CELLULAR MICROTISSUES SPONTANEOUSLY FORMED IN A MICROFABRICATED DEVICE FOR ANGIOGENESIS

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ABSTRACT

We report here on a microdevice for the spontaneous and simultaneous formation of microtissues -or microscale spheroids- in a fast, controlled and reproducible way. Spheroids are relevant constructs for tissue engineering applications, and notably for studying the process of angiogenesis which is of crucial importance for both tissue regeneration and understanding/manipulating tumor vascularization. Our microdevices consist of PDMS microwell arrays whose surface properties are optimized (PDMS composition and applied coating agent) for both promoting cellular aggregation into spheroids and facilitating spheroid removal out of the device.

KEYWORDS: Tissue Engineering, Spheroids, Angiogenesis, PDMS microarray.

INTRODUCTION

Angiogenesis -or the sprouting of a vascular bed- is a key-process promoting mass transport and tissue development. Understanding/controlling angiogenesis in the context of a complex tissue microenvironment is crucial (i) for the vascularization of newly engineered tissues (tissue regeneration) and (ii) in oncology to manipulate tumor vascularization. Angiogenesis is classically studied in hydrogel-based cellular constructs using strictly capillary specific cell types [1]. More physiological models such as microtissues are more relevant to study capillary development and the reciprocal interaction with the local environment in normal or pathological conditions [2]. Conventional methods to produce multicellular spheroids (hanging drop method [3], micromass culture [4]) suffer from a number of limitations including (i) poor control and reproducibility, (ii) tedious and time-consuming procedures and (iii) low production yield of microtissues. Figure 1 represents a spheroid prepared from hMSCs (human mesenchymal stem cells) and HUVECs cells (endothelial cells) using the hanging-drop technique to study the process of angiogenesis. The present work aims at (i) developing an alternative methodology for a better and controlled production of spheroids and (ii) subsequently applying it for studying angiogenesis.

EXPERIMENTAL

Microwell arrays are fabricated from PDMS using conventional molding technique against a silicon/SU-8 mold. Various PDMS compositions are used here: 10:0.5, 10:1 and 10:3 prepolymer:curing agent ratios [5]. After curing circular PDMS microchips (Ø 2.1 cm) are cut, placed in 6-well plates and subsequently
coated with various agents. We particularly investigate here BSA (10-50 mg/mL) and PEG (MW 300-35,000; 10-50 mg/mL) coatings, and compared them to negative (no coating) and positive (fibronectin coating) controls. Approximately $10^6$ cells are seeded on every chip. After 1-2 days of culture, resulting spheroids are removed when possible out of the chips.

**RESULTS AND DISCUSSION**

Using a microwell array tissue formation is straightforward and requires reduced amounts of cells and biological factors. We investigate here the material properties of the microarray for firstly promoting the formation of spheroids.

**Table 1. Preparation of microtissues in coated PDMS microwells: cellular aggregation vs. adherence depending on the PDMS composition and the coating.**

<table>
<thead>
<tr>
<th>Coating PDMS composition</th>
<th>Ø</th>
<th>Fibronectin</th>
<th>BSA 10 mg/mL</th>
<th>BSA 50 mg/mL</th>
<th>PEG 300 10 mg/mL</th>
<th>PEG 35,000 50 mg/mL</th>
<th>Agarose</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:0.5</td>
<td>Adherence + Aggregation</td>
<td>Adherence ++ Aggregation</td>
<td>Adherence +++ Aggregation</td>
<td>Adherence +++ Aggregation</td>
<td>Adherence No Aggregation</td>
<td>Adherence No Aggregation</td>
<td></td>
</tr>
<tr>
<td>10:1</td>
<td>Adherence + Aggregation</td>
<td>Adherence ++ Aggregation</td>
<td>Adherence +++ Aggregation</td>
<td>Adherence +++ Aggregation</td>
<td>Adherence + Aggregation++</td>
<td>Adherence + Aggregation++</td>
<td></td>
</tr>
<tr>
<td>10:3</td>
<td>Adherence +++ Aggregation</td>
<td>Adherence +++ Aggregation</td>
<td>Adherence +++ Aggregation</td>
<td>Adherence +++ Aggregation</td>
<td>Adherence + Aggregation+++</td>
<td>Adherence + Aggregation+++</td>
<td></td>
</tr>
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Both the composition of the PDMS and the nature of the coating greatly influence cellular adherence and aggregation, as indicated in Table 1. The results range from strong to no adherence on the surface, and cellular assembly from isolate cell “suspension” to extensive cellular aggregation. Best efficiency in spheroid formation is observed with a 10:0.5 PDMS composition and a 35,000 MW PEG coating. PDMS 10:0.5 gives the lowest cellular adherence while 35,000 MW PEG at 50 mg/mL promotes cellular aggregation into microtissues.
Figure 3: Photograph of the microwell array after seeding of cells (hMSCs) at a density of $10^6$ cells/chip.

Figure 4: Photograph of the microchip 36 h later: cells have aggregated into spheroids nicely sitting in the wells.

The size of the resulting spheroids is tunable, from 25 to 100,000 cells, depending on the size of the microwells and the cell seeding density and it lies in the hundreds of micron range which is more suitable for imaging purposes. We also investigate here the removal of the spheroids from the wells for further off-chip studies of the microtissues. This is successfully achieved after up to 1.5 day of culture on the chip, with an efficiency of < 50% for PBS-treated (negative control) and > 90-95% for BSA (10 mg/mL) or PEG (MW 35,000; 50 mg/mL) coatings.

CONCLUSIONS

We describe a novel methodology using a microwell array for the spontaneous formation of a large amount of spheroids. We are currently investigating the preparation of hybrid spheroids based on a co-culture of hMSCs and HUVECs for studying angiogenesis and the formation of a vascular network, after eventual stimulation with biological factors. On other aspects, till now our studies focused on coated PDMS but we are investigating other materials to suppress the use of a coating.

REFERENCES


