No.48

TECH NOTE

High Density Culture on 2D MicroHex Microcarriers

In mass production of biological substances it is desirable to reach the highest possible concentration of the crude product in order to minimise the down stream processing cost. With anchorage dependent cells it is only possible to reach a certain limited cell density (cells/cm²). In microcarrier cultures the cell density (cells/ml) can be improved by increasing the carrier density (cm²/ml), provided the cell density on the carriers (cells/cm²) is not reduced at higher carrier density. In this Tech Note we report cell densities in 2D MicroHex cultures at carrier densities ranging from 3.75 to 22.5 cm²/ml.

Materials:

2D MicroHex, Nunc A/S
Microcarrier stirrer, Techne Ltd.
1l bottle (culture up to 500 ml), Techne Ltd.
MEM E supplemented with 10% FCS
L-929 cells
NucleoCounter, ChemoMetec

Methods:

Three cultures were compared using up to 400 ml/flask and MicroHex at density of 3.75, 7.5 and 22.5 cm²/ml respectively. The cells were inoculated at the same density, 2 x 10^4 cells/cm². The required amounts of carriers were added to the final volume of medium, which was pre-warmed. The required amounts of cells were added and the stirring started.

Table 1.
Culture conditions:

Carrier Density (cm²/ml)	Stirring speed (rpm)	Medium change[1] (day)
3.75	35	2
7.5	35	2
22.5	40[2]	1, 2, and 3

[1] *350 ml/change*
[2] *Stirring speed was increased to obtain uniform carrier distribution.*

Results:

The cell density was determined daily by means of a NucleoCounter.

Table 2.

3.75 cm²/ml		
Day	Cells/cm²	Cells/ml
0	2.0 x 10^4	7.5 x 10^4
1	2.1 x 10^4	7.8 x 10^4
2	4.5 x 10^4	1.7 x 10^5
3	1.3 x 10^5	4.7 x 10^5
4	1.5 x 10^5	5.7 x 10^5

Table 3.

7.50 cm²/ml		
Day	Cells/cm²	Cells/ml
0	2.0 x 10^4	1.5 x 10^5
1	2.7 x 10^4	2.0 x 10^5
2	5.9 x 10^4	4.4 x 10^5
3	1.0 x 10^5	7.8 x 10^5
4	1.5 x 10^5	1.1 x 10^6

Table 4.

22.50 cm²/ml		
Day	Cells/cm²	Cells/ml
0	2.0 x 10^4	4.5 x 10^5
1	2.5 x 10^4	5.6 x 10^5
2	5.2 x 10^4	1.2 x 10^6
3	8.3 x 10^4	1.9 x 10^6
4	8.5 x 10^4	1.9 x 10^6

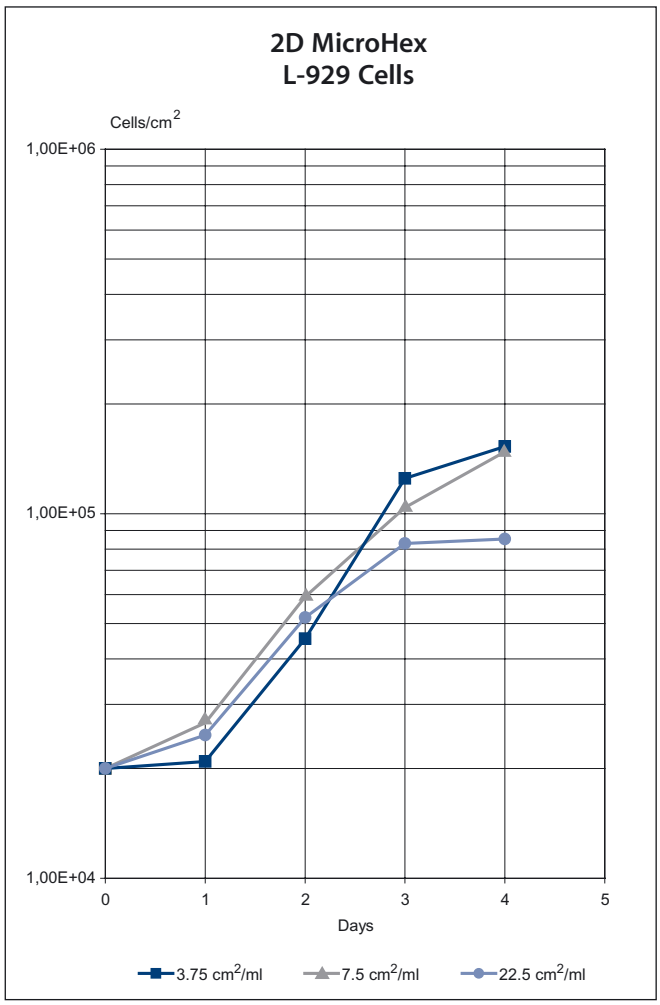

Figure 1.

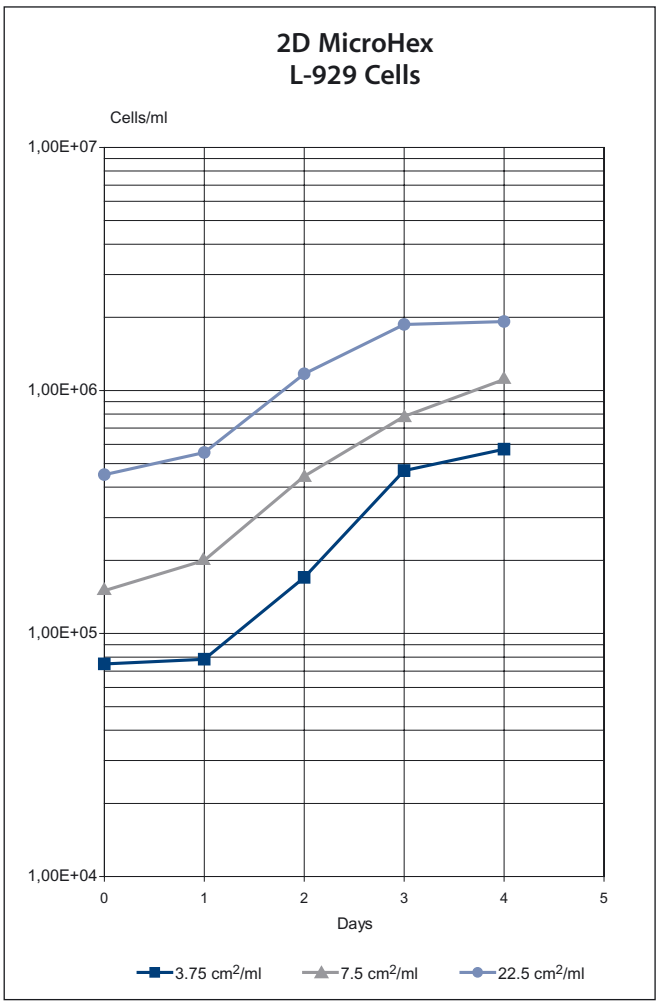

Figure 2.

From fig. 1 it can be seen that the growth rate is practically identical for carrier densities ranging from 3.75 to 22.5 cm²/ml. The density in the 22.5 cm²/ml culture is slightly lower due to the fact that daily medium change is insufficient for reaching 100% confluence.

Fig. 2 shows the cell yield in relation to the reactor volume (cells/ml).

Table 5 shows the total number of cells produced per ml of totally spent medium.
It should be noticed that a much more efficient utilisation of the medium could be obtained in a perfusion culture than in a feed batch culture, i.e. more cells could be produced per ml of medium.

Nunc A/S
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Table 5.

Media consumption		
cm²/ml	Medium total, ml	Cells/ml
3.75	750	3.1 x 10^5
7.50	750	5.9 x 10^5
22.50	1450	5.3 x 10^5

Nunc A/S · Denmark · Tel +45 4631 2000 · infociety@nunc.dk · www.nuncbrand.com
Nalge Nunc International · USA · Tel +1 585 586 8800 · nnitech@nalgenunc.com · www.nuncbrand.com
Nunc GmbH & Co. KG · Germany · Tel +49 6 111 86740 · nunc@nunc.de · www.nunc.de
Nalge Nunc International KK · Japan · Tel +81 3381 63355 · info@nalgenunc.co.jp · www.nalgenunc.co.jp

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