APPLICATION NOTE

Zellwerk GmbH·HiPer-Gruppe

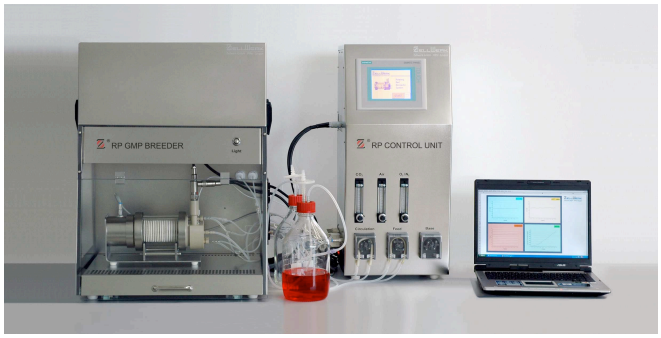
Removing cells from Sponceram® carrier discs used in the Z® Technology

Adherent growing cell lines as well as primary cells are easily attached to the surface of Sponceram® macro pores without any previous coating. The seeding cell suspension is absorbed into the pores of the carrier discs resulting in an even cell distribution over the total surface area. During initial stages of cultivation cell division occurs and within 1-2 weeks the first structures of an extra cellular matrix (ECM) can be observed under a microscope.

As this process continues in the Z® RP Technology, additional ECM is generated eventually forming a gelatinous layer over the complete carrier disc. The thin ceramic carrier discs (typical thickness/diameter of 3mm/65mm) offer a large surface (more than 8 m²) for ECM-embedded cells. Zellwerk GmbH has developed methods to ensure that up to 80% of these viable cells can be harvested and used for further experiments and analytic determinations.

Introduction

It should be noted that removal of cells during the early phase of cultivation, when little or no ECM exists, differs completely from harvesting viable cells in a later phase, were they are embedded in their ECM. Each cultivation has its own individual characteristics but by using different types of removal solutions and procedures developed by Zellwerk it is possible to harvest cells cultured on Sponceram® carrier discs used in the Z® RP Bioreactor with a high level of viability and yield.



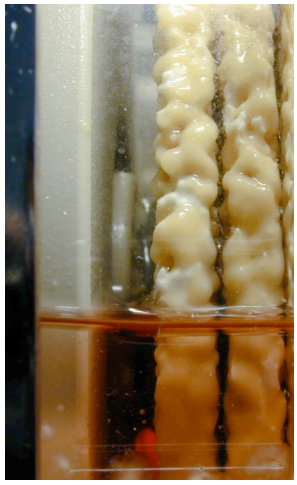
Z® RP Technology with Z® RP control unit, Z® RP GMP Breeder and Z® RP rotating bed bioreactor equipped magnetic drive and Sponceram® ceramic carrier discs

Cell removal during the first phase of cultivation

Removal of cells during the initial cultivation phase can be carried out in the same way as for cells growing in Petri dishes or culture flasks using trypsin or other commercially available protease solutions. Medium should be pumped out of the reactor vessel while the carrier discs are rotating at 10 rpm and the vessel temperature maintained at 37°C. Carrier discs should be rinsed twice for 10 min with 200 ml of culturing medium (free of serum and supplements). After the medium is removed, 200 ml of 0,1 - 0,25 % trypsine solution should be added followed by 15 min of incubation. Remove the trypsine solution, add trypsine neutralizing solution and centrifuge at 300 x g. The cell pellet is now suspended in medium and can be used further. Trypsination should be continued until no further cells are present in the solution.

Harvesting of cells embedded in ECM

Harvesting ECM-embedded cells (see figure) from Sponceram® in the Z® RP Bioreactor should proceed as follows:
Medium should be pumped out of the reactor vessel while the carrier discs are rotating at 10 rpm and the vessel temperature maintained at 37°C. Carrier discs should be rinsed twice for 10 min with 200 ml of culturing medium (free of serum and supplements). This should be repeated until all cell debris and dead cells are removed from the bottom of the reactor vessel. 200 ml of the selected removal solution should be added to the reactor vessel.



ECM embedded HEP-G2 cells after 100 days

Examples of different types of removal solutions than can be used for some common cells are listed in table 1.
In case of an unknown quality of ECM it may be useful to test different removal solutions using a single ECM covered carrier disc, which can be washed and incubated in a Petri dish.

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Start the removal procedure by first increasing the disc rotation speed to 50 rpm for 5 min followed by reduction to 10 rpm for a further 25 min. Repeat this procedure for 1-3 hours until most of the ECM is removed from the disc. Remove the suspension; centrifuge at 300 x g and resuspend the cell pellet in medium for further use. If the pH-value during incubation decreases to 6,8 or lower (cells starving), incubation should be stopped, cell suspension harvested and further removal continued using fresh medium.

This procedure should be repeated several times, depending on the number of cells still being counted after each removal run.

In the repeated removal procedures incubation periods should be longer (6 to 18 h). In these runs the 5 min rotation at 50 rpm should be carried only in a three to six hours period. In aliquots of all suspensions the number of living and dead cells should be counted (microscopic counting chamber, Trypan Blue staining).

Removal solution storage and use

10 ml of vials concentrated removal solution should be stored at -20°C. At this temperature they will be stable at least 6 months. „Ready to use“ solutions should be freshly prepared before starting cell harvesting. The contents of each 10 ml vial selected (see table 1) should be dissolved in 500 ml of Dulbecco´s medium containing a high glucose content (3,500 mg/l) and warmed to 37°C before use.

Table 1: Detecting Solutions

No.	Type	Effectiveness	Harvest of ECM-embedded cells, used for
1	ZW-DT-02	mild	CHO K1; CHO DHFR-; HEK; BHK, L 929; umbilical cord stem cells
2	ZW-DT-04	strong	chondrocytes; osteoblasts; hepatocytes; fibroblasts; muscle cells
3	ZW-DT-08	broad	In case of no success with solutions 1 and 2
4	ZW-DT-12	specific, mild	Avoiding destruction of cell surface receptors

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