Encapsulation as a powerful tool for cell therapies and GMP biomanufacturing

Ch. Heinzen, A. Berger and C. Fux.
Inotech Biotechnologies Ltd, Eulerstrasse 55, 4003 Basel, Switzerland, heinzen@inotech.ch

Introduction

Cell transplantation and cell therapies require powerful tools for optimal in-vivo function and protection from host immun system. Encapsulation of cells in biocompatible microcapsules enables easy transplantation in-vivo and additionally, protect cells during cryo conservation. Microcapsules with diameters smaller than 0.7 mm are required in order to avoid diffusion limitations. For long-term use of capsules in-vivo as e.g. for diabetes and cancer treatment, polymer material used for microcapsules should show high mechnical resistance and biocompatibility.
Where animal cells are used as biocatalysts to produce pharmaceutical proteins, as e.g. monoclonal antibodies, hormones, cytokines and viral vaccines, nowadays attention is focused on the development of improved process technologies with **higher productivity** by increasing cell density and improved expression systems. Encapsulation in microcapsules having a resistant membrane is a technique not only to protect cells from shear stresses and toxic metabolites but also to attain high cell density.
In this presentation we will focus of biotechnological applications of the Morquio disease.

The mucopolysaccharidosis (MPS) are part of the group of lysosomal storage disease. Mucopolysaccharides are stored within the body and consist of polymer sugar molecules. They constitute the connective tissue of our body. Keratansulfates are one example of Mucopolysaccharides.
In general, all biochemical structures and molecules are continuously produced and degraded, so also the mucopolysaccharide are used up, then decomposed in the somatic cell and finally excreted from the body. In case of MPS type IV A disease there is a shortage or lack of this specific enzyme for the decomposition of the Mucopolysaccharides Keratansulfat: it is the enzyme **N-Acetylgalactosamine-6-Sulfate Sulfatase (GALNS)**.
The degraded Mucopolysaccharides are stored more and more in the lysosomal cells causing malfunction of the cells. By the time the stored material increases and this results in an intensification of the disease symptoms. First of all the bones and the skeleton were attacked, but furthermore malfunctions of other organs are affected, i.e. heart, eyes, liver, teeth etc.

With the use of **enzyme replacement therapy**, the Morbus Morquio type IV A patients will get the following assistance: the GALNS-enzyme, which does not work because of a gene defect, will be produced biotechnologically by a cell-line in a bio reactor. Subsequently this enzyme will be prescribed by a weekly infusion to the patient. The recombinant manufactured enzyme takes over the function of the not naturally working GALNS enzyme and replaces the missing enzyme function.
Due to the fact that the GALNS-enzyme is very thermolabil, the production process in a bioreactor has to be a perfusion process. A very potential way of defining a perfusion process is the use of encapsulated CHO-cells producing the GALNS-enzyme (***FermentaCaps***).

Medical applications of encapsulation utilised in cell therapies are of high interest. Many diseases are caused by the inability of the body to produce the necessary amount of a specific molecule, such as a hormone, growth factor, or enzyme. Cell therapy offers an enormous potential for the treatment of such diseases. Encapsulated cell systems consisting of living cells immobilised and protected inside micro- or macrocapsules, are implanted into a patient, where the cells produce the required therapeutic substances. A selection of the most important diseases that could be treated by encapsulated cells (Kühtreiber, 1999) are:

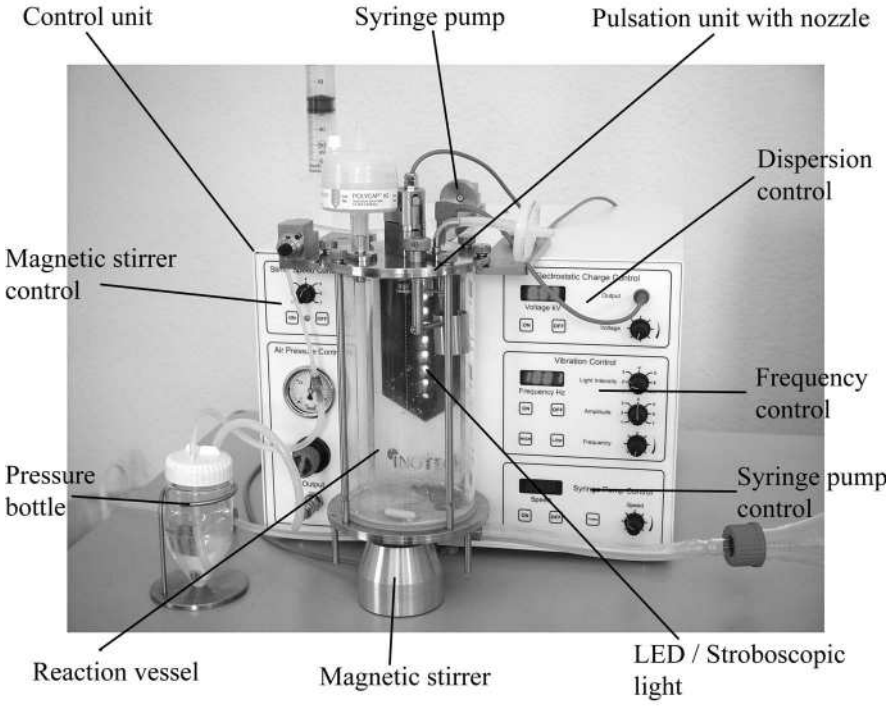
Alzheimer's, ALS, Affective disorders, Huntington's, Hypoparathyroidism, Hemophilia, Anemia, Enzymatic defects, Liver failure, Syringomyelia, Infertility, Atherosclerosis, Muscular dystrophy, Wound healing, AIDS, Cancer, Diabetes, Kidney failure, Spinal cord injuries, Chronic pain, Strokes, Dwarfism, Epilepsy, Parkinson's.
As a **second generation drug,** encapsulated CHO-cells producing the GALNS-enzyme will be implanted directly into the patient providing the enzyme to the patient at a constant concentration level. The patient does not need to receive weekly any enzyme infusions.

Material and methods
The encapsulation process used by Inotech is based on the laminar jet break-up technology (Hulst, 1985), enabling to produce monodisperse capsules with diameters of 0.5 mm which is important for successful cell encapsulation. A sinusoidal disturbance is superimposed by vibrating the nozzle or pulsating the fluid. Due to an instability criterion, the jet breaks up into monodisperse droplets (Rayleigh, 1878). Capsules can be produced in coating alginate capsules with poly-L-lysine and an additional alginate layer and in dissolving the inner alginate bead with Na-Citrate (Lim & Sun, 1980). Another encapsulation polymer is cellulose-sulphate by gelating the droplets in pDADMAC-solution showing membranes depending on defined retention times of said gelating process (Dautzenberg, 1999).

Inotech encapsulation device

The main parts of the Encapsulation lab scale device with a concentric nozzle are shown in Figure 1. All parts of the instrument which are in direct contact with the capsules can be sterilised by autoclaving. The product to be encapsulated (hydrophobic or hydrophilic liquid) is put into the syringe or the product delivery bottle. The liquid is forced to the nozzle by either a syringe pump or by air pressure. The liquid then pass through a precisely drilled sapphire-nozzle and separate into equal sized droplets on exiting the nozzle. These droplets pass an electrical field between the nozzle and the electrode resulting in a surface charge. Electrostatic repulsion forces disperse the capsules as they drop to the hardening solution.

Capsule size is controlled by several parameters including the vibration frequency, nozzle size, flow rate, and physical properties of the polymer-product mixture. Optimal parameters for capsule formation are indicated by visualisation of real-time droplet formation in the light of a stroboscope lamp. When optimal parameters are reached, a standing chain of droplets is clearly visible.



Once established, the optimal parameters can be pre-set for subsequent capsule production runs with the same encapsulating polymer-product mixture. Poorly formed capsules, which occur at the beginning and end of production runs, are intercepted by the bypass-collection-cup.

Fig. 1: Inotech encapsulation device IE-50R.

Methods

Fermentacap cultivation:
GALNS cells were encapsulated with Sodium-Alginate with a density of 1.5E6 cells/mL. In total 290E6 cells were encapsulated whereby 200mL capsules could be harvested. The 200mL capsules were cultivated in 300 mL HP5 Media in a Spinner System with the following parameters: pO2: 30%, pH: 7.3 – 7.0, Temperatur: 37°C, Gasflow: 3 – 10 L/h, head space; Stirrer speed: 80 rpm with glassball
After 6 days of cultivation, the perfusion run was started with an exchange of 50 mL/ day media. The feeding media was HP6 supplemented with 300 mg/dL D-Glucose and 2mM L-Glutamine. Each fourth day, the supernatant were harvest and frozen down to -20°C. The perfusion was stopped after 27 days of cultivation.

T-Flask cultivation
3E5 GALNS cells were seeded into a T-175 flask and cultivated until confluency with HP1 supplemented with 10% FBS. After the cells were confluent, each day the media was exchanged with HP1 without FBS. The harvested supernatant was frozen down to -20°C.

Material

- 1.5 % Sodium-Alginat solution (Inotech cat.# IE-1010)
- HP1 ((Cell Culture Techologie cat.# CHP5-04)
- HP5 (Cell Culture Techologie cat.# CHP5-04)
- HP6 (Cell Culture Techologie cat. # CHP6-4)
- L-Glutamine (Sigma, G7513)
- L-Glucose (Sigma)
- FBS, virus tested (PAA cat # A15-331)

Results

The following figure 2 shows the comparison between a fermentacap and a T-Flask production of GALNS.¨

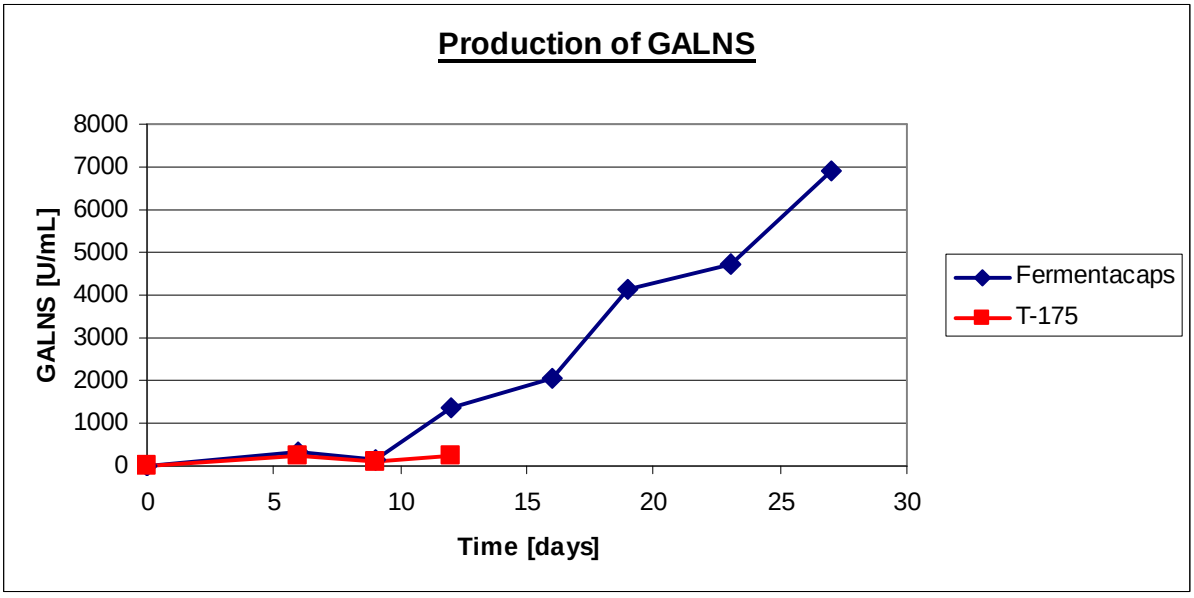


Figure 2: Production of GALNS with fermentacaps or in T-175 flask

Figure 3 shows the growth of encapsulated cells within the capsules.

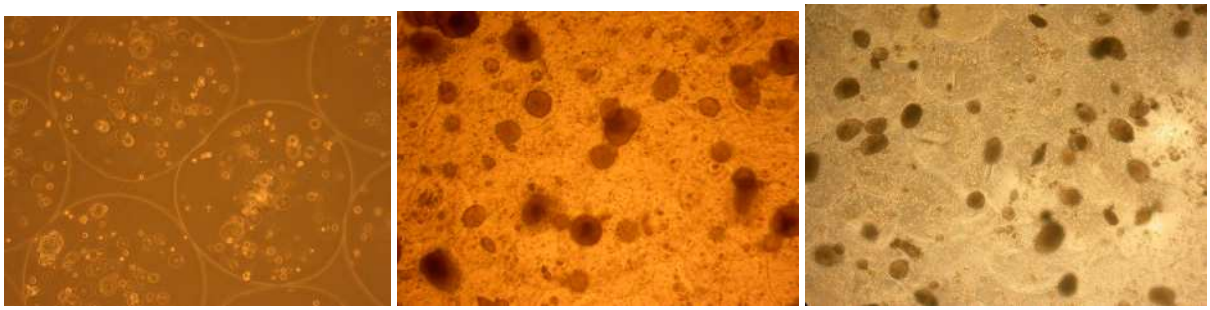


Figure 3:

6 days in culture (start of perfusion)	19 days in culture	27 days in culture (stop of fermentacap run)

Conclusion

We could show that the use of encapsulated cells for the biotechnological production of an enzyme for treatment of Morquio shows a huge potential.
The volumetric productivity could be enhanced from 250 U/mL in T-Flask (maximal productivity in T-flask) to 7000 U/mL in fermentacaps due to the fact that the total cell density could be increased. The fermentacap system shows a big advance as the cultivation time can be increase to 1 month instead of 2 weeks in T-flask and the harvest periods can be decreased (less manipulation needed).

Further work will be executed to optimize the settings for fermentacap cultivation and production of enzyme and to perform preclinical proof-of-concept results for treatment of Morquio by use of encapsulated cells which will be transplanted into patients.

References

Dautzenberg H et al. (1999), Development of cellulose sulphate based polyelectrolyte complex microcapsules for medical applications. Ann NY Acad Sci 875, 46 – 63.

Hulst, A.C. et al. (1985), A new technique for the Production of Immobilized Biocatalyst in Large Quantities, Biotechnol. Bioeng. Vol. 27, pp. 870 – 876.

Kühtreiber. W.; Lanza, R.P. and Chick, W.L. (1999) Cell encapsulation technology and therapeutics. Birkhäuser, Boston.

Lim F. and Sun A.M. (1980). Microencapsulated Islets as Bioartificial Pancreas, Science, Vol.210, pp. 908-910.

Rayleigh (1878), On the stability of jets, Proc. London Math. Soc., Vol. 10, pp. 4 - 13.