Electronic Supplementary Material (ESI) for RSC Advances.


**Electronic Supplementary Information**

# Micro-Respirometry of Whole Cells and Isolated Mitochondria.

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## Methodology

### Cell Culture Methodology

ARPE-19 cells (ATCC, CRL-2302, passage 31) were grown on 75 $cm^2$ polystyrene flasks (T75 flask; Corning Life Sciences, Oneonta, NY) in 50% Dulbecco's modified Eagle's medium low glucose base media (DMEM; Corning Cellgro, Manassas, VA), 50% F-12 supplement (Life Technologies, Grand Island, NY), 1% antibiotic/antimycotic mix (Thermo Fisher Scientific, Waltham, MA) with 10% fetal bovine serum (Atlas Biologicals, Fort Collins, CO). Bovine Retinal Endothelial Cells (BREC) were isolated and cultured as previously described[2] in 10% Fetal Bovine Serum (FBS) Complete Media with 1% Antibiotic/Antimycotic (AA) (Gibco; ThermoFisher; Waltham, MA). Passages 4-8 were used for all experiments. At 100% confluence, cells were trypsinized (0.25% trypsin-EDTA) (Thermo Fisher Scientific) and counted using the Trypan blue exclusion cell viability method (Sigma Aldrich). Cells were either plated on the MfR well or stored, in suspension, on ice for adherent or non-adherent measurements, respectively.

### Mitochondrial Isolation and Assay

ARPE-19 cells were trypsinized, resuspended in ice cold mitochondrial isolation buffer (MIB) (200 mM sucrose, 50 mM mannitol, 5 mM MOPS, 1 mM EGTA, 5 mM $K_2HPO_4$, pH 7.5) and homogenized using a Teflon pestle (20 strokes at 3000 RPM). Homogenate was centrifuged at 600 g for 10 min and supernatant was collected. Pellet was resuspended in 3 mL of fresh ice-cold MIB, homogenized and centrifuged two more times collecting supernatant every time. The pooled supernatants were centrifuged at 7,000 g for 10 min, discarding supernatant. The pellets were resuspended in 200 µL of MIB, pooled and centrifuged again for 10 min at 7000 g, repeating three times. Final

pellet was resuspended in a minimal volume of ice-cold MIB and stored on ice prior to use. All steps were performed at 4° C.

Table S1

**Table S1**: **Oxygen permeability of selected polymers**. Barrer = $10^{-10}$ $cm^3$ cm $cm^{-2}$ $s^{-1}$ $cmHg^{-1}$, mean ± SD (N=4). † - measured at 23° C. ‡ - measured at 37° C.

| Polymer | Permeability (Barrer) |
|---|---|
| **Reported** | |
| Poly(dimethylsiloxane) (PDMS) | 610[1] |
| Polyethylene (PE) | 23[1] |
| Poly(tetrafluoroethylene) (PTFE) | 4.2[1] |
| Poly(methyl methacrylate) (PMMA) | 0.09[3] |
| Polyetheretherketone (PEEK) | 0.13[1] |
| **Measured** | |
| PEEK | 0.143 ± 0.001 |
| VeroClear | 0.125 ± 0.007† |
| | 0.218 ± 0.006‡ |

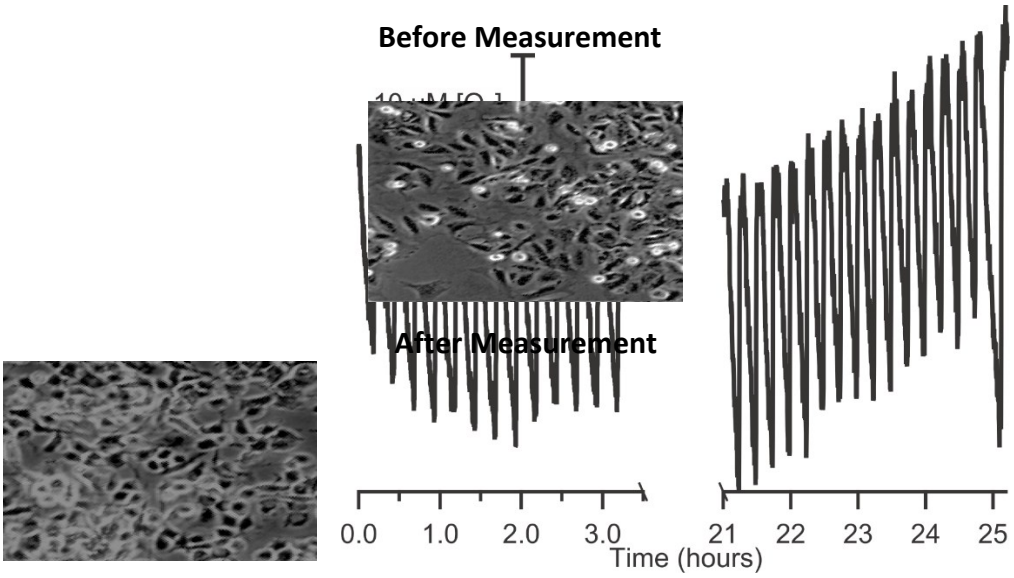


**Figure S1. Biocompatibility of the MfR.** Photomicrographs of BRECs grown on the and after (bottom) ~25 hours of continuous measurement (right). The experiment multiple times, with the continuous measurements longer than 24 hours without activity.

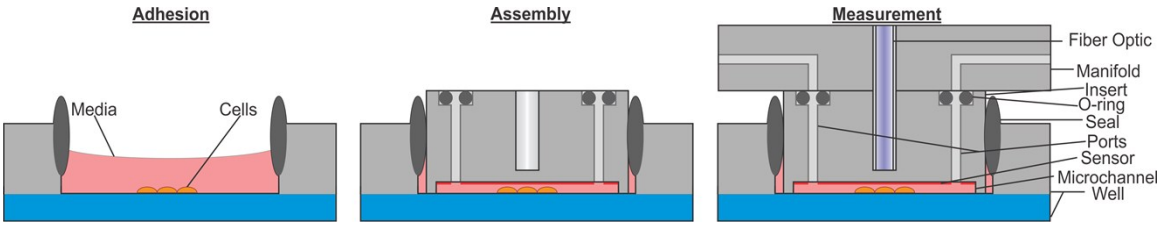


**Figure S2**. **Schematic of the MfR for adherent samples**. Cells are cultured on the glass base of the well, open to the incubator atmosphere (Adhesion). Assembly and Measurement refer to sequential application of MF chip and manifold immediately prior to $R_{O2}$ determination.

**References**

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