

## Generation of Human Umbilical Cord Stem Cells with a

### Bioreactor System

The Z<sup>®</sup> RP Technology developed by Zellwerk defines a new type of cell cultivation device. The unique rotating bed allows efficient cultivation of cell lines and primary cells. The Z<sup>®</sup> RP Technology enables users to expand all types of primary cells and yield larger amounts of these cells in vital status compared to common cultivation techniques. Human keratinocytes, chondrocytes, endothelial cells, hepatocytes e.g., as well as cells from many human tumor tissues can be expanded in the Z<sup>®</sup> RP. Detachment from the porous Sponceram<sup>®</sup> support is easily achieved. The Z<sup>®</sup> RP cultivation technology leads to tissue-like cell organization in very high density, accompanied by significant production of extra cellular matrix. Equipped with a new Z<sup>®</sup> RP control unit, a Z<sup>®</sup> RP GMP-Breeder and Z<sup>®</sup> RP Bioreactor the Z<sup>®</sup> RP system is suitable for GMP/GCP-compliant production of living cells for therapeutic applications. This application note describes the cultivation and expansion of an adult stem cell population deriving from human umbilical cord tissue.

### Introduction

New cell therapy approaches generate a growing need for ex vivo preparations of vital primary cells. A few therapeutic regimens requiring large amounts of autologous and allogeneous cells already exist and are performed routinely (bone marrow transplantation, autologous chondrocyte transplantation, keratinocyte preparation for covering skin lesions). Many experimental cell therapies are under development and will enter clinical stages soon (cell transplantation for vaccinations, stem cell transplantation to regenerate dysfunctional or damaged tissues, e. g.). Production of cell preparations must follow stringent protocols and requires a high standard quality management. GCP/GMP criteria must be fulfilled even in a bedside, clinical surrounding. The new EU directive based on "Com (2003) 340 final" has to be considered.



Z<sup>®</sup> RP bioreactor operated in a Z<sup>®</sup> RP GMP-Breeder with Z<sup>®</sup> RP Control Unit

The Z<sup>®</sup> RP Technology opens a new way to generate large amounts of viable cells for therapeutic use and allows the conduction of cell therapy protocols fulfilling GCP/GMP prerequisites. Starting with a population of 10<sup>7</sup> cells a 100 to 1000-fold multiplication can be achieved in reasonable time, using the Z<sup>®</sup> RP bioreactor.



Your agent for Zellwerk<sup>®</sup> Cell Culture systems:

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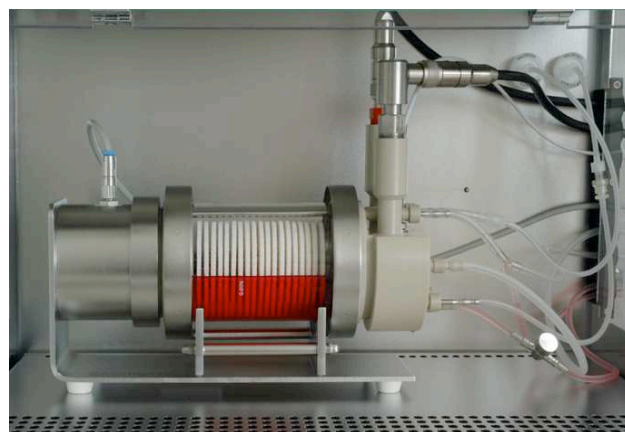
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Z<sup>®</sup> RP bioreactor equipped with Sponceram<sup>®</sup> carrier discs. The configuration with spacer was applied for cultivation of umbilical cord stem cells described in this note.

### Experimental

Adult stem cells (umbilical cord endothelial progenitor cells) were prepared from the cord of a newborn baby (piece of 100-200 mm length). A central vessel of the cord was washed 3 times with medium 1 to remove blood residues. Medium 2 was then poured into the vessel, closed at the end. The cord piece was incubated at 20 °C for 30 min in medium 2. During incubation it was furled gently a few times to support enzyme action. After incubation the cord piece should be washed 3 times with medium 1, the gathered solutions were centrifuged for 5 min at 300 x g. The cell pellet was suspended in 10 ml medium 3 and incubated at 37 °C for 12 h in a 25 cm<sup>2</sup> culture flask. Cord endothelial cells adhered after incubation, remaining erythrocytes were removed by gentle washing with medium 3.

#### Culture Media

Medium	
Medium 1	Dulbeccos medium + 2 mg gentamycin/l
Medium 2	Dulbeccos medium + 2 mg gentamycin + 100 U/ml collagenase II
Medium 3	AmnioGrow plus, PAN Biotech Amniomax C 100, Invitrogen

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Stem cells were isolated by detaching with usual trypsin solution, centrifuging the suspension at 300 x g and re-suspension in medium 3. Proliferation was induced by seeding the suspended cells in 75 ml culture flasks. Cells were fed with medium 3 over 5-10 days and then gathered by detaching with trypsin solution, centrifuging (300 x g) and re-suspension in 10 ml medium 3. During this time around 1 to 2 x 10<sup>7</sup> cells were grown.

The gathered cells were transferred into a Z<sup>®</sup> RP bioreactor equipped with 4 Sponceram<sup>®</sup> discs and corresponding spacers. Transfer was performed by injecting the cell suspension through the designated port. Culturing conditions are described in Table.

#### Culturing Conditions

Temperatur:	37,0 °C
Bed rotation:	2 rpm
Medium circulation:	30 ml/min
pH-value:	7,2
Overlay air:	0,1 ml sterile air/min
DO-value:	85 %

After 3 h the cells adhered on the Sponceram<sup>®</sup> carriers, 100 ml medium 3 was pumped into the reactor vessel and medium circulation was started. DO- and pH-values were controlled.

Medium feed was conducted according to glucose consumption measured on a daily basis. Glucose concentration was determined by an YSI 2700 Select Biochemistry Analyser with glucose oxidase sensor. Glucose concentration in the harvested medium was kept above 300 mg/l.

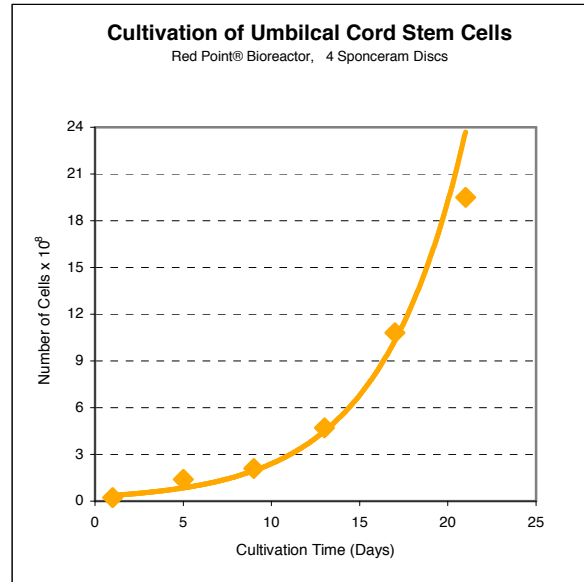
Cultivation was stopped after 22 days, carrier were overgrown with stem cells at this time. Cells are harvested by removal of medium 3 from the reactor vessel, adding of 200 ml of detaching solution (ZW-DT-02) and setting the bed rotation to 50 rpm for 5 min at 37°C. The cell suspension was then transferred into centrifuge tubes and centrifuged 5 min at 300 x g. This detaching procedure was repeated.

The combined cell pellets were re-suspended in 50 ml of medium 3 and centrifuged again. The washing action was repeated. Cells were ready for further use, an aliquot was counted (microscopic counting chamber, Trypan Blue staining).

### Results

Approximately 1,8 x 10<sup>9</sup> viable umbilical cord stem cells were recovered after the last washing step from the 4 Sponceram<sup>®</sup> discs. The figure shows the growth curve calculated on the basis of daily glucose consumption and living cells counted at the end of the cultivation. Umbilical cord stem cells for clinical trials were isolated and expanded to therapeutically significant numbers in a Z<sup>®</sup> RP cell cultivation system. Cell cultivation and all manipulations were conducted in the sterile working environment of a Z<sup>®</sup> RP GMP Breeder.

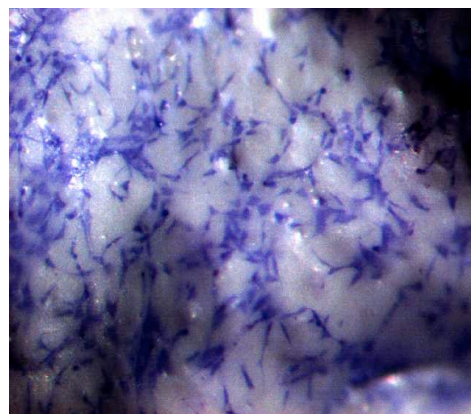
The rotating bed design - gentle moving of attached cells alternately through the medium and the atmosphere above - significantly improves oxygen supply and avoids any cell stress. Cells grow fast and to high densities, generating



Growth curve of umbilical cord stem cells during a cultivation period of three weeks

typical tissue like clusters of cells embedded in their own ECM. Fully equipped with Sponceram<sup>®</sup> discs up to 10<sup>10</sup> viable cells can be harvested in one culturing run of a Z<sup>®</sup> RP Bioreactor. No other reactor system and cell carrier type is described in the literature possesses the ability to harvest comparable amounts of fully viable anchorage-dependent cells.

Unique is not only the quantity of viable cells that can be harvested. Moreover, cells surrounded by ECM show special qualities compared to suspended cells or even to mono-layer cells in culture flasks. A growing number of recent studies report on the importance of ECM in cell culturing, playing a fundamental role for spreading, dividing, crawling, tissue like organisation, differentiation of cells, e. g.. ECM-embedded cells are more suitable for use in cell therapies and tissue engineering.



Microscopic image of umbilical cord stem cells, adhered on Sponceram<sup>®</sup> carrier discs shortly after seeding