VERTICALLY ENCODED TETRAGONAL HYDROGEL MICROPARTICLES FOR MULTIPLEXED BIOMOLECULE DETECTION

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ABSTRACT

Encoded hydrogel particles have drawn many attentions in diagnostics as the particles can be used for high-performance multiplexed assays. Here, we present encoded tetragonal hydrogel microparticles that are comprised of vertically distinct code and probe regions, and incorporated with quantum dots (QDs) in the code regions. By virtue of the particle geometry, particles can be synthesized at a high production rate in vertically stacked micro-flows using hydrodynamic focusing lithography. The particle coding capacity can be largely expanded by changing wavelengths of QDs and the number of code layers. We also demonstrate the multiplexed capability of the particles in a DNA assay.

KEYWORDS: Quantum dots (QDs), Hydrodynamic focusing lithography (HFL), Multiplexed assay

INTRODUCTION

Multiplexed assays detect multiple targets in a single assay, and thus enable rapid detection of target biomolecules\textsuperscript{1,2}. However, existing multiplexed methodologies suffer from high cost, low sensitivity and specificity. Recently, the barcoded hydrogel microparticles have been developed to overcome these limitations, and introduced as a powerful tool that provides high sensitivity and specificity in the multiplexed detection\textsuperscript{3}. Yet, these hydrogel microparticles are synthesized with a 1-D array at relatively low production rates because the synthesis process requires precise mask alignments on parallel laminar co-flows. In addition, the multiplexed assay capability of these particles is reduced due to limited code numbers. Here, we present encoded tetragonal hydrogel microparticles that consist of code and probe regions in vertically patterned compartments, and bear quantum dots (QDs) in the code regions. These particles are created by hydrodynamic focusing lithography (HFL) that can be used to synthesize striped particles in vertically stacked micro-flows\textsuperscript{4}. As the HFL process allows for the 2-D array synthesis, the particles are fabricated at a much higher production rate than the barcoded particles. The tetragonal hydrogel particles are encoded by QDs conjugated with hydrogel network in the code regions. A large number of codes can be generated by changing the wavelengths of the QDs and increasing the number of code layers. As a demonstrative application of the particles, we perform the multiplexed detection of two different DNA targets. With the experiments, we confirm that the particles can be used in more readily accessible high-performance bioassays without compromising the multiplexed assay capability of the barcoded particles.

EXPERIMENTAL

To synthesize encoded tetragonal hydrogel microparticles, we prepared a two-layered PDMS microfluidic chip utilizing replica molding and soft lithography methods. We then use the flow focusing technique to create stable stacked micro-flows inside the device. In the stacked flows, we synthesized tetragonal hydrogel microparticles encoded with QDs via HFL (Figure 1A-B). To prevent leaking, QDs were methacrylated, and photochemically conjugated with hydrogel networks during the UV
polymerization. As proof-of-concept identification, we tagged probe regions with green fluorescent microbeads. Also, we proved that the heights of the code and probe regions were readily adjusted by controlling flow rates of each monomer stream. Previously, a hydrodynamic resistance model has been used to predict the height of each region corresponding the flow rates\(^6\). However, we note that this model is not suitable unless inlet pressures are controlled very precisely. To address this, we developed a new model that employed estimated pressure ratios instead of the measured pressure ratios (Figure 1C).

**RESULTS AND DISCUSSION**

By virtue of 2-D array particle synthesis in HFL, we were able to fabricate QD-encoded tetragonal hydrogel microparticles at a high production rate. Compared to the typical SFL approaches\(^7\), the particle production rates are increased by a factor of more than 200\(^4\). Utilizing QDs as encoding materials, we fabricated encoded tetragonal hydrogel microparticles with three different QDs (blue, red and green) in the code region (Figure 2A). Also, we synthesized particles consisting of two sub-layers in the code region (Figure 2B). The code numbers in the particles can be greatly expanded through the combination of QDs with different wavelengths in the sub-layers.

**Figure 2: Tetragonal hydrogel microparticles encoded with different wavelength of QDs**

For the demonstration of the multiplexed detection, we synthesized two types of encoded tetragonal hydrogel microparticles; each type was incorporated with a distinct QD in the code region and a DNA probe (i.e., ssDNA oligomer) in the probe region. We chose the same DNA probes and targets that were used in bioassays with the barcoded particles\(^8\). After the multiplexed assay, we confirmed that our particles showed high sensitivity and specificity akin to the barcoded particles in either presence and/or absence of the target DNAs (Figure 3A-B). Figure 3B shows calibration curve of target 1.
CONCLUSION

We have presented QDs-encoded tetragonal hydrogel microparticles that are comprised of vertically distinct compartments for code and probe regions. As the particles are synthesized with a 2-D array, the particle throughput was greatly improved compared to that of the barcoded particles. For the particle code, we photochemically immobilized QDs in the code region. The numerous codes can be generated by using QDs with different wavelengths and increasing the number of code layers. We also demonstrated that encoded tetragonal hydrogel microparticles can be exploited in high-performance multiplexed assays. Using two examples of DNA targets and probes, we showed that the particles exhibited bioassay performance similar to that of the typical barcoded particles. Based on the previous literatures on the barcoded particles, it is implied that encoded tetragonal hydrogel microparticles can be utilized to detect other biomolecules such as miRNAs, mRNAs, and proteins in a highly sensitive and specific manner. We believe that the encoded tetragonal hydrogel particle can be a practical and powerful platform for multiplexed bioassays.

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