MULTIPLEXED DRUG SCREENING USING PARTIPETTING

Su Eun Chung, Jiyun Kim, Dongyoon Oh, Younghoon Song, Sunghoon Kwon
Seoul National University, Korea

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 pipietting 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.

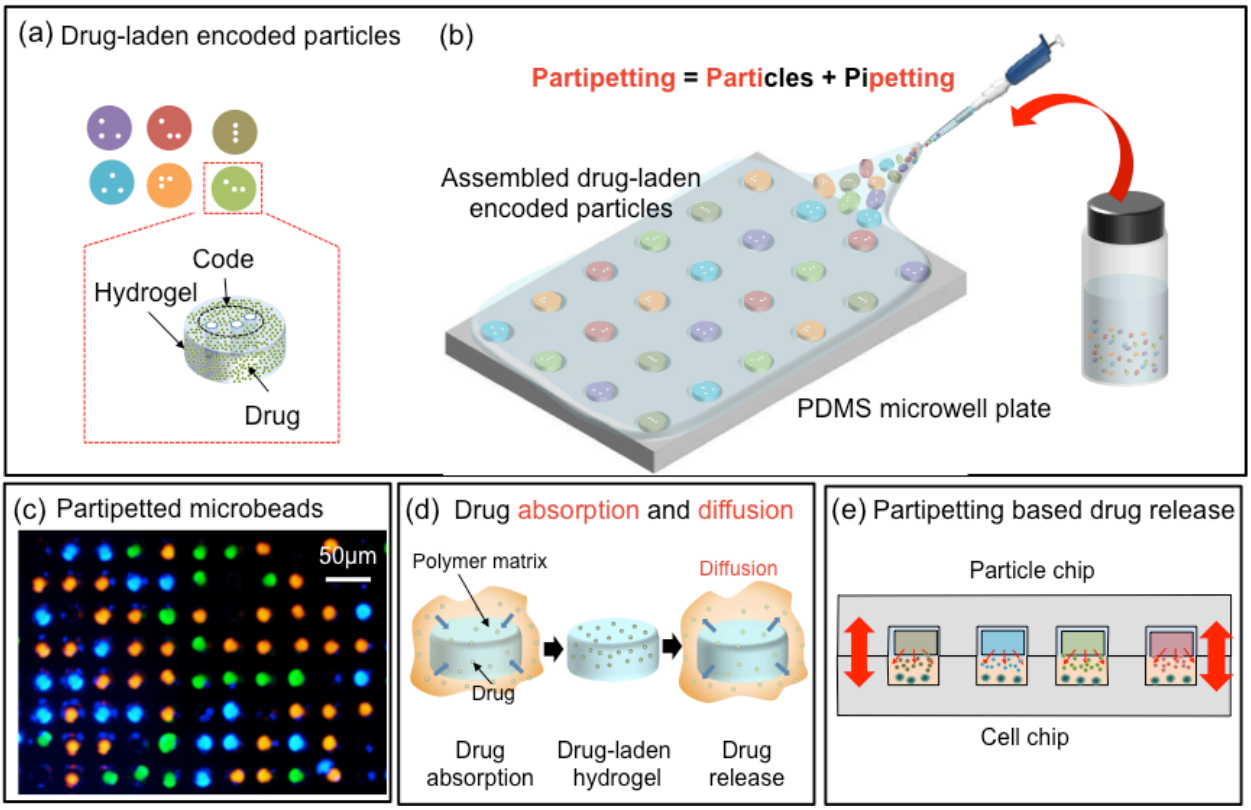


Figure 1 Concept of partipetting (a) Drug-laden encoded microparticles (DEP). Each code represents a type of absorbed drug in the hydrogel matrix (b) A single pipetting of various DEP to the microwell platform (partipetting = particles + pipetting) (c) Partipetting of fluorescent microbeads (d) Drug 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)).

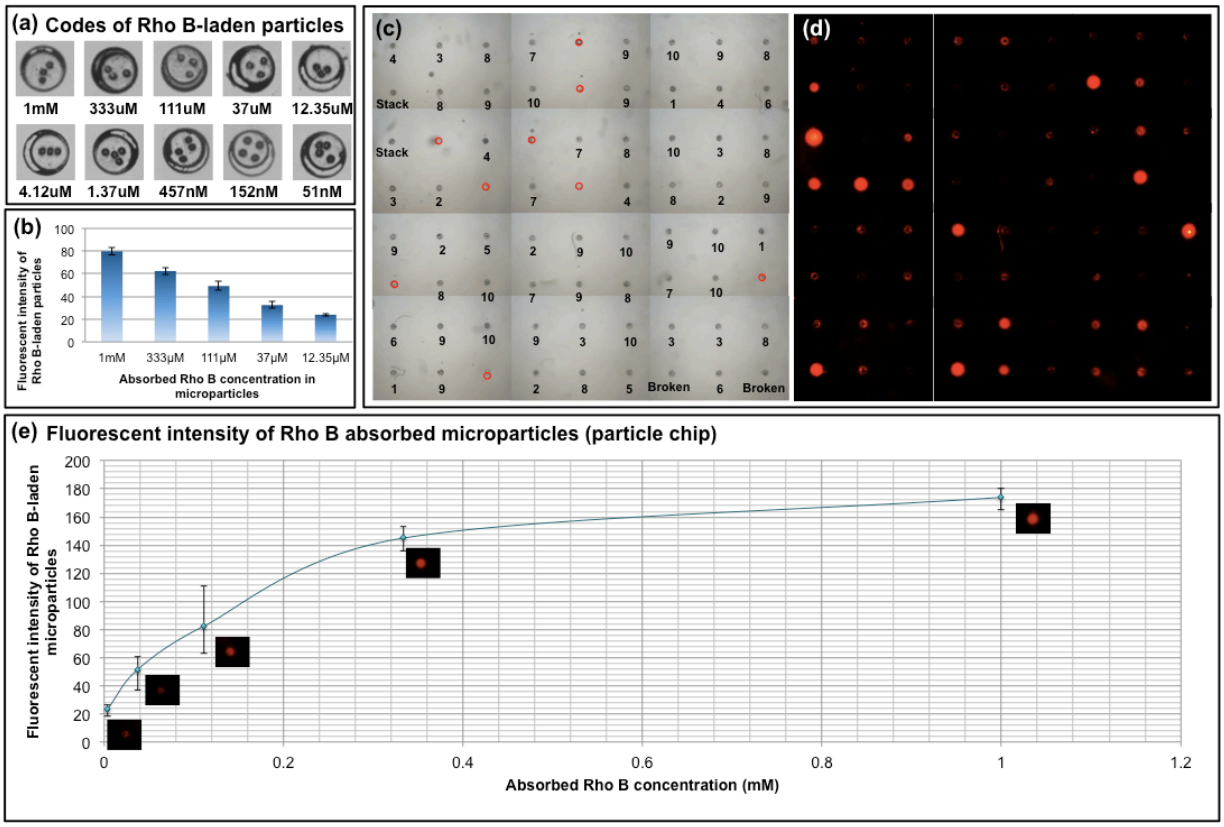


Figure 2 Rhodamine B-laden encoded particles (REP) for the analysis of diffusional drug release in partipetting-based assay platform (a) REP of 10 different codes (b) Inter-particle variation by fluorescent intensity of 5 different concentrations of REP (c) Partipetted 10 different concentrations of REP in microwell platform (d) Fluorescent image of partipetted REP in (c) (e) Fluorescent intensities of REP with 5 different concentrations

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.

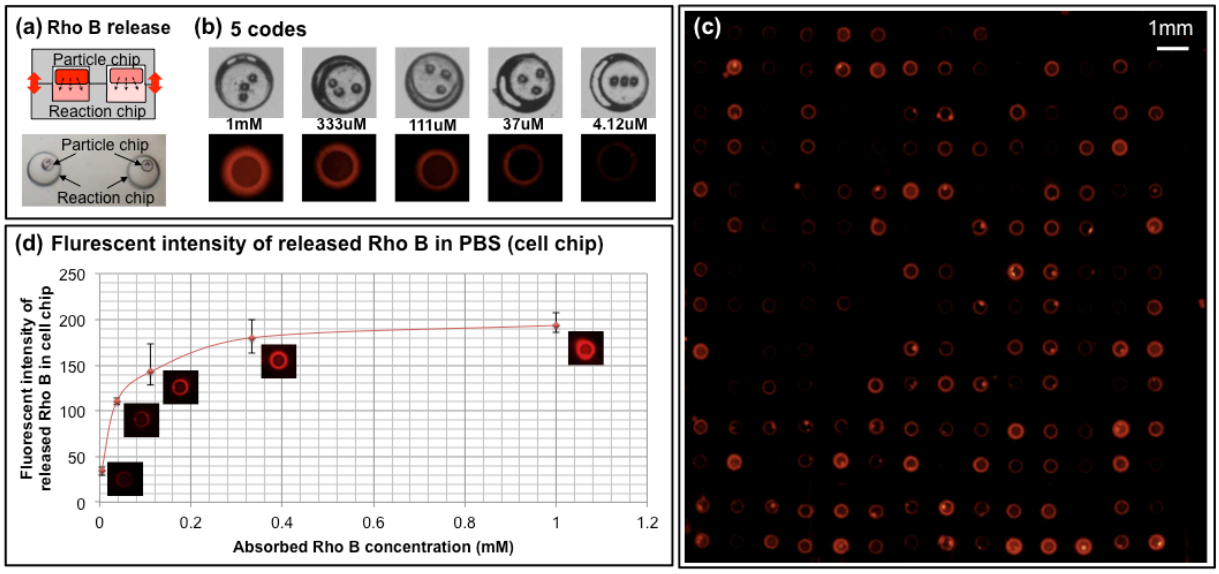
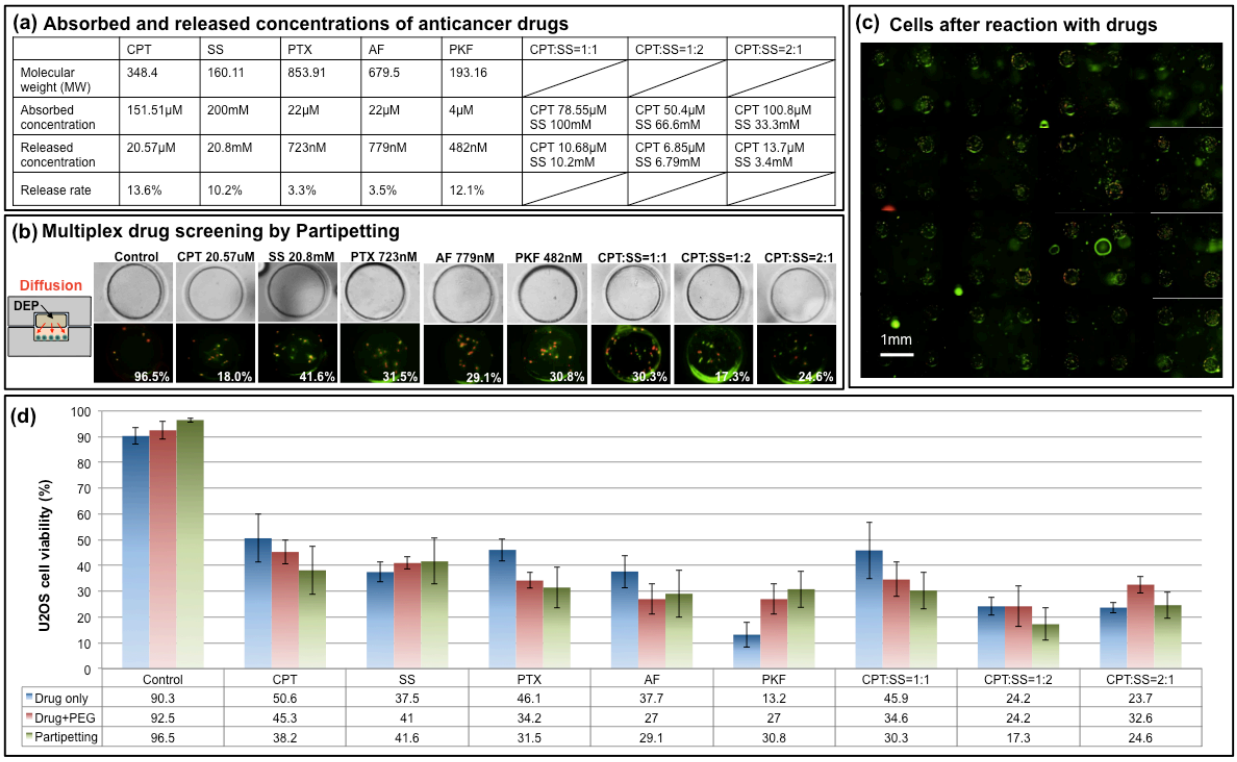


Figure 3 Diffusional drug release in a large area (a) Assembled particle chip and reaction chip. From REP, Rho B is diffused out to PBS in reaction chip. (b) 5 concentrations of released Rho B (c) Reaction chip with 5 concentrations of Rho B dissolved PBS after diffusional Rho B release (d) Fluorescent 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.



	Control	CPT	SS	PTX	AF	PKF	CPT:SS=1:1	CPT:SS=1:2	CPT:SS=2:1
Drug only	90.3	50.6	37.5	46.1	37.7	13.2	45.9	24.2	23.7
Drug+PEG	92.5	45.3	41	34.2	27	27	34.6	24.2	32.6
Partipetting	96.5	38.2	41.6	31.5	29.1	30.8	30.3	17.3	24.6

	CPT	SS	PTX	AF	PKF	CPT:SS=1:1	CPT:SS=1:2	CPT:SS=2:1
Molecular weight (MW)	348.4	160.11	853.91	679.5	193.16			
Absorbed concentration	151.51μM	200mM	22μM	22μM	4μM	CPT 78.55μM SS 100mM	CPT 50.4μM SS 66.6mM	CPT 100.8μM SS 33.3mM
Released concentration	20.57μM	20.8mM	723nM	779nM	482nM	CPT 10.68μM SS 10.2mM	CPT 6.85μM SS 6.79mM	CPT 13.7μM SS 3.4mM
Release rate	13.6%	10.2%	3.3%	3.5%	12.1%			

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

[1] S.E. Chung et al, MicroTAS 2011 (2011).
[2] J. Wu *et al.*, Biomaterials, **32**, 841 (2011).
[3] S.E. Chung, *et al.*, Applied Physics Letters, **91** (2007).
[4] H. Lee and J. Kim *et al.*, Nature Materials, **9** (2010)
[5] S.H. Lee *et al.*, Lab on a Chip, **11**, 4 (2011)

CONTACT

Sunghoon Kwon, +82-2-880-1736 or skwon@snu.ac.kr