MICROFABRICATION OF SHAPED MM-SCALE TISSUES TO STUDY VASCULAR DEVELOPMENT USING A MODULAR BOTTOM-UP APPROACH

N.C. Rivron,[1] S. Le Gac,[2] E. Vrij,[1] J. Rouwkema,[1] D. Wijnperlé,[2] R. Truckenmuller,[1] A. van den Berg[2] and C.A. van Blitterswijk[1]

[1] Tissue Regeneration, Institute for Biomedical Technology and
[2] BIOS Group, MESA+ Institute for Nanotechnology,
THE NETHERLANDS

ABSTRACT

Recapitulating developmental mechanisms *in vitro* necessitate models of intermediate complexity, between simple 2D culture and complex *in vivo* models, which integrate both physical and molecular cues. Here, we describe a cheap and simple bottom-up microfabrication method to build 3D millimeter-scale tissues with geometric shapes. These tissues are suitable for long-term culture in tissue-based assays and as implants for clinical applications. In a case study, we recapitulate some mechanisms of vasculogenesis, the assembly of capillary blood vessels.

KEYWORDS: Tissue engineering, microwell array, vasculogenesis, self-assembly

INTRODUCTION

In vitro investigation of developmental mechanisms requires the use of novel, relevant and flexible tissue models. These tissues must present an intermediate complexity, integrate cues that are found *in vivo*, such as physical and chemical cues and must be built in a controlled way. In that context, we propose a novel, cheap and versatile methodology for the production of mm-sized shaped tissue models for both tissue engineering and clinical applications. Tissues are prepared using a bottom-up approach via the modular assembly of micrometer-sized building blocks into larger pieces of tissues (fig. 1).

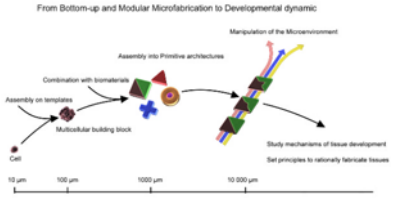

Figure 1: The bottom-up approach. Multicellular building blocks are used to build primitive compartments prone to remodeling.

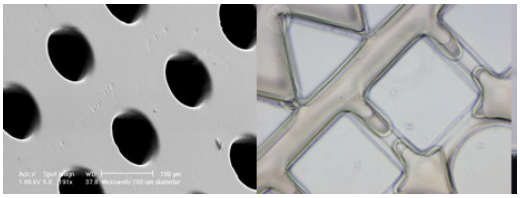

Figure 2: Molding tools for the bottom-up approach. Left: enlarged view of a microwell array (100 µm diameter); right: agarose mold for the preparation of mm-sized shaped tissues.

EXPERIMENTAL

Chip fabrication. Microwells arrays are fabricated in agarose with diameters of 100-400 µm and depth up to 300 µm. A first silicon mold is fabricated using Deep Reac-

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tive-Ion Etching and used for the production of a second PDMS mold which is subsequently employed to create 4% agarose microwell arrays. Shaped wells arrays are fabricated in agarose with a projection area of 10^6 and 2.5 10^5 μm^2.

Tissue production and retrieval. A highly concentrated cell suspension (hMSCs, HUVECs, C2C12, mESC, mEF, CHO) is seeded directly on the microwell array and accumulated by gravity into the wells. After 1-2 days, microtissues are flushed from the microwell array, filtered, centrifuged, resuspended and plated on the shaped well arrays. Tissues are cultured in their respective mediums.

Angiogenesis studies. Vasculogenic assays are performed as previously described [3]. Briefly, microtissues of hUVEC and hMSC are formed separately on the microwell array and seeded in a 1:1 ratio in a shaped well array.

RESULTS AND DISCUSSION

Microtissues are firstly massively produced using microwell chips that fit in standard Nunc well-plates (fig. 2). Every chip integrates up to 20,000 independent wells for the spontaneous and simultaneous preparation of large amounts of reproducible microtissues [1]. These tissues form in the individual wells typically within 24 hours after seeding the cells on the microchip (fig. 3), and their size (30-250 µm) is tunable by adjusting the well size (diameter) and the cell seeding density (fig. 4). Besides, the microtissues can easily be retrieved from the chip (fig. 3) for their further utilization or characterization. We have demonstrated the production of building blocks using various cell lines: hMSCs, HUVECs, C2C12, mESC, mEF, CHO. These different cell types exhibit distinct assembly behaviors in the microwells, with the formation of pancake-shaped or spherical microtissues and with various levels of condensation.

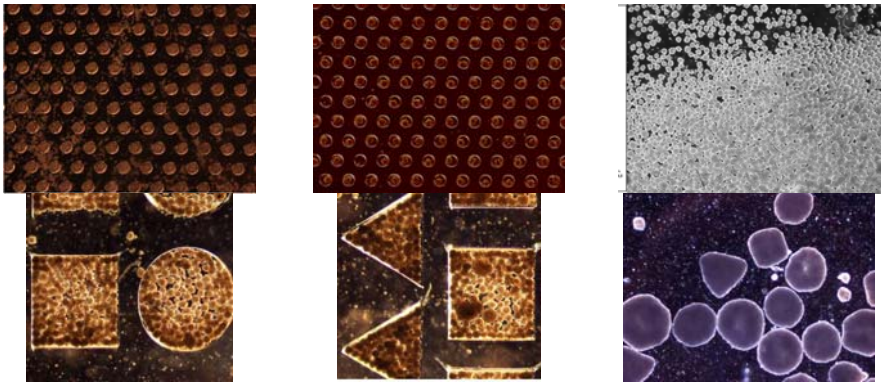

Figure 3: Bottom-up formation of mm-sized shaped microtissues; from left to right and from top to bottom: (1) seeding of cells in a microwell array; (2) assembly of cells into well-defined µm-sized tissues; (3) microtissue retrieval out of the microwells for further utilization; (4) microtissues seeding in larger structures; (5) microtissue self-assembly in larger pieces of tissues; (6) resulting shaped mm-sized tissues that can easily be manipulated

The second step in our modular approach is the formation of mm-sized tissue using these building blocks. After retrieval from the microwell chips, micrometer-sized tissues are seeded in mm-sized structures where they assemble into larger and shaped tissues (up to 1 mm^3) within a day (fig. 3). These large tissues are stable and can be kept under culture for up to several weeks for the investigation of long-term processes such as differentiation and the formation of complex tissues.

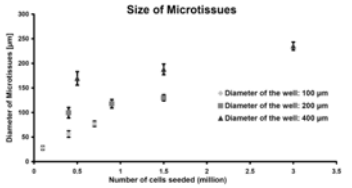

Figure 4: Production of microtissues with a tunable size; use of microwells with a 100-400 µm diameter and cell seeding density of 0.1-3 millions.

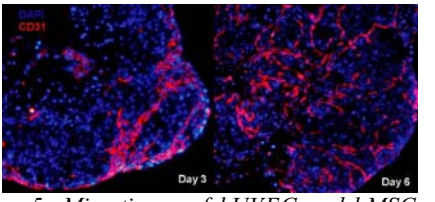

Figure 5: Microtissues of hUVEC and hMSC segregate according to the differential adhesion hypothesis (DAH) [4] (left) and later support the sprouting of a network typical of vasculogenesis [3].

We focus here on vascular development and the assembly of capillary blood vessels or vasculogenesis. Subsequently, for the second assembly stage, we use a mixture of microtissues made from HUVECs and hMSCs cells [3]. Interestingly, while building blocks had been randomly mixed before seeding, a process of cellular re-organization is clearly observed in the tissue: at day 3, HUVECs cells are forming the outside layer of the tissue while hMSCs are located inside; and at day 6, HUVECs cells invade the core of the tissue where they form a primitive capillary network (fig. 5) which is typical of vasculogenesis [3].

CONCLUSIONS

We present a simple, cheap, yet versatile method to produce mm-sized tissue units for tissue-based assays and clinical applications. We demonstrate the possibility to culture different cell types on a long term and to study vasculogenesis, the assembly of blood capillaries. This versatile method is a powerful way to develop more complex *in vitro* tissue models in a bottom-up approach. It proved useful to recapitulate mechanisms of vascular development and thus build pre-vascularized implants.

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