BIOCOMPATIBILITY OF OSTE POLYMERS STUDIED BY CELL GROWTH EXPERIMENTS

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ABSTRACT

The recently introduced OSTE polymer technology has shown very useful features for microfluidics for lab-on-a-chip applications. However, no data has yet been published on cell viability on OSTE. In this work, we study the biocompatibility of three OSTE formulations by cell growth experiments. Moreover, we investigate the effect of varying thiol excess on cell viability on OSTE surfaces. The results show poor cell viability on one OSTE formulation, and viability comparable with polystyrene on a second formulation with thiol excess below 60%. In the third formulation, we observe cell proliferation. These results are promising for cell-based assays in OSTE microfluidic devices.

KEYWORDS: Microfluidics, OSTE, Off-stoichiometry thiol-ene, lab-on-a-chip, biocompatibility, cell growth

INTRODUCTION

Compared to traditional plate and well formats of cell-based assays, microfluidic cell-based assays provide a higher degree of microenvironmental control and a higher throughput, permitting a deeper understanding of cell biology and cell interaction, often in a low-cost and simple format [1]. Cell culturing is an example of a promising microfluidic application, in which clever microfluidic designs yield high throughput by small sample volumes, and allow applying stimuli gradients to cell arrays in a single microfluidic chip [1].

PDMS soft lithography is the prevalent polymeric platform for microfluidic fabrication in academia, but long curing times, and lack of bonding techniques compatible with surface biofunctionalization make its industrial application questionable [2]. Moreover, PDMS gas permeability, although often important for cell development, is accompanied by absorption of many small molecules of interest, such as fluorophores and pharmaceuticals, into the PDMS, and subsequent leaching of those molecules after injection leaves an undefined environment for following cell studies [1].

Off-stoichiometry thiol-ene (OSTE) is a novel polymer technology designed for rapid and simple fabrication of labs-on-a-chip for biological and medical applications [3]. OSTE has already shown features such as: a wide range of tailor-made mechanical properties, micro-structuring via soft lithography [3], and/or direct lithography [4], along with robust surface modification [5] and bonding. Moreover, we have previously demonstrated unassisted bonding of OSTE microfluidic layers at low temperature to biofunctionalized materials, enabled by the thiol excess feature of OSTE [6]. In addition, microfluidic integration of OSTE with QCM [7] and with photonic transducers [8] has also been demonstrated. These properties, in combination with the minimum absorption and low leachability of small molecules into and from OSTE, make OSTE a promising candidate material for cell-based assays on-chip [3].

Here, we report the first study of cell viability on the native surface of OSTE polymers, by growth of human kidney and liver cells on the surface.

EXPERIMENTS:

To investigate cell viability on OSTE polymers, we performed two cell growth experiments on three OSTE formulations.

In a first experiment, we studied cell proliferation on two OSTE formulations, with two thiol excess levels for each formulation. The thiol excess levels used were 50 and 70%, yielding formulations with functional group molar ratios of 1:1.5 and 1:1.7 allyl:thiol respectively, with a 0.1 wt% photoinitiator. The two OSTE formulations used were:

- **OSTE 3T4A:** OSTE tri-thiol/tetra-allyl. Thiol: tris[2-(3-mercaptopropionyloxy) ethyl] isocyanurate (Waco Chemical Inc.). Allyl: tetraallyloxyethane (Tokyo Chemical Industry Co.). Photoinitiator: ethyl-2,4,6-trimethylbenzoylphenylphosphinate (BASF AG).
- **OSTE 4T$_1$3A:** OSTE tetra-thiol$_1$/tri-allyl. Thiol: pentaerythritol tetrakis (2-mercaptoacetate) (Sigma-Aldrich). Allyl: triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (Sigma-Aldrich). Photoinitiator: ethyl-2,4,6-trimethylbenzoylphenylphosphinate (BASF AG).

We cast the polymers on polystyrene Petri dishes, with only half of the surface coated with the polymer, leaving an uncoated half of the dish for control of leachability of chemicals from the OSTE (Fig. 1). We prepared two Petri dishes per material, along with two Petri dishes with no coating as control samples. We poured the pre-polymer on one half of a Petri dish (Fig. 1.1). After covering the pre-polymer with a plastic foil to avoid oxygen inhibition of the free radical polymerization, we UV-cured the samples with a collimated NUV light source (wavelength peaks at 365, 405, and 436 nm; 13 mW/cm^2 for 15 seconds) (Fig. 1.2). After curing, we peeled the plastic foil off (Fig. 1.3). Finally, after sterilization with ethanol, we plated HEK293A human kidney cells (Fig. 1.4) in cell medium (DMEM) containing

4.5 g/L glucose, 4 mM L-glutamine and sodium pyruvate, supplemented with 100 U/mL penicillin and 100 µg/mL streptomycin, with 10% Fetal Bovine Serum (FBS). The medium was not exchanged during the experiment.

We cultured the cells for 6 days at 37ºC and 5% CO_2, with visual inspections every 24 hours during the first 3 days, followed by a last inspection on day 6. The inspections were carried out by using an immersion microscope, taking micrographs of 10 equidistant visualization points on each sample and thus yielding a quantification of the density of viable cells.

In a second experiment, we investigated how different levels of thiol excess in OSTE affect cell viability on the surface of OSTE. We seeded human hepatocellular carcinoma cells (HEP G2 cells) on samples containing a third OSTE formulation. The samples featured thiol excess levels of 0, 30, 60, and 90% (i.e. mixed in functional group molar ratios allyl:thiol of 1:1, 1:1.3, 1:1.6, and 1:1.9 respectively), n=6. The OSTE formulation studied was:

- **OSTE 4T$_2$3A**: OSTE tetra-thiol$_2$/tri-allyl. Thiol: pentaerythritol tetrakis (3-mercaptopropionate) (Sigma-Aldrich). Allyl: triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (Sigma-Aldrich). No photoinitiator was used.

The OSTE samples were polymerized in the bottom of the wells of a 96-well polystyrene plate (Fig. 2). We pipetted 70 µl OSTE pre-polymer into the wells (Fig. 2.1) and UV-cured the samples at 254 nm and 900 mJ/cm^2 (Fig. 2.2). Empty wells (uncoated polystyrene surfaces) served as controls. After sterilizing the 96-well plate with ethanol, we seeded the cells in the wells using an α–MEM complete medium which consisted of 10% FBS, penicillin and streptomycin (Fig. 2.3). The medium was exchanged every second day.

After 5 days incubation at 37 °C and 5% CO_2, we stained the cells by adding 200 µl of 2.5 µg/mL Alamar-blue solution [9] to each well (Fig. 2.4) . The plate was then incubated for 3 h. Viable cells reduce the stain into a fluorescent form which has an emission peak at ~590 nm (Fig. 2.5). Non-viable cells do not reduce the stain, leaving it non-fluorescent. Using a fluorometer (FLUOstar Galaxy, BMG Lab technologies) we obtained the fluorescence intensity, acting as a measure for cell viability.

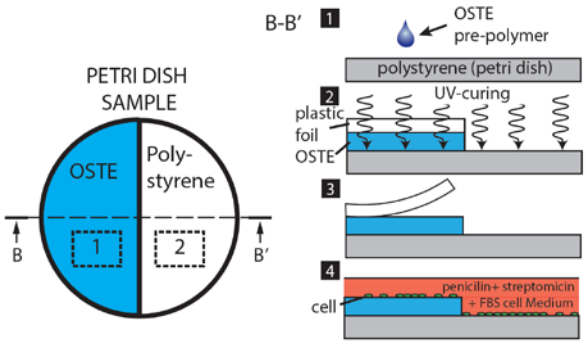

Figure 1. Setup for experiment 1. Left: top view of a polystyrene Petri dish surface with one half OSTE coated and the other half unaltered. Areas 1 and 2 refer to the observation points depicted in Table 1. Right: surface modification protocol. Cells were grown in the Petri dishes for 6 days and inspected daily for the first 3 days.

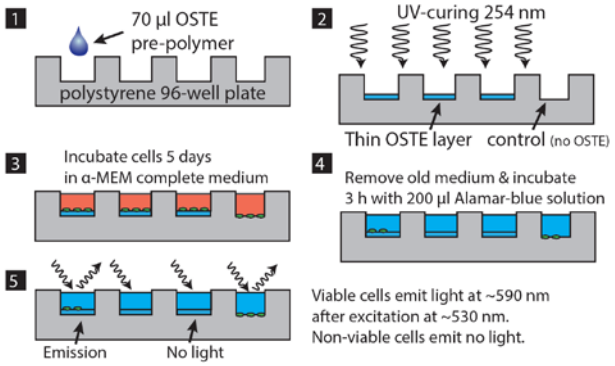

Figure 2. Setup for experiment 2. (1) OSTE was pipetted into the polystyrene 96-well plate. After (2) UV-curing of OSTE, (3) cells were grown for 5 days. The cell viability was obtained via (4) Alamar-blue staining, followed by (5) measurement of emitted light intensity using a fluorometer.

RESULTS:

Table 1 illustrates the HEK293A cell growth in experiment 1. On the OSTE 4T$_1$3A samples, we observed low viability of cells on the OSTE half, slightly higher on the polystyrene half. In contrast, we observed higher cell viability on OSTE 3T4A, similar on both OSTE and polystyrene halves of the samples. However, the rate of cell growth on the polystyrene control samples was twice as fast as on the best of the OSTE samples (OSTE 3T4A), which suggests leaching of uncured monomer or photoinitiator into the culture medium. We observed that cell viability was significantly higher for the samples with 50% thiol excess compared to 70% thiol excess, for both formulations, which supports the hypothesis of larger monomer leaching at higher thiol excess levels. The presence of active thiol groups on the surface could also play a role.

Fig. 3 shows HEP G2 cell fluorescence intensity after 5 days of incubation in experiment 2. The results show that cell viability on OSTE 4T$_2$3A, for thiol excess lower or equal to 60%, is not different from polystyrene.

CONCLUSIONS:

We have performed cell growth experiments on native OSTE surfaces. In a first experiment, we compared HEK293A cell viability on the surface of two OSTE formulations with 50 and 70% thiol excess for each formulation. In a second experiment, we studied the effect of the thiol excess level (0, 30, 60 and 90%) on HEP G2 cell viability on surfaces of a third OSTE formulation.

Cells that were seeded on OSTE 4T$_1$3A showed poor viability for every level of thiol excess that was investigated. The cells grown on this OSTE formulation gradually died after 2 days of culture.

In contrast, by changing the thiol monomer (OSTE 4T$_2$3A) and using no photoinitiator, we observed cell viability on OSTE similar to that on polystyrene, for OSTE samples with a thiol excess lower or equal to 60%.

Furthermore, the formulation called OSTE 3T4A showed cell proliferation for every thiol excess level that was investigated. However, although the cell density was high during all 6 days of culture, the cell viability on both halves (OSTE and polystyrene) of the OSTE 3T4A samples was lower than on control samples. We believe the inferior viability to be caused by leaching of chemicals (uncured monomers or photoinitiator) from the polymer to the cell medium. The presence of active thiol groups on the surface could also play a role.

These results are promising for further cell-based studies using OSTE polymers, where in particular the two materials OSTE 3T4A and OSTE 4T$_2$3A (with low thiol excess values), have been shown to support cell proliferation.

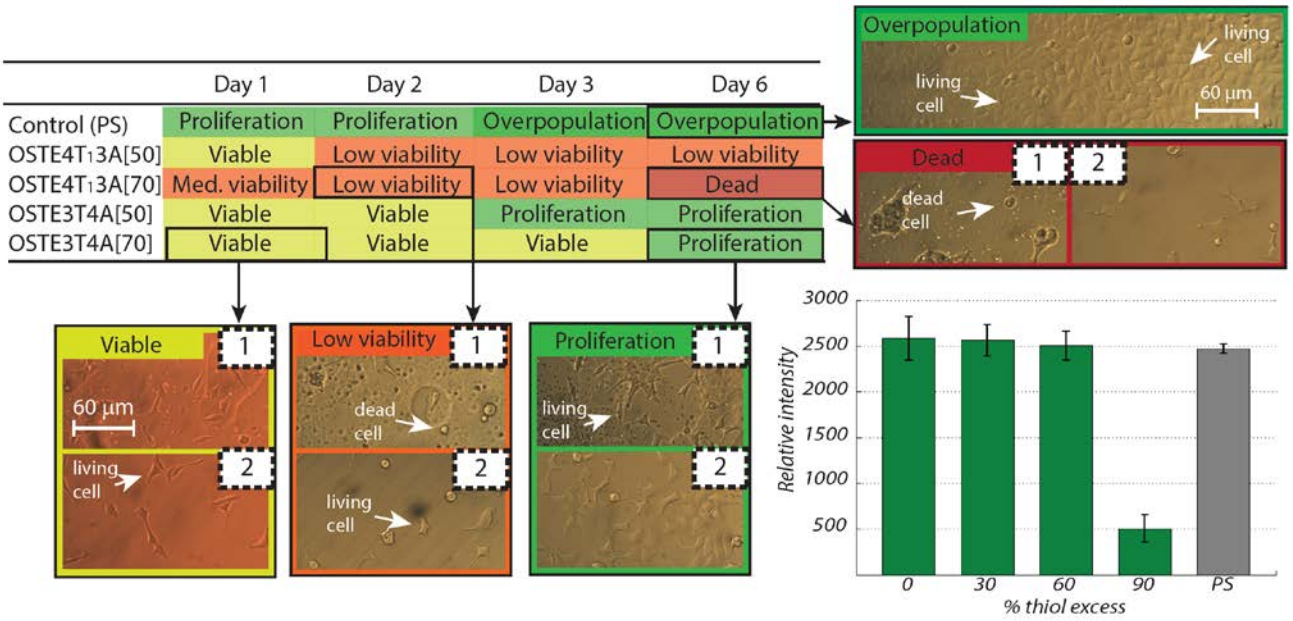

Table 1. Results of experiment 1: OSTE 4T$_1$3A and OSTE 3T4A, both with 50 and 70% thiol excess (numbers in brackets). The linked pictures show representative micro-graphs of the cells during growth. The numbers in squares indicate the site of observation: (1) is the polymer, and (2) the polystyrene. Cells adhering to the surface are living, while round semi-attached cells are either dying (apoptotic) or dividing.

Figure 3. Results of experiment 2 (n=6): Fluorescence intensity from cells grown for 5 days on OSTE 4T$_2$3A with varying level of thiol excess. The intensity from cells on OSTE with ≤60% thiol excess was not different from polystyrene (PS).

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