ethoxylated trimethylolpropane triacrylate instead of PFPE.

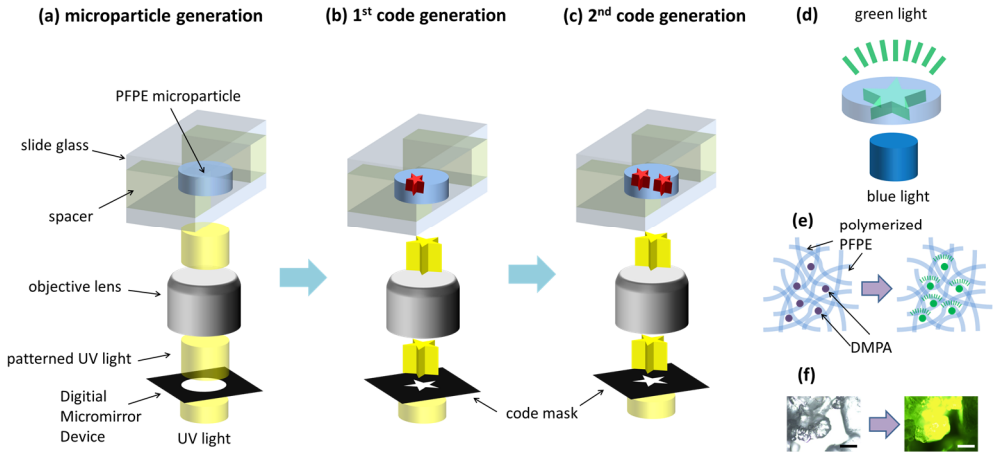

Figure 1: *Schematic diagram of the generation of code-changeable encoded microparticles by using an OFML system.* ***(a)–(c)*** *Microparticle generation and multiple encoding.* ***(d)*** *Code reading.* ***(e)*** *DMPA photoinitiator in the polymer network.* ***(f)*** *Actual image of photoluminescent DMPA photoinitiator. Scale bar is 200 um.*

RESULTS AND DISCUSSION

Figure 2 shows the characteristics of our encoding method. First, our encoding method has high coding capacity. A variety of graphical codes can be produced, as shown in Figure 2(a). Without physically replacing a mask with another, picture files for codes can be selectively loaded onto the digital micromirror device. Patterned UV light is then irradiated onto the particle by using our laboratory-made software. Second, the generated codes have long durability. Figure 2(b) shows that the encoded particles maintain their photoluminescence for at least seventeen days. Therefore, differently encoded particles can be distinguished from one another. In addition, the code intensity can be varied. Figures 2(c) and (d) indicate that code intensity depends on the initiator concentration in the PFPE resin and on UV irradiation time. A higher concentration of DMPA photoiniatiator and longer UV irradiation time increase the photoluminescence intensity of codes. Therefore, the photoluminescence of codes can be adjusted by using these two parameters. Figure 2(e) shows an example of three-level codes in the microparticle. These codes were produced by varying UV irradiation time.

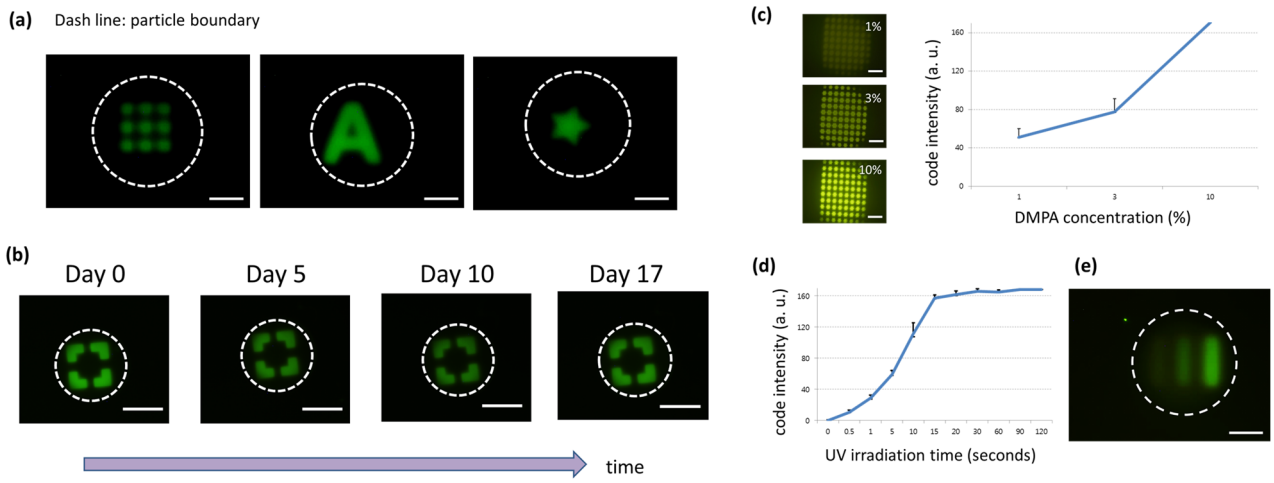

Figure 2: *Characteristics of the encoding method that uses DMPA.* ***(a)*** *Differently shaped codes can be created on the microparticles.* ***(b)–(e)*** *These codes have long durability, and their intensity can be controlled through DMPA concentration and UV irradiation time. Scale bar is 150 um.*

Figure 3 highlights the key feature of our encoding method, which is code-changeability. In this figure, three squares in a column are sequentially encoded in the same microparticle. This feature can be used to record the history of biochemical reactions in the bead-based assay, such that the code can be modified after each reaction.

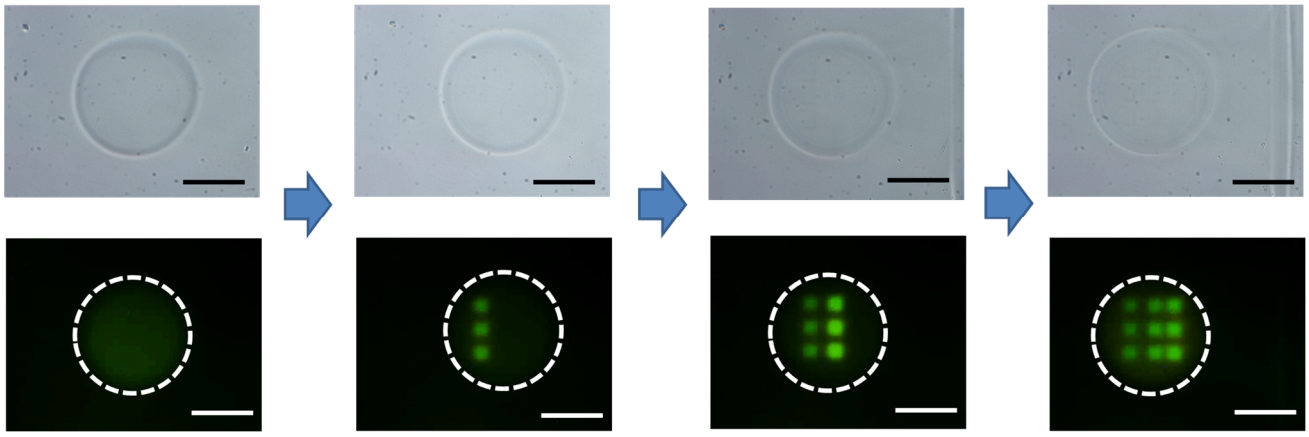

Figure 3: *Code-changeability of encoded microparticles. Three squares in a column are sequentially encoded in the same particle. This feature enables the microparticle to record a history of biochemical reactions. Scale bar is 100 um.*

The recognition of particle codes when such particles are randomly dispersed in solutions is important. Moreover, particles need to be separated from solutions for washing or solution exchange. Thus, magnetic nanoparticles were inserted into particles, as shown in Figures 4(a) and (b). After the magnetic chains were formed by the application of a magnetic field to the magnetic nanoparticle solution surrounding the particle, these chains were photopolymerized by UV light to produce double-layered particles. Given that the magnetic chains align along the magnetic field, the double-layered particle can be rotated by changing the direction of the magnetic field. Hence, the codes of particles can

be easily recognizable regardless of their initial orientation. Moreover, these particles are movable because the magnetic nanoparticles are attracted by a magnetic field. Such movement enables particles to be separated from solutions and facilitates solution exchange and particle washing.

To demonstrate that our code-changeable microparticles can be used in a multi-step microparticle-based assay, the particles need to have functional groups. A silica coating can be applied to the particles, as shown in Figure 4(c). The silica coating enables the particles to have functional groups. We utilized the modified Stöber method to coat particles with silica [4, 5]. Particles that contain the DMPA photoinitiator were silica-coated, and a scanning electron microscope (SEM) image shows the silica coating around particles. The silica-coated microparticles remained writable. Code "K1" was created in the silica-coated particle. In addition to the generation of functional groups, silica coating prevents the DMPA photoinitiator from diffusing out from the core and enhances microparticle resistance to strong acid and base environments. In summary, a microparticle that contains DMPA photoiniator was silica-coated to generate functional groups for peptide attachment and for the particle-based assay.

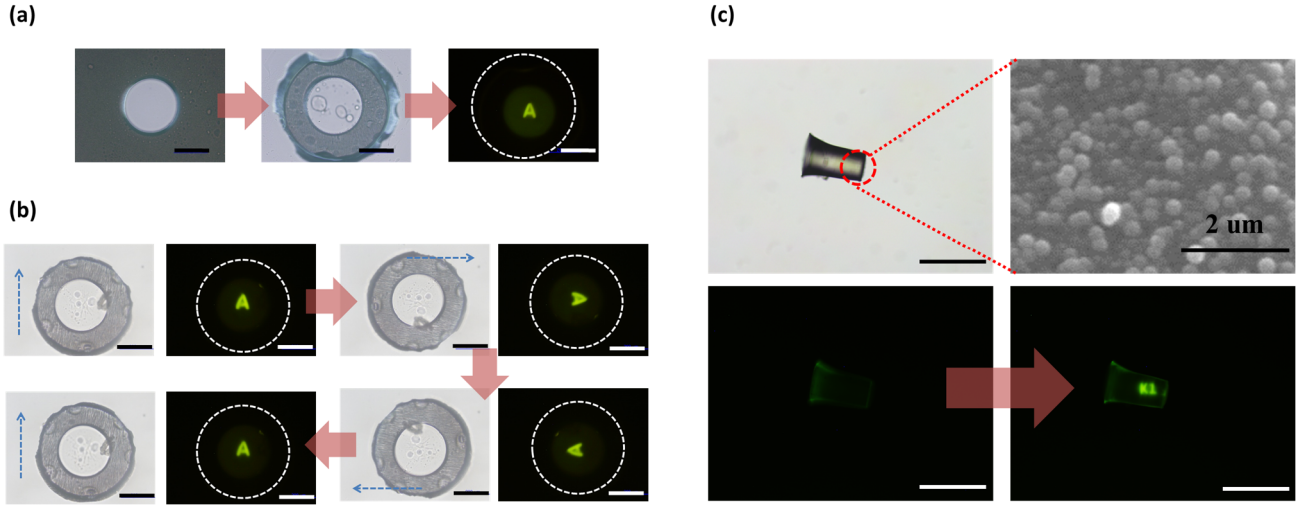

Figure 4: ***(a)*** *Generation of a magnetic nanoparticle-laden code-changeable microparticle.* ***(b)*** *Rotation of a code-changeable microparticle by a magnetic field. Blue arrows indicate the direction of magnetic chains.* ***(c) (Top left)*** *Bright-field image of a silica-coated writable microparticle.* ***(Bottom left)*** *Fluorescent image of the particle.* ***(Top right)*** *SEM image of the silica layer of a writable microparticle.* ***(Bottom right)*** *Fluorescent image of the encoded particle. Scale bar is 200 um.*

CONCLUSION

A new encoding method that utilizes a photoluminescent photoinitiator was demonstrated. We expect that this encoding method will be useful for multi-step microparticle-based assays.

ACKNOWLEDGEMENTS

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