A biopharmaceutical plant on a chip: continuous micro-devices for the production of monoclonal antibodies

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We report a proof-of-principle for the use of micro-devices as continuous bioreactors for the production of a glycosylated pharmaceutical product (a monoclonal antibody). We culture CHO cells on the surface of PDMS "zigzag" shaped micro-channels textured with semi-spherical cavities and coated with fibronectin. We observe a steady-state productivity of 6,240 mg L^{-1} day^{-1} , at least 100 times higher than that observed in commercial scale systems.

Supplementary Materials and Methods

Cells, culture media, and culture conditions. We used a CHO-S clone (denominated here C8F4-A2) derived from a CHO-S cell line (Invitrogen, Carlsbad, CA, USA) that had been engineered to produce a biosimilar of the monoclonal antibody Infliximab.[17] Cells were cultured using a medium mixture containing CD OptiCHO™ (Invitrogen, Carlsbad, CA, USA) and CHO CD EfficientFeed™ B (Invitrogen, Carlsbad, CA, USA) supplemented with 200 mM L-glutamine (Invitrogen, Carlsbad, CA, USA).

Micro-fluidic device fabrication. Micro-fluidic devices (Figure 1) were manufactured through mechanical micromachining[10] in a vertical milling machine (MAKINO F3, CA, USA; position accuracy of 0.0015 mm). The zigzag channel (Figure 1b-f) was design in CAD-CAM and

fabricated from a rectangular PMMA wafer (6 mm wide, with a micro end mill with a 0.410 µm cutting diameter). Each zigzag micro-channel was 44 mm long, 0.5 mm wide, and 0.1 mm deep, with a 1 mm diameter reservoir (Figure 1).

The upper section of each micro-device (i.e. the lid) was built from PDMS (Sylgard 184, Dow Corning Corp., Midland, MI, USA; Figure 1b). PDMS and curing agent were mixed to obtain a prepolymer with a 10:1 weight ratio. The PDMS was cured at 120°C for 20 min and then peeled from the mask to obtain a 5 mm PDMS wafer. Reservoirs were drilled into the PDMS surface with a sharpened blunt-tip needle. PDMS and PMMA surfaces were treated with a handheld corona treater (Electro-Technic-Products Inc., Chicago, ILL, USA, model BD20A) for 15 sec to achieve an efficient bonding between both surfaces and to convert their nature from hydrophilic to hydrophobic. In addition, PDMS and PMMA layers were sealed together using liquid PDMS around the borders and then thermally bonded by incubation in an oven at 120°C for 20 min. Subsequently, tubes were inserted into the reservoirs and glued in place with liquid PDMS. Micro-devices with their corresponding tubes were also thermally bonded at least 24 hours before use.

Surface treatment. Devices were constructed with a rough surface through mechanical micro-machining. Roughness consisted of semi-spherical cavities with an average diameter of 15 µm (see Figure 1a). In addition, the surface of some micro-devices, both smooth and textured, was coated with fibronectin (FN) (according to the procedure described later). Two different types of zigzag micro-devices were tested: a textured surface without FN coating (TF^-) and a textured surface with FN coating (TF^+).

Continuous micro-reactor experimental set-up. For dispensing of solutions or continuous feeding of culture media, micro-devices were connected to a Harvard 33 twin syringe pump (Harvard

Apparatus, Inc., Holliston, MA, USA) using Tygon hose (Figure 1f). Micro-devices were sterilized by continuous feeding with 70% ethanol at 10 µL min^{-1} under UV light. In the experiments where FN (20 µg/mL) was used, 100 µL of FN (Invitrogen, Carlsbad, CA, USA) was injected into the micro-channel and incubated at 37°C in a humidified atmosphere for 1 h. Excess FN was then removed from the micro-channel by injecting 300 µL of PBS into the micro-device. Prior to cell inoculation, CD OptiCHO™ medium was infused into all micro-devices. For each experiment, micro-devices were inoculated with a cell suspension with a viable density of $2x10^6$ cells mL^{-1}, delivered at 5 µL min^{-1}. After inoculation, in the first stage of culture, micro-devices were incubated under static conditions for 96 h at 33°C in an incubator with a humidified 8% CO_2 atmosphere. The extent of cell proliferation was monitored photographically. Pictures were taken every day with a Carl Zeiss 200 inverted fluorescence video microscope (Carl Zeiss, Jena, Germany) equipped with a 20X microscope objective.

After static culture for 96 h, micro-devices were subjected to continuous flow. A medium mixture (CD OptiCHO™ and CHO CD EfficientFeed™ B) was injected continuously for one to 2 days at a flow rate of 5 µL min^{-1} or 6 µL min^{-1} while the micro-devices were maintained at 31°C inside an incubator with a humidified 8% CO_2 atmosphere. Samples were collected on day four (at the end of the batch culture stage), and every 12 h afterward during continuous culture. These samples were subsequently used for determination of mAb concentrations using an ELISA method.

Velocity field calculation. The velocity fields within the micro-channel under the condition of continuous flow at 5 µL min^{-1} were estimated using Computational Fluid Dynamics (CFD) simulations. The commercial software Comsol® was used to generate the geometry of the zigzag micro-channel, to create its tetrahedral mesh consisting of 411,801 elements, and to solve the

Navier Stokes equations (for a laminar flow condition) at each mesh node to obtain local velocity field. A non-slip condition was imposed at the channel boundaries. The viscosity and density of the culture media were assumed to be 0.89 cP and 0.96 g/mL, respectively.

mAb quantification. Monoclonal antibody amounts in samples were quantified using an enzyme-linked immunosorbent assay (ELISA) assay.[17] Briefly, 100 µL/well of antigen (TNF-α at 5 µg L^{-1} from BioSource™; Invitrogen, Carlsbad, CA, USA) were added to 96-well plates, which were then incubated overnight at room temperature. The antigen was removed and the plates were washed twice with PBC-Tween 0.05% (pH 7.2 ± 0.2) and twice with phosphate-buffered saline (PBS; Invitrogen, Carlsbad, CA, USA). Blocking-Buffer (Pierce, N502, Rockford, IL USA) was added and the plates were incubated 1 hour at room temperature and washed as previously described. Samples diluted in PBS and standards were then added at 100 µL/well and the plates were incubated for 1 hour at room temperature. The TMB substrate (Pierce, N301) was then added at100µL/well and the plates were incubated for 15 minutes in darkness at room temperature; the reaction was stopped by adding 1M H_2SO_4 at 50 µL/well and the plates were read at a 450 nm (OD_{450}) in a microwell reader (Biotek, Winooski, VA, USA). A standard curve of concentration vs. OD was generated and sample concentrations were interpolated.