PARTIPETTING FOR MULTIPLEXED BIOASSAY IN MICROWELLS
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ABSTRACT
We demonstrate the cell-based assay microwell platform for multiplexed drug screening with a single pipetting of various encoded drug-laden microparticles. The encoded drug-laden hydrogels are fabricated in high-throughput manner and assembled in a microwell platform. Each drug is released from the hydrogels and reacted with target cells in microwells by assembling particle chip and cell chip. Our method allows handling one hundred thousand different chemicals in one chip with a single input.

KEYWORDS: Microwells, Drug-laden hydrogels, Assembly

INTRODUCTION
In drug screening, more than one hundred thousand different chemicals in a compound library are handled to determine a final drug candidate. Conventionally, well plates such as 96- or 384-well plates are used. With these well plates, introduction of chemicals into each well is cumbersome works, because it requires a lot of pipetting works. For example, if we want to deal with 100k different chemicals, 100k different pipetting events are necessary. Recently, drug screening methods in micro scale have been attempted using microfluidics. Microfluidic based assay has the capability of multiple chemical environments on a single chip. However, it also requires the same number of inputs as types of chemicals used in the experiments. We present a novel bioassay method in microwell platform by pipetting encoded drug-laden microparticles.

EXPERIMENTAL
Fig. 1(a) schematically illustrates concept of our multiplexed bioassay platform. For high-throughput drug screening, various encoded drug-laden hydrogels are fabricated, assembled, and reacted with target cells. The hydrogels are fabricated in high-throughput manner using OFML system. Drugs are doped inside polymeric hydrogel mesh during the fabrication process. Fabricated particles are collected in a single pool. These drug-laden hydrogels are provided and assembled into the microwell plate. Drugs kept inside hydrogels are released to target cells when hydrogels meet cell media by diffusion. After an appropriate drug release time, hydrogels are removed and reacted cells are observed to figure out which drugs are effective to target cells. Since, there is a necessity for pipetting lots of chemicals at once to increase drug screening throughput, we propose a new concept of pipetting technique, ‘partipetting’ as shown in Fig. 1(b). It is the combination of ‘particles’ and ‘pipetting,’ thus ‘partipetting’ means pipetting particles into the assay platform. The partipetting process reduces repetition of drug introduction into the platform and enables high-throughput drug screening. Figure 1(c) shows microparticles assembly method, a scraper-assisted assembly to remove excessive particles on the surface of the microwell plate, and Fig. 1(d) is an example of partipetting using various fluorescent microbeads.

![Figure 1: Partipetting based multiplexed assay platform (a) Schematic concept and process of partipetting based assay platform (b) Partipetting. Fabricated various encoded drug-laden hydrogels are pipetted into the well plate (c) Scraper-assisted assembly to fill the particles into the wells and remove excessive particles on the surface (d) Example of partipetted microbeads in a well plate. Three different fluorescent microbeads are assembled in microwells](image-url)

RESULTS AND DISCUSSION
Each hydrogel, which is a drug-laden polymer microparticle, has its identical code. Note that drugs are kept inside polymer mesh in dried form as shown in Fig. 2(a). Since each particle contains identical drug, a single partipetting can provide...
millions of chemical laden hydrogels with different codes to microwell platform (Fig. 1(b)). Unlike conventional drug screening technique, a single pipetting is used for the introduction of various drugs. We fabricated these various drug-laden hydrogels in high-throughput manner using optofluidic maskless lithography (OFML) system [1-2]. OFML system enables encoding on hydrogels by manipulating DMD pattern during the fabrication process. Figure 2(b) shows the process of drug-ladening into polymer matrix. First, only PEG-DA (photopolymer solution) is photopolymerized with codes for drug types in OFML. After the fabrication of hydrogels, particles should be washed completely with ethyl alcohol (EtOH), because uncured polymer could be toxic to the cells. Then, the solution is replaced to drugs. In this process, drugs are absorbed into the bare microparticles. The remnant drug solutions are also washed with EtOH. Fabricated microparticles are dried after the removal of buffer solution to prevent further drug release due to the excessive washing.

The protection of drugs in hydrogel matrix should be confirmed for particles in oil solution. Figure 2(e)-(f) show the drug release test in oils and PBS. To ensure that chemicals are not released inside oil solutions, Rhodamine B-laden hydrogels is gently vortexed overnight and the light absorbance of oil without hydrogels is observed using UV spectrophotometer (Fig. 2(e)). Only PBS solution has a peak, which means that Rh-B is not released in oils. After the removal of oils, PBS is added as a buffer solution for the drug release. The light absorbance of PBS after the drug release proves that all the chemicals are released to the replaced PBS buffer solution after the removal of oil. Therefore, it has been confirmed that even though the drug-laden particles were originally stored in oil solution, drug molecules finally diffuse out to buffer media.

In partipetting based bioassay experiments, two different microwell platforms are used separating particle chip and cell chip to prevent drug release before the cue. Since drug-laden hydrogels are stored in shrunken and dried form, dried microparticles swell when the cell culture medium touches them, and then, drugs start to diffuse from the swollen hydrogels. Therefore, particle chip and cell chip are separately prepared by partipetting, and they assembled together when the user need to do drug screening.

For cell chip, cells are introduced into the PDMS microwell platform by scraper-assisted assembly technique as shown in Fig. 3(a). Cells in culture medium is provided into the microwell plate and after the settling of the cells, the scraper sweeps the surface of the plate to remove excessive cells on the substrate. Fig. 3(b) shows assembled cells in microwell. In addition, Figure 3(c) shows particle chip prepared by partipetting in a large area. Drug-laden microparticles are mixed with mineral oil and partipetted on the PDMS microwell plate. This oil-assisted partipetting method shows high assembly yield about 96% in large area over 100 wells (Fig. 3(c)).

The assembly method of two chips is very simple. Prepared particle chip and cell chip are sandwiched together face by face in drug release step [3]-[4](Fig. 3(d)). Drug release starts from the hydrogel matrix by chemical diffusion phenomenon right after two chips are assembled. Figure 3(e)-(f) show heterogeneous staining of cells by partipetting based assay method. Two different cell staining drugs, green and red, are laden during the shape-encoded hydrogel fabrication process.

To apply our partipetting based assay platform to multiplexed drug screening, we also simply demonstrated cancer cell and anti-cancer drug reaction in microwell platform. We used U2OS cancer cells and Camptothecin (CAM) as anti-cancer drug. Two different concentrations (1mM and 1μM) of CAM have been absorbed into encoded microparticles and these particles are partipetted into microwell platform. By sandwiching particle chip with CAM-laden microparticles and cell chip, we
acquired CAM reacted cancer cells in microwells (Fig. 3(g)). Cells that stain positive for FITC Annexin V (green) and negative for PI (red) are undergoing apoptosis. In contrast, cells that stain positive for both FITC Annexin V and PI are either in the end stage of apoptosis, are undergoing necrosis, or are already dead. After 6 hours drug release from the hydrogels into the cancer cells, 1μM CAM absorbed particles, which have final release concentration of 300nM measured by UV spectro meter, gave 83% viability of U2OS cells which correspond to the measured cell viability of 78–88% using conventional method in 96 well-plate.

**CONCLUSION**

The ‘partipetting’ has been demonstrated as an innovative technique to provide efficient bioassay platform for multiplexed drug screening. We envision this technique will give important flexibility in multiplexed drug screening.

**ACKNOWLEDGEMENTS**

This work was partly supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST)(2011-0016491) and supported by the National Research Foundation of Korea (NRF) Grant funded by MEST (2010-0029797).

**REFERENCES**


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