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Macroporous microcarriers for introducing cells into a microfluidic chip

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Macroporous gelatin beads (CultiSpher™ microcarriers) provide a convenient method for rapidly and reliably introducing cells cultured *ex situ* into a microfluidic device, where the spheres create a 3D environment for continued cell proliferation. We demonstrate the usefulness of this technique with a proof-of-concept viability analysis of cardiac cells after treatment with doxorubicin.

Toxicity research using animals is being replaced by stem-cell-derived *in vitro* toxicity assays (IVTA), which is encouraged by industry, for reducing costs in pharmaceutical development, by regulatory agencies, for improving safety, and by animal-welfare organisations, for reducing the number of research animals required.[1,2] Access to improved IVTA utilising human organ-specific cell types has accelerated this development. Contrary to other IVTA, the microfluidic devices offer precise temporal and spatial control of the biochemical environment and the possibility to mimic physical effects found *in vivo*, for example shear forces or material elasticity,[3] as well as efficiency due to the minimal quantities of both cells and reagents required.[4]

Growth of surface-dependent cells on porous and non-porous beads is a well-proven technology for culturing large volumes of cells in suspension, providing significant surface area for cell adhesion.[5-8] Macroporous gelatin beads have previously been used to culture cardiomyocytes,[5] but, to our knowledge, this type of support has not been reported as a construction element in a microfluidic device.

The microfluidic system was fabricated in PDMS with common soft-lithographic techniques[9] and contains a central 4500x300x200 µm^3 cell-culture area lined with pillars to prevent microcarriers (MCs) from escaping through the adjacent media perfusion channels. As seen in Fig.1, the reactor is constructed of two pieces, with the culture chamber in the lower part and channels for media perfusion in the upper part. Holes for fluidic connections were punched in the upper part and the two halves were plasma treated, aligned, sealed, and thereafter baked at 80 °C for 10 min to complete bonding. The cell culture media (CCM) inlet was equipped with a bubble trap employing the gas permeability of a 500 µm-thick PDMS membrane to release gas while CCM continues through the microfluidic system underneath.

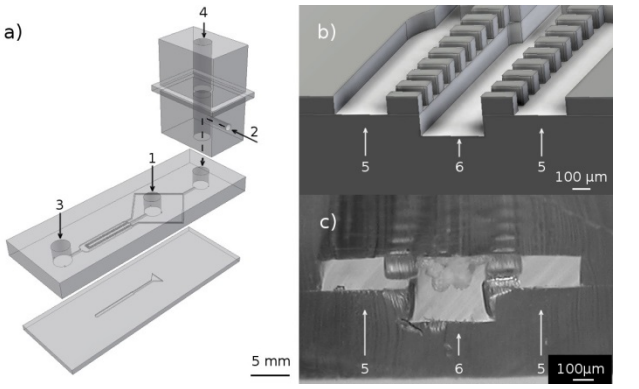

Fig. 1 (a) Microfluidic system for studying cells cultured *ex situ* in gelatin spheres. The cell-laden spheres are introduced through the inlet channel (1). Cell media was flown into the system through port 2, and out through port 3. Port 4 was used to remove any gas bubbles. Media channel (5) and cell culture chamber with spheres (6) shown in cross-sectioned drawing (b) and micrograph (c) of reactor.

90% of standard CultiSpher™ MCs (Percell Biolytica AB, Sweden) have a diameter of 130–380 µm. To reduce the required reactor ceiling height, hydrated carriers were sieved first with a 104 µm stainless-steel mesh. Small carriers were then removed using an 80 µm–wide mesh resulting in carriers ranging between about 80–104 µm in diameter. The MCs were washed twice with PBS and twice with cell culture media before use.

Embryonic chicken cardiomyocytes (ECCM), used up to passage five, were seeded together with MCs in a culture flask at a ratio of 250 cells/CM, and cultured for 3 days. All cell work was performed at 37 °C, 5% CO_2 using Dulbecco's Modified Eagle Medium (Gibco) supplemented with 10% Foetal Bovine Serum, non-essential amino acids, and penicillin-streptavidin.

Before cell introduction, the microfluidic devices were autoclaved and perfused with ethanol and CCM, each for 30 min. The devices were visually inspected by video microscope (LabSmith SVM340) to verify that the beads packed uniformly, as shown in Fig. 2.

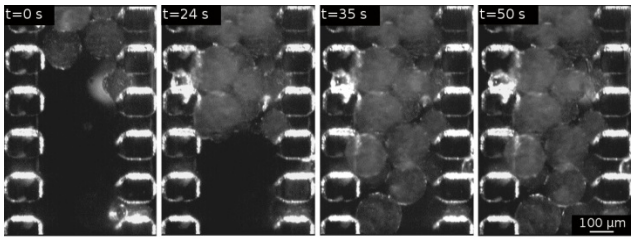

Fig. 2 Sequence of four images showing the microcarriers captured by PDMS pillars (squares at edges of each image) as they fill the device. These images are oriented so that the cell carriers and media flow from bottom-to-top.

Three reactors were prepared and filled with ECCM precultured with MCs in the same cell culture flask. Cells were injected by hand using a syringe at an estimated rate below 500 µL/min, demonstrating the robustness of the cell constructs. The viability of the cells was determined using a Live/Dead Assay (LDA) (Molecular Probes) with calcein AM and ethidium homodimer-1 diluted in PBS to 2 µM and 4 µM, respectively. The assay reagents were introduced into the reactors via the CCM inlet for 30 minutes, Fig. 3a shows the resulting confocal microscope image (Zeiss Axio Observer.Z1, LSM700) in one of the reactors directly after the cells were introduced. Live cells on the surfaces of the MCs appear green due to esterase activity in the cytoplasm. The remaining two reactors were incubated (37 °C and 5% CO_2, CCM provided at 1 µL/min) for 4 days. One was then stained with the LDA and visualized as shown in Fig. 3b, where the edges of the MCs are not seen as clearly, indicating that the cells continued to grow both into and between the MCs. The final reactor was exposed to 20 µM doxorubicin for 15 hours, followed by the same LDA stain. The lack of green dye and abundance of red dye attached to the DNA of the dead cells in the image in Fig. 3c demonstrate that the cells did not survive doxorubicin exposure.

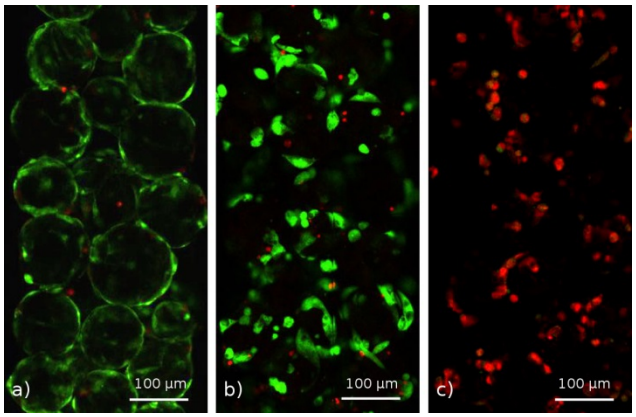

Fig. 3 Confocal image of Live/Dead-stained ECCM in microcarriers cultured for three days before introduction into the microsystem. Green marks the cytoplasm of living cells while red indicates the DNA of dead cells. Microreactor visualized when staining performed (a) directly after introduction of cell construct, (b) after four days of microsystem culture, and (c) after four days of microsystem culture and exposure to 20 µM doxorubicin for 15 h before imaging.

Conclusions

We present a robust method to culture and rapidly introduce cells into a microfluidic system using macroporous gelatin MCs. By allowing cells to attach to MCs *ex situ* before injection into microfluidic devices, the cells can be produced in relatively large and homogenous batches that fill many reactors, providing tremendous flexibility in terms of cell handling and parallelization, while reducing the time required for on-chip culturing and any subsequent variations and complications that may arise. Furthermore, this method introduces the cells in a format that makes them easy to trap in a microfluidic device and provides a 3D environment for their growth and study.

Although we have simply shown the applicability of this strategy with primary ECCMs and the anti-cancer therapeutic doxorubicin, the reported use of similar carriers in macroscopic systems suggests that our method should also be appropriate for studies with human cells. Even primary cells, including keratinocytes[10] and myoblasts[11] have reportedly been seeded on CMs at a density of 50 and 250 cells/bead, respectively. Furthermore, the system offers considerable opportunity to study more complex systems such as cocultures (mixed beads) or stratified systems in which beads populated with varying cell types are introduced sequentially into the microfluidic system.

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Notes and references

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