LOCALIZED GENE DELIVERABLE ENCODED MICROPATCH IMMOBILIZED WITH VIRAL VECTOR FOR MULTIPLEX HIGH CONTENT SCREENING

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ABSTRACT

We present a new concept of delivering gene with encoded micropatch for multiplex transfection assay with high flexibility. We fabricated encoded polymeric microparticles and immobilized viral vector on the particle surface. After prefabricating heterogeneous encoded viral micropatches with batch process, they were dispensed on cells cultured in a well. With one dispensing of heterogeneous viral micropatches, GFP, RFP gene are spatially delivered to cells from the viral vectors immobilized on an encoded polymeric microparticle in a well. We immobilize ad-GPCR viral vector on the microparticles for expressing G-protein coupled receptor (GPCR), which is popular target in drug discovery. By dispensing these micropatches to cells in a well plate, heterogeneous GPCR were locally expressed to cell membrane under the micropatches. We verified GPCR internalization with an assay based on the viral vector micropatch.

KEYWORDS: Multiplexed Bioassays, G-protein coupled receptor, Encoded microparticle, Transduction

INTRODUCTION

In pharmaceutical industry, screening the biological activity of millions of compounds at high throughput remains very challenging. Moreover in order to obtain more biologically relevant data from screening work, cell based assay are required, which require more complex approaches. Development of a high throughput screening technique based on robotic system has been able to partially solve these challenges. However, small volume liquid handling and repetitive redundant processes remain a challenge to automate[1, 2]. Reducing pipetting time and cost can be accomplished by using a particle based multiplex assay concept. Here, we present a new concept, "Partipetting" that multiple encoded microparticles are simultaneously delivered to the reaction sites instead of repetitive pipetting for the drug screening process.

We designed an encoded micropatch immobilizing viral vector. Encoded viral micropatches are partipetted to cells cultured layer to locally deliver a vector to cells. The encoded micropatches are placed on the cell layer and infect the cells with viral vector (Figure 1). We used graphical code for the encoding scheme, which is simple but powerful. Each encoded micropatch contains adenovirus on the surface as shown in Figure 1.

RESULTS AND DISCUSSION

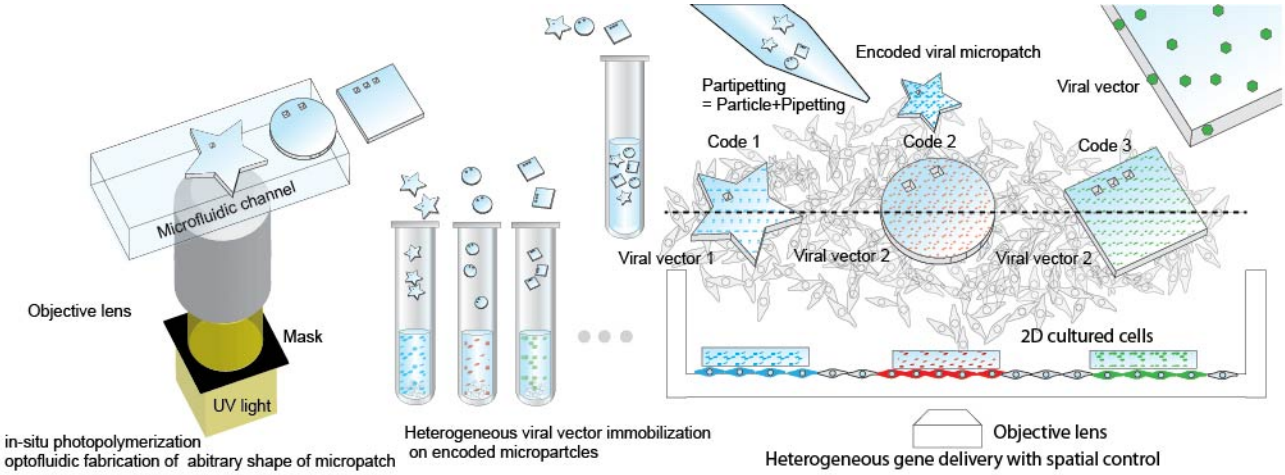

Figure 1: Schematic diagram of multiplex transduction by encoded micropatches. Fabrication process of encoded micropatch for gene delivery. Micropatches were photopolymerized in a microfluidic device with micro-patterned UV light through objective lens. Viral vectors are immobilized on an encoded micropatch. After fabricating various gene deliverable encoded micropatch, they were inserted into cell cultured well plate, which we called it "partipetting". Encoded micropatches were placed on the cells and viral vector were locally delivered to cell. We can identify gene transduced to cell by micropatch's code. Scale bar : 300um

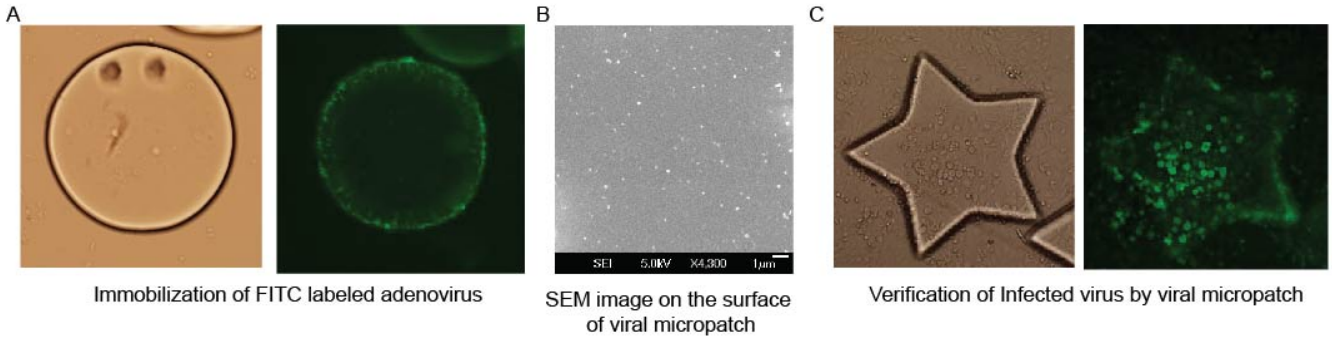

Figure 2 A. FITC-labeled adenovirus were immobilized to verification of viral vector immobilization. B. SEM image on the surface of viral micropatch. (Scale bar: 1um) C. Verification of infection from adenovirus using FITC-labeled antihexon antibody.

The key feature of the encoded micropatch is local gene delivery with a code according to the shape of the micropatch. Various shapes of micropatches were used for viral transduction as can be seen in Figure 3. We used ad-RFP viral vector, which can deliver a gene expressing RFP in a cell. We found RFP expressed cell with the micropatch's shape. By using this feature, heterogeneous genes were locally delivered to cells. We fabricated heterogeneous micropatches immobilized with GFP and RFP expressing genes and randomly spread them on cells cultured well plate. As shown in Figure 3b, heterogeneous gene delivery was successful using viral vector micropatches and they express GFP and RFP in cell area covered by each encoded micropatch.

In order to synthesize encoded micropatches, we used optofluidic maskless lithography system, which can continuously fabricate arbitrary shaped polymeric microparticles in a microfluidic device [3]. After fabricating microparticles with polyethylene glycol diacrylate and acrylic acid, we deposit positively charged polymer for virus immobilization on the microparticles. We confirm immobilization of viral vector by using FITC labeled adenovirus (Figure 2b). Additionally, immobilized virus were observed with SEM.

For solving the real world problem of multi-target cell drug screening based on G-protein coupled receptor (GPCR), which is the most common target in drug discovery, G-protein coupled receptor in Glucagon receptor family tagged with GFP were locally expressed after localization of gene delivery using gene deliverable micropatch (Figure 4). This fluorescent protein tagged GPCR could be used in high content assays based on GPCR internalization.

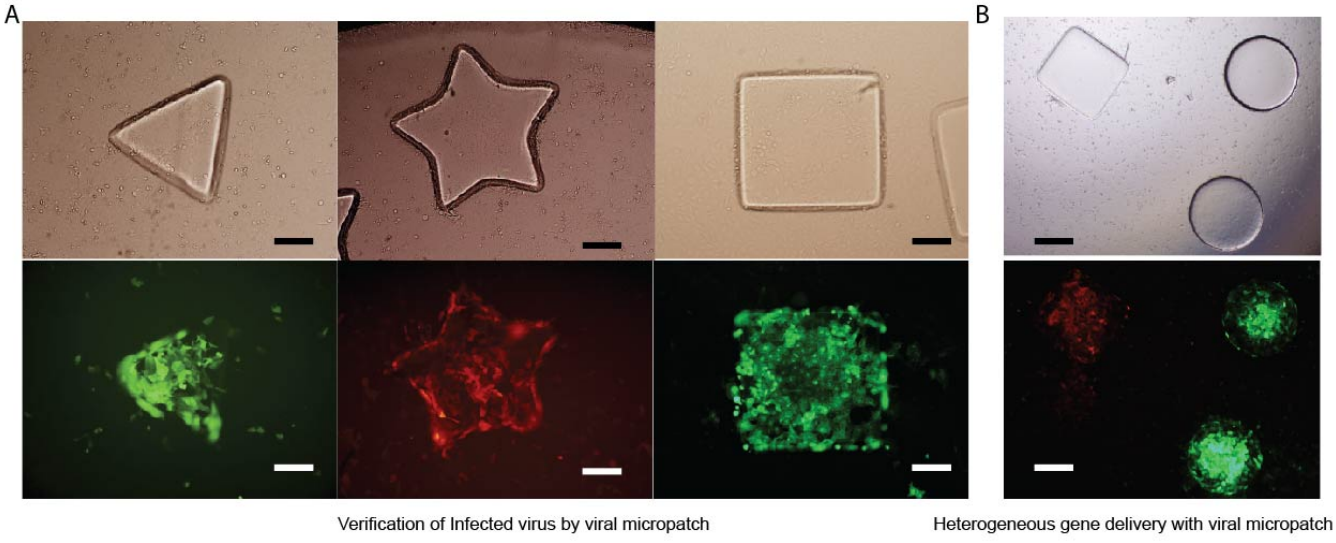

Figure 3 A. Arbitrary shaped local transduction using shape-coded micropatch immobilized with Ad-RFP, Ad-CMV-GFP viral vector. (Scale bar 300um) B. Heterogeneous viral vector transduction using two types of shape-coded micropatch immobilized with Ad-RFP viral vector and Ad-CMV-GFP viral vector. (Scale bar 600um)

EXPERIMENTAL

Micropatch preparation

After injecting prepolymer (PEGDA 34%, Acrylic acid 60% and Darocur 1173 5% NVP 1%) into a PDMS microfluidic device, micropatches was polymerized with patterned UV light reflected by digital micromirror device. The micropatches were washed out in ethanol for 1 hour in order to remove unpolymerized monomer in the microparticle. Micropatches were treated with a solution comprising 5 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), 5 mM N-hydroxysuccinimide (NHS), and 50 mM sodium bicarbonate in deionized water for 1 hour. Adenovirus solution (100ul, 10^9 pfu/ml) was slightly voltexed with 3000ea microparticle at 4°C for 6 hours.

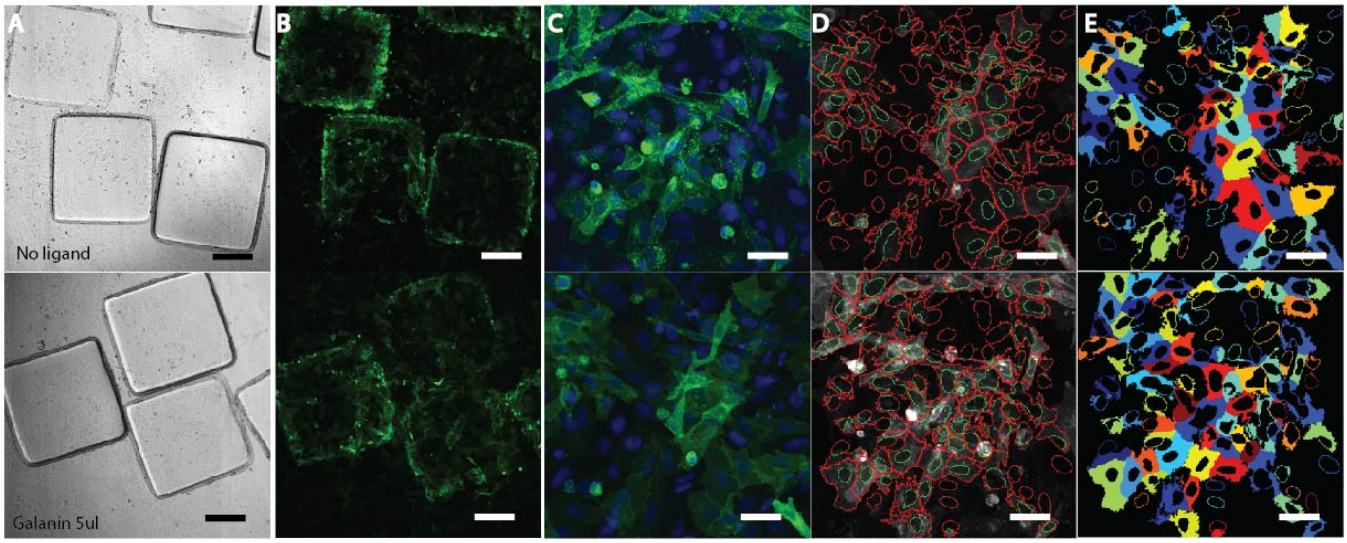

Figure 4 G-protein coupled receptor (GPCR) internalization assay with U2OS cell transduced by viral micropatches. Viral micropatches immobilized with Ad-GAL were dispensed to 96 well plate. B. Fluorescent microscope image that transduction are successfully done. C. GPCR internalization occurs only after adding of Galanin agonist at the yellow rectangular region in Fig.4B. D,E Image analysis using cell profiler (http://www.cellprofiler.org) (Scale bar : 300um)

CONCLUSION

We presented the concept of partipetting, which is that encoded microparticles are simultaneously delivered to the reaction sites instead of pipetting for drug screening process. We designed an encoded micropatch immobilizing viral vector and this encoded micropatches are distributed to cell cultured layer to locally deliver a vector to cells. We found genes are locally delivered to cells with information of gene in the microparticle based identifier. We envision that this encoded particle based gene delivery system can revolutionize the multi-target gene transduction screening process in high content screening. This technique is a scalable concept, which means that we can parallelize and expand multiplicity of assay by increasing the number of encoded micropatch types.

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