COMPOSITE MATERIAL DIAPHRAGM ARRAYS FOR MECHANOBIOLOGICAL STIMULATION OF CULTURED CELLS
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ABSTRACT
This paper reports on a novel device array designed with composite materials intended to enhance cell adhesion to a mechanically active substrate, while maintaining the mechanical properties and fabrication advantages of using PDMS microdevices. Layered polyurethane-PDMS films are suspended over a cavity and distended by the application of a cyclic pressure waveform. The resulting deformation is characterized. Significant improvements in adhesion of primary cells to mechanically active substrates are demonstrated.

KEYWORDS: Mechanical stimulation; substrate deformation; polyurethane; suspended films

INTRODUCTION
Preliminary work presented at MicroTAS 2009 [1] demonstrated an array of PDMS diaphragms distended by cyclic pneumatic pressure. Cells cultured on the diaphragms experienced a cyclic substrate stretch and were tested for mechanobiological response. Polyurethane (PU) was used as the diaphragm material since PDMS only loosely adsorbs matrix proteins while PU covalently binds matrix proteins and improves long-term cell culture [2]. However, the PU material experiences creep under cyclic load, resulting in varying mechanical stimulation being applied to the cultured cells over time. In order to maintain the mechanical properties of the deforming substrate, while enabling long-term culture in a mechanically dynamic microenvironment, we developed composite diaphragms in which cells attach to a thin (< 1 μm) layer of PU bound to a 45-100 μm thick PDMS layer (Figure 1). The PDMS layer provides elasticity and mechanical stability to the diaphragm, while PU enhances matrix protein binding.

Figure 1: Device overview. (A, B) Composite films consisting of a thick layer of PDMS and a thin layer of PU are (A) suspended over an actuation cavity; and (B) distended by the application of a cyclic pressure waveform. (C) High-throughput system consisting of 12 wells, each containing 9 culture films, fabricated on a 3” × 2” glass slide.

EXPERIMENTAL
As shown in Figure 2, composite films are fabricated by dip-coating a dummy slab of PDMS in a 0.5 wt% PU solution in tetrahydrofuran. Uncured PDMS is then spin-coated onto the resulting thin PU layer after oxygen plasma treatment. The composite diaphragm is then cured and plasma bonded to an array of wells connected to a pressure source. The dummy PDMS slab is then peeled away and discarded. The devices can then be sterilized by UV-irradiation and treated with oxygen plasma to covalently bind adhesive matrix proteins to the surfaces.

Strains were characterized using confocal microscopy imaging to track the deformation of fluorescent beads deployed on the device surface (Figure 3). Radial and circumferential strains were calculated based on the following displacement-dependent radial ($\sigma_r$) and circumferential ($\sigma_\theta$) stress functions[3]:

\[
\sigma_r = -\frac{6D}{h^2} \left( \frac{d^2w}{dr^2} + \frac{\nu}{r} \frac{dw}{dr} \right) + \frac{1}{r} \frac{d\phi}{dr}
\]

(1)

\[
\sigma_\theta = \frac{6D}{h^2} \left( \frac{1}{r} \frac{dw}{dr} + \nu \frac{d^2w}{dr^2} \right) + \frac{d^2\phi}{dr^2}
\]

(2)

where $D$ is the flexural rigidity of the circular film, $h$ and $r$ are the film thickness and radius of the films and $w(r)$ and $\Phi(r)$ are functions of the measured deflection and geometric parameters of the film.
RESULTS AND DISCUSSION

Radial and circumferential strain profiles were obtained using analytical models (Eq. (1)&(2)) for the actuation of a 1 mm diameter diaphragm of thicknesses 100 and 45 µm at 6 and 11 kPa respectively (Figure 3; Table 1). By limiting the region of interest (ROI) to a 600 µm diameter circle (Figure 3C), strain uniformity within 1.7% was achieved, at two nominal strain values of 3 and 12%.

The diaphragm deformation times in response to an application of step-pressures (1 Hz) were also determined. The actuation and relaxation time constants were 0.17 ± 0.01s and 0.37 ± 0.01s, respectively (Figure 4A). These parameters allow for a physiologically relevant stimulation profile, consistent with the cyclic loading produced in the cardiovascular system. Diaphragms did not experience observable creep over 100,000 actuation cycles at 12% maximal strain (Figure 4B).

Adhesion of primary fibroblast cells to mechanically active PDMS diaphragms was compared with the PDMS-PU composite diaphragms. Cell adhesion was quantified by counting the number of cells remaining in the ROI (defined in Figure 3), after 24 hours of 1 Hz cyclic stimulation at 12% maximal strain (Figure 4C) using PDMS and PDMS-PU composite diaphragms. After this time period, the number of cells remaining adhered to PDMS-only diaphragms decreased to 20%, while nearly 80% of the cells remained adhered to the PDMS-PU composite diaphragms.

Table 1. Summary of device parameters and characterization results

<table>
<thead>
<tr>
<th>Radius</th>
<th>Thickness</th>
<th>Pressure</th>
<th>Strain (within a 600 µm diameter ROI)</th>
<th>Nominal strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mm</td>
<td>100 µm</td>
<td>6 kPa</td>
<td>Radial: 3.4 ± 0.3 %</td>
<td>~3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Circumferential: 3.4 ± 0.3 %</td>
<td></td>
</tr>
<tr>
<td>1 mm</td>
<td>45 µm</td>
<td>11 kPa</td>
<td>Radial: 12.4 ± 0.2%</td>
<td>~12%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Circumferential: 11.3 ± 1.7%</td>
<td></td>
</tr>
</tbody>
</table>
CONCLUSION

Our data show that mechanically active PDMS diaphragms are poorly suited as cell culture substrates in long-term cell mechanobiology studies. However, by engineering the composite membrane structure described here, we have successfully maintained the ease of fabrication associated with PDMS devices and enhanced the system’s suitability for longer-term mechanically dynamic cell culture.

REFERENCES


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