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Transfection in perfused microfluidic cell culture devices: A case study

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ABSTRACT

Automated microfluidic devices are a promising route towards a point-of-care autologous cell therapy. The initial steps of induced pluripotent stem cell (iPSC) derivation involve transfection and long term cell culture. Integration of these steps would help reduce the cost and footprint of micro-scale devices with applications in cell reprogramming or gene correction. Current examples of transfection integration focus on maximising efficiency rather than viable long-term culture. Here we look for whole process compatibility by integrating automated transfection with a perfused microfluidic device designed for homogeneous culture conditions. The injection process was characterised using fluorescein to establish a LabVIEW-based routine for user-defined automation. Proof-of-concept is demonstrated by chemically transfecting a GFP plasmid into mouse embryonic stem cells (mESCs). Cells transfected in the device showed an improvement in efficiency (34%, n = 3) compared with standard protocols (17.2%, n = 3). This represents a first step towards microfluidic processing systems for cell reprogramming or gene therapy.

1. Introduction

There is a need to develop systems for the safe and economical production of cell therapies [1]. Autologous cell therapies only require a small starting cell population from a patient blood or skin sample. To derive iPSCs this somatic population needs to be transfected with pluripotent factors and maintained in stable long-term culture. Once derived, these cells can be further expanded and differentiated in downstream processing steps for transplantation back into the patient. First clinical trials for such autologous therapies are already underway in Japan for the treatment of macular degeneration. For this therapy, a small sheet consisting of retinal pigment epithelial (RPE) cells (5×10^4 cells) is transplanted into the patient's retina [2,3]. A scale-down approach to bioprocessing particularly benefits treatments that require low cell input for transplant, such as required for an RPE-retina graft [4].

A key goal in bioprocessing is process integration to simplify unit operations, shorten residence times and reduce footprints [5,6]. Integration can have additional advantages in cell processing, such as increasing cell viability by reducing the need for enzymatic detachment [7]. A recent example of integration in cell processing was demonstrated with cell expansion and differentiation in a single stirred reactor, where micro-carriers have been used to convert human pluripotent stem cells (hPSCs) into cardiomyocytes or neural progenitors. This was achieved with a high cell yield, low chance of contamination and a controlled aggregate size [6,8]. A scale-down approach to autologous cell therapy presents an ideal platform to test and validate integration of unit operation steps. Transfection and long-term culture are important steps specific to iPSC therapies that could benefit from integration.

Electroporation is often regarded as having the highest efficiency of currently available micro-scale transfection approaches [9]. For example, cells were recently cultured on a porous polycarbonate substrate and transfected by localised electroporation to maintain high cell viability [10]. Integration of electrodes, however, typically increases the complexity of both device design and control [11]. Chemical transfection is a simpler method, and efficacy and viability continue to improve with each new commercial reagent [12]. It is important that transfecting agents are introduced in an automated fashion to minimise environmental fluctuations or operator bias, which are more likely to occur with manual procedures, and ultimately to improve robustness and reproducibility of the transfection process. A number of microfluidic culture devices have demonstrated chemical transfection of cultured cells [13–16]. Examples include a digitally controlled cell-microchip with parallel circular culture chambers [13], and a self-contained system with near-chip peristaltic micro-pumps [16], both designed for combinatorial cell-based assays.

Integrated transfection devices described thus far compromise on two aspects essential for long-term stem cell culture: a uniform

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culture microenvironment and real-time analysis of growth kinetics and transfection outcome. To avoid compromise we integrate a two-position valve to automate the injection of a transfection reagent upstream of a microfluidic device that we previously developed and characterised for the long-term perfusion culture of adherent stem cells [17]. Our device offers uniform medium flow over the cell culture chamber and control over the dissolved gas concentrations [17,18], and a fully automated and on-line culture monitoring system [19]. Furthermore, we successfully demonstrate transfection of mouse embryonic stem cells (mESCs), and we compare the efficiency of the transfection in the microfluidic device with a well-established manual culture protocol.

2. Materials and methods

2.1. Fabrication of the microfluidic cell culture device

The microfluidic culture device was fabricated according to Macown et al. [17]. Gaskets, gas-permeable lids and the microfluidic chip were cast from poly(dimethylsiloxane) (PDMS, Sylgard 184, Dow Corning, USA). A rigid polycarbonate holder in a screw-down aluminium frame was used to compress the microfluidic chip, which contained the fluidic channels and the culture chamber, against a tissue-culture polystyrene (TC-PS) microscope slide (260225, Elektron Technology Ltd, UK). The culture surface was 0.52 cm^2 and the lid defined the height of the perfusion chamber at 450 μm, giving a chamber volume of ~25 μL.

2.2. Pressure-Driven pumping system

The pressure-driven pumping system consisted of a gas supply (21% O_2, 5% CO_2, N_2; BOC, UK) connected to a flow control system (OB1, Elveflow, France) which fed into a medium reservoir (DURAN® bottle with GL-45 cap, Schott AG, Germany). The pressure was regulated by feedback control for a set flow rate of 5 μL min^{-1}. The outlet of the medium reservoir was connected to a flow sensor (MFS 2, Elveflow, France), which fed into a low pressure, six-port injection valve (C22-3186EH, VICI AG International) with a 50 μL injection loop (Fig. 1A). The flow control system and injection valve were automated using LabVIEW (National Instruments, USA). The microfluidic culture device connected with the injection valve *via* a 10 cm long, 0.0635 mm inner diameter (ID) tubing (PEEK, IDEX Health & Science, USA). The device parts, medium reservoir and tubing were sterilised by autoclave and assembled in a biosafety cabinet under sterile conditions.

2.3. Cell culture

Mouse ESC were maintained as previously reported by Macown et al. [17]. The TC-PS slide was coated with 0.1% (w/v) gelatin (G1890, Sigma-Aldrich, UK) solubilised in Dulbecco's Phosphate Buffer Solution (D1408, Sigma-Aldrich, UK) for 15 min at room temperature. The mESC culture medium consisted of knock-out Dulbecco's modified Eagle medium (10829-018, Life Technologies, UK) supplemented with 15% v/v fetal bovine serum (26140-079, Life Technologies, UK). Priming and seeding of the microfluidic cell culture device was performed as described by Macown et al. [17]. Briefly, a suspension of mESCs (in culture medium) were seeded by pipette at a density of 2×10^5 cells.cm^{-2}. Cells were allowed to attach for 6 h in a 37 °C incubator before the start of perfusion. During perfusion the device was placed on the stage of an automated microscope (Eclipse Ti-E, Nikon Instruments, UK) at 37 °C in a cage incubator (Okolab, Italy).

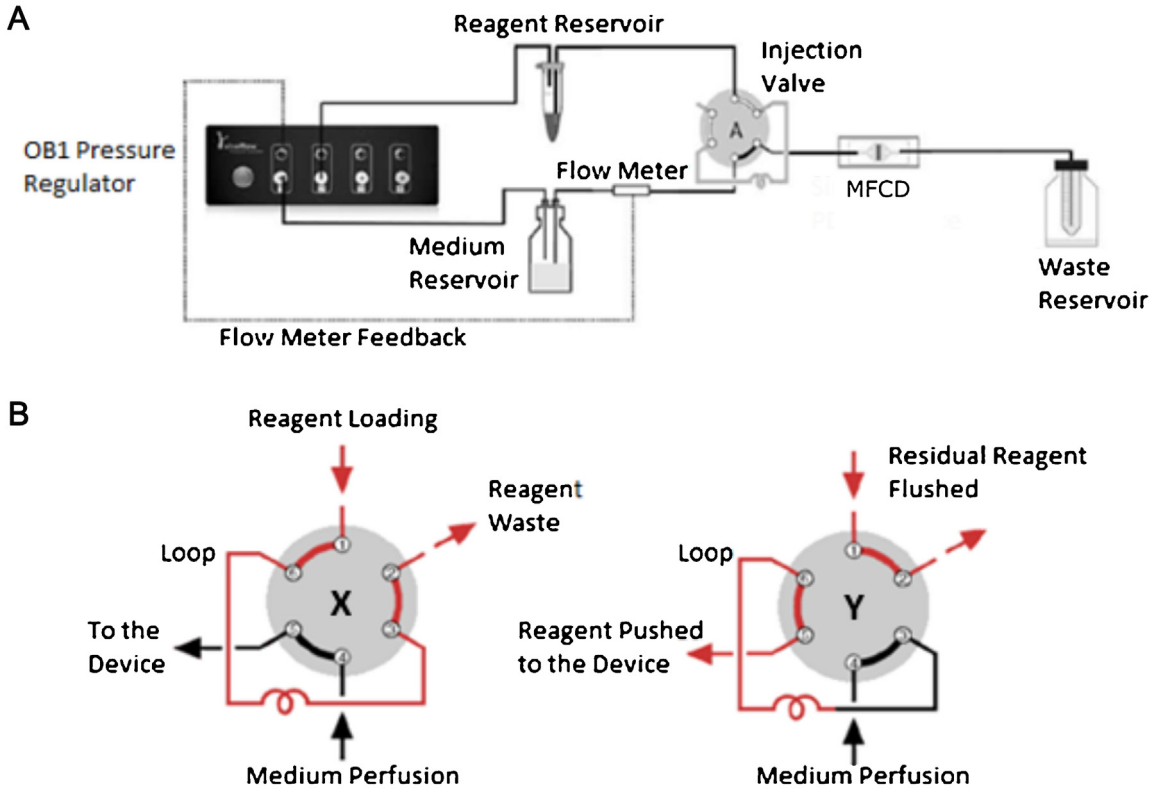

Fig. 1. (A) A schematic of the perfusion system with feedback from the flow meter controlling the flow of culture medium into the microfluidic cell culture device (MFCD). Transfection mixture from the reagent reservoir is pushed into the injection loop by gas from the pressure regulator; once it has filled the loop, the valve will switch and reagent will move into the chip. (B) The two possible positions of the injection valve: X = reagent loading into loop, and Y = reagent injection into the microfluidic cell culture device.