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Shaping living tissues using microfabricated structures

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ABSTRACT

In this paper, we present a technique for shaping the morphology of living Multi Cellular Tumour Spheroids (MCTS) by using micro-engineered structures. To that aim we created biocompatible, high aspect ratio, polydimethylsiloxane (PDMS) microstructures, that are compatible with the size of MCTS. We show that these microstructures can conform MCTS into pre-determined shapes by confining them, under cell culture conditions. This moulding of a living growing tissue by PDMS microstructures needs optimization of surface conditions, in order to favour the wetting of the mould by the cells, while preventing their attachment to the confining structures. An adequate treatment of PDMS with non-adhesive coating turned out to be mandatory for combining a successful shaping and a soft unmoulding of the engineered tissue. After unmoulding, the tissues are still viable and can be further cultured. Our work demonstrates that very sharp corners can be moulded on originally spherical aggregates of cells and that freestanding cell architectures of arbitrary shapes can be generated. This bio-moulding process can therefore be used for the investigation of the rheological properties of tissues as well as morphogenesis mechanisms by measuring the post-moulding evolution of the engineered shape.

1. Introduction

Shaping tissues into structures that mimic their in vivo architecture is the core of tissue engineering. On one hand, the motivation is mainly driven by the application of such processes for regenerating in vivo damaged tissues. The ultimate goal would be to tune the whole morphogenesis of a functional tissue or organ by providing a patterned scaffold, capable to control the architecture and fate of cells. Moreover, tissue engineering offers a very interesting tool to study drug permeability [1], and mechanical properties. On the other hand, the need for designing a 3D cellular environment, for fundamental investigations of cell and tissue biology, has been very well identified. Indeed, conventional cell culture on 2D substrates elucidated the inner mechanisms of adhesion and mechano-sensing of cells. However, the alarming fact that cells in vivo do not experience 2D but 3D cell–environment interactions, questions the real significance of these methods in living conditions and call for new methods of investigation, in 3D cell culture [2]. As an example, it is known that during morphogenesis many cellular rearrangements occur and it is believed that the mechanical and spatial factors contribute in deciding cell fate. It has been found that tissue geometry can affect cell differentiation patterning [3] and cell proliferation [4]. The local curvature of a cell tissue has been also shown to play an important role and therefore, it seems interesting to engineer in vitro cell tissues where arbitrary shapes and local curvatures can be imposed. This could open a new methodology for a better understanding on the mechanisms by which shape cues are used by tissues for generating reproducible architectures.

The fabrication of tissues in different shapes could be achieved using different techniques. Scaffold based techniques rely on using three dimensional matrices made of solid polymers or hydrogels, containing growth factors and peptides, on which cells attach and take the shape of the scaffold [1,5–7]. Conversely, scaffold free techniques, often called bio-printing have also been developed. They are based on the manipulation of spheroids as building blocks. Spheroids are multicellular cell aggregates naturally exhibiting a spherical shape and they can be produced in different sizes and from different cell lines. Their use in bio-printing allowed the formation of tubular structures and vascular branched trees by spheroid fusion [8,9]. Lithographic and microfluidic based techniques have also been implemented for forming complex 3D tissues [10–12]. In this paper we present an alternative method

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for moulding 3D living tissues with very precise shapes. We show that very sharp corners with very low curvature radii can be formed without altering the viability of the cell aggregate. The basic idea of bio-moulding, shown in Fig. 1, is to grow a multi-cellular spheroid inside a PDMS confining structure serving as a mould. After unmoulding, the result is a perfectly shaped cell-aggregate that is very much different from its original spherical shape. We believe that this process is complementary to the set of methods previously described and may open new perspectives for investigating "shape-sensing" inside tissues.

2. Materials and methods

2.1. Fabrication process of the confining PDMS microstructures

The PDMS structures used in this study were obtained by casting PDMS (10:1 ratio of pre-polymer:reticular agent) (Sylgard184) inside a resist master mould, (5 h at 80 °C). The main challenge of this fabrication process was to produce PDMS structures exhibiting a height comparable with the spheroids to be moulded. Our method of fabrication, previously described in [13], involves UV-photolithography on a 300 μm thick layer of SU-8 photoresist followed by the replication of high aspect ratio PDMS structures. The final PDMS structures used in this work were 300 μm high and are made of pillars of 1:10 aspect ratio, square confining walls of 100 μm thickness and mixed microstructures including pillars of 100 μm diameter and walls of 100 μm thickness with a spacing of 30 μm, all compatible with cell culture experiments.

2.2. Surface treatment of the PDMS microstructures

In order to learn how cell adhesion influences our bio-moulding process, we have used two kinds of PDMS surface treatments, in the purpose of enhancing cell attachment to PDMS or inhibiting it. To change PDMS surface to adhesive, it was firstly exposed to oxygen plasma for 30 s at 200 W and incubated with a fibronectin (Sigma) solution at 40 μg/ml for 1 h at room temperature and then rinsed with Di-ionised water to remove excess fibronectin. To change PDMS surface to anti-adhesive, we used Pluronic® F-127 (Sigma). This triblock amphiphilic copolymer is known to prevent cell adhesion thanks to a Poly Ethylene Glycol chain on one side and strong attachment to PDMS on the other side through hydrophobic interactions. We prepared this compound at a concentration of 2% in Di-ionised water at 4 °C. The PDMS chip, directly after unmoulding, was incubated in this pluronic solution for 30 min at room temperature, followed by a removal of the solution and rinsing in Di-ionised water. For both surface treatments, the culture medium is added after the final rinsing step, followed by spheroid deposition inside the structures.

2.3. Principle of the bio-moulding process

Colon cancer cells (HCT116) and mammary cancer cells (MCF7) spheroids are produced in poly-HEMA coated 96 well plates. Three days following the seeding of the cells, spheroids of 300 μm are formed. They are then deposited one by one in individual microstructures by a simple deposition using the micropipette or a gentle handling with a microtweezer. Spheroids are then left to grow under cell culture conditions at 37 °C and 5% CO_2 for a few days period, depending on the spheroid's rate of growth. During growth, the spheroid will get in contact with the PDMS confining microstructures and upon 2 days, it will occupy the confinement space. The PDMS structures then shape the spheroid without inducing any damage, toxicity or any trace of cell death. When the spheroid is structured by the PDMS microdevice, it can be removed by lifting it gently from the bottom using a microtweezer. As well, depending on the size and shape of the PDMS structure, for example in the case of a square PDMS microdevice (Fig. 3a), the spheroid can be directly pipetted out of the microdevice using a micropipette. The structured spheroid maintains its shape right after the removal from the microdevice, as cells do not attach to the PDMS surface. The resulting tissue contains the imprints of the PDMS microdevice as seen clearly in the SEM (scanning electron microscope) image of Fig. 1.

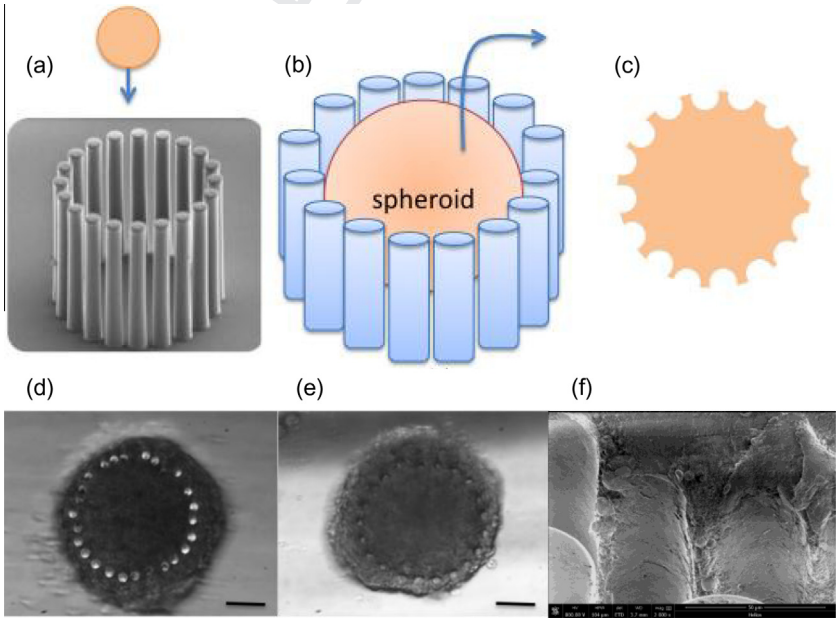

Fig. 1. Principle of bio-moulding living spheroids. (a) A spheroid is placed at the centre of a confining PDMS microdevice, (b) under cell culture conditions, the spheroid grows and gets in contact with the microstructures, (c) the living spheroid, moulded by the microstructures is then removed from the device, (d) optical microscopy image showing a spheroid of HCT116 cells growing in the microdevice, (e) a bottom view of the spheroid after removal from the device showing the imprints of the pillars, (f) a SEM image showing the clear imprints of the pillars on the surface of the spheroid (scale bar: 100 μm).

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