METHODS FOR ADVANCED CELL CULTURE IN MICROWELLS UTILIZING AIR BUBBLES

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ABSTRACT

We present several novel and unique advanced cell culture methods utilizing air bubbles together with microwells to provide substantial control over cellular microenvironments and organization as well as to demonstrate a method for high throughput drug screening and cytotoxicity assays using collagen film covered microwells.

KEYWORDS: Microwell, Air Bubble, Collagen Film, Hepatocyte, Drug Screening, Cytotoxicity Assay

INTRODUCTION

Nowadays, cells are still routinely cultured in laboratories on flat, two-dimensional (2D) substrates since the introduction of the first cell culture flask in 1923 [1]. These flat, 2D cell culture substrates offer simplicity and ease of use. However, they do not provide a tissue or *in vivo*-like microenvironment for the cells. As a result, a substantial gap is present between the behavior and function of the cells *in vivo* and *in vitro*, which is a serious problem that *in vitro* cell culture is facing today. Although, over the years, a number of other cell culture formats have been developed, such as membranes, roller bottles, spinner flasks, etc., these formats were not designed to offer control over cellular behavior and morphologies, but rather they only provide throughput and scale-up. In recent years, other technologies have been applied to cell culture systems to provide more control over local microenvironments, for example, by using microfluidic technologies to provide well defined diffusion gradients and precise control over metabolites [2-10]. Although these microfluidic devices were able to provide biologically relevant data, microfluidic cell culture devices have not been widely used, which is likely due to the complexity and training required to operate these devices. In this study, we present several novel and unique advanced cell culture methods utilizing air bubbles together with microwells to provide substantial control over cellular microenvironments and organization. We also demonstrate a method for high throughput drug screening and cytotoxicity assays using collagen film covered microwells.

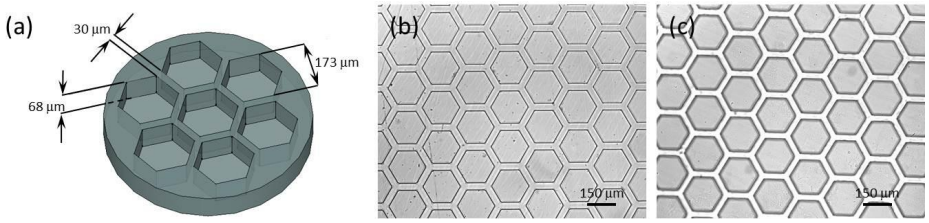

Figure 1: (a) Schematic diagram of a honeycomb microwell substrate. Images of honeycomb microwell substrate fabricated out of (b) poly(dimethylsiloxane) (PDMS) and (c) polystyrene by soft lithography using a photoresist-patterned silicon mold and by hot embossing using a patterned PDMS mold [11], respectively.

EXPERIMENTAL

We chose flat-bottomed hexagonal "honeycomb" microwells as an example to help demonstrate our novel and unique air bubble-assisted cell seeding and culture methods (Fig. 1). Although other microwell formats such as round or flat-bottomed circular microwells could also be used, honeycomb microwells are simple to fabricate with high packing and surface areas. In addition, we designed the honeycomb microwells to be compatible with standard microplate format which allows them to be used with standard laboratory equipment and conventional biological protocols with little to no training required. By combining air bubble mask cell seeding methods in microwells with appropriate surface treatments and materials (Fig. 2), cells can be exposed to different physical microenvironments which can direct them into

drastically different morphologies and behaviors. The presence of air bubbles, which spontaneously formed when polar solvents such as cell culture media are loaded, enables highly controllable cell patterning and organization of seeded cells.

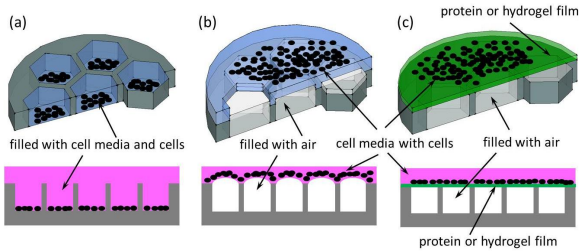

Figure 2: Schematic diagrams of different honeycomb microwell cell seeding methods. Top and bottom panels are three-dimensional (3D) and cross-sectional views, respectively. (a) Filled microwells: media and cells are seeded in honeycomb microwells. (b) Air bubble masked: air bubbles act as a mask for patterning and seeding cells. (c) Film covered: gel film formed on top of honeycomb microwells with the aid of air bubble mask and cells can be seeded on the gel film.

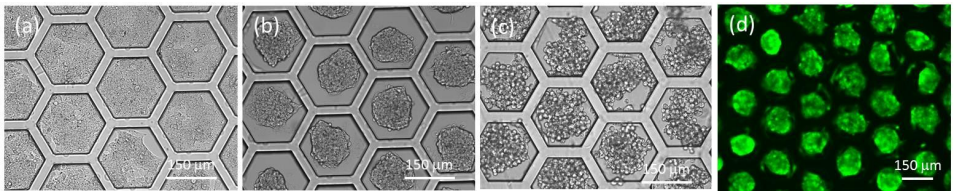

Figure 3: C3A cell cultured in filled microwells. (a) – (c) Bright field and (d) fluorescent images of cells on the bottom of the honeycomb microwells after 72 hours of cell culture. C3A cell cultured in the polystyrene honeycomb microwells (a) that were treated and (b) non-treated with oxygen plasma. (c) Cells embedded in a collagen gel were cultured in the non-oxygen plasma treated PDMS honeycomb microwells. (d) Fluorescent image of cells cultured in (b) stained with calcein-AM to study their viability. Cell seeding density was 30,000 cells per microplate well in all cases.

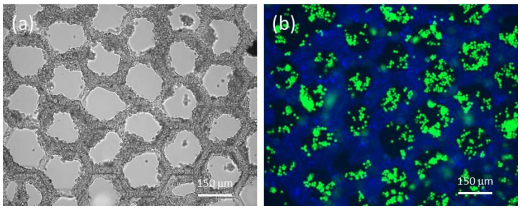

Figure 4: (a) Bright field image of C3A cells cultured on collagen coated top walls of PDMS honeycomb microwells after 24 hours. (b) Top wall-patterned C3A cells were nuclear stained with DAPI (blue) and C3A cells cultured in the honeycomb microwells were viability stained with calcein-AM (green). Top walls of the honeycomb microwells were patterned with collagen using the air bubble masking technique. Cell seeding density for each cell seeding step was 30,000 cells per microplate well.

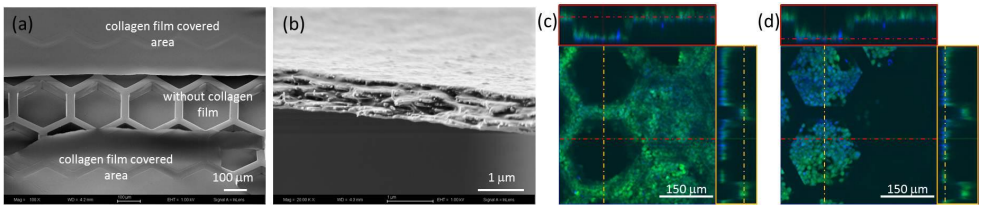

Figure 5: Scanning electron microscope (SEM) images of (a) collagen film covered PDMS honeycomb microwells and (b) a portion of the collagen film. (c) and (d) Confocal images of C3A cells stained with calcein-AM (green) and DAPI (blue). Cells were cultured (c) on top of collagen film and (d) on the bottom of the PDMS honeycomb microwells after 24 hours of cell culture. Cell seeding density was 30,000 cells per microplate well.

RESULTS AND DISCUSSION

We used C3A cells as an exemplary cell model to obtained different cell morphologies (Fig. 3), to patterned first cell networks on the top walls of PDMS honeycomb microwells and subsequent co-culture of second cell type on the bottom of the honeycomb microwells (Fig. 4), and to obtained a cell monolayer on suspended collagen film (Fig. 5). Furthermore, by combining air bubble mask cell seeding methods with suspended collagen film in microwells, we also demonstrate a method for high throughput drug screening and cytotoxicity assays using collagen film covered microwells (Fig. 6 and 7).

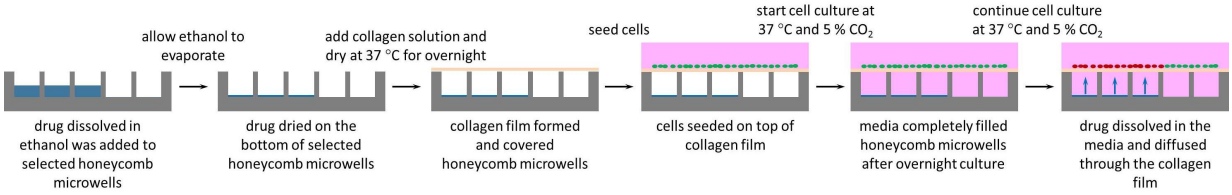

Figure 6: Schematic diagrams depicting the working principle of drug release using suspended collagen film on honeycomb microwells.

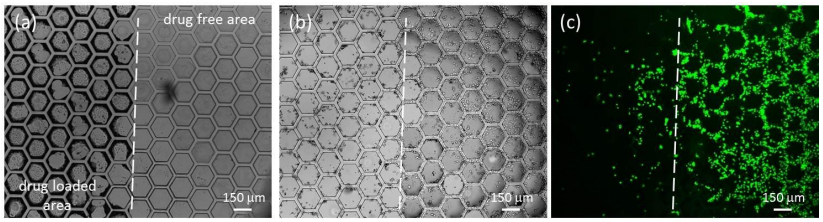

Figure 7: (a) Bright field image of honeycomb microwells after loading and drying of Nefazodone (the drug) on the bottom of selected honeycomb microwells. (b) Bright field and (c) viability stained fluorescent images of C3A cells after 24 hours of cell culture with dried Nefazodone on the bottom of selected honeycomb microwells. The white dashed lines are the boundary between the drug loaded (left) and drug free (right) areas. Cell seeding density was 10,000 cells per microplate well.

CONCLUSION

We demonstrated several novel and unique air bubble mask cell seeding methods for advanced cell culture utilizing air bubbles together with microwells to direct cell behavior, organization, morphology, and function in a number of distinct formats. In addition, these air bubble mask cell seeding methods together with microwells could provide an easy way for high throughput drug screening and cytotoxicity assays as different drug compounds could be pre-loaded and dried in selected microwells and then released during cell culture.

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