

Ultrasound as an effective method to isolate intact mitochondria from CHO cells for physiological studies

J. Bahnemann, S. Kayo, A-P. Zeng

*Institute of Bioprocess and Biosystems Engineering,
Hamburg University of Technology, Germany*

Mitochondria play key roles in energy metabolism and cell physiology. The properties and metabolic capacity of mitochondria in suspension cultures of mammalian cells have been seldom studied despite their importance for the production of recombinant proteins.

Current metabolic studies of mammalian cells generally examine the cells as a whole, regardless of the compartmentalization of metabolism. Within the frame of a systems biology study of mammalian cell culture we are making efforts to expand our knowledge of *in vivo* dynamics of key metabolic reactions in the conjunction of glycolysis and tricarboxylic acid cycle which take place in both, cytosol and mitochondria. For this purpose, a fast and sensitive isolation of mitochondria is needed.

In this work, four methods for the disruption of Chinese hamster ovary (CHO-K1) cells are evaluated regarding their influence on mitochondrial integrity and yield. Sonication has been found to be a rapid and sensitive isolation procedure of mitochondria which resulted in high yields with CHO cells being disrupted using an ultrasound bar. Mitochondria released from the cells are then separated from the remaining cell homogenate by differential centrifugation in a sucrose containing medium. Yields of 14.0 ± 0.3 mg raw mitochondrial protein per $1 \cdot 10^8$ cells were obtained. These mitochondria are morphologically intact, with membrane integrities of 67% (outer membrane) to 94% (inner membrane). In addition, the functionality of isolated mitochondria was proven by measurement of oxygen consumption rate. By comparing this approach to mitochondria isolation methods using Dounce homogenization, digitonin permeabilization, or electroporation for cell disruption we demonstrated that the ultrasound approach provides the highest yield of isolated mitochondria. Furthermore, this procedure is relatively rapid (≈ 45 s for disruption), provides highly efficient cell disruption, and is more sensitive regarding mitochondrial integrity. Thus, ultrasound is well suitable for the preparation and investigation of mitochondria of suspension cell culture.

Development of living cell microarrays as miniaturized test systems

Rebecca Bongartz, Frank Stahl, Thomas Scheper

*¹Gottfried Wilhelm Leibniz University Hannover, Institute for Technical Chemistry,
Callinstr. 5, 30167 Hannover, Germany*

The present work supports the development of new methods for biomarker detection. Therefore, cell microarrays were developed as miniaturized protein detection systems for diagnostics and biotechnological purposes e.g. recombinant protein production.

First of all, microarray surface optimization was performed regarding enhanced cell adhesion and growth rates due to combine surface modifications with cell culture substrates. Viability and proliferation of different model cell lines as well as primary cells cultivated on the produced cell microarrays were monitored with 4', 6-diamidino-2-phenylindole (DAPI) and CellTiter-Blue[®] cell viability assay. Furthermore, quantum dot labeled aptamers and antibodies against different cancer screening proteins were implemented as detection molecules by in-situ hybridization on the self produced cell arrays, to demonstrate that a high specific analysis of biomarkers in living cells on microarrays is possible. In summary, the so produced living cell microarrays allow a complete systematical analysis of cellular functions and pathophysiological mechanisms.

A Novel Process Technology for Automated Natural Killer Cell Expansion

Markus Granzin, Mareke Brüning, Iris Spiegel, Sabine Müller,

Volker Huppert, Jürgen Schmitz

Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Natural killer (NK) cells are a promising tool for cancer therapy. They possess the ability to detect and eliminate abnormal cells and using NK cells for therapeutic cell transfer in the clinic requires high numbers of these cells. Therefore we developed a method for automated large-scale expansion of human NK cells.

Automation of NK cell expansion was done by use of the CliniMACS® Prodigy instrument, a novel technology for cell processing in a clinical environment. Automated Ficoll density gradient centrifugation was performed to remove erythrocytes and granulocytes from buffy coat samples of healthy donors and to obtain peripheral blood mononuclear cells (PBMC). This automated method yielded a suitable number of PBMC and a better depletion of granulocytes compared to the manual PBMC preparation. PBMC were cultivated with Interleukin 2 and OKT-3 leading to an expansion of NK cells, but also of NK like T cells and T cells.

NK cell numbers up to $5,1 \times 10^8$ were reached with expansion factors of 54-208 fold and NK cell purities of 5-52% after three weeks. Pure NK preparations are preferred with regard to analysis of clinical outcome and to reduce the risk of graft versus host disease possibly caused by contaminating T cells. Removing co-cultured T and NK like T cells by CD3-depletion during the expansion phase was possible and resulted in a higher purity of NK cells (56 - 96%). The cells retained their proliferative capacity after depletion and expansion factors of 30-156-fold were obtained. Flow cytometry analysis showed comparable expression of important, activating NK cell receptors (CD16, NKp30, NKp46 and NKG2D) for manually or automatically expanded NK cells either from CD3-depleted or undepleted cultures.

Our results show that large scale automation of NK cell expansion is possible by use of the CliniMACS® Prodigy and CD3 depletion of unwanted T and NK like T cells during culture is feasible. So, this a forward-looking approach to support the clinical use of NK cells in the treatment of cancer.

Production and Characterization of Alginate-based Microcapsules Produced by a Vibrational Encapsulation Device for Cell Immobilization

F. Rüdinger¹, Dror Seliktar², T. Scheper¹

*¹Institut für Technische Chemie, Leibniz Universität Hannover,
Callinstraße 5, 30167 Hannover*

*²Faculty of Biomedical Engineering, Technion, Israel Institute of Technology,
Technion City, Haifa 32000, Israel*

Immobilization of living cells in alginate is a well-established technology in an increasing range of different applications. The most important system is the entrapment of cells into an alginate gel matrix. The dripping method with a syringe is still a popular technique to form beads. Due to diffusion limitations of nutrients and products other techniques are investigated to produce smaller microcapsules of equal size.

Next to alginate beads modified hydrogel biomaterials can be used for 3-D cell encapsulation of mammalian cells and tissue engineering. The polymers can be cross-linked with RGD peptides or fibrinogen to achieve cell adhesion. Moreover nutrients maintenance is optimal for these demanding cells. In the recent work it is shown that fibroblasts can be cultivated in PEG and Pluronic[®] F127 fibrinogen-based hydrogels. Thus they represent a high potential for 3-D cell cultivation.

For the production of microbeads from alginate an encapsulation device with a laminar jet break-up technique is used. Microbeads were analyzed by microscopy depending on the nozzle size, frequency, flow rate, voltage and viscosity of the polymer solution. Monodisperse microbeads in a range between 160 – 400 µm with a standard deviation < 5 % are generated.

Furthermore core-shell-microbeads with a shell of alginate and a hydrogel core were produced to combine the advantages of the simple gelation process of alginate with the cell proliferation support of the hydrogel. Therefore it is possible to design a scaffold for 3-D cell cultivation with a high production rate.

Cytotoxic effects of nanoparticles on mammalian cells

Franziska Sambale¹, Frank Stahl¹, Thomas Scheper¹, Detlef Bahnemann¹,

¹Gottfried Wilhelm Leibniz University Hanover, Institute for Technical Chemistry,

Callinstr. 5, 30167 Hanover, Germany

It is important to understand the potential risk that nanoparticles pose to humans because a wide range of nanoparticles of different types and different properties are currently being synthesized that need to be examined concerning their toxicity. As a constantly increasing amount of technological products become available to the customers, concerns have been raised regarding the harm and the potential health risk of nanoparticles. For this reason it is important to screen any kind of nanoparticles towards their toxicity.

Concentrating on the possible adsorption of the nanoparticles into the human organism, via the skin and via the respiratory tract, the effects on different cell lines and human stem cells have to be examined. In order to measure the active metabolism indicating cell viability the MTT assay is used in this study. Moreover the cytotoxicity of nanoparticles can be detected by observing changes in cell morphology.

Zinc oxide nanoparticles have been examined regarding their toxic effect on different mammalian cell lines. First results demonstrate a significant deterioration of the cell viability after cultivation with zinc oxide. Furthermore phase contrast studies revealed that zinc oxide treated cells lost their normal morphology into a rounded one and were detached from the surface.

Untersuchung von verkapselten humanen T Lymphozyten als Vorstufe zum bioartifiziellen Lymphknoten

D. Schmoldt¹, V. Jérôme¹, und R. Freitag¹, K. Zambrano², H. Hübner², R. Buchholz².

¹Lehrstuhl für Bioprozesstechnik, Universität Bayreuth, Deutschland; ²Lehrstuhl für Bioverfahrenstechnik, Friedrich-Alexander Universität Erlangen-Nürnberg, Deutschland

Die Entwicklung eines bioartifiziellen Lymphknotens bedarf Materials, an dem Zellen absorbieren können, und welches für eine lokale Konzentrierung von Wachstumsfaktoren und Zytokinen geeignet ist. Vorhergehende Arbeiten zeigten, dass bei der Kultivierung humaner primärer T Lymphozyten in den Polyelektrolytkapseln, die aus Natriumzellulosesulfat (NaCS) und Polydiallyldimethylammoniumchlorid (PDADMAC) bestehen, hohe Zelldichten erreicht werden konnten, und dass diese keinen negativen Einfluss auf das Wachstum und die Proliferation der Zellen haben. Darüber hinaus ist das verwendete Kapselmaterial biokompatibel, gegen Scherkräfte sehr stabil (z.B. bei der Kultivierung in einem Wirbelschichtreaktor) und bietet aufgrund des geringen *molecular weight cut off* (MWCO) der Membran (unter 10 kDa) eine für die Entwicklung von T Zellen Subpopulationen optimale Mikroumgebung. Daher ist der nächste logische Schritt zur Rekonstruktion eines Lymphknoten-ähnlichen Gewebes eine Kokultivierung mehrerer Zelltypen (z.B. T Lymphozyten und Monozyten) in diesen Polyelektrolytkapseln. Dabei sollte die Rolle der zellulären Mikroumgebung mittels zell- und molekularbiologischen sowie immunologischen Methoden wie FACS, qPCR, RT-PCR und ELISA untersucht werden. Das Kapselinnere als Mikroumgebung der Zellen wird auf das Vorhandensein und die Menge der für das Wachstum und die Proliferation der Zellen notwendigen Faktoren (z.B. Zytokine, Transkriptionsfaktoren) auf der zellulären Ebene sowie die Expression dieser auf der Transkriptionsebene überprüft. Auf diese Weise werden neue Einblicke in die Funktion der Mikroumgebung bei der Ausbildung eines Lymphknotens gewonnen, die insbesondere im Bereich *Tissue Engineering* ihre Anwendung finden könnten.