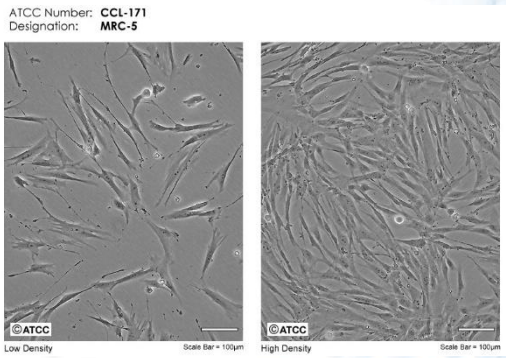
MRC-5 (Medical Research Council -5) Human Diploid Cell Culture using Tide Motion Bioreactors

Background

- Lung tissue obtained from 14-week fetus; Karyotype is 46,XY
- Fibroblast-like cell
- Population doubling to senescence is 42-48 passages (Jacobs,JP,1976)

Gross morphology



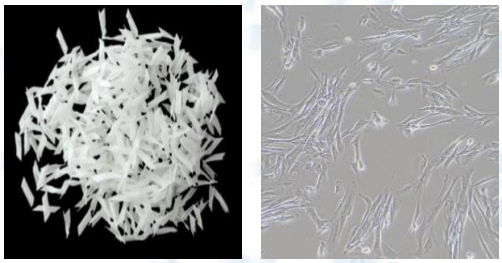
Applications

Large scale vaccine/viral production:

Adenovirus	Hep A/Hep B	Human Rabies
DTaP-IPV/Hib	MMR (MMR-II)	Varicella
Hep A	MMRV	Zoster (shingles)
Hep B		

Culture of MRC-5 cells

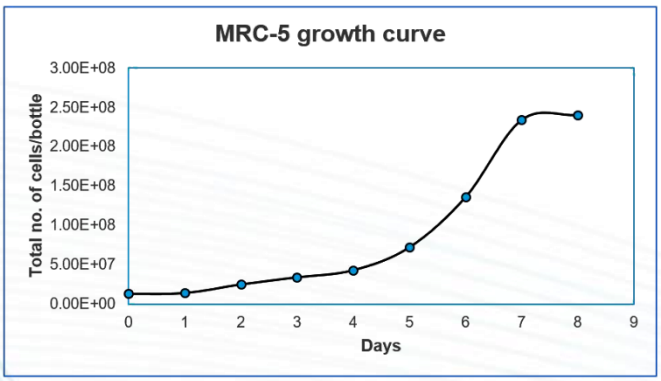
MRC-5 diploid cells are an extremely challenging cell line to culture to high densities in solid 3-D matrices. Given their wide application in production of an array of human vaccines, (Jordan and Sandig, 2014) we have optimized the culture conditions using our Tide motion Bioreactors (CelCradle ™ -500A) which use BioNOC ™ II macrocarriers as the matrix. These cells have been cultured to high densities in these matrices that mimic a 3-D *in vivo* environment. Cells have been grown to approximately 19-fold the seeding densities.



MRC-5 cell growth supported by BioNOC ™ II macrocarriers which provide a large surface area for growth
(L)- BioNOC ™ II macrocarriers
(R)- MRC-5 cells under 4x magnification

Cells are grown to typically high densities using a batch mode of culture in serum-containing medium as represented in (A) using these bench-top bioreactors of 500ml scale. Stained cells observed microscopically are represented in (B). The scaleability and surface area for MRC-5 cell growth in different volume bioreactors is as represented in (C).The technology therefore, is very robust for linear scalability.

A



Media	DMEM + 15% FBS, 1% Penicillin-Streptomycin
Culture Period	Recommended Harvest at 7th/8th day
Seeding Density	15,000 cells per carrier (12.75 million cells per bottle)
Harvested	282,000 cells per carrier (240 million cells per bottle)
Fold change	19-fold

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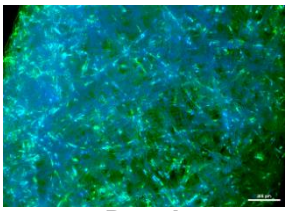
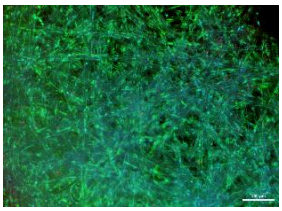
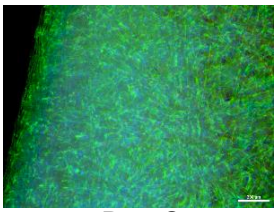
B



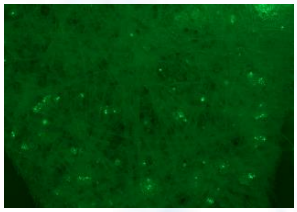
Day 1
40,000 cells/carrier



Day 2
85,000 cells/carrier



Day 3
282,000 cells/carrier



After harvest

Fluorescein diacetate (FDA) staining of live MRC-5 cells grown in DMEM containing 15% FBS and viewed under 4x magnification

C

Scalability

Model	Fixed Bed volume (L)	Cell numbers at harvest
CelCradle™	0.1	$2.4x10^8$
TideXcell™-002	2	$4.8x10^9$
TideXcell™-020	20	$4.8x10^{10}$
TideXcell™-100	100	$2.4x10^{11}$

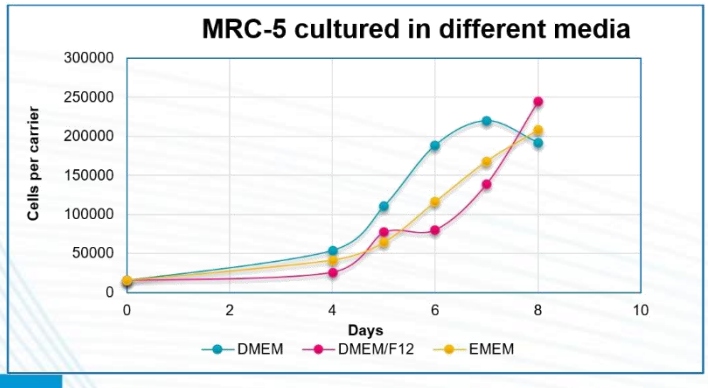
Surface Area for Cell Growth (in DMEM)

Model	Fixed Bed volume (L)	Cell culture surface area in m^2
CelCradle™	0.1	0.28
TideXcell™-002	2	5.6
TideXcell™-020	20	56
TideXcell™-100	100	280

Optimum media for MRC-5 cell growth

MRC-5 cells have been reported to be cultured in EMEM, DMEM, DMEM: F12 media. Our results indicate a robust cell growth in DMEM supplemented with 15% FBS as reported above. This is a cost-effective solution as well, in terms of obtaining high cell densities with significantly less expensive media. The cell growth pattern of MRC-5 cells cultured on BioNOC ™ II using different media is as shown in(D).

D



Recommendations for MRC-5 culture

- MRC-5 cells show optimum growth in DMEM medium supplemented with 15%FBS
- A low seeding cell density of 15,000 per carrier results in optimum growth and expansion of up to 19 fold
- The 7/8th day of culture is optimum for harvest in DMEM
- Culture of MRC-5 cells for virus production is optimum using passage 20-27 wherein 18-20 fold cell expansion is observed
- A simple DOE to test cell growth in 3D using different media such as DMEM, EMEM and DMEM:F12 supplemented with 15% FBS suggests that DMEM is optimum for cell expansion, recovery, better preservation of cell morphology and cost economy with almost comparable cell numbers.
- Application of these culture conditions in Tidemotion bioreactors demonstrates good cell expansion which can be used for production of cell banks/ viruses including Oncolytic Viruses and could form the basis for large-scale manufacturing.

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References

1. Jacobs, JP (1976).The Status of Human Diploid Cell Strain MRC-5 as an Approved substrate for the Production of Viral Vaccines. *Journal of Biological Standardization*. 4 (2): 97–99.

2. Jordan I and Sandig V (2014). Matrix and Backstage: Cellular Substrates for Viral Vaccines**. 6(4): 1672–1700**

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