Proliferation of lymphocytes immobilized in polyelectrolyte capsules.

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INTRODUCTION AND OBJECTIVES

For the *in vitro* cultivation of eukaryotic cells quite a number of systems have been developed. Depending on the end application they range from static systems like culture flasks or wells to dynamic systems like shake flasks, roller bottles or bioreactors. However, each one of these culture methods has its pros and cons. Dominating technical problems to overcome are to achieve high cell concentrations in spite of low growth rates and to provide a proper environment (temperature, pH, nutrient supply) for the highly sensitive cells. In this study, different cell lines (human and murine) were encapsulated. Capsules were prepared via a complex coacervation reaction from sodium cellulose sulfate (NaCS) and polydiallyldimethylammonium – chloride (polyDADMAC). This contribution presents the first step towards a strategy for high density cultivation of primary lymphocytes in polyelectrolyte capsules and the idea to recreate the *in vivo* microenvironment.

MATERIALS AND METHODS

Cell lines

Four different cell lines were tested. Jurkat human lymphoma cell line was kindly provided by the Chair of Process Biotechnology, University of Bayreuth, Germany. The Jurkat cells were grown in complete RPMI medium containing 2 mM l-glutamine, 10% FBS, with 5% CO2 and 37°C. CH27-LX murine B cell lymphoma derived from spleen cells (Bishop and Haughton, 1968), B62.c IL-7-dependent murine pre-B cell line (Milne et al 2004), and CD40-LM modified fibroblast were kindly provided by Dr. Schuh, Div. of Molecular Immunology, Department of Internal Medicine III, University of Erlangen, Germany. The murine B cell lines were grown in complete RPMI medium (RPMI 1640) containing 2 mM l-glutamine, 1 mM sodium pyruvate, 50 mM 2-Mercaptoethanol, 10% FBS, with 5% CO_2 and 37°C.

Encapsulation

Cells were resuspended in NaCS (1.5-2.2 wt% in PBS pH 6.3) (Euroferm GmBH, Erlangen, Germany). This resulting suspension was dropped into a stirred solution of polyDADMAC (1.2-2.0 wt% in PBS pH 6.3 (low M_w 20 wt%, Sigma-Aldrich, Steinheim, Germany). After hardening the capsules were washed with PBS and with medium prior to cell culture. Microencapsulation (500-700µm) was performed using the Inotech Encapsulator IEM-40 (Inotech AG, Dottikon, Switzerland) based on the principle of a vibrating nozzle. The 2-3mm capsules were produced by simple dropping through a thin capillary tube (0.2mm of diameter). Encapsulated cells were incubated in baffled flasks under constant orbital shaking (80-100 rpm). Cell culture conditions were the same as non-encapsulated cells and precultures.

Cell growth and viability

Cell densities and vitalities of the encapsulated and non-encapsulated cells were quantified using a trypan blue based exclusion assay. Capsule samples were incubated for 0.5-1h at 37°C in a cellulase solution (1-2 wt%, Onozuka Merk KGaA, Darmstadt, Germany).

Capsule oxygen profile measurement

The measurements were performed under culture conditions on a single capsule (2-3mm diameter) using a implantable oxygen microsensor based on dynamic quenching (IMP-PSt1, Presens GmbH, Regensburg, Germany). A two point-calibration was performed before each measurement using air and nitrogen saturated cultivation media, respectively. Each capsule was fixed within the measurement chamber using a suction support (The device was specially developed for this application). The oxygen sensor was inserted in the capsule and moved towards the center in definite steps using a precision screw. Oxygen partial pressure data were recorded spatial-dependent with Oxy-4-Software (Presens GmbH, Regensburg, Germany).

Diffusion coefficients

Diffusion of glucose and albumin through the membrane from the capsule into PBS was measured. For this, capsules with holes and without holes were produced (Fig 3). Firstly, a defined amount of capsules were weighted and added to a glucose solution (5.6 mM in PBS) 1:7 or albumin solution (1g/L in PBS) 1:2. After 24h and 100h of incubation respectively, the capsules were washed and transferred to PBS. Samples of 100 µl of the solution were collected at a defined time interval. The glucose concentration was quantified by the hexokinase method (GLUCOSE liquiUVmono, Human GmbH, Germany) and the albumin by the Bradford Method (Roti®-Quant, Roth, Karlsruhe, Germany). Diffusion coefficients were calculated from the kinetic curves according to the mathematical model of Lewinska et al. (Lewinska et al., 2002).

RESULTS AND DISCUSSION

Cell proliferation

In all experiments and for all cell lines the capsules' material showed no adverse effects on the cells and the capsules preserved their stability for up to 50 days (Fig 1). Specific growth rate of the immobilized cells in a fed batch process was similar to suspended cells; however peak cell densities were higher (Table 1).

Table 1: Comparison of the growth parameters of suspension cultures and capsule culture of different cell lines (μ_{max} in h^{-1}; *peak cell density in ml^{-1})

Cell type	Parameter	Susp. Batch	Susp. Fed-Batch	Capsules**
Jurkat	μ_{max}	0.029	0.029	0.027
	pcd*	$1.7x10^6$	$6.5x10^6$	$1.1x10^8$
CH27-lx	μ_{max}	0.045	0.051	0.051
	pcd*	$1.1x10^6$	$3.3x10^6$	$1.0x10^8$
B62.c	μ_{max}	0.051	0.049	0.048
	pcd*	$1.0x10^6$	$2.0x10^6$	$0.8x10^8$

**Peak cell density in $ml_{Capsule}^{-1}$

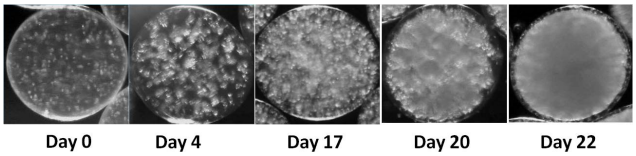

Figure 1: Phase contrast images of microcapsules (0.7 mm diameter) during a cultivation of 30 days

Mass transport

Taking into account the cellular growth in the capsules, and based on the transport rates and diffusion coefficients of oxygen for a steady state, a cell concentration profile in the capsule was calculated (Figure 4). Cells grow homogenously inside the capsules until limited; i.e nutrients or oxygen (Fig 1 and Table 1-μ_{max}). However, when the cell concentration rises, the cells close to the membrane are supplied better than the ones in the center of the capsule (Fig 4 – right).

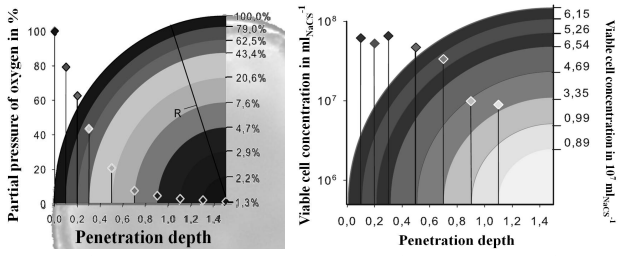

Figure 2: Measured oxygen partial pressure in the capsules (left). Graphic representation of the cell concentration profile in a capsule, calculated based on the oxygen data (right)

To evaluate the nutrients supply in the capsules, the transport rates of oxygen, glucose and albumin were determined. The measurements showed on one hand that glucose diffusion depends mainly on the surface-to-volume ratio, whereas albumin diffusion depends rather on the amount of holes in the membrane (Fig 2 & Fig 3). The arrows show circular imperfections (holes) caused by deceases cells that were incorporated into the membrane during the encapsulation process.

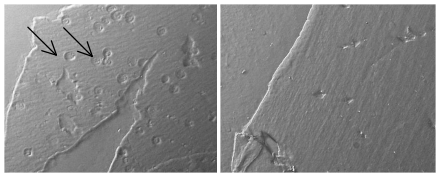

Figure 3: Phase contrast images of the capsule membrane. With holes (left); without holes (right).

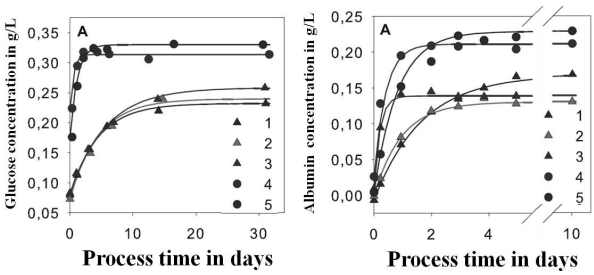

Figure 4: Mathematical modeling of the transient mass transport of glucose (left) and albumin (right) through the capsule membran following Lewinska et al 2002. (1 and 5 represent capsules without holes)

CONCLUSIONS

In the case of high cell density cultures, in comparison to the conventional systems, an extra diffusion barrier between the cells and the culture media is necessary to protect the cells from shear forces or to prevent wash out in continuous cultures. Capsules of different diameters from 500-700µm up to 2-3mm were produced and tested in order to examine the influence on growth, viability and other biological parameters of the immobilized cells.

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