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In-situ NIR-laser mediated bioactive substance delivery to single cell for EGFP expression based on biocompatible microchamber-arrays

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ABSTRACT

Controlled drug delivery and gene expression is required for a large variety of applications including cancer therapy, wound healing, cell migration, cell modification, cell-analysis, reproductive and regenerative medicine. Controlled delivery of precise amounts of drugs to a single cell is especially interesting for cell and tissue engineering as well as therapeutics and has until now required the application of micro-pipettes, precisely placed dispersed drug delivery vehicles, or injections close to or into the cell. Here we present surface bound microchamber arrays able to store small hydrophilic molecules for prolonged times in subaqueous conditions supporting spatiotemporal near infrared laser mediated release. The micro-chambers (MCs) are composed of biocompatible and biodegradable polylactic acid (PLA). Biocompatible gold nanoparticles are employed as light harvesting agents to facilitate photothermal MC opening. The degree of photothermal heating is determined by numerical simulations utilizing optical properties of the MC, and confirmed by Brownian motion measurements of laser-irradiated micro-particles exhibiting similar optical properties like the MCs. The amount of bioactive small molecular cargo (doxycycline) from local release is determined by fluorescence spectroscopy and gene expression in isolated C2C12 cells via enhanced green fluorescent protein (EGFP) biosynthesis.

1. Introduction

Microchambers (MC) allowing smart encapsulation are utilized in a wide variety of applications, including not only drug delivery, but also anti-corrosion, electronics, tissue engineering and food conservation [1]. MCs offer the possibility to encapsulate active components, cells, particles [2] and have potential for drug delivery [3,4]. Presently, MC arrays are assembled either via 3D lithography (via a multistep system of 2D lithographic processes), composite layer-by-layer systems, or polyelectrolyte multilayers (PEM) [1,2,5]. The fabrication of these structures is, despite the availability of layered deposition robots [6], time and work demanding [1,5]. Recent PEM based deposition systems need 40–60 bilayers and require 36–60 h for fabrication [5], while larger structures (mm scale) require up to 1000 bilayers and are fabricated on the time scale of weeks [1]. Such long and complicated fabrication procedures obviate the mentioned uses and advantages, since most industrial and scientific practices prefer cheap (cents) and fast (timescale of seconds) production processes.

Besides the slow fabrication processes, cargo loading and effective sealing is another time consuming step, which is commonly performed on the timescale of dozens of minutes to hours [7]. Entrapment of small molecules (MW $<$ 1000) remains a challenge, especially if biologically benign delivery systems [8,9] are considered. A previously reported biodegradable solid supported drug delivery system was of sub-mm size and only capable of encapsulating polymers or hydrophobic compounds in a quantity of ~0.1–0.5 mg [10]. In case of a power failure, the sealing mechanism ceased to function, causing fatal burst release of the entire payload [10]. Other systems composed out of biocompatible materials were in a similar size range, however utilized non-biologically-benign release stimuli [1,2,5]. Similar problems hold true for dispersed delivery systems [11–13]. Recent works on molecular membrane composition and structure of polymeric encapsulation systems demonstrated the need for hydrophobic wall materials to encapsulate small hydrophilic molecules [14,15]. Polylactic acid (PLA) is a

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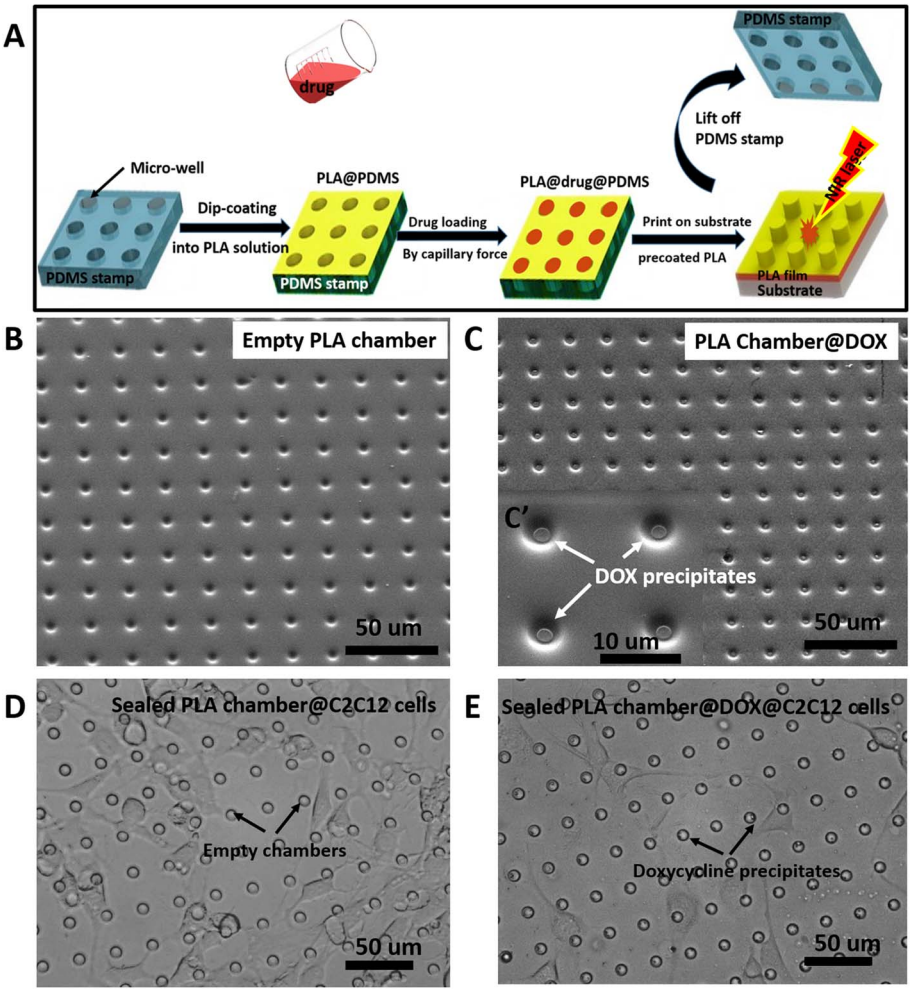

Fig. 1. Preparation method, loading and sealing of PLA MC, PLA@PDMS refers to PLA MCs residing on the PDMS stamp. A) PLA MC arrays are prepared by a single dip-coating step of a PMDS stamp into PLA solution, small hydrophilic molecular cargos like RhB and DOX were loaded (symbolized by beaker with drug solution with which the thin films were loaded) by solution drying only within the MCs. Sealing is performed by transfer printing the MCs onto a flat PLA film while NIR laser is used as the release stimuli; B) empty, C) DOX filled PLA MCs, C' is a magnified section of C showing the DOX precipitate. Mouse myoblast (C2C12) cell growth on top of D) empty and E) DOX filled PLA MCs.

hydrophobic polymer regularly utilized in sealing macroscopic structures due to its outstanding barrier-properties [16]. At the same time PLA offers outstanding biologically-benign and biodegradable properties making it suitable for implants and it is classified as generally recognized as safe (GRAS) by US Food and Drug Administration (FDA) [17]. Recent miniaturized PLA based MC arrays were only shown to allow cargo release with the use of high intensity focussed ultrasound (HIFU) [18,19]. HIFU based release stimuli are unable to address specific cells due to simultaneous opening of hundreds of chambers in an area of 500 × 500 μm^2 [18,19].

Drug delivery to isolated cells remains a challenge. This is especially the case if structures of cells (e.g. every second cell), resting on a solid support are to be targeted. Such applications require until now extensive investments in equipment. In this study, we encapsulate small hydrophilic fluorescence dye Rhodamine B (RhB) and the bioactive antibiotic molecule doxycycline (DOX) within individual PLA MC. These molecules are released via photothermal opening of isolated MCs. The controlled addressing of single target cells allows in addition release from controlled patterns of cells within large cell populations. The effective amount of administered bioactive substance delivered to isolated cells is determined by enhanced green fluorescent protein (EGFP) [20] expression in selected target cells upon DOX exposure, which is controlled via the *tet-on* system in the genetically modified cells. It is also measured by the fluorescence spectra of released RhB.

2. Materials and methods

2.1. Patterned PDMS stamp fabrication

For patterned polydimethylsiloxane (PDMS) stamp (Elastosil RT 602, Wacker, München, Germany) fabrication, the PDMS base and curing agent were mixed at a ratio of 9:1, degassed for 30 min using a vacuum, poured onto the silicon masters and cured for 1 h at 70 °C. The silicon masters were produced via standard optical lithography (Shenzhen semiconductor, Shenzhen, China), whereby spherical pillars were exposed on the silicon masters. Two silicon masters were utilized in this study, both with a pillar height of 4 μm, while silicon master 1 utilized a pillar diameter of 10 μm with a centre to centre distance of 25 μm, and silicon master 2 utilized a diameter of 5 μm with same centre to centre distance (supporting information (SI) Fig. S10).

2.2. PLA microchambers fabrication

Biopolymer PLA (3 mm granule, Mw ~ 60,000) purchased from Sigma, (Merck, Darmstadt, Germany) was used. PLA was dissolved at 1, 2, 3, and 5 wt% (m%) in chloroform. PDMS stamps with microwell arrays on surface were dipped for 5 s into PLA solution and removed at pull-out speeds from 0.5 to 80 mm/s using two homemade electrical devices. Device one is using an air pressure valve [6] withdrawing the PDMS stamp at 80 mm/s, and device two used a computer controlled