Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary information

Chip Fabrication

The elaborate two-step wet etching process was developed to combine higher (>50 μm) with shallower structures (sub-micrometre), while accompanying the adhesion challenges for masking small features using low and more controlled etch rates, which require prolonged chemical exposure. In the first step, all features were etched in 0.21 mm thin D263 glass (Schott, Germany), with a depth of 1 μm, using wet chemical etching (masking layer: sputtered 50 nm chromium; etchant: buffered hydrogen fluoride (BHF, BASF, Germany) with an etch-rate of approximately 60 nm·min^{-1}). Etching for 16.5 min resulted in a channel depth of approximately 1000 nm. The use of a single 50 nm chromium layer, instead of the classical 200 nm chromium-gold layer, increased adhesion between photoresist and glass, necessary for prolonged BHF etching. Additionally, it reduced top heavy overhanging masking material sensitive to mechanical stress. In the second step, the chamber and the surrounding channels were created, also using wet chemical etching (masking layer: sputtered 15 nm chromium and 200 nm gold; etchant: 10% hydrogen fluoride (HF, BASF, Germany) with an etch-rate of around 1 μm·min^{-1}). Since HF is not buffered, etch rates are unpredictable and decline in time. Therefore, the etching was performed in several steps and the height of the structures measured after each step using a profilometer (DEKTAK, USA), until a depth of 75 μm was reached. In the top glass substrate, 1.1 mm D263 glass, via-holes were powder-blasted, using 29 μm alumina particles, and both glass substrates were thermally bonded at 570°C in an oven (1 h with 6 h up and 6h down ramp). After bonding the wafers were cut in 2 cm x 2 cm dies with a dicing saw followed by polishing (Fig. 2B).

Perfluoroalkoxy tubing oxygen mass transfer

With a flow rate of the medium in the 1 μL·h^{-1} range, the pressure gradient over the wall of the perfluoroalkoxy (PFA) tubing, between air and liquid, is negligible, making diffusion dominant in mass transfer. The diffusion coefficient of O_2 through PFA, is approximately 22 x 10^{-8} cm^2·s^{-1} (at 25°C),[1] as compared to water 2.4 x 10^{-5} cm^2·s^{-1} (at 25°C) and air 0.22 cm^{-2}·s^{-1} (at 25°C). In a 20% oxygen environment, medium contains approximately 200 μM dissolved oxygen at equilibrium, or 1.2 nmol in 6 μL. To reach this oxygen level in water, it would take approximately 5 minutes through 360 OD and 150 ID PFA tubing at 1 μL·h^{-1} in a 20% O_2 air environment (at 25°C and 1 atm), as demonstrated in Fig. 1 by finite element analysis (COMSOL).[2] In conventional, static flask culture, the change in oxygen content could require > 3 h before an equilibrium is reached, which makes absorption of oxygen into medium the limiting factor.[3] Since the surface-to-volume ratio in the tubing (160 mm^2 / 6 mm^3 ≈ 27 m^{-1}) is much larger than in classical culture conditions, the equilibrium is reached faster. Therefore, a residence time of 6 h selected for the experiments in the presented study is more than sufficient to ensure that the desired oxygen tension in the medium is reached.

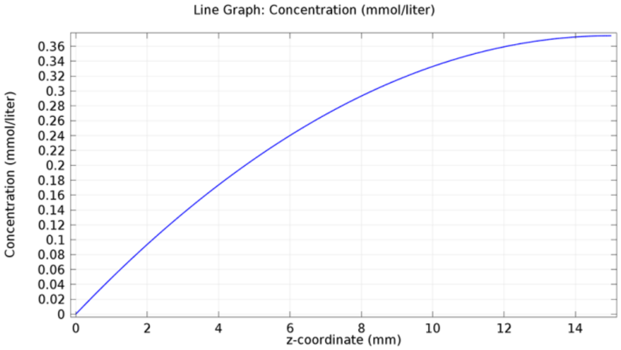

Fig. 1 Graph depicting finite element analysis (COMSOL), using incompressible laminar flow (Navier-Stokes) and diluted species diffusion, of a 15 mm piece of PFA tubing (360 μm OD and 150 μm ID) in a 20% oxygen air environment and flowing water at a flow rate of 1 μL·h^{-1}(at 25°C and 1 atm).

References

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2. A. Oller, C. Buser, M. Tyo, and W. Thilly, *J Cell Sci*, 1989, **94**, 43–9.
3. C. Allen, *Am J Physiol Lung Cell Mol Physiol*, 2001, **281**, 1021–27.