ENCODED DROPLET MICROCARRIER FOR FORMATION AND ISOLATION OF DROPLET IN A MICROFLUIDIC DEVICE

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ABSTRACT
We present an encoded amphiphilic microcarrier including microwell of femtoliter volume and microfluidic system for simultaneous dispensing quantified femtoliter liquid on amphiphilic microcarrier. The encoded amphiphilic microcarrier is composed of hydrophobic hexagonal outer structure and hydrophilic inner structure. We demonstrated fabrication and assembly of encoded amphiphilic microcarriers in a microfluidic device. Femtoliter aqueous volume was simultaneously loaded on encoded amphiphilic microcarriers by serial loading of aqueous liquid solution and oil solution. We controlled femtoscale dispensing volume by regulating microwell’s diameter and depth. This technique would be applicable to high-throughput multiplexed assay based on suspension-based assay with quantified liquid dispensing on a microcarrier in a microfluidic environment.

KEYWORDS: Encoded microcarrier, Droplet carrier

INTRODUCTION
Particle-based multiplex technologies have arisen over past a decade for simultaneous multiplexed assay in medical diagnostic, drug screening, and combinatorial chemistry. This technology provides sample volume reduction, favorable target-probe binding kinetics, and simultaneous detection of multiple molecular targets in a single sample comparing to conventional planar array technology[1-3]. While conventional planar array technology uses location for identifying molecular probe, particle-based multiplexed assay carries a unique identifier, such as spectral, graphical, biological, and physical code. In encoding scheme, it is important to increase code number and reduce encoding cost and time. We presented an encoded droplet microcarrier for formation and isolation of aqueous single droplet in a microfluidic device. This technique is the creative and effective method to carry quantified monodisperse droplet on the microcarrier with graphical code, which can be versatile for various applications such as drug-discovery, drug-screening and multiplex assay.

RESULT AND DISCUSSION

Figure 1: High-throughput loading of femtoliter droplets on free-floating amphiphilic microcarriers. (a) Fluidic self-assembly of amphiphilic microcarriers with high density at anchors in microfluidic device. (b) Injecting aqueous solution to microfluidic device. Color aqueous dye was introduced into microfluidic device. (c) Injecting oil solution into the device. Oil solution sweeps aqueous solution and leaves isolated femtoliter droplet on amphiphilic microcarrier. Quantified aqueous droplets are loaded on amphiphilic microcarriers. (scale bar: 100 µm)
Figure 2: Fabrication process of amphiphilic microcarrier in a microfluidic channel. (a) Injecting hydrophobic oligomer in a microfluidic device and illuminating UV light on the channel with hexagonal shape including a hole at the center in order to photopolymerize hexagonal outer structure. (b) Washing the channel out with ethanol and drying on a 60°C hot plate for 30 mins. (c) Injecting hydrophilic oligomer into microchannel again and illuminating circular patterned UV light to fill a hole in the hexagonal structure. Washing uncured oligomer and drying on a 60°C hot plate for 30 mins.

The droplet carrier is composed of hexagonal hydrophobic area and circular hydrophilic area. The height of circular hydrophilic area is lower than the height of hexagonal hydrophobic area, therefore the cup shape is formed to confine the droplet in the center of the amphiphilic droplet carrier. Hydrophilic area plays a role of attracting aqueous solution in immiscible solution and isolating it in a cup shape (Fig.1a). The droplet microcarrier includes a graphical code such as Arabic number or bar-codes on hexagonal rim structure to identify a droplet loaded on the microcarrier. Aqueous blue dye solution was introduced in a microchannel, where amphiphilic droplet microcarriers were assembled (Fig.1b). Immiscible oil phase was introduced in the microfluidic channel using syringe pump and isolated droplets in droplet microcarriers. Immiscible phase wipes the channel and isolates droplet in an amphiphilic microcarrier (Fig.1c).

Amphiphilic droplet microcarriers were fabricated through process as shown in Fig.2. First, hexagonal structure with hole was fabricated with UV curable hydrophobic polymer using photolithography technique in a microfluidic device. We used optofluidic maskless lithography system for photolithography on a microfluidic channel[4]. The hexagonal patterned UV light was illuminated on the channel and polymerize the hexagonal shaped polymer. The microdevice was washed out with ethanol and UV curable material was injected in the microchannel. We repeated the photolithography process again to fill the hole in hexagonal structure previously fabricated in the first step.

In order to regulate the size of droplet isolated on the microcarrier, we change the size of hydrophilic area (Fig.3a,c). The fluorescent intensities were measured at each microcarrier capturing a droplet. (Fig.3b) Controlling the shape of hydrophilic areas surrounded by hydrophobic wall can control the shape of loaded droplet. Star, rectangle, and triangle shape droplet were formed in a droplet microcarrier, confined by hydrophobic wall (Fig.3d).

Figure 3: Control the quantity and pattern of loaded solution on the amphiphilic microcarrier (a) Fluorescence microscope image of assembled amphiphilic microcarriers with four different diameters of hydrophilic inner structure. Four different volumes of fluorescent drops were loaded on the amphiphilic microcarriers. (scale bar: 100µm) (b) Fluorescence intensity of four different diameter hydrophilic (x-axis: radius, y-axis: fluorescence intensity) (c) Fluorescence microscope images of amphiphilic microcarriers with four different diameters of hydrophilic inner structure. (scale bar: 50µm) (d) Arbitrary shapes of loading site on the amphiphilic microcarrier. Liquid droplets are loaded forming star, rectangle, and triangle shape by the shape of loading site. (scale bar: 50µm)

To verify that the liquid droplet is formed right after loading it into the amphiphilic microcarrier, a fluorescent microparticle suspension was loaded on an amphiphilic microcarrier within femtoliter droplet (Fig.4a). The dotted hexagon indicates the hydrophobic outer structure. By tracing one polystyrene bead in a yellow dotted circle area, the movement of beads rotation about the axis can be clearly verified (Fig.4b). This technique provides new solution for loading quantified droplet on encoded microcarrier. We envision that the encoded droplet microcarrier could be applied to new high-throughput screening technologies in a microfluidic device.
EXPERIMENTAL

Fabricating microfluidic channel
A soft-replica mold process was used as a method for fabricating microfluidic channels. PDMS(polydimethylsiloxane) was poured on an SU-8 mold and baked 150 °C for 15 minutes. The PDMS was peeled off and bonded with PDMS coated glass after corona treatment of both surfaces. The width and height of the microfluidic channel is 840µm by 80µm.

Preparing fluorescent beads suspension
Spherotech fluorescence polystyrene beads (high intensity yellow, 1µm) are mixed with deionized water and 30% (w/v) dextran from Leuconostoc spp.(Mr ~500,000, Sigma)

Loading solution process on amphiphilic microcarrier
After uncured polymer was removed by washing 5mL ethanol for 1min, liquid solution are introduced into microfluidic-channel using syringe pump. The flow rate of syringe pump is 10µL/min. The mineral oil (0.1µL) is sequentially injected into microfluidic channel at 10 µL/hour. For oil solution, mineral oil is mixed with 1% span80 and fluorocarbon oil (FC-40, Sigma) mixed with surfactant (Krytox, Dupont)

CONCLUSION
We have presented free floating amphiphilic droplet microcarriers composed of a hydrophobic hexagonal outer structure and hydrophilic inner structure for simultaneous loading of multiple femtoliter volumes. The hydrophobic outer structure is used as a diffusion barrier when droplet microcarriers are assembled in a microfluidic device and provides sufficient mechanical strength in order to prevent distortion during the packing process. We controlled carrier volume by altering the width and thickness of the hydrophilic inner structure by modulating UV dose during photopolymerization. Using droplet trapping in the amphiphilic microcarriers, microbeads suspension were loaded and rotated with immiscible oil, which offers a potential mixing scheme. The aim of future research is to create full test tube functionality, not limited only to differential volume loading, but also multiplexing of contents in different droplet carriers.

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