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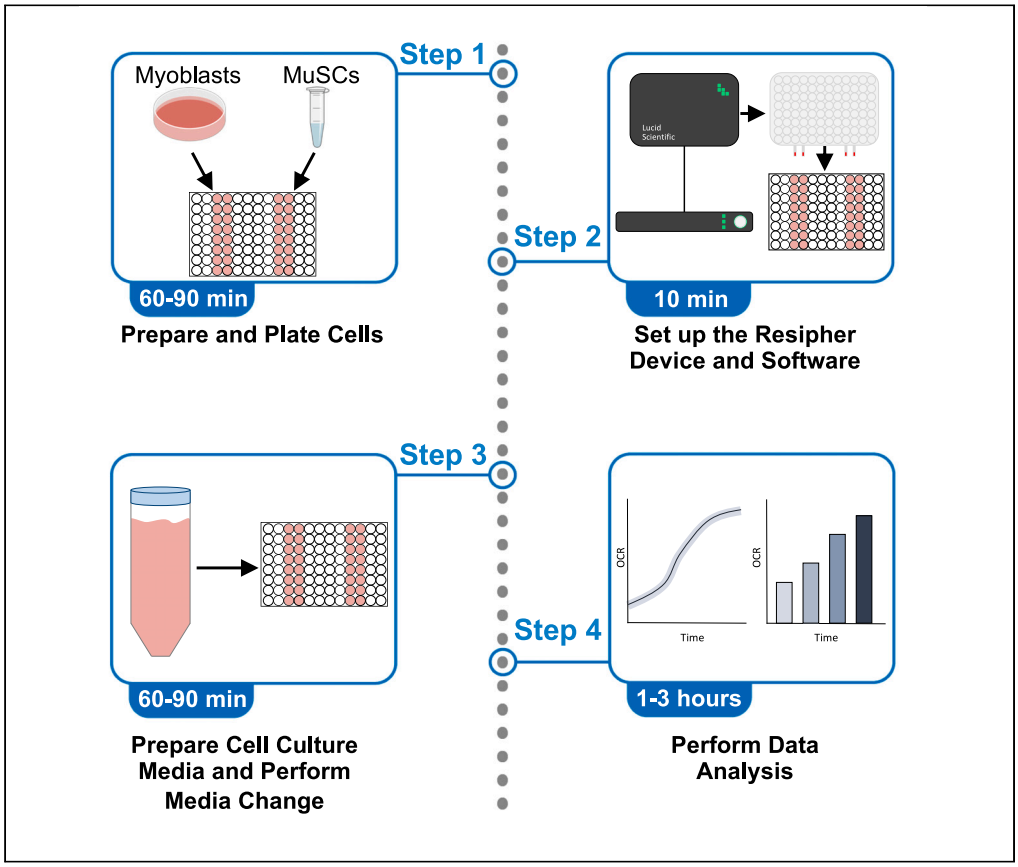

Mitochondrial function is typically assessed by measuring oxygen consumption at a given time point. However, this approach cannot monitor respiratory changes that occur over time. Here, we present a protocol to measure mitochondrial respiration in freshly isolated muscle stem cells, primary skeletal muscle, and immortalized C2C12 myoblasts in real time using the Resipher platform. We describe steps for preparing and plating cells, performing media changes, setting up the software and device, and analyzing data. This method can be adapted to other cell types.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Protocol to measure cellular oxygen consumption in real time using Resipher

Procedures for culturing myoblasts and muscle stem cells to assess oxygen consumption

Instructions to quantify and represent oxygen consumption data

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Protocol to monitor live-cell, real-time, mitochondrial respiration in mouse muscle cells using the Resipher platform

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SUMMARY

Mitochondrial function is typically assessed by measuring oxygen consumption at a given time point. However, this approach cannot monitor respiratory changes that occur over time. Here, we present a protocol to measure mitochondrial respiration in freshly isolated muscle stem cells, primary skeletal muscle, and immortalized C2C12 myoblasts in real time using the Resipher platform. We describe steps for preparing and plating cells, performing media changes, setting up the software and device, and analyzing data. This method can be adapted to other cell types.

For complete details on the use and execution of this protocol, please refer to Triolo et al.[1]

BEFORE YOU BEGIN

Preparation of cell-culture reagents

Timing: ~70 min

1. Obtain cells of interest.

Note: The present protocol is focused on assessing mitochondrial oxygen consumption in immortalized C2C12 cells, primary myoblasts obtained from mice, and primary muscle stem cells isolated from mice. Both the muscle stem cells and primary myoblasts were obtained using fluorescence-activated (FACS) or magnetic-activated (MACS) cell sorting, as previously described.[1,2] This method of assessing oxygen consumption can also be generally applied to other cell populations but will be subject to cell-type specific modifications.

2. Prepare the appropriate media required for cells of interest.
   a. For C2C12 proliferation media, measure 5 mL of FBS and add 45 mL of DMEM that is supplemented with 1% penicillin – streptomycin in a 50 mL tube.
   b. For muscle stem cell media and primary myoblast proliferation media, measure 10 mL of FBS and add 40 mL of HAMS-F10 that is supplemented with 1% penicillin-streptomycin, and 2.5 ng/ μL of basic fibroblast growth factor (bFGF) in a 50 mL tube.
   c. Ensure media is mixed well.

Note: Optimal results are achieved when made and used fresh.

3. Place media in cell culture incubator for 1 h.

⚠ CRITICAL: The media being used must be placed in the cell-culture incubator in which the oxygen consumption will be measured in. Place the cap on the tube but do not screw it closed. This will allow the tube contents to equilibrate to the environment. It is important that the media equilibrates to the cell-culture conditions so that the equipment calibrates efficiently and calibration time is minimized.

4. Warm 1×PBS and TrypLE at 37°C.

Preparation of Matrigel and coating 96-well assay plate

Note: This is required for both muscle stem cells and primary myoblast cells

5. Prepare a working solution of Matrigel by diluting a 5 mg/mL Matrigel stock solution 1:10 with serum and antibiotic free, cold, DMEM.

Note: Matrigel may come at different concentrations from suppliers, thus, we create a 5 mg/mL Matrigel stock in serum and antibiotic free DMEM, that is aliquoted and stored at −20°C.

Note: It is important to thaw and keep the Matrigel stock on ice to prevent coagulation. Furthermore, ice-cold P1000 tips should be used to pipette the Matrigel stock. To ensure sterility of these pipette tips, we place 3–5 tips in a sterile 50 mL tube that is kept in the freezer.

Note: This working solution of Matrigel can be stored at 4°C and re-used for up to two weeks.

6. Place 50 μL of the working Matrigel solution into each required well of a 96-well Nunclon Delta-Treated (NUNC) plate.

Note: The 32-well Resipher device described in this protocol uses the wells in columns 1-12 of rows 2, 4, 9 and 10.

⚠ CRITICAL: The plates suggested in this protocol are required to ensure a proper fit of the Resipher sensing lids.

7. Incubate Matrigel-containing plates for 30 min in a cell-culture incubator.
8. Remove residual Matrigel solution via pipetting.
9. Immediately add 100 μL of 1× PBS.
10. Aspirate 1× PBS.
11. Add 100 μL of 1× PBS.

Note: For efficiency, we use a multi-channel pipette for the above steps.

Note: Matrigel coated plates can be made in advance, sealed with Parafilm and stored at 4°C for up to one week. If prepared in advance, check on plates to ensure the PBS has not dehydrated.

⚠ CRITICAL: Matrigel coated plates should never be allowed to dry. It is integral to the success of the experiment to remove the residual Matrigel solution after coating and add 1× PBS as rapidly as possible.

12. Whether using Matrigel coated plates that were prepared immediately before the experiment or in advance, the coated plates should be placed in a cell-culture incubator for 1 h prior to the experiment to ensure the plate is at 37°C.

Institutional permissions

Animal protocols were approved by the University of Ottawa's Animal Care Ethics Committee and adhered to the guidelines of the Canadian Council on Animal Care. Mice used to isolate primary muscle cells were housed and maintained at the Animal Care and Veterinary Service (ACVS) of the University of Ottawa. Please ensure that these permissions are acquired based on the relevant institution and national guidelines.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
1× Phosphate buffer solution (PBS)	Wisent	Cat# 311-010-CL
Dulbecco's modified Eagle's medium	Wisent	Cat# 319-005-CL
HAMS F-10 medium	Wisent	Cat# 318-050-CL
TrypLE Express	Gibco	Cat# 12605010
Penicillin-Streptomycin	Wisent	Cat# 450-201-EL
Fetal bovine serum	Wisent	Cat# 080-150
Horse serum	Wisent	Cat# 065-150
Basic fibroblast growth factor (bFGF)	PeproTech	Cat# 100-18B
Matrigel matrix basement membrane	Corning	Cat # 354234
4′,6-Diamidino-2-phenylindole (DAPI)	MilliporeSigma	Cat# D9542
Experimental models: Cell lines		
C2C12 myoblast cells	ATCC	Cat# CRL-1772; RRID:CVCL_0188
Primary myoblast cells from C57BL/6 × Sv129	Triolo et al.[1]	N/A
Muscle stem cells (MuSCs) from wild-type OPA1-floxed-Pax7CreERT2 mice	Baker et al.[2]	N/A
Experimental models: Organisms/strains		
Mouse: C57BL/6 × Sv129, male, 8–10 weeks old	Cogliati et al.[3]; Varanita et al.[4]	N/A
Mouse: OPA1-floxed-Pax7CreERT2, male, 8–10 weeks old	Baker et al.[2]	N/A
Software and algorithms		
Lucid Scientific Software	Lucid Scientific	–
Other		
Nunc MicroWell 96-well, Nunclon delta-treated, flat-bottom microplate	Thermo Scientific	Cat # 167008
32-well Resipher sensing lid	Lucid Scientific	N/A
32-well Resipher device	Lucid Scientific	N/A
Resipher Hub	Lucid Scientific	N/A

MATERIALS AND EQUIPMENT

C2C12 Proliferation Media		
Reagent	Final concentration	Amount
Fetal Bovine Serum	10% (v/v)	5 mL
Penicillin-Streptomycin	1% (v/v)	500 µL
DMEM	N/A	44.5 mL
Total	**N/A**	**50 mL**
Make fresh and use fresh.		

⚠ CRITICAL: Media should be warmed in the cell culture incubator in which the experiments will be taking place to minimize equipment calibration time.

Muscle Stem Cell and Primary Myoblast Proliferation Media		
Reagent	Final concentration	Amount
Fetal Bovine Serum	20% (v/v)	10 mL
Penicillin-Streptomycin	1% (v/v)	500 µL
bFGF	2.5 ng/ µL (m/v)	–
HAMS-F10	N/A	39.5 mL
Total	**N/A**	**50 mL**
Make fresh and use fresh.		

⚠ CRITICAL: Media should be warmed in the cell culture incubator in which the experiments will be taking place to minimize equipment calibration time.

C2C12 Differentiation Media		
Reagent	Final concentration	Amount
Horse Serum	2% (v/v)	1 mL
Penicillin-Streptomycin	1% (v/v)	500 µL
DMEM	N/A	48.5
Total	**N/A**	**50 mL**
Make fresh and use fresh.		

⚠ CRITICAL: Media should be warmed in the cell culture incubator in which the experiments will be taking place to minimize equipment calibration time.

Primary Myoblast Differentiation Media		
Reagent	Final concentration	Amount
Horse Serum	2% (v/v)	1 mL
Penicillin-Streptomycin	1% (v/v)	500 µL
DMEM	48.5% (v/v)	24.25 mL
HAMS-F10	48.5% (v/v)	24.25 mL
Total	**N/A**	**50 mL**
Make fresh and use fresh.		

⚠ CRITICAL: Media should be warmed in the cell culture incubator in which the experiments will be taking place to minimize equipment calibration time.

STEP-BY-STEP METHOD DETAILS

Part 1: Preparation of cells for live-cell respirometry

Timing: ~60 min

This portion of the protocol will outline the preparation of three different cell-types for measurement of in-vitro respiration using the 32-well Resipher Device. Once reagents and coated plates are prepared and incubating in a cell-culture incubator, as described above, cells are prepared as described below. The protocol described here was optimized to be used with 5,000–10,000 C2C12 cells, 20,000 primary mouse myoblasts and 100,000 freshly isolated muscle stem cells. Furthermore, we perform experiments with biological replicates run in duplicate or triplicate (space permitting).

1. Prepare a cell suspension.

Note: Since C2C12 and primary mouse myoblasts are adherent cells, cell suspensions are prepared by standard trypsinization procedures. Subsequently, the samples are spun at 100 × *g* for 5 min and resuspended in the appropriate proliferation culture media. Once cells reach confluency, they can be induced to differentiate via changing to differentiation media following the steps outlined in **Part 2** of this protocol.

Note: The muscle stem cells (MuSCs) collected from MACS or FACS are in suspension with collection buffer in a 1.5 mL tube. These cells should be spun at 1700 RCF for 2 min and resuspended in fresh myoblast media.

2. Count cells.

Note: We use a hemocytometer, but other counting procedures can be used.

3. Plate cells into the appropriate wells of the NUNC-96 well plate and add media such that the total volume per well is 175 μL.

⚠ CRITICAL: The volume of media used per well must remain the same, as the diffusion distance for oxygen must be constant between conditions for appropriate analysis.

⚠ CRITICAL: The following steps must be completed in a timely manner, as the longer the reagents sit outside the experimental incubator, the longer it will take the Resipher Device to calibrate and provide accurate recordings of oxygen consumption. We find that adding media to each well using a multichannel pipette with media in a media reservoir, followed by the addition of cells works best to minimize time.

4. Let cells settle and adhere before moving to Part 2 of this protocol.

⚠ CRITICAL: The Resipher is optimized for adherent cells, although cells in suspension may be used with guidance from Resipher technical support. Allowing the cells to settle is integral to the experiment, to 1) prevent cells from sticking to the sensing probes and 2) to limit the impact that the sensing probes may have on cell adherence, as they move up and down within the well (500 μm range).

Note: We wait a minimum of 4 h for C2C12 and primary mouse myoblasts to adhere.

Note: MuSCs take long to adhere on their own, so we spin the 96-well plate at 200 x g for 1 min allowing the centrifuge to stop without brakes.

Part 2: Using the Resipher device and software

Timing: 10 min

5. Place the Resipher Sensing Lid (Figure 1A) onto the 96-well NUNC plate.

⚠ CRITICAL: This should be done extremely gently, ensuring that the sensors are not damaged.

6. Place the experimental plate into the cell culture incubator and gently place the Resipher Device onto the Sensing Lid (Figure 1A).

⚠ CRITICAL: The magnetic chip of the Sensing Lid should align with the magnetic strip on the Resipher Device.

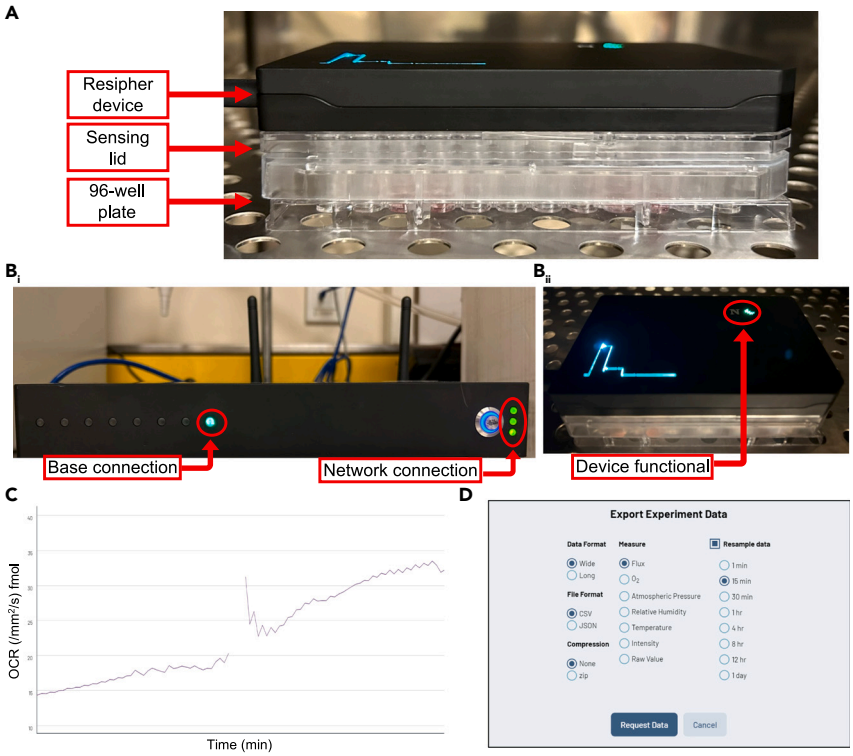

Figure 1. Resipher set-up to assess real-time oxygen consumption rate

(A) Image depicting the Resipher Device arrangement, showing the 96-well plate, with the Resipher Sensing lid and Device in place.

(B_i) Image of the Resipher Base Station, with green lights (circled in red). The left light is indicative of a proper connection between the Base Station and the Resipher Device. The right three lights indicate that the Base Station is appropriately connected to the network and that the software is working.

(B_{ii}) Image of the Resipher Device with a green light (circled in red) to indicate the device is properly set up.

(C) Representative graphical image from the Lucid Scientific Software showing a gap in recordings during a media change.

(D) The export settings utilized in the current protocol.

7. Turn on the Resipher Base Station. Observe the green lights on the Resipher Base Station and the Resipher Device. This indicates the machine is working appropriately (Figure 1B, red circles).
8. Open the Resipher Software on a computer (https://lab.lucidsci.com/) and set up the experiment, adding the pertinent details to the experiment.

   Note: Set up the plate layout as per software instructions. Representation of data can be stratified by experimental conditions of interest (i.e., cell type, plating density, treatments etc.). This information can then be used to help organize the data during analysis stage.

9. Start the experiment on the Resipher Software.

Part 3: Changing cell media

Timing: ~90 min

When removing the experimental plate from the cell-culture incubator to perform media changes, the system will require time to stabilize its recordings. This portion of the protocol addresses how we perform media changes to minimize machine re-calibration time.

Note: In the present protocol, media changes are performed when changing from proliferation to differentiation media after cells have reached confluency and every 48-h thereafter, as denoted in Figure 3A.

Note: The below steps can also be utilized if adding treatments to cells.

10. Pre-incubate fresh media in the cell-culture incubator, with the lid placed on top, for 60 min to equilibrate to the conditions.
11. Gently remove the Resipher Device from the Resipher Sensing Lid.
12. Remove the experimental plate and the tube containing the fresh media from the cell-culture incubator and place in a sterile tissue culture hood.
13. Pour the media into a media reservoir.
14. Using a multichannel pipette, rapidly remove media from the wells.
15. Using a multichannel pipette, rapidly add 175 μL of the fresh media to each well.
16. Replace the Resipher Sensing Lid and the Resipher Device as described in step 5 and 6 above.
17. On the Lucid Lab Software, click "Add a Note" to denote the media change.

Note: Whenever the Resipher Device is disconnected from the Sensing Lid, this will appear as a break in the recording on Lucid Lab Software (Figure 1C), and data will not be recorded. Thus, it is important to ensure that media changes / cell treatments are done so as to not interfere with measurements required at time points of interest.

Part 4: Exporting and analyzing oxygen consumption data

Timing: Experiment dependent

Following the conclusion of an experiment, raw data can be exported and saved as a ".CSV" or ".JSON" file. There is a multitude of data that can be exported, which can provide insight into any discrepancies in measurements (see troubleshooting for more detail). These measurements include oxygen flux (i.e., OCR), O_2 concentration, atmospheric pressure, relative humidity, temperature, intensity and raw values. For quantification, export "flux". The values are reported as fmol/mm^2/s.

18. Export "flux" measurements as a .CSV file type (Figure 1D).

Note: There are options to export oxygen consumption at different resampling time intervals. We export data using 15-min intervals. This allows the user to evaluate if any discrepancies exist within the time-range of interest due to environmental and/or technical issues.

Note: The resultant file may not be organized based on experimental arrangement, thus, to simplify analysis, users can copy the data into a separate Excel file in an organized fashion (i.e., replicates of the same condition arranged in parallel rows or columns).

19. Generate representations and summaries of the data, which can be performed in various ways as demonstrated in Figures 2 and 3.

⚠ CRITICAL: The data representations described below can be presented as 1) not normalized to cell number (Figure 2), which provide information on global changes within an experimental condition or 2) normalized to the number of nuclei (Figure 3), which provides additionally useful information, especially in situations where cell manipulations or treatments may influence proliferation rate or cell death.

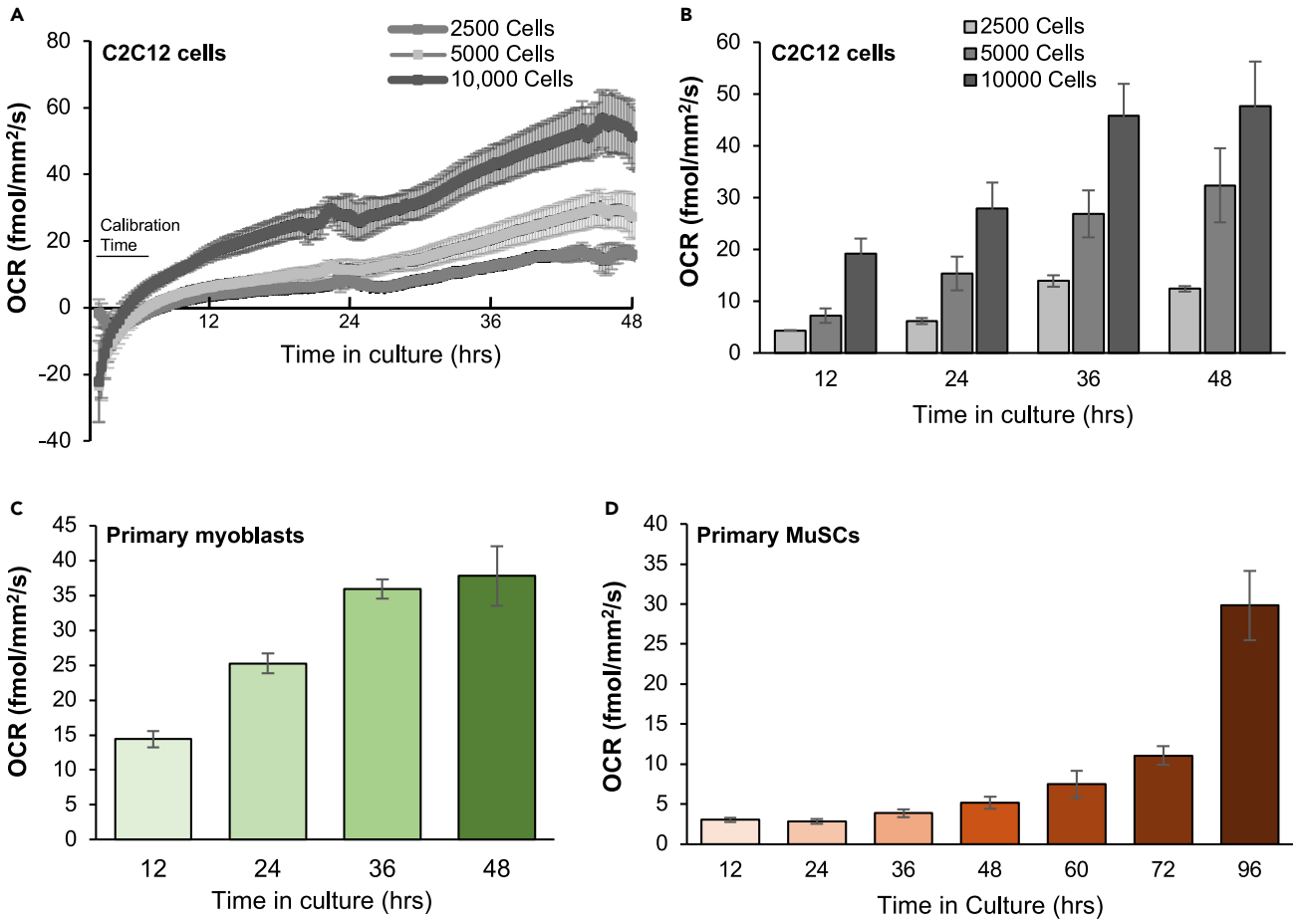

Figure 2. Oxygen consumption rate in C2C12 myoblast cells, primary mouse myoblasts and primary MuSCs

(A) Tracing of live cell-respirometry oxygen consumption rate (OCR) during 48-h of C2C12 myoblast proliferation, whereby differing amounts of C2C12 cells were plated. Traces represent the mean of 2-5 technical replicates. Error bars represent ±SEM. "Calibration Time" represents the period in which the Resipher device is undergoing calibration at the onset of an experiment.

(B) Quantification of OCR in proliferating C2C12 myoblasts. 2500 cells *N* = 2; 5,000 and 10,000 cells N = 5 technical replicates, mean ± SEM.

(C) Quantification of OCR in proliferating primary mouse myoblasts. *N* = 4 biological replicates, mean ± SEM.

(D) Quantification of OCR in mouse MuSCs. *N* = 5 technical replicates, mean ± SEM.

Note: To generate cell counts tandem plates that are treated identically to the experimental plate should be utilized. A tandem plate should be created for each time point in which data will be summarized.

Note: Tandem plates are fixed at these pre-determined time points followed by nuclei staining, visualized using fluorescent microscopy and counted. We visualize 3 ROI's per well using a 10× objective, manually count each nucleus per ROI and subsequently calculate the number of nuclei per well. Raw OCR values are then normalized to nuclei counts.

Note: We manually count nuclei to ensure accurate selection of each nucleus, as nuclei are densely packed in confluent myoblasts, and differentiating myotubes, and automated software may misrepresent counts.

a. Present tracings of OCR over time can be presented as shown in Figures 2A and 3A.

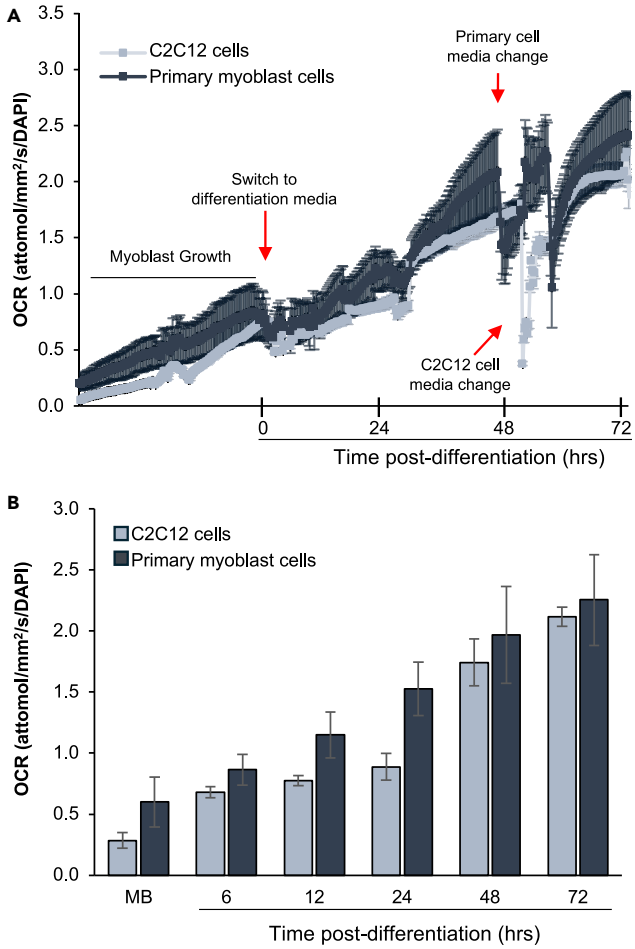

Figure 3. Oxygen consumption rate during differentiation in C2C12 myoblast cells and primary mouse myoblasts

(A) Tracing of live cell-respirometry oxygen consumption rate (OCR) per nuclei (DAPI), during periods of myoblast growth and differentiation in C2C12 and primary myoblasts. Trace represents the mean of 4–5 biological replicates. Error bars represent ±SEM. Notes on graph represent changes in experimental conditions.

(B) Quantification of OCR in myoblasts (MBs) and during differentiation in C2C12 and primary mouse myoblasts. n = 4–5 independent experiments, mean ± SEM.

Note: To do so, OCR recordings from each 15-min interval can be plotted over time. Each data point represents the average OCR for technical or biological replicates, with error bars representing the variability between replicates.

b. Generate bar graphs that summarize the data at time points of interest as depicted in Figures 2B–2D and 3B.

Note: This is done by calculating the average of the recordings between technical or biological replicates during the 1-h interval for time points of interest.

Note: As an example, the data that is exported contains measurements made in 15-min intervals. Thus, the average of the last four time points leading up to the time interval of interest.

EXPECTED OUTCOMES

The expected outcomes below are based on previous findings[1] and unpublished observations. The measurements shown represent oxygen consumption rates (OCR) in cells that are not normalized to the number of nuclei (Figure 2) and those that are normalized (Figure 3).

Oxygen consumption rates in proliferating C2C12 myoblasts

We typically find that in proliferating C2C12 myoblasts, OCR ranges from ~5 fmol/mm^2/s to ~45 fmol/mm^2/s, increasing as cells are proliferating. Data in Figures 2A and 2B depict the impact of plating density and time on OCR in C2C12 myoblasts (Figures 2A and 2B). Specifically, 3 cell densities were evaluated, 2500 cells/well, 5000 cells/well and 10000 cells/well. These data reveal that 1) the Resipher is sensitive to the number of cells plated and 2) OCR is increased as cell number increases. (Figures 2A and 2B).

Oxygen consumption rates in proliferating primary mouse myoblasts

The average OCR of proliferating primary myoblasts, plated at 20,000 cells/well ranges from ~15 fmol/mm^2/s to ~35 fmol/mm^2/s, when confluency is reached (Figure 2C).

Oxygen consumption rate in freshly isolated and cultured muscle stem cells (MuSCs)

MuSCs have very low metabolic activity, as they are exiting from a quiescent state. As such, the average OCR of freshly isolated MuSCs, plated at 100,000 cells/well is ~3 fmol/mm^2/s (Figure 2D). As these cells begin to activate and enter proliferative stages (0–72 h in culture), there is a modest increase in OCR to ~10 fmol/mm^2/s. As the cells transition into primary myoblasts, at 96 h in culture, the OCR of these cells increases to ~30 fmol/mm^2/s.

Oxygen consumption rates during myogenic differentiation

During the early phases of differentiation of both C2C12 and mouse primary myoblasts there is a considerable increase in OCR when normalized to the number of nuclei, which stabilizes after 48–72 h (Figures 3A and 3B).

LIMITATIONS

The protocols described have been optimized in cultured cells derived from mice. It is important to note that these cells do not reside in their native environment (in-vivo), and thus interpretations from the data generated must be made carefully. As such, the data derived with this protocol can be used in addition to other assays that examine mitochondrial respiration in a native environment (i.e., ex-vivo tissue preparations). In addition, the reproducibility of the findings using the Resipher system for muscle cells derived from other host species, including humans, remains unexplored.

A further limitation of the Resipher Device is that the maximum number of wells that can be used is 32 per experiment. This may limit the number of replicates and/or conditions that can be tested in a single experiment, as it is recommended to perform experiments in triplicates. However, it should be noted that when comparisons between different trials on the device were made, we have not seen significant differences between the same conditions (i.e., 24 h proliferating primary myoblasts from two different Resipher plates; data not shown).

Furthermore, although the information gathered from monitoring live-cell respiration is valuable, it does not provide insight into the efficiency of the mitochondria (i.e., maximal respiratory capacities). Thus, to gain insight into organelle capacity, mitochondrial stress tests, such as those run on the Seahorse Device, or mitochondrial ATP production measures, should be considered in conjunction with the data generated in the protocol described above.

Finally, the data generated from the Resipher Device in the protocol described above does not reveal the nutrient source being utilized for OXPHOS in the cells being studied

(i.e., carbohydrate -derived electron donors vs. fatty acid-derived electron donors). As such, to understand which metabolic intermediates are being utilized by mitochondria further experiments are required.

TROUBLESHOOTING

Problem 1

Unsteady recordings / large fluctuations in recordings following step 9.

Potential solution

This is commonly caused by changes in the cell culture incubator environment. We typically see this during periods where users are actively opening and closing the incubator door to conduct cell work. To minimize this there are two possible solutions.

- Instruct users to carefully open the incubator door, and remove required components rapidly, being cautious not to slam the door on closure.
- Contain the Resipher device in an independent incubator that is not used for any other purpose during the experimental time course.

Problem 2

Calibration times are longer than anticipated (i.e., greater than 2 h). As seen in the first 4-h of recordings in Figure 2A, it is common for calibration to take some time at the beginning of an experiment, following step 9. This is also common following media changes / treatment of cells, following step 16.

Potential solution

As discussed within the protocol itself, it is essential that users work rapidly during steps that take place outside of the tissue culture incubator. Some guidelines beyond those discussed above, that may assist include the following:

- As cells are in suspension (prior to adhering), there may be fluctuations in the recordings. Users may want to considerer gently centrifuging the culture plate to enhance adherence. However, since this will remove the cells from the experimental environment it may also lead to a prolonged calibration time as the media and cells are re-introduced to the tissue culture incubator.
- Rather than immediately beginning the experiment following passaging of cells, users may want to allow cells to adhere and adapt to the tissue culture incubator conditions prior to beginning the experiment.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Mireille Khacho (mkhacho@uottawa.ca).

Technical contact

Further information regarding technical aspects of this protocol should be directed to and will be fulfilled by the technical contact, Dr. Mireille Khacho (mkhacho@uottawa.ca).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate any unique data sets or code.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

M.K. conceptualized and led the study. M.T. and M.K. developed and optimized the protocols and designed the experiments. M.T. performed the experiments and quantified the data. M.T. wrote the manuscript and designed the figures. M.K. contributed to writing the manuscript, generating the figures, and editing and finalizing the paper and figures.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Triolo, M., Baker, N., Agarwal, S., Larionov, N., Podinić, T., and Khacho, M. (2024). Optic atrophy 1 mediates muscle differentiation by promoting a metabolic switch via the supercomplex assembly factor SCAF1. iScience *27*, 109164. https://doi.org/10.1016/j.isci.2024.109164.

2. Baker, N., Wade, S., Triolo, M., Girgis, J., Chwastek, D., Larrigan, S., Feige, P., Fujita, R., Crist, C., Rudnicki, M.A., et al. (2022). The mitochondrial protein OPA1 regulates the quiescent state of adult muscle stem cells. Cell Stem Cell *29*, 1315–1332.e9. https://doi.org/10.1016/j.stem.2022.07.010.

3. Cogliati, S., Frezza, C., Soriano, M.E., Varanita, T., Quintana-Cabrera, R., Corrado, M., Cipolat, S., Costa, V., Casarin, A., Gomes, L.C., et al. (2013). Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. Cell *155*, 160–171. https://doi.org/10.1016/j.cell.2013.08.032.

4. Varanita, T., Soriano, M.E., Romanello, V., Zaglia, T., Quintana-Cabrera, R., Semenzato, M., Menabò, R., Costa, V., Civiletto, G., Pesce, P., et al. (2015). The OPA1-dependent mitochondrial cristae remodeling pathway controls atrophic, apoptotic, and ischemic tissue damage. Cell Metab. *21*, 834–844. https://doi.org/10.1016/j.cmet.2015.05.007.