AN AUTOMATED AND MULTIPLEXED MICROFLUIDIC BIOREACTOR PLATFORM WITH TIME-LAPSE IMAGING FOR CULTIVATION OF EMBRYONIC STEM CELLS AND ON-LINE ASSESSMENT OF MORPHOLOGY AND PLURIPOTENCY MARKERS

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ABSTRACT

An automated and multiplexed microfluidic bioreactor platform with time-lapse imaging for cultivation of embryonic stem (ES) cells is reported. Media temperature is controlled in the storage container and before entering the microbioreactors. Furthermore, the temperature in the microbioreactor is regulated to maintain cells at culture temperature over prolonged periods of time. Flow control is implemented in the platform to vary flowrates and to switch between two different media bottles in one experiment. Morphology and pluripotency of mouse embryonic stem (mES) cells were monitored with the integrated time-lapse imaging.

KEYWORDS: Time-lapse imaging, microbioreactor, embryonic stem cells (ESCs), cell culture monitoring

INTRODUCTION

Data-rich experimentation requires monitoring and controlling of important process parameters. Various input parameters such as media composition, medium replacement times and temperature affect the proliferation or differentation of embryonic stem (ES) cells. Output from the cells, for example changes in their morphology and pluripotency/differentiation needs thus to be monitored/controlled to correlate the results with the tested parameter. To monitor and control all these input and output parameters, particularly for long-term experiments, automated fluid handling, time lapse imaging and tight control of the microenvironment are required. Different systems have been developed, which address the optical monitoring, but do not include automated liquid handling. For example, a microfluidic culture system compatible with plate readers has been recently reported to measure fluorescent intensities [1]. However, analysis of cell morphology, a daily routine task in cell culture, has to be carried out on a microscope. A charged-coupled device (CCD) has also been directly attached to a microfluidic cell culture device for counting cells [2]. For high quality imaging, a microfluidic culture chamber with a transparent heater has been implemented on an inverted microscope to monitor HeLa cell cultures at defined temperatures [3]. We present a multiplexed microfluidic platform which combines automated brightfield and fluorescent imaging with flow control. This will allow the on-line study of the complex and dynamic cellular processes, under controlled culture conditions.

EXPERIMENTAL

The platform consists of a 'microscope module' and a 'media handling module', both automated via a LabView™ routine as shown in Figure 1A. The microscope module includes three microfluidic bioreactors, a flow splitter to feed the three microfluidic bioreactors, a media preheat element to heat up cooled media and a box for the temperature control of the microbioreactors as shown in Figure 1B.

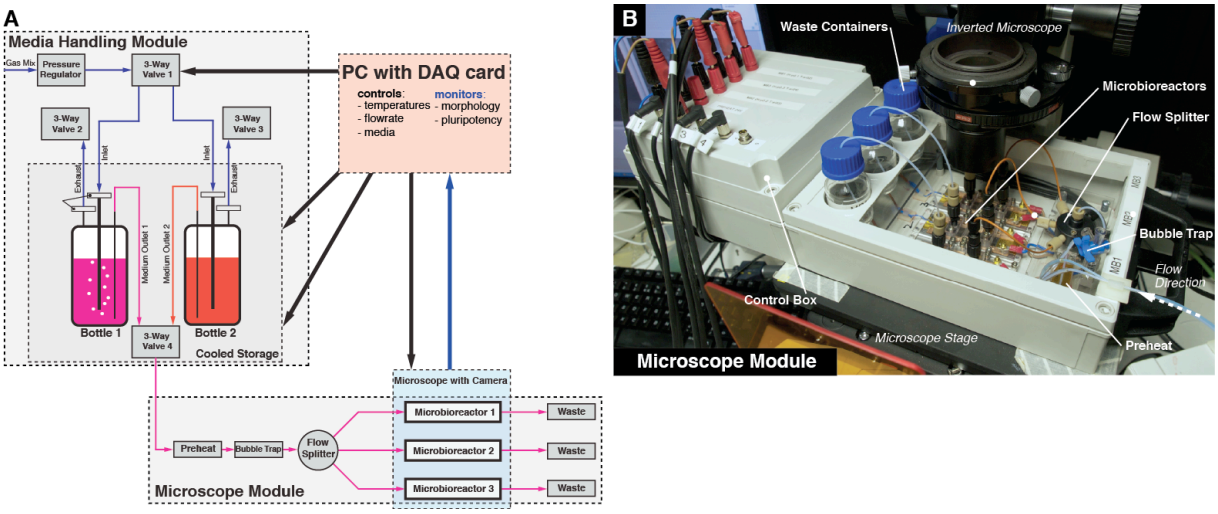

Figure 1: (A) schematic representation of all modules and components of the microfluidic ES cell culture platform. (B) shows the microscope module and its components.

The microfluidic bioreactors used for this platform are based on a previously presented design where we demonstrated perfusion culture of feeder-attached human ES cells [4]. In this design as shown in Figure 2A and 2B, the temperature in each microfluidic bioreactor can be controlled individually. An indium tin oxide (ITO) microscope slide is

used as a resistive heating element and - coated with gelatin - serves as the culture surface for ES cells. A temperature feedback control loop implemented in LabView™ monitors and controls the resistive heating by pulse-width modulation. The culture chamber of each bioreactor is split in two compartments to accommodate two mESC lines with a different green fluorescent protein (GFP) marker each.

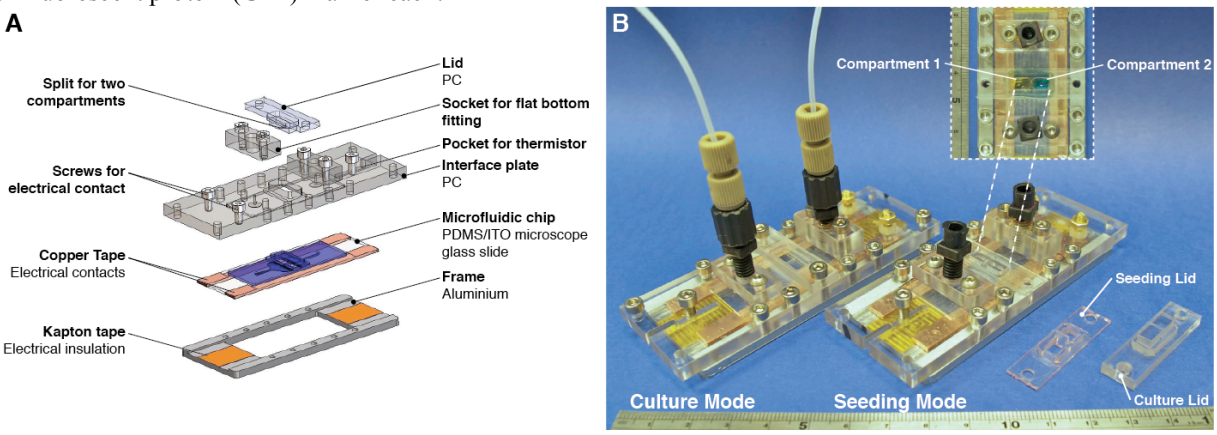

Figure 2: (A) Exploded view of all parts of the microfluidic bioreactor for the cell culture platform. (B) shows the two modes of operation, seeding and culture mode of the microbioreactor [4], and the corresponding lid. The microfluidic bioreactor has two compartments to seed different populations, and both compartments can be directly accessed with standard laboratory pipettes.

The media handling module includes two media bottles, a cooling plate, a pressure regulator and a set of valves as shown in figure 1A. To avoid media decomposition during long term cultures, the media bottles are stored on the cooling plate (using the same temperature control loop as for the microbioreactors). The pressure regulator is used to control the head pressure in the media bottle and therefore the media flow. A set of valves is used to switch between the media bottles. An inverted microscope with a CCD camera was used for imaging cell morphology and fluorescent markers. Phase contrast images were automatically taken every 15 minutes with a LabView™ routine. Images of the fluorescent markers were taken daily.

All three microbioreactor and medium flasks were autoclaved before each experiment. Tubing was sterilized by flowing ethanol through for 20 min followed by a PBS wash. Sterile filters were used to seal the inlet and outlet for the pressurized gas. Prior to seeding, an 0.1% (w/v) gelatine solution was used to coat the glass slides. Two mESC lines were used, an Oct4-GFP reporter line to monitor pluripotency seeded in one compartment, and a Sox-1-GFP reporter line for early neuronal differentiation in the other. Cells were statically seeded with a pipette to a seeding density of approximately 100,000 cells/cm^2. 24h after seeding, the microbioreactors were changed from seeding mode into culture mode by sealing the culture chambers with a lid. Tubing was connected first to the media bottles and then to the microbioreactors, which had been placed into the microscope module in a laminar flow hood. After priming, the microscope module was transferred to the inverted microscope and mounted on the microscope stage. The temperature for all microbioreactors was set at 37°C and the flowrate controlled to maintain approximately 300 µl/h in each microbioreactor .

RESULTS AND DISCUSSION

The microfluidic bioreactor platform consists of 6 culture chambers. Each chamber can be accessed individually with a laboratory pipette and different types of cells can be seeded without cross-contamination. Culture programs with physical parameters such as temperatures and flow rates can be automatically read into the LabView™ routine and altered manually during the experiments. Flow rates and temperatures can be accurately controlled as shown in Figure 3A and B.

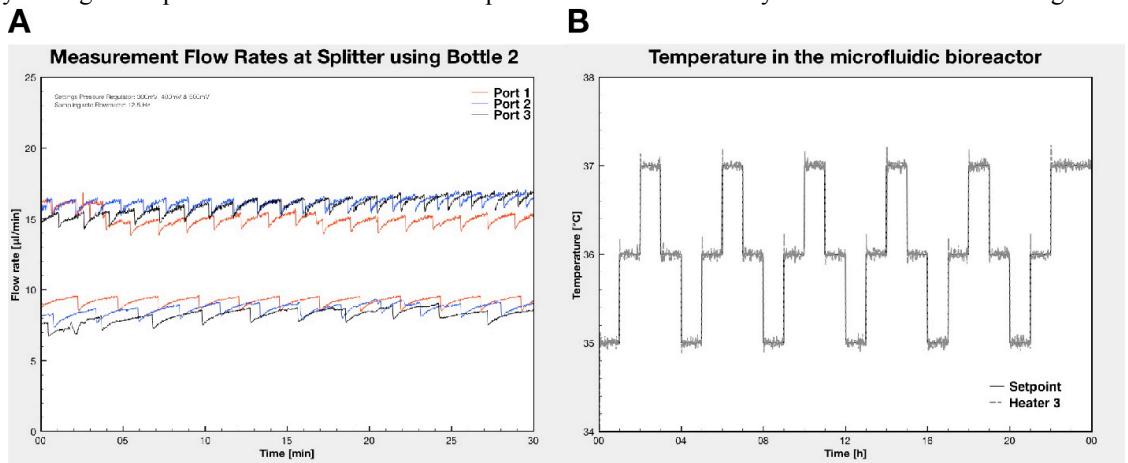

Figure 3: Flow rates after the flow splitter using bottle 2 at different head pressures measured with a flow sensor (A). Temperature of the culture surface in a microfluidic bioreactor (B).

Preliminary results with the microfluidic platform show that the cells grow under low shear perfusion and that cell proliferation and marker expression can be monitored (Figure 4A and B). During culture, cells moved over the culture area as shown in Figure 4C and D. Such information on cell migration and proliferation can only be obtained from real time cell culture monitoring, which illustrates the importance of an automated time-lapse imaging routine.

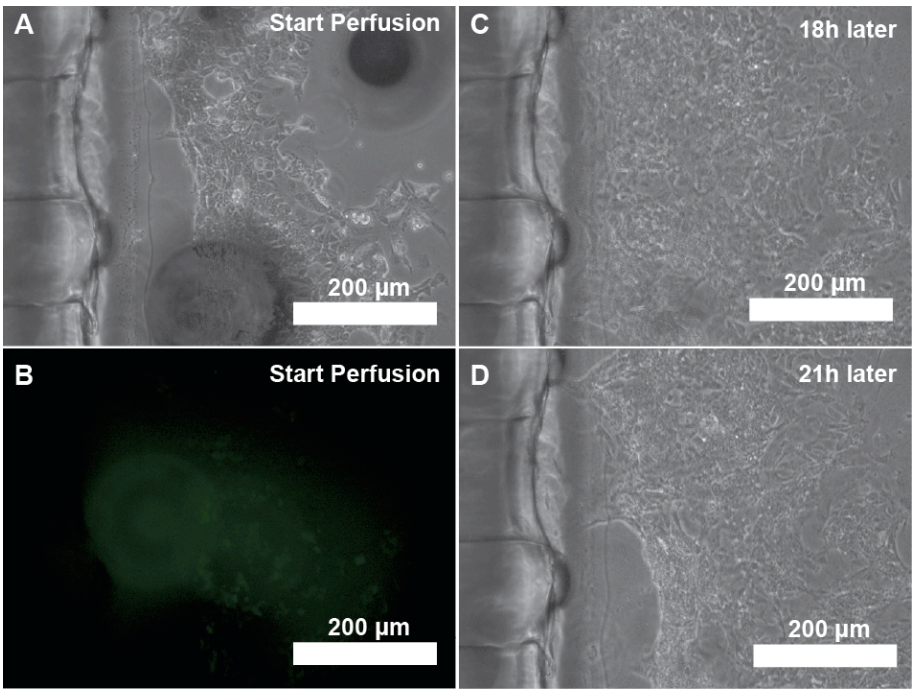

Figure 4: Representative images of a cell culture compartment. Phase contrast images at different time points (A,C,D) and fluorescent Oct-4 marker image (B).

CONCLUSION

An automated, versatile microfluidic platform for long-term cell culture has been devoloped to monitor cell morphology, migration and proliferation from phase contrast images, and pluripotency and differentiation markers from fluorescence images. The microbioreactors have a culture chamber with two compartments which allows to monitor simultaneously and under identical culture conditions two GFP-reporter mES cell line. A deeper insight in cellular processes and data-rich experiments can be gained by modulating flow, extracellular matrix and media composition using such a microfluidic platform with its integrated optical monitoring. Future work includes the implementation of advanced imaging algorithms for on-line analysis and long-term experiments.

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REFERENCES

[1] Y. Wen, X. Zhang and S.-T. Yang, "Microplate-reader compatible perfusion bioreactor array for modular tissue culture and cytotoxicity assays", *Biotech. Prog.*, DOI10.1002/btpr.423, 2010.

[2] A. Ozcan and U. Demirci, "Ultra wide-field lens-free monitoring of cells on-chip", *Lab Chip*, vol. 8, pp. 98-106, 2008.

[3] S. Petronis, M. Stangegaard, C. B. Christensen and M. Dufva, "Transparent polymeric cell culture chip with integrated temperature control and uniform media perfusion", *Biotechniques*, vol. 40, pp. 368-375, 2005.

[4] M. Reichen, L. Ruban, F. S. Veraitch and N.Szita, Human embryonic stem cell culturing in a microfluidic perfusion system with a reversibly sealable lid, Proc. Micro Total Analysis Systems 2009, pp. 1829-1831, (2009).

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