MULTIPLEXED DRUG SCREENING USING PARTIPETTING
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ABSTRACT
We demonstrate multiplexed drug screening with a single pipetting of various drug-laden encoded microparticles. The encoded drug-laden hydrogels are drug absorbed photopolymerized microparticles with individual codes corresponding to drugs inside. Using ‘partipetting’ method, which is a single pipetting and assembly of various encoded drug-laden microparticles into microwell platform, we first analyzed drug absorption and release characteristics of drug-laden encoded microparticles using fluorescent dye. Then, we tested 8-plex drug screening in microwell platform with U2OS cancer cells and 8 different anti-cancer drugs. Since our method allows handling one hundred thousand different chemicals in one chip with a single input, we expect possibility in high-throughput drug screening of million different drugs.

KEYWORDS
Drug screening, Microwells, Drug-laden microparticles, Encoded particles

INTRODUCTION
In drug screening, more than one hundred thousand different chemicals in a compound library are handled to determine a final drug candidate. Conventional microtiter plates such as 96- or 384-well plates require a lot of pipetting events to test various drugs. For example, if we want to deal with 100k different chemicals, it requires 100k different pipetting events. Previously, we developed a novel bioassay method named ‘partipetting’ (Fig. 1) using encoded drug-laden microparticles to reduce pipetting times for drug screening [1]. It is the combined word of ‘particles’ and ‘pipetting,’ thus ‘partipetting’ means pipetting particles into the assay platform. By a single pipetting of drug-laden particles, we easily fabricate screening platform with millions of drug particles. It reduces repetition of drug introduction into the platform and enables high-throughput drug screening. As applications of partipetting technique, we demonstrate multiplexed drug screening using cancer cells and anti-cancer drugs.

EXPERIMENT
First, to figure out the release characteristics of molecules in our assay platform for drug screening, we observed fluorescent dye (Rhodamine B) diffusion from hydrogels to buffer media in microwells.

Figure 1 Concept of partipetting (a) Drug-laden encoded microparticles (DEP). Each code represents a type of absorbed drug in the hydrogel matrix (b) A single pipetting of various DEP to the microwell platform (partipetting = particles + pipetting) (c) Partipetting of fluorescent microbeads (d) Drug absorption into hydrogel matrix and diffusion from DEP (e) Partipetting based drug release
Figure 2(a) shows 10 different concentrations of Rho B absorbed encoded microparticles. All the microparticles with various Rho B concentrations are partipetted to the particle chip, and each hydrogel is assembled one by one in a single well with the assembly yield of 88% (Fig. 2(b)-(c)).

For the characterization of Rho B release dynamics, we prepared PBS filled reaction chip as another PDMS microwells. The reaction chip and the particle chip are assembled by sandwiching face to face [1-2], thus the absorbed dye in microparticles is released and mixed with PBS in microwells (Fig. 3(a)). Figure 3(c) shows the fluorescent variations according to the released Rho B with each different concentration from original hydrogels to PBS filled microwells.

Figure 3 Diffusional drug release in a large area (a) Assembled particle chip and reaction chip. From REP, Rho B is diffused out to PBS in reaction chip. (b) 5 concentrations of released Rho B (c) Reaction chip with 5 concentrations of Rho B dissolved PBS after diffusional Rho B release (d) Fluorescent intensities of 5 different concentrations of released Rho B to PBS in reaction chip.
The release dynamics of fluorescent dye in microwells is also characterized. The fluorescence of each Rho B reacted PBS has been measured and relative intensity has been plotted to characterize diffusional release rate based on the absorbed concentrations of fluorescent dyes (Fig. 3(d)).

Based on this release dynamics, we applied partipetting method to multiplexed drug screening. Fig. 4 shows 8-plex screening results using U2OS cancer cells and 8 different anti-cancer drugs. As shown in Fig. 4(d), partipetting based screening results are compatible to conventional screening results in microtiter plates.

![Figure 4](image)

**Figure 4.** 8-plex drug screening (a) Absorbed and released concentrations of anti-cancer drugs (b) Microscope image of anti-cancer drugs reacted cells in a single microwell. Green fluorescence is cells with undergoing apoptosis (c) 8 anti-cancer drugs reacted U2OS cancer cells in microwells (d) Cell viability of 8-plex drug screening by partipetting

Partipetting is an innovative technique to provide efficient bioassay platform applying to high-throughput multiplexed drug screening. We envision it will give important flexibility in multiplexed drug screening.

**REFERENCES**


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