Digital Drug Dosing: Dosing in Drug Assays by Light-Defined Volumes of Hydrogels with Embedded Drug-Loaded Nanoparticles

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Digital drug dosing: Dosing in drug assays by light-defined volumes of hydrogels with embedded drug-loaded nanoparticles

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***Abstract*— Polyethylene glycol (PEG)-based hydrogels are widely used for biomedical applications, including matrices for controlled drug release. We present a method for defining drug dosing in screening assays by light-activated cross-linking of PEG-diacrylate hydrogels with embedded drug-loaded liposome nanoparticles in freely definable areas of micro wells.**

I. Introduction

Synthetic hydrogels are widely applied as biomaterials for in vivo and in vitro use [1], with a lot of research focusing on PEG-based hydrogels as easily accessible materials with low non-specific protein attachment. PEG-based hydrogels have often been produced with embedded (bio)molecules for later release, with the caveat that small molecule release typically occurs at short time scales similar to the compound diffusion times in free solution. Encapsulation in nanoparticles may delay or even prevent compound release until a specific trigger signal is applied. Thus, the embedding of drug-loaded nanoparticles into PEG-based hydrogels is appealing for nanoscale compound containment in a macroscale hydrated framework. The use of reactive PEGs provides further design options by enabling spatially selective gel formation through patterned light activation of the cross-linking initiator. Here, we demonstrate the fabrication of a photo-polymerized ´smart` hydrogel for in vitro drug release purposes, where compound-loaded nanoparticles (liposomes) are embedded within hydrogel matrices at defined locations in microwells and the cargo release is induced by applying a proper trigger.

II. Results and Discussion

Hydrogels resulted from near-UV light (410 nm) exposure of an aqueous solution of polyethylene glycol diacrylate (PEGDA) and a previously described photo-initiator [2]. A computer controlled commercial light projector defined the lateral shape of the hydrogel sheets in a 12-well plate (Fig. 1A), while the sheet thickness was controlled by the solution height. Liposomes loaded with calcein (50 µM) were used to evaluate the liposomes integrity when embedded into hydrogels. Free calcein or calcein-loaded liposomes were embedded by addition to the solution prior to light exposure. Fig. 1B (lower row) shows the fast diffusion of free calcein from the hydrogel into the supernatant while the hydrogel containing calcein-loaded liposomes (upper row) remain colored over time. By applying a trigger such as a detergent (e.g. Triton), calcein is released from the liposomes within minutes as revealed by the colored supernatant. The amount of released calcein is measured by recording the fluorescence in the supernatant (Fig. 1C). The liposomes have previously been used as drug releasing system both *in vitro* and *in vivo*, where the chemical trigger for the cargo release is substituted by a cell-compatible signal (e.g. temperature). In summary, the simple arbitrary shaping of PEG-based hydrogels by a commercial projector and the high stability of the embedded compound-loaded liposomes enable digital control of the drug dosing in neighboring microwells and easy scaling to smaller microwell systems (at least 384- and possibly 1536-well plates) for high throughput screening assays.

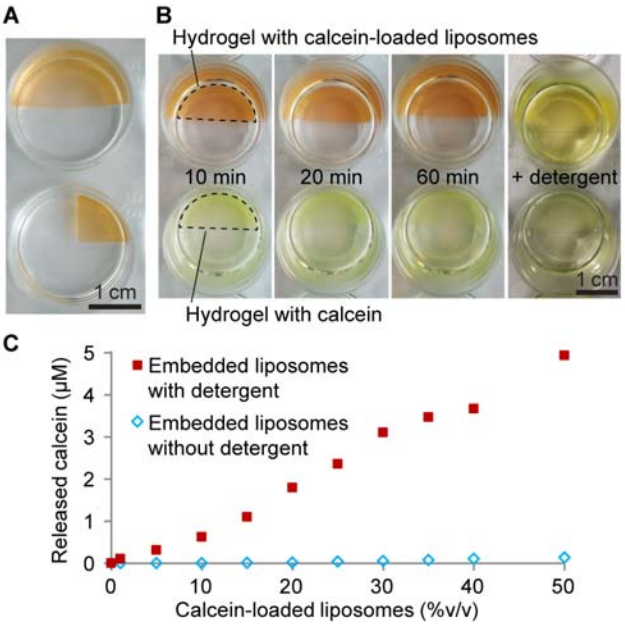

Figure 1. (A) Shaped PEG hydrogel volumes with embedded calcein-loaded liposomes in a 12-well plate obtained after 1 min of light exposure. The absence of green fluorescence is due to calcein self-quenching at high concentrations (50 µM). (B) Calcein release into the supernatant over time: calcein-loaded liposomes (*top*) and free calcein *(bottom)*. (C) Monitoring of calcein release as function of liposome concentration (%v/v) over a period of 8 hours in the presence and absence of 1%w/v Triton.

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