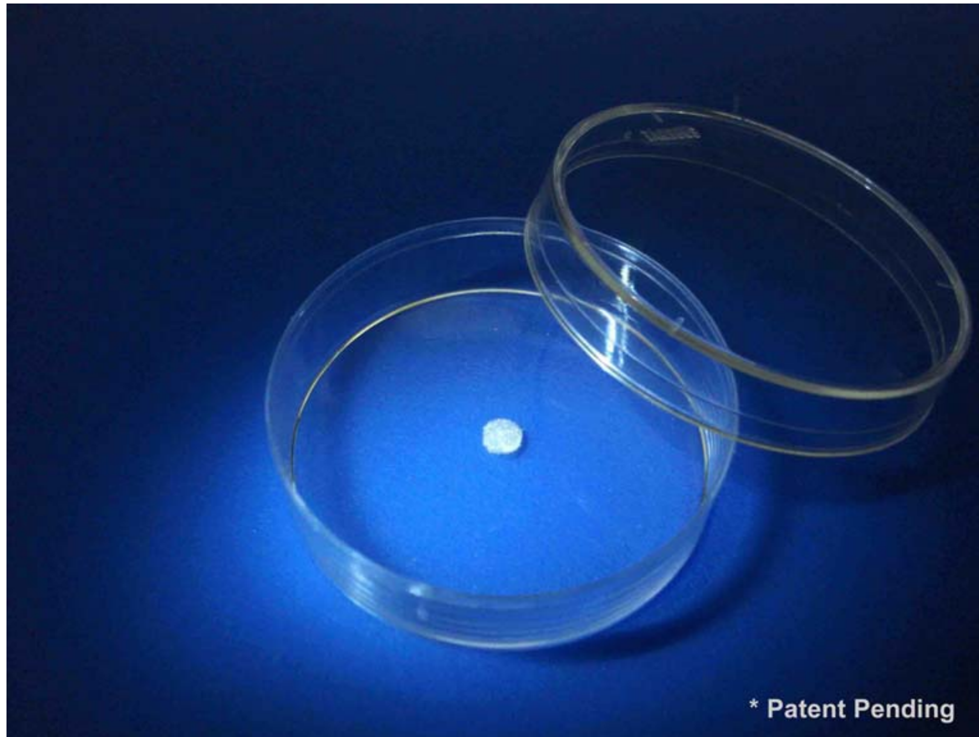
ExCel Matrix Biological Devices P. Ltd.
Hyderabad

3D Cell Culture
Microcarrier Culture
Cell Culture Bio-Assays
ExVivo Organ Model
Tissue Engineering

Dot-Cult®

A Convenient 3D Cell Culture Method*



Three dimensional (3D) cell culture of human and animal cells mimic natural tissue interactions with its environment better than 2D monolayer culture for any practical purposes.

Matrigel, collagen and reconstituted extracellular matrix as hydrogels are popular among cell biologists to create tissue model due to their ability to mimic extracellular micro-environment[1,2,3]. Porous scaffolds and hydrogels of biodegradable polymers are preferred for tissue engineering research due to robust handling properties[4,5].

Finding a common biomaterial that has both ECM mimicry and engineering properties has been difficult[1]. Further, delivering the biomaterial as cost effective ready-to-use devices has not been achieved[6].

Dot-Cult® is designed to provide 3D cell culture in popular and convenient Petri dish and multiwell plate formats.

3D culture is achieved in specified ECM Analog® scaffold coated area (a dot) of Dot-Cult® instead of entire surface of Petri dish. Uncoated area of Petri dish is treated for non-adhesion of cells to prevent any 2D-monolayer growth of cells. ECM Analog® coated area is kept optimal to provide proportionate number of cells to the culture volume.

Consequently, Dot-Cult® for 3D cell culture remains identical to a Petri dish in terms of convenience, acquaintance, no prior training or exposure, medium requirement, duration of culture, trypsinization and handling robustness.

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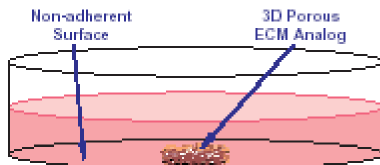
Highlights of Dot-Cult® and 3D Cell Culture Procedure

Dot-Cult®

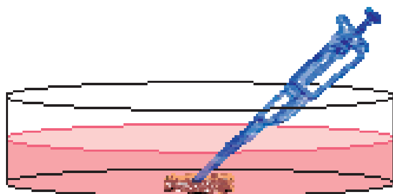


Highlights

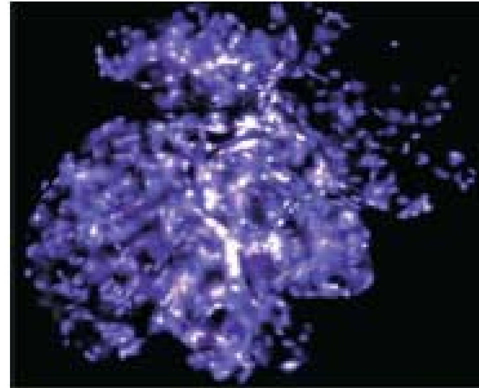
- Just add cells, media, and incubate.
- Change media as per monolayer culture.
- Harvest cells by usual trypsinization.
- Long term 3D cell culture.
- Multiple cell types can be inoculated in single plate to create organ like cultures.

General Method

1. Take out ready to use Dot-Cult® from sterile packaging under sterile laminar flow hood.
2. Prepare cell suspension in complete medium.
3. Adjust the cell number as per the culture duration and cell multiplication for inoculation.
4. Add required amount of cell suspension in Dot-Cult® under sterile conditions.



5. Incubate in appropriate conditions, e.g. at 37°C, 5% CO_2 for attachment on the 3D dot of ECM Analog® for overnight in incubator.
6. After cell attachment on 3D dot of Dot-Cult® unattached cells are washed away twice with buffer and culture is replenished with complete medium.
7. Alternatively cells in high concentration can be inoculated directly on the 3D dot in small volume (100µL maximum). Keep cells at 37°C, 5% CO_2 to attach for 2-3 hr and replenish with required amount of medium afterwards.
8. ECM Analog® coating can be pre soaked if required with PBS/ medium for 24 hours before cell inoculation. Discard soaking medium before inoculation.
9. Routine observations can be made using typical microscope. Bright field is likely to be more convenient than phase contrast.



Dense growth of cells as visualized under UV using DAPI for staining nuclei of cells

Precautions and Notes

1. Ensure that there is no residual protease enzyme in the cell suspension used for inoculation of Dot-Cult®. Protease enzyme will dissolve the ECM Analog®.
2. Cell number needs to be adjusted depending upon duration of experiment, cell multiplication etc. For example, if cell have low multiplication potential or not required to multiply, cells may be inoculated in high concentration (0.1 million to a few million cells/ mL culture volume), i.e. typical saturation density of monolayer cultures.
3. Lid of Dot-Cult® dish is not shown in the illustrations for convenience.
4. Coated ECM Analog® may detach as small particles. It can be washed out during medium change.

References

1. Linda Griffith, Acta Mater. 48, 2000, pp 263-277.
2. Kleinman H. K. et al. Biochemistry, 21, 1982, pp 6188–6193.
3. Elsdale T, Bard J, J Cell Biol, 54, 1972, pp 626-637.
4. Lutoff MP, et al. Nature Biotech, 23, 2005, pp 47-55.
5. Mano JF, et al. J. R. Soc. Interface, 4, 2007 pp 999–1030.
6. Editors, Nature, Vol. 424, 2003, pp 861.

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