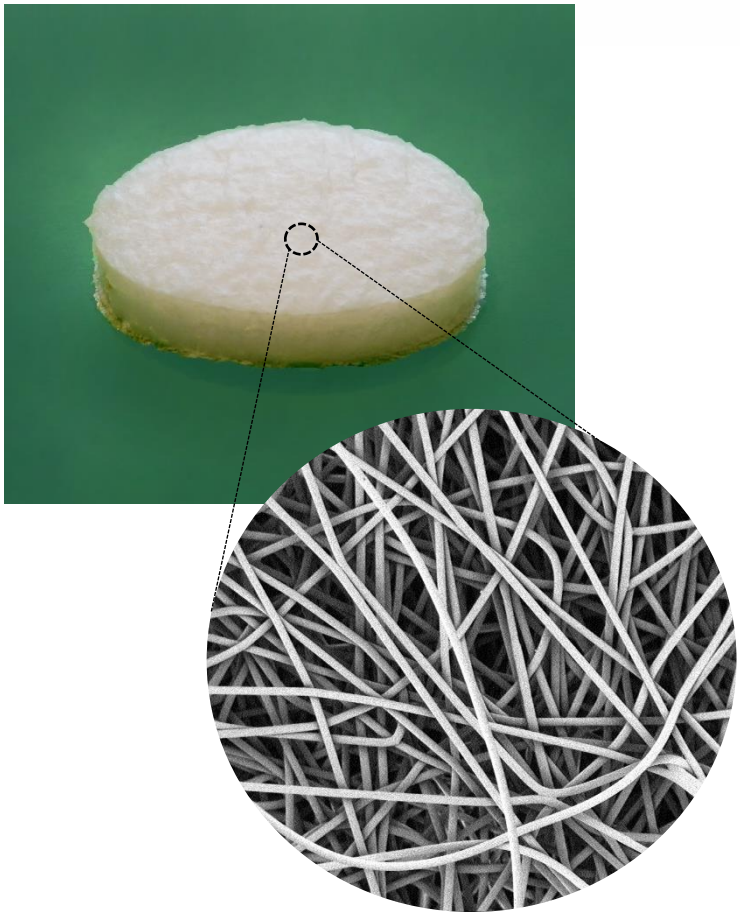
ELECTROSPINNING® COMPANY

Mimetix® Air Product Manual



INSTRUCTION FOR USE

The Electrospinning Company Ltd, Unit 5 Zephyr Building, Eighth Street, Harwell Campus,
Didcot, Oxfordshire, OX11 0RL, UK
info@electrospinning.co.uk www.electrospinning.co.uk

Contents

1. Description

Mimetix® Air scaffolds are highly porous electrospun microfibre scaffolds that facilitates 3D cell culture. The scaffolds are non-biodegradable in *in vitro* applications and contains no animal origin material.

- Material: medical grade Polycaprolactone (PCL)
- Fibre diameter: 6-8 micron
- Porosity: 90-95% porous with a pore size of ~ 100 µm.
- Thickness: 1.5 mm
- Format: Material is produced as sheets and can be supplied as individual discs or in well plates at room temperature according to requirement.
- Treatment: Supplied plasma treated for enhanced cell penetration and irradiated by gamma or e-beam.
- Product use: For Research Use Only. Not for use in diagnostic procedures.

2. Important Information

- Store in room temperature in the dark.
- Product shelf life: 2 years.
- Perform all procedures aseptically, in a laminar flow hood.
- Optimizing culture inoculation and incubation conditions may improve performance.

3. Culturing Cells on Mimetix Air Scaffolds in Well Plates

a. Materials Required (not provided)

- Culture vessels containing adherent cells
- Complete growth medium, pre-warmed to 37°C
- Phosphate Buffered Saline (PBS), containing no calcium and magnesium
- Dissociation reagent such as trypsin
- Disposable sterile tubes containing complete growth media
- 37°C incubator

b. Plating Loose Disks

The following is a general protocol for culturing cells on plasma treated sterile Mimetix Air scaffolds. If the scaffolds are received as disks (without well plate), place the disks in non-tissue culture treated or ultra-low attachment well plate. Media volumes may be optimised for specific cell type and disk sizes. All amounts are given on a per well basis.

c. Recommended Cell Seeding Method

- Grow cells in appropriate media and culture conditions. Harvest cells and centrifuge at x 300 g for 5 minutes to obtain cell pellet. Resuspend the cell pellet in required quantity of pre-warmed complete growth media and count the number of cells using a haemocytometer or an automated cell counter.
- Mix cells gently by pipetting and seed the required cell number in the cell seeding media volume mentioned below.

Well Plate	Seeding Density*	Cell Seeding Media Volume (µl)	Final media volume (ml)
24	0.06 x10^6	100 -200	1
12	0.2 x 10^6	200-400	2
6	0.4 x 10^6	500-600	3

*cell-seeding density should be optimised based on the proliferation rate of the cells and the duration of the study.

- Allow the cells to adhere to the scaffolds at 37 °C in an incubator for 1-2 h.
- Following cell attachment, flush the well with appropriate quantity of media to obtain final media volume as mentioned above.
- Place the well plates back in the incubator at 37 °C.

d. Cell Retrieval Process

- To recover cells from the scaffolds, gently remove and discard the spent media from the well plates.
- Wash the scaffolds x3 with PBS to ensure all the media has been removed from the scaffolds.
- Add the required quantity of trypsin.

Well plate	Trypsin quantity (µl)
24	50
12	100
6	200

- Immediately after adding the trypsin, swirl the plate to ensure that the entire surface of the scaffold is covered.
- Allow cells to dissociate for 1-2 minutes. Gently pipette a few times to encourage the cells from within the scaffold to detach and gently tap on the sides of the plate to encourage dissociation.
- Transfer the trypsinized cells to fresh sterile tubes containing complete growth medium.
- Add fresh media to the same scaffolds and gently pipette a few more times to ensure all the cells are retrieved from the scaffold and add it to the same sterile tube containing the cells.
- Centrifuge the tubes at x 300 g for 5 minutes to obtain cell pellet.

e. Cell Lysis Procedure

- The pierce IP cell lysis buffer (Thermo Fisher Scientific) is compatible with Mimetix Air scaffolds.
- Remove the cell culture media from well plates and wash the cells with phosphate buffered saline.
- Add ice cold lysis buffer to the cells according to the manufacturer's instructions and incubate on ice for 5-10 minutes with periodic mixing.
- Transfer the lysate along with the scaffold to a microcentrifuge tube.
- Vortex the tubes for 30s and centrifuge at ~ 13,000 x g for 10 minutes to pellet the cell debris.
- Transfer the supernatant to new tube for DNA, RNA and protein analysis.

4. Culturing Cells on Mimetix Air scaffolds - Stirred Tank Systems

For stirred tank systems the following protocol provides the best seeding method.

a. Materials Required (not provided)

- Sigmacote® available only from Sigma Aldrich
- Glass tubes with perforated caps for gas exchange
- Roller Mixer
- Stirred tank system
- Culture vessels containing adherent cells
- Complete growth medium, pre-warmed to 37°C
- Phosphate Buffered Saline (PBS), containing no calcium and magnesium
- Disposable sterile tubes containing complete growth media
- 37°C incubator

b. Preconditioning the Vessels

To ensure that cells do not adhere to the spinner flasks, use Sigmacote to siliconize the surface of the spinner flask and the glass tube used for seeding cells on the scaffolds.

Apply Sigmacote to the entire vessel surface area and aspirate.

Place the vessels in the fume hood and rinse with distilled water after 24h and autoclave prior to use.

c. Preconditioning the Scaffolds

i. Non-Plasma treated scaffolds

Place the scaffolds in the Sigmacote treated tube and flush the tube with complete growth media. Place the tube in the fridge at 4 ºC for 48 h. On the day of seeding, discard the conditioned media and follow the recommended seeding protocol.

ii. Plasma treated scaffolds

Plasma treated scaffolds can be used directly for seeding cells.

d. Recommended Cell Seeding Protocol

The following protocol is based on seeding primary Mesenchymal stem cells. To obtain best results, the protocol needs to be optimised for incubation time and seeding density based on the cell type. The dimensions of the scaffold used for the optimization was 6mm x 1mm thickness.

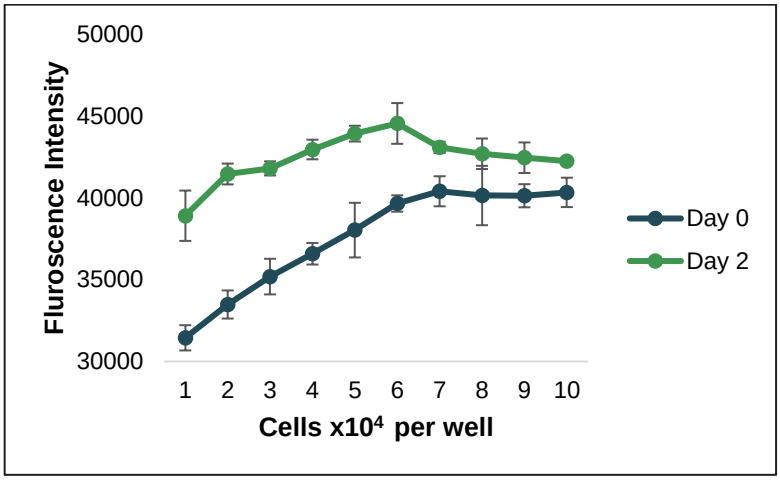
To seed cells on the scaffolds, place the required number of scaffolds in a glass tube that is preconditioned with Sigmacote. Add required quantity of cells based on the dimensions and the quantity of the scaffolds. For 6 mm x 1 mm scaffolds, $2x10^4$ cells/scaffold was added. A total of 20 scaffolds were seeded in 10 ml of media. The tubes were sealed and placed on a roller mixer and allowed to gently mix for 8 h at 37 ºC in an incubator. After 8 h, transfer the entire contents of the tube to stirred tank and add required quantity of media. For a 50 ml stirred tank, up to 20 scaffolds with the above-mentioned dimensions can be used.

5. Performance of Assays on Mimetix Air Scaffolds

a. Cell viability measurement

Mimetix Air scaffolds are compatible with a variety of resazurin based cell viability reagents. To assess cell viability and proliferation, use Alamar blue® or Prestoblue® directly on the scaffolds and follow manufacture protocol for the respective assays.

To demonstrate the performance of Alamar blue, non-plasma treated scaffolds were seeded with 3T3 cells in 24 well plates. The plates were incubated for 6 h to enable all the cells to adhere to the scaffolds. The scaffolds were then transferred into a new 24 well plate containing Alamar blue and fresh media in 1:10 ratio. The plates were incubated for 2 h and fluorescence was observed in Tecan infinite® 200 plate reader. The scaffolds were then washed with PBS and placed in Hanks balanced salt solution to remove all the dye. Fresh media was added to the scaffolds and placed in the incubator at 37º C. On day 2, the metabolic activity of the cells was measured following the same procedure.



The graph shows the metabolic activity of 3T3 cells on the scaffolds over 2 days.

Note: The dye may stain the scaffold and skew the results. It will be beneficial to obtain background absorbance on blank scaffold. To perform time point assay, place the scaffolds in balanced salt solution until all the dye has leached out following each absorbance/florescence measurement. Failure to ensure that all the dye has washed out will result in high background reading.

b. DNA quantification

To determine the quantity of DNA, 2 x10^4 3T3 cells were seeded on non-plasma treated scaffolds and 2D tissue culture treated well plates in 24 well plates. The plates were incubated for 24 hours and the cells were lysed using the above-mentioned protocol. Picogreen assay was performed according to manufacturer's instructions and fluorescence was measured using Tecan Infinite 200 PRO plate reader. The experiment was performed in triplicates.

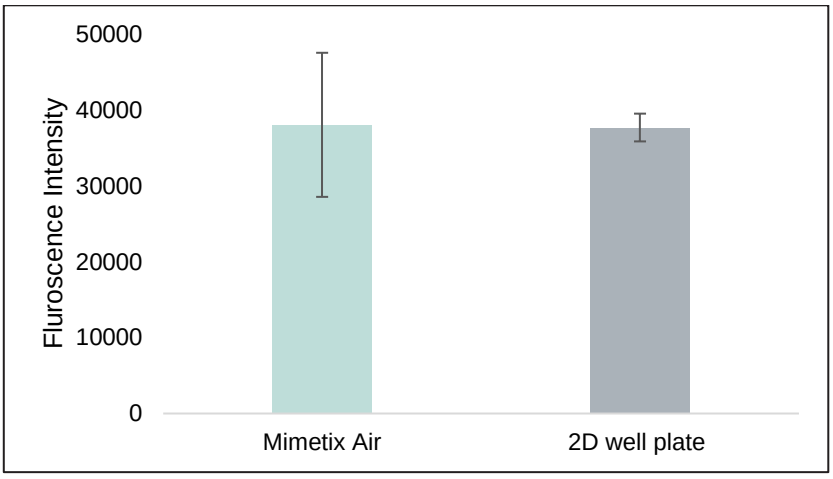


Figure 1 shows similar DNA quantity in both Mimetix Air and 2D well plate.

6. Imaging Cells on Scaffolds

Cell viability can be qualitatively assessed by using the LIVE/DEAD assay Kit on Mimetix Air scaffolds. To visualise cells adhered to the scaffolds follow manufacturer's instructions to prepare the reagents and add required quantity of reagent to the scaffolds in well plates. Incubate the cells for 45 minutes at 37º C. Following incubation, remove the scaffolds from the well plates and place them on glass slides. Add 10 -15 µl of reagent to the glass slide to keep the scaffolds wet. Cover the scaffolds with cover slips.

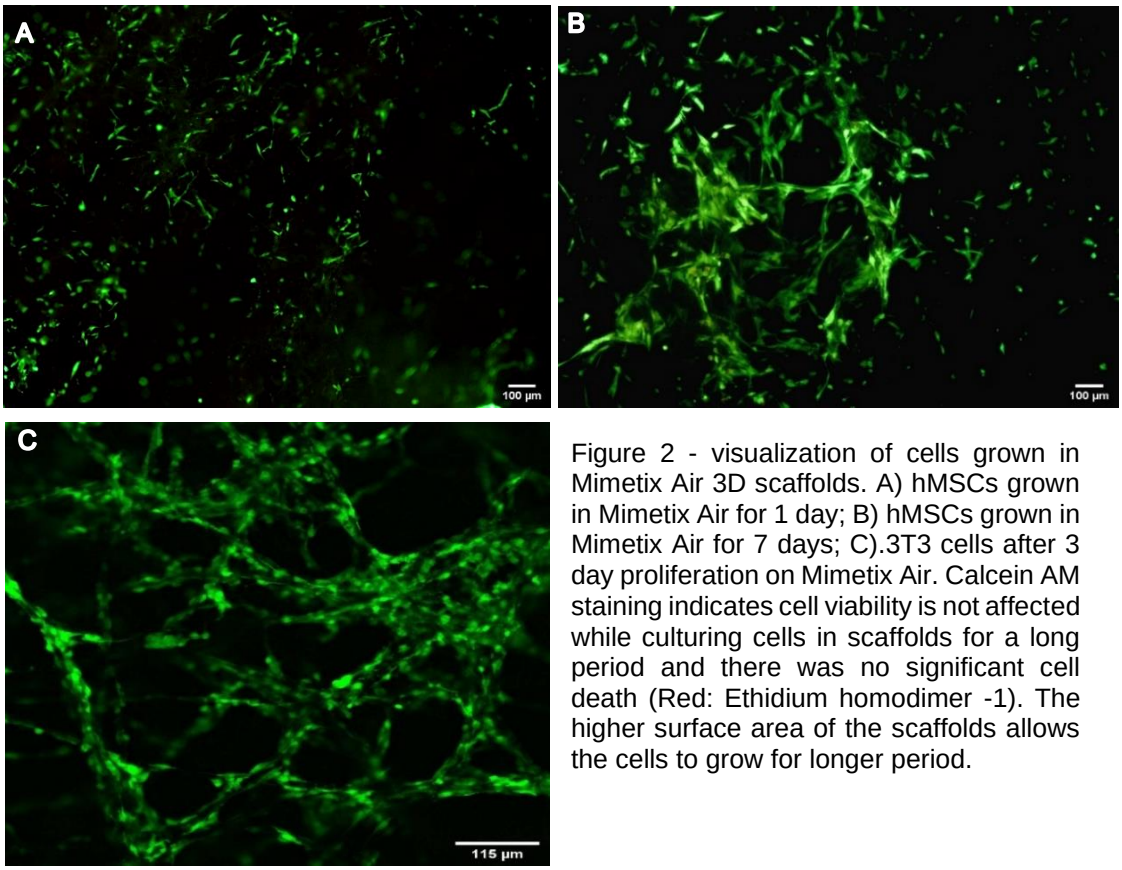


Figure 2 - visualization of cells grown in Mimetix Air 3D scaffolds. A) hMSCs grown in Mimetix Air for 1 day; B) hMSCs grown in Mimetix Air for 7 days; C).3T3 cells after 3 day proliferation on Mimetix Air. Calcein AM staining indicates cell viability is not affected while culturing cells in scaffolds for a long period and there was no significant cell death (Red: Ethidium homodimer -1). The higher surface area of the scaffolds allows the cells to grow for longer period.

To purchase Mimetix® products or to view the full portfolio, please contact our distributor AMS Biotechnology.

a. Fixing and Staining Cells on the Scaffold

The following protocol is suitable for fixing, staining and imaging cells in Mimetix Air scaffolds.

- Discard media and wash the cells twice with PBS.
- Add 4% paraformaldehyde for 30 minutes at room temperature to fix the cells.
- Following fixation wash the cells twice with PBS and permeabilise cells with 0.1% Triton X-100 in PBS for 5 minutes.
- Wash the cells twice with PBS and block using 0.5% BSA in PBS for 1h.
- Following blocking wash the cells twice with PBS and add primary antibody at the dilution recommended by the manufacturer and incubate for 1h.
- Wash the cells twice with PBS and add the secondary antibody following the manufacturer's instructions.
- Finally wash the cells twice with PBS.
- For DAPI and Actin staining, ReadyProbes® Reagents from Life Technologies (#R37606 and #R37110, respectively) work very well with the Mimetix scaffold.

7. FAQ

- **What are Mimetix Air scaffolds?**

  Mimetix Air scaffolds are support matrices for the growth of adherent cells (mammalian and animal cells). Mimetix Air scaffolds are optimised for use in stirred tank and perfusion bioreactor, but they can also be used in well plates.

- **What are Mimetix Air scaffolds made of?**

  Mimetix Air scaffolds are made of medical grade Polycaprolactone (PCL) polymer. They are fabricated by electrospinning the polymer to create microfibre scaffolds with 90-95% porosity.

- **What is the size of the scaffolds?**

Fibre Diameter	6-8 µm
Thickness of the scaffold	1.5 mm (other thicknesses available)

- **Can the scaffold dimensions be optimised to requirement?**

  Yes, the material is produced as a sheet which is then laser cut to specific dimensions.

- **Which cells have been successfully grown on Mimetix Air scaffolds?**

Primary cells	Cell line
Human Mesenchymal stem	3T3
Human Embryonic stem	INS-1
Human dermal fibroblast	
Human muscle satellite	
Human Adipose-Derived Stem Cells	

- **Are the disks reusable?**

  The reuse of the disks is not recommended, similar to other scaffolds and microcarriers currently available in the market.

To purchase Mimetix® products or to view the full portfolio, please contact our distributor AMS Biotechnology.