OMEGA^{MP} 3D Skeletal Muscle Device

User Guide

Contents

5524 Saint-Patrick, Office 460
Montreal, Quebec, Canada
H4E 1A8

CONTACT US AT INFO@ENUVIO.COM

v2.2

General Information

Unpacking

Thank you for purchasing eNUVIO's OMEGA^{MP} devices. All the items contained within the shipping vial have been packaged under sterile conditions. To maintain sterility, it is recommended to unpack the contents of the shipping vial in an aseptic environment (e.g. in a biological safety cabinet). The shipping vial (and label) is completely autoclavable and can be repurposed (it is also recyclable).

The OMEGA^{MP} device kit contains:

- 4 x OMEGA^{MP} devices (individually packaged)

OMEGA^{MP} devices are packaged sterile and are ready to use in cell culture.

Before Starting

It is recommended to prepare all reagents and tools required to carry out the protocol in its entirety prior to opening and removing the device from its sealed packaging. If the sterility of the device is suspected to be compromised, the device can be re-sterilized using a plasma cleaner, UV/ozone cleaner or autoclave. OMEGA^{MP} devices are designed to fit into the wells of standard 12-well microplates. **These microplates are not included in the device kit.**

OMEGA^{MP} devices are compatible with a variety of common downstream experimental procedures including:

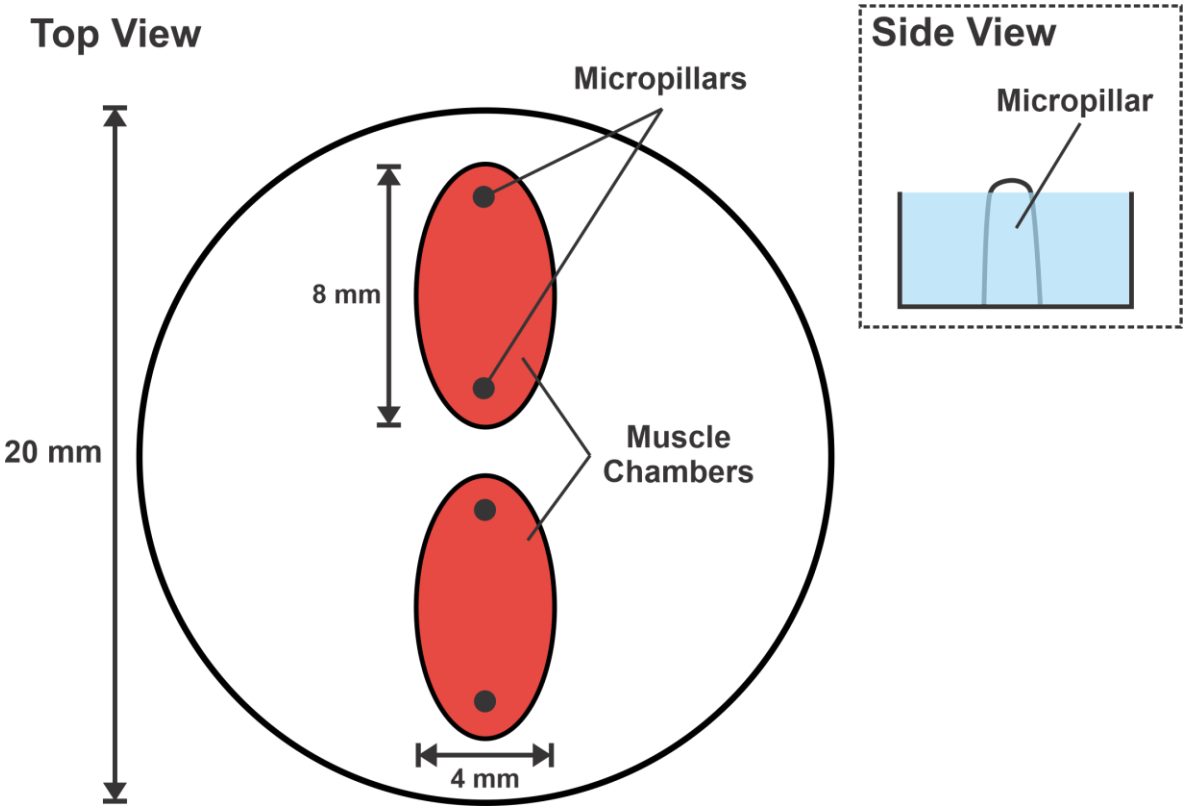
a) Cell fixation and immunohistochemical staining
b) Brightfield and fluorescence microscopy*
c) Calcium imaging*
d) RNA/Protein extraction and analysis (e.g. Western blotting)
e) Patch-clamp electrophysiology
f) Muscle contractility/force measurements

Surface Coating

All surfaces of the OMEGA^{MP} are made from uncoated polydimethylsiloxane (PDMS). An anti-fouling (non-adherent) surface coating is required to prepare the PDMS surface for the formation of 3D muscle microtissues. This antifouling agent prevents seeded myoblasts from settling and adhering to the chamber base and walls during seeding. Several biocompatible antifouling coating options exist, many of which are based on the antifouling properties of polyethylene glycol (PEG) or polyethylene oxide (PEO) polymers. For example, poloxamer surfactants (e.g. Pluronic® F-127†), poly-L-lysine grafted PEG (PLL-g-PEG) and PEG-siloxane compounds can all be used as antifouling reagents for cell culture applications.

* *Note that OMEGA^{MP} base is ~650 µm thick and may not be suitable for imaging with short working distance objectives.*
† *Pluronic® is a registered trademark of BASF*

OMEGA^{MP} Schematic



OMEGA^{MP} Specifications

Seeding volume: 35 - 40 µL
Chamber surface area: ~0.25 cm^2
Micropillar height: 2.7 mm
Micropillar width: 1 mm
Device diameter: 20 mm
PDMS base thickness: ~500 µm
Number of chambers per device: 2

Cell Seeding Information

Cell Seeding Density

The surface area of the chamber of the OMEGA^{MP} device is ~0.25 cm^2. For the successful formation and maturation of 3D skeletal muscle microtissue, optimal seeding density should be determined. A seeding density of between 7.5 x 10^6 - 15 x 10^6 cells/mL of myogenic progenitor cells is suggested.

Seeding cultures: General Information

The following protocol was designed to use FACS-sorted primary myogenic progenitors to generate the 3D skeletal muscle structure. In this case, successful microtissue formation has been shown using a 95:5 myoblast(CD56+):fibroblast(CD56-) ratio. Importantly, the general methodology presented below is also compatible with other sources of skeletal muscle progenitors (e.g. iPSC-derived skeletal muscle, immortalized myoblast lines, and primary myoblasts). Successful differentiation and maturation of 3D skeletal muscle microtissues does depend heavily on the initial seeding density, therefore it is strongly recommended that plating densities be optimized for each cell type/source to be used in these devices.

After counting cells and collecting the appropriate number of cells by centrifugation to be used for seeding the muscle chambers, it is recommended to extract as much medium as possible from the cell pellet to prevent media carryover into the hydrogel-ECM seeding mixture.

Seeding cultures: Timing

The differentiation and maturation rate of each culture will strongly depend on the origin and type of cells that are being utilized. Depending on their source, seeded hydrogel-embedded myogenic progenitors generally start to form microtissues within 1 - 3 days, continue to mature over the course of 14 days, and can be kept for >4 weeks in culture.

Troubleshooting Cultures

1) Seeded cells form visible clumps or aggregates within the chamber

   3D skeletal muscle microtissues form best when they are seeded in a homogenous manner. That is, the cells are spread evenly within the seeding hydrogel and throughout the chamber. If this is not the case, there is a tendency for cells to clump, aggregate and form spheroid-type structures. This type of observed aggregation can also occur if the hydrogel-ECM is not sufficiently homogenous. For example, this can be a result if the hydrogel mixture is not being thoroughly mixed, or if it polymerizes during mixture preparation (too early) or too quickly. To avoid this, it is highly recommended to keep all plasticware, including pipette tips, that contact the hydrogel-ECM mixture cold during preparation. The device should be kept on ice during plating to ensure seeding homogeneity within the chamber.

2) Muscle microtissue fails to form

   There are several reasons why skeletal muscle microtissue might fail to form. The most common cause of microtissue formation failure is due to low cell density. Seeding the correct number of cells per chamber is critical for successful formation of the microtissue. Microtissue formation can also fail if the physical hydrogel properties are incorrect. The hydrogel scaffold is essential for the myoblasts to rearrange correctly to

form the muscle microtissue. Usually, this is due to one of the components of the seeding mixture being erroneously omitted from the seeding mixture.

3) Muscle microtissue detaches from one of the micropillars

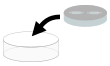
Detachment usually occurs after the microtissue has formed and begins to create tension between the micropillars. Under tension, the micropillars bend and the microtissue slips off the top (usually only one side). This type of detachment occurs because the seeding volume was too high, forming a thicker muscle microtissue that sits closer to the top of the micropillars. Reducing the mixture seeded volume by 3 – 5 µL solves this problem.

Protocol

Device Setup and Coating

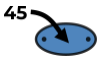
1) Under aseptic conditions and using a sterile blade or scissors, cut open the package of the OMEGA^{MP} device.

2) Using sterile flat-tipped tweezers or another suitable tool, carefully remove the device from its package, taking note of its orientation.



3) Place each device into a well of a standard 12-well plate or other suitable container (well diameter must properly accommodate the 20 mm device).

4) Proceed with coating the chambers for seeding cells.



5) Add 45 µL of 5% Pluronic® F-127 in PBS to each chamber.

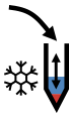
6) Seal the plate with parafilm and place at 4°C for at least 12 hours.

Seeding of Myogenic Progenitors

7) Prepare hydrogel mixtures on ice using pre-cooled pipette tips. The OMEGA^{MP} device should already be cool from the coating incubation, and should be kept cool during seeding by placing the plate on ice.

8) Prepare at least 90 µL of final hydrogel mixture per device (2 chambers per device; each chamber has a seeding volume of ~35 - 40 µL). Prepare the final hydrogel mixture **on ice without thrombin**

9) Prepare and pellet the appropriate number cells required for the plating. Final seeding density should be between 7.5 x 10^6 and 15 x 10^6 cells/mL. For example, to seed **two chambers** at 7.5 x 10^6 cells/mL, pellet 600,000 cells. After centrifuging to pellet the cells, make sure to remove as much media as possible to minimize carryover into the muscle chambers. **Place the cell pellet on ice**.



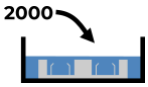
10) Add 80 µL (i.e. for 2 chambers when using 40 µL seeding volume per chamber) of the prepared hydrogel mixture to the cell pellet and resuspend thoroughly to produce a single cell suspension.

11) Aspirate the coating solution from each muscle chamber. **Washing the chambers is not necessary**.

12) Add thrombin (0.2 U/mg of fibrinogen) to the hydrogel/cell mixture and **immediately** resuspend using a cold pipette tip (use a 200 µL tip).

13) Promptly seed 35 - 40 µL of the hydrogel mixture into each muscle chamber. Ensure the seeding volume distributes uniformly within the chamber and does **not completely submerge the micropillars.**

14) Incubate the dish for 5 minutes at 37°C to allow the hydrogel/cell suspension to fully polymerize. During incubation, prepare the myoblast seeding media.

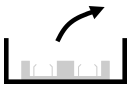


15) Gently add 2 mL of myoblast seeding media to the well to completely submerge the device in its entirety.

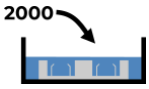
16) Return the plate to the incubator.

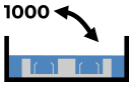
Differentiation and Maintenance

17) After 24 hours, verify that the culture is not adhering to the inner vertical walls of the chamber. If so, these can be gently detached using a small pipette tip.



18) After 2 days of incubation, verify that cultures have remodeled. Exchange the myoblast seeding medium with 2 mL of **freshly prepared myoblast differentiation medium**





19) Over the course of incubation, perform half-media changes every 2 days with myoblast differentiation medium.

Reagents and Solutions

(all solutions should be sterile)

Final Hydrogel Mixture

- DMEM
- 4 mg/mL bovine fibrinogen
- 20 % v/v ECM (e.g. Geltrex® or Matrigel®)
- 0.2 units of thrombin/mg of fibrinogen

Myoblast Seeding Medium

- Ham's F-10 nutrient mix
- 20 % fetal bovine serum (FBS)
- 1.5 mg/mL 6-aminocaproic acid
- 1X Antibiotic-antimycotic or Pen/Strep

Myoblast Differentiation Media

- DMEM (1 g/L glucose)
- 2 % horse serum
- 10 µg/mL insulin
- 2 mg/mL 6-aminocaproic acid
- 1X Antibiotic-antimycotic or Pen/Strep

Insulin

Available as a sterile solution at 10 mg/mL.

6-Aminocaproic acid

Prepared at 50 mg/mL in sterile water.

ECM (Geltrex®/Matrigel®*):

Follow manufacturers' recommendation for preparation and storage.

Fibrinogen (bovine)

Prepare at 10 mg/mL (can be up to 33 mg/mL) in sterile 0.9% w/v NaCl (saline). Dissolves slowly over several hours at 37°C. Filter-sterilize by gently passing through a 0.22 µm filter (do not apply too much force, and do not use a 0.1 µm filter). Aliquots of 1 mL can be stored for 6 months at -20°C. Thaw on ice before use.

Thrombin (human plasma)

Dissolve between 25 - 100 U/mL in 0.1 µm-filtered 0.1% BSA in PBS. Mix the solution thoroughly. Prepare aliquots of 50 µL and store at -80°C. Thaw on ice before use.

Pluronic® F-127†

Prepare at 5 % w/v in PBS. Filter sterilize with a 0.2 µm filter and store at 4°C.

**Matrigel® and Geltrex® are registered trademarks of Corning and Thermo Fisher Scientific, respectively*
†Pluronic® F-127 is a registered trademark of BASF