Characterization of Oxygen Nanobubbles and in vitro evaluation of retinal cells under hypoxic conditions

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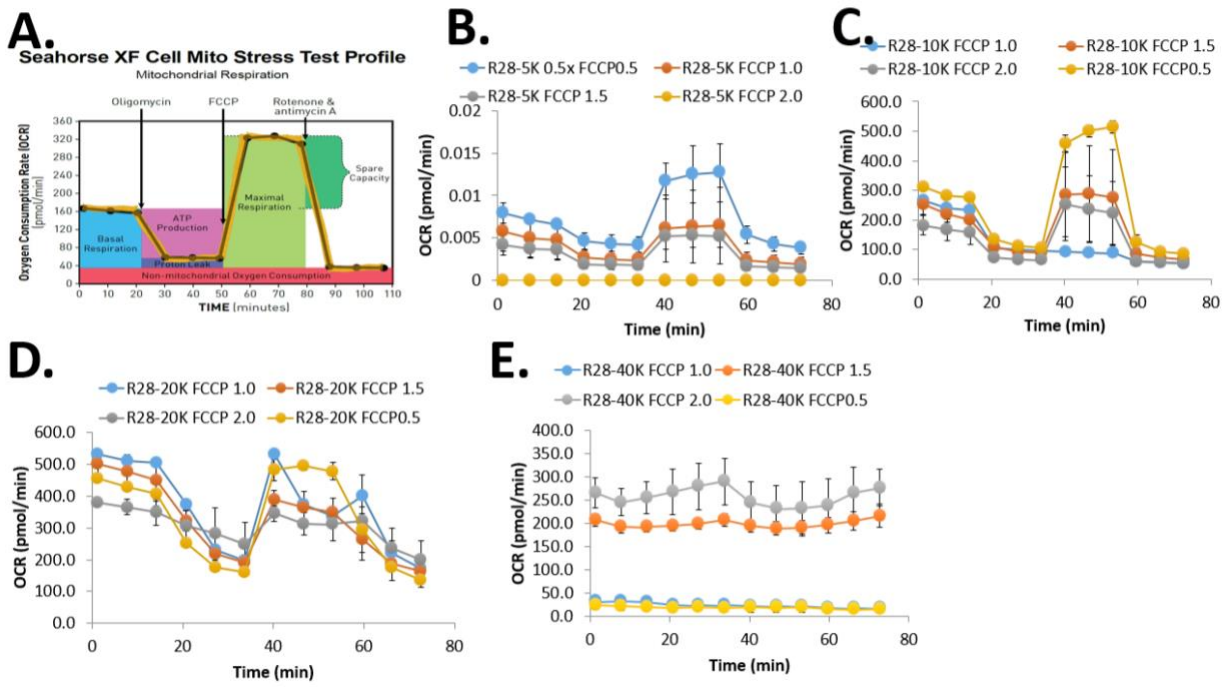

Figure S1: Mitochondrial Stress Test R28 Optimization. (A) The typical mitochondrial stress test profile. R28 cells were seeded at (B) 5,000 cells/well, (C) 10,000 cells/well, (D) 20,000 cells/well, or (E) 40,000 cells/well and exposed to 6 hours of hypoxia followed by treatment of 0.5, 1.0, 1.5, or 2.0 μM of carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP). Ideal Mitochondrial stress profiles have starting OCR between 100-200 pMol/min. The highest maximal respiration peak at the determined cell concentration defines the FCCP concentration. Optimization showed that a cell concentration of 5,000 cells/well matched the typical profile, and the FCCP concentration of 1.5 μM showed the largest maximal respiration for R28 cells.

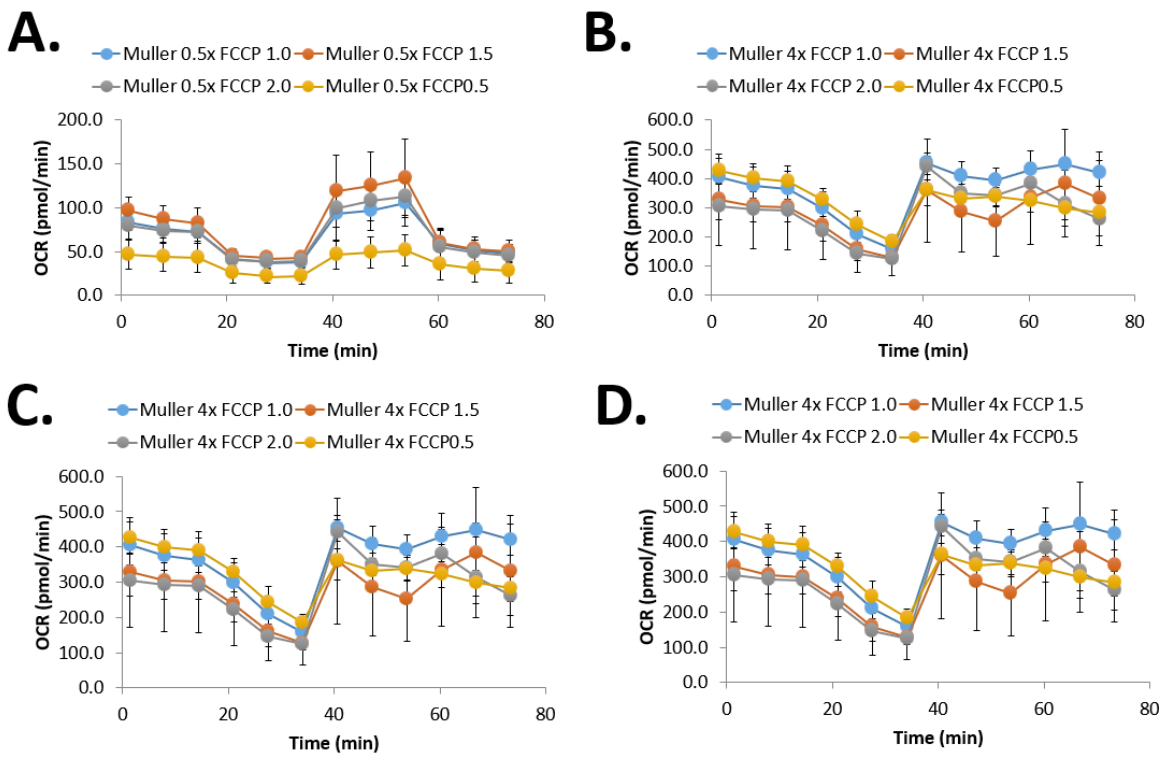

Figure S2: Mitochondrial Stress Test Muller Optimization. Muller cells were seeded at (A) 5,000 cells/well, (B) 10,000 cells/well, (C) 20,000 cells/well, or (D) 40,000 cells/well and exposed to 6 hours of hypoxia followed by treatment of 0.5, 1.0, 1.5, or 2.0 µM of carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP). Ideal Mitochondrial stress profiles have starting OCR between 100-200 pMol/min. The highest maximal respiration peak at the determined cell concentration defines the FCCP concentration. Optimization showed that a cell concentration of 5,000 cells/well matched the typical profile, and the FCCP concentration of 1.5 µM showed the largest maximal respiration for Muller cells.

Table S1: Body Weight Change. Days post injection (dpi). Group 1: Saline injection. Group 2: ONB injection.

Subject Name	Group	Baseline	1 dpi	7 dpi
101	1	3.21	3.16	3.25
102	1	2.98	2.98	3.11
103	1	3.06	3.03	3.09
104	2	2.98	2.99	3.06
105	2	3.12	3.15	3.14
106	2	3.10	3.11	3.21

Table S2: Clinical Signs Observed. Days post injection (dpi). Group 1: Saline injection. Group 2: ONB injection. No abnormalities detected (NAD).

Subject Name	Group	0 dpi	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi
101	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
102	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
103	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD
104	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD
105	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD
106	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD

Table S3: General Ophthalmic Exam Evaluation.

Rabbit ID	Eye	Group	Treatment	Time point	IOP Mean	CC	CS	CD	PA	CO	CA	KP	AC	HY	AF	FS	IH	IE	IS	ALC	PLC	LO	VC	VH	RV	PP	Total clinical score
101	OD	N/A	N/A	Baseline	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	N/A	N/A	Baseline	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	OD	N/A	N/A	Baseline	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	N/A	N/A	Baseline	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	OD	N/A	N/A	Baseline	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	N/A	N/A	Baseline	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	OD	N/A	N/A	Baseline	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	N/A	N/A	Baseline	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105	OD	N/A	N/A	Baseline	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	N/A	N/A	Baseline	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	OD	N/A	N/A	Baseline	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	N/A	N/A	Baseline	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	OD	1	Saline	Day 3	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	1	Saline	Day 3	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	OD	1	Saline	Day 3	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	1	Saline	Day 3	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	OD	1	Saline	Day 3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	1	Saline	Day 3	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	OD	2	ONB	Day 3	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	2	ONB	Day 3	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105	OD	2	ONB	Day 3	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	2	ONB	Day 3	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	OD	2	ONB	Day 3	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	2	ONB	Day 3	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	OD	1	Saline	Day 7	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	1	Saline	Day 7	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	OD	1	Saline	Day 7	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	1	Saline	Day 7	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	OD	1	Saline	Day 7	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	OS	1	Saline	Day 7	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
104	OD	2	ONB	Day 7	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	2	ONB	Day 7	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
105	OD	2	ONB	Day 7	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	2	ONB	Day 7	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
106	OD	2	ONB	Day 7	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OS	2	ONB	Day 7	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

CC: Conjunctival congestion; CS Conjunctival swelling; CD: Conjunctival discharge; PA: Corneal vascular pannus; CO: Corneal opacity; CA: Corneal opacity area; KP: Inflammatory KP; AC: Aqueous cell; HY: Hypopyon; AF: Aqueous flare; FS: Fibrin strands; IH: Iris hyperemia; IE: Iris exfoliation; IS: Iris synechia; ALC: Anterior lens capsule deposit; PCL: Posterior lens capsule deposit; LO: Lens opacity; VC: Vitreous cell; VH: Vitreous haze; RV: Retinal vasculitis; PP: Papillitis; Total clinical score: Sum of findings.