MicroMatrix™ 36

Cell Culture System

INSTRUCTION MANUAL

Catalog # MM012011

Revision A

For Research Use Only

www.microstem.com

MATERIALS PROVIDED

Catalog # MM012011

Component 1: MicroMatrix™ 36 slide (1)
Component 2: Four-chambered cell culture tray (1)

STORAGE CONDITIONS

Store at –20°C upon receipt. Keep the components in the foil pouch until ready to use.

ADDITIONAL MATERIALS REQUIRED

Sterile 1× phosphate buffered saline solution (PBS)
Cell culture media
Phase contrast microscope
4% paraformaldehyde (PFA) prepared in 1× PBS
Milli-Q H_2O

Ordering Information and Technical Services

P.O. Box 910594
San Diego, CA 92191

Telephone	(800) 819-2534
Order Toll Free	(800) 819-2534
Technical Services	(800) 819-2534
Fax	(800) 819-1514
E-mail	sales@microstem.com
World Wide Web	www.microstem.com

LIMITED PRODUCT WARRANTY

MicroStem product warranty limits to replacement of this product. No other warranties expressed or implied are provided by MicroStem. MicroStem shall not be liable from damages arising out of the use, the results of use, or the inability of use of this product.

INTRODUCTION

Mammalian cells exist in unique microenvironments *in vivo* that affect their behavior (growth, differentiation, death, etc.) and function. When cells are cultured *in vitro*, it is necessary to recapitulate such microenvironments for physiologically relevant growth. Among many factors affecting cell behavior *in vivo*, extra-cellular matrix proteins (ECMPs) play a particularly significant role in determining cellular functions. Currently, finding the physiologically relevant ECMP for cell culture is a trial and error process requiring large numbers of cells as well as a significant time and cost commitment. MicroStem's MicroMatrix™ 36 cell culture system (patent pending) permits 36 single and combinations of ECMPs to be tested simultaneously using a small number of cells in a short time period.

MicroStem's MicroMatrix™ 36 cell culture system contains a microscope glass slide functionalized with a proprietary hydrogel on the surface. Thirty-six ECMP conditions are deposited onto the hydrogel surface as printed array spots. Each spot has a diameter of 400 μm and is printed in replicates of 9 (Figure 2). ECMPs are localized within each spot without cross diffusion from neighboring spots, so each spot represents an independent 'well' or experiment. Cells of interests are seeded onto the slide in a 4-chambered culturing tray (included) and allowed to be cultured in the incubator for a desired amount of time. Cell morphology, attachment, and growth can be visualized using a bright field microscope. Specific cellular behaviors can be monitored by staining the cells (live and/or fixed) on the slide using specific fluorescence-based markers. Fluorescence signal can be detected using a fluorescence microscope imaging system, a high content imaging system or a DNA array scanner.

The MicroMatrix™ 36 cell culture system is simple to use in comparison to performing the same experiment in multi-well tissue culture plates. The MicroMatrix™ 36 cell culture system is ready to use, as ECMPs are pre-coated on the slide as printed spots; no multi-well protein coating process is required. Cell seeding, fixing, washing, and processing steps are done in one single solution exchange, eliminating multi-well solution handlings. Cell attachments are usually observed within 24 hours for most cell types.

PROTOCOL

Seeding cells onto the slide

1. Prepare 250,000 desired cells in 5 ml complete culture growth media (50,000 cells/ml). Seeding density of the cells is cell and experiment dependent, may require optimization by the user.

2. Remove the MicroMatrix™ 36 system, still in its foil pouch, from the −20°C freezer and put it under a sterile laminar flow hood. Open the foil pouch and remove the contents. First, take out the cell culture tray from the plastic bag and remove the lid. Then, open the cap on the slide holder and gently remove the slide from the slide holder. Take precaution while removing the slide; use sterile technique, such as wearing sterile gloves or using a sterile forceps, and only touch the edge of the slide. Put the slide in one of the chambers of the cell culture tray. Make sure the printed spot side is up (numbers and letter on the printed side of the slide should be legible).

3. Immediately add 5 ml of sterile 1× phosphate buffered saline solution (PBS) onto the slide. Make sure that the whole slide is immersed in the PBS solution and avoid trapping air bubbles underneath the slide. Wash the slide by rocking it gently back and forth several times and then aspirate the PBS solution away.

   Do not touch the surface of the slide.

4. Immediately add 5 ml of the desired culture media (same media used for culturing cells) and make sure the whole slide is immersed in the media. Wash the slide by rock it gently back and forth several time and then aspirate the media solution away.

   Do not touch the surface of the slide.

5. Mix the prepared 250,000 cells briefly by gently pipetting up and down and immediately add the cells directly onto the slide. Evenly distribute the cells by moving the pipette back and forth while dispensing the cells onto the slide. Replace the lid over the plate.

   Do not touch the surface of the slide.

6. Carefully transfer the 4-well tray containing the slide to the incubator and culture the cells at 37ºC, 5% CO_2 to allow cells to attach to the slide.

   Note: For cell lines, 12 hours should be sufficient to see cell attachment. For cells freshly isolated from tissue or cells previous frozen, it may require up to 2 days for significant cell attachment.

   Note: If a cell proliferation study is desired, reduce the cell number to allow extra space on the spot for cells to divide. We recommend starting with 100,000−150,000 cells depending on the size and morphology of the cells. The bigger the size of cells, the smaller number of cells is needed.

Washing and observing cells on the slide

1. After a minimum 5-hour incubation to allow sufficient cell attachment, remove the tray from the incubator and place it under the phase contrast microscope for initial observation, starting at the 5× objective lens. Gently move the plate back and forth to distinguish the non-adherent floating cells from the attached cells. This step can be repeated multiple times until sufficient cell attachment and desired cell morphology is observed.

   Note: Because of the non-fouling nature of the hydrogel surface on the MicroMatrix™ slide, cells will preferentially attach to the spots where appropriate ECMPs are coated. Cells are confined within the spots.

2. Remove the non-adherent floating cells by aspirating the media away, making sure the slide surface is not disturbed. Wash the slide twice with 5 ml culture media each, and then leave the slide in 5 ml of culture media.

3. Observe the cell attachment under the phase contrast microscope for each block of spots. Each block represents a set of 9 replicate ECMP spots. For ECMP identity of each block, see Figure 3.

4. Cells can be fixed at this stage or used for further experimentation, such as proliferation studies, differentiation studies, drug toxicity studies, etc. Cells can be cultured on the slide for an additional 2 days before fixing.

Fixing cells on the slide

1. Remove the culture media by aspirating the media away. Wash the cells on the slide twice with 5 ml of cold PBS.

2. Fix the cells on the slide by adding 5 ml of cold 4% paraformaldehyde (PFA) prepared in 1× PBS. Leave the slide in the PFA solution at 4^{o}C for 5 minutes, then incubate at room temperature for an additional 10 minutes.

3. Remove PFA solution and then wash the slide twice with 5 ml of PBS.

4. Slide can now be stained or stored in PBS at 4^{o}C for future staining.

Nuclear- and Immuno- staining cells on the slide

1. Stain the cells on the slide using an appropriate staining protocol.

   - For nuclear staining, many commercially available reagents, such as Hoechst, PoPo-3, DraQ5, etc. are suitable. Follow the manufacturer's instructions for staining.

   - For specific antibody staining, follow the manufacturer's instructions for immuno-cytochemistry dilution recommendations and procedures. After finishing antibody stainings, wash the slide twice (5 minutes per wash) with 5 ml of Milli-Q H_2O. Then, remove the slide from the plate and dry the slide on the rack in the dark.

2. Load the slide onto a fluorescence microscope for direct observation. If a high content imaging system or a DNA array scanner is used, please contact MicroStem, Inc. (sales@microstem.com, 800-819-2534) for instrument specific form factors (high content imaging systems) and the *.gal* file (DNA array scanner).

   Note: Do NOT use any mounting media, such as Vectashield.
   Do NOT use inverted position for observation commonly used for oil lenses.

APPLICATIONS

MicroMatrix™ 36 cell culture system has been successfully used to culture the following cell types:

Cell Lines:	**Primary Cells:**
MCF 7	Mouse Cardiac Progenitor cells
CHO	Rat Neo-natal Cardiomyocytes
HUVEC	Human Oligodendrocytes Precursor Cells
Human Fibroblast	Human Mesenchymal Stem Cells
MG-63	Human Hepatocytes
Jurkat	Human Embryonic Stem Cells
Vero	Human Liver Stellate Cells
MDCK	Human Lung Cancer Cells (including $CD133^+$ cells)
A549	

MicroMatrix™ 36 cell culture system has been successfully used to perform the following studies:

- Taxol efficacy studies
- EMT (Epithelial Mesenchymal Transition) studies of cancer cells
- Hepatocyte toxicity studies
- Mesenchymal stem cell differentiation studies
- Cancer stem cell proliferation studies

MCF 7 **Human Hepatocytes**

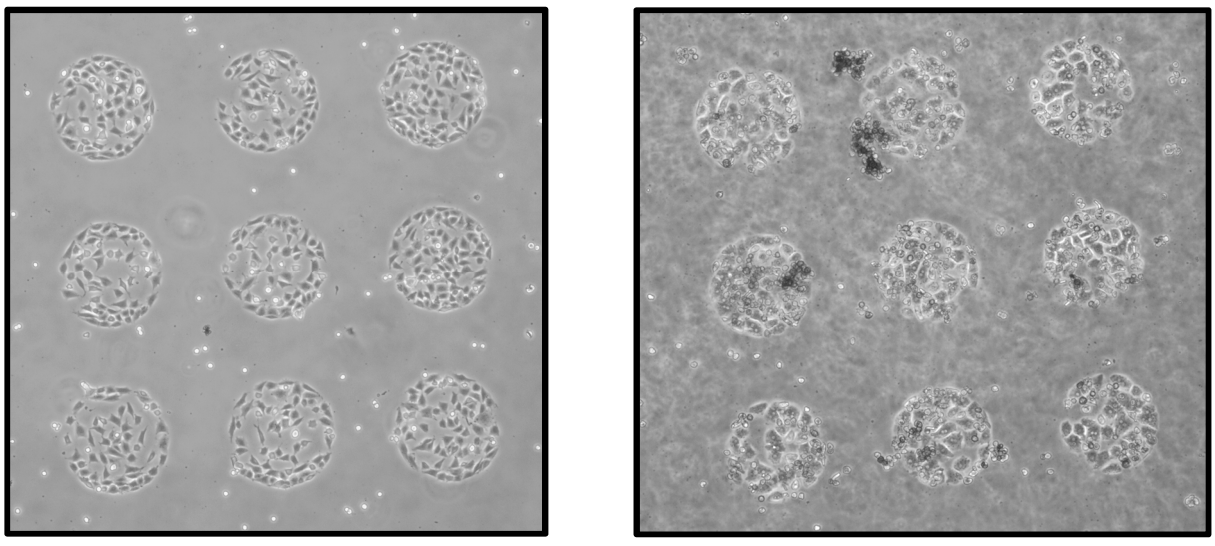

Figure 1: Examples of cell attachment within a single condition (9 replicate spots) on a MicroMatrix™ 36 slide. Left image is MCF 7 cells. Right image is human hepatocytes. Images were taken on a 5× objective lens on a Leica DMIL microscope system.

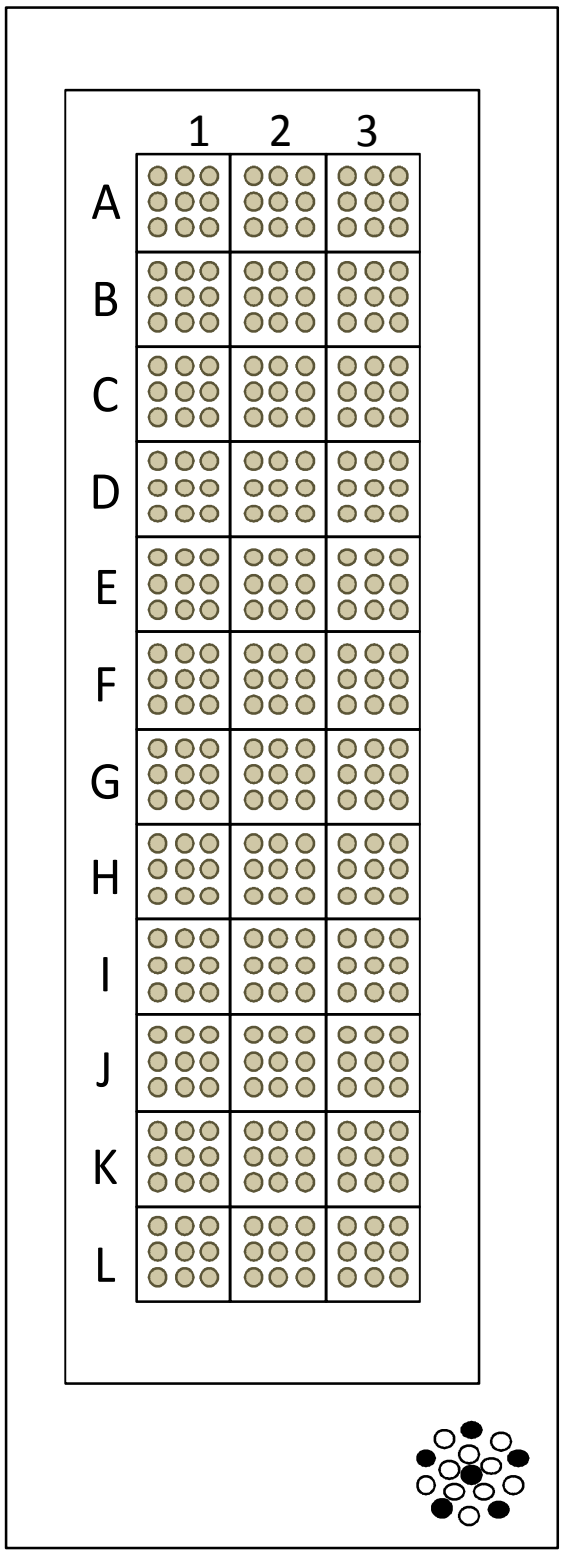

Figure 2: MicroMatrix™ 36 slide design. Each block contains a unique ECMP condition with 9 replicate spots. Spot diameter is 400 µm. Distance between spots in each block is 500 µm from center to center. Each block is 4.5 mm in diameter.

	1	2	3
A	Collagen I (250µg/ml)	Collagen I (125µg/ml) Collagen III (125µg/ml)	Fibronectin (125µg/ml) Collagen IV (125µg/ml)
B	Collagen III (250µg/ml)	Collagen I (125µg/ml) Collagen IV (125µg/ml)	Fibronectin (125µg/ml) Collagen VI (125µg/ml)
C	Collagen IV (250µg/ml)	Collagen I (125µg/ml) Collagen V (125µg/ml)	Fibronectin (125µg/ml) Laminin (125µg/ml)
D	Collagen V (250µg/ml)	Collagen I (125µg/ml) Collagen VI (125µg/ml)	Fibronectin (125µg/ml) Vitronectin(125µg/ml)
E	Collagen VI (250µg/ml)	Collagen I (125µg/ml) Fibronectin (125µg/ml)	Laminin (125µg/ml) Collagen IV (125µg/ml)
F	Fibronectin (250µg/ml)	Collagen I (125µg/ml) Laminin (125µg/ml)	Laminin (125µg/ml) Collagen VI (125µg/ml)
G	Laminin (250µg/ml)	Collagen I (125µg/ml) Vitronectin (125µg/ml)	Vitronectin (125µg/ml) Collagen IV (125µg/ml)
H	Vitronectin (250µg/ml)	Collagen I (125µg/ml) Topoelastin (125µg/ml)	Vitronectin (125µg/ml) Collagen VI (125µg/ml)
I	Tropoelastin (250µg/ml)	Fibronectin (83.3µg/ml) Laminin (83.3µg/ml) Collagen I (83.3µg/ml)	Vitronectin (125µg/ml) Laminin (125µg/ml)
J	Collagen IV (125µg/ml) Collagen VI (125µg/ml)	Fibronectin (83.3µg/ml) Laminin (83.3µg/ml) Collagen IV (83.3µg/ml)	Vitronectin (125µg/ml) Topoelastin (125µg/ml)
K	Collagen III (125µg/ml) Collagen V (125µg/ml)	Vitronectin (62.5µg/ml) Laminin (62.5µg/ml) Collagen I (62.5µg/ml) Collagen IV (62.5µg/ml)	Topoelastin (125µg/ml) Collagen IV (125µg/ml)
L	Negative Control BSA (250µg/ml)	Fibronectin (62.5µg/ml) Laminin (62.5µg/ml) Collagen I (62.5µg/ml) Collagen IV (62.5µg/ml)	Topoelastin (125µg/ml) Collagen VI (125µg/ml)

Figure 3: ECMP components of each block. Concentrations indicated in parentheses represent the concentration of the ECMP in the solution that is spotted on the slide.

REFERENCES

Brafman D., Shah KD, Fellner T, Chien S, Willert K. (2009) "Defining Long-Term Maintenance Conditions of Human Embryonic Stem Cells With Arrayed Cellular Microenvironment Technology." Stem Cells Dev., 18(8): 1141-1154.

Brafman, D, de Minicis S, Seki E, Shah KD, Teng D, Brenner D, Willert K, Chien S. (2009) "Investigating the role of the extracellular environment in modulating hepatic stellate cell biology with arrayed combinatorial microenvironments". Integr. Biol., 1(8): 513 – 524.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information is provided on MicroStem, Inc. website at *http://www.microstem.com.* MSDS documents are not included with product shipments.

TRADEMARK

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