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# Encapsulation of Living Cells with Polymeric Systems

*Christian Schwinger,*[1] *Albrecht Klemenz,*[2] *Karsten Busse,**[1] *Jörg Kressler*[1]

[1] Institute of Bioengineering, Department of Engineering Science, Martin-Luther-University Halle-Wittenberg, D-06099 Halle, Germany
[2] Institute of Medical Physics and Biophysics, Department of Medicine, Martin-Luther-University Halle-Wittenberg, D-06097 Halle, Germany
E-mail: karsten.busse@iw.uni-halle.de

**Summary:** Microencapsulation of cells producing recombinant proteins or hormones leads to immunoprotection and immobilization in culture or *in vivo*. We are investigating three different strategies for the production of calcium cross-linked alginate beads of a small size with immobilized and immunoprotected mammalian cells: a) the AirJet technology (coaxial gas flow extrusion), b) the vibrating nozzle technology, and c) the JetCutter technology. A alginate/poly-L-lysine/alginate complexation was used as the polymeric system. All three methods may be used for production of homogeneous beads with a diameter of approximately 350 μm. While the vibrating nozzle technique was limited to an alginate viscosity of 0.2 Pa·s or less, the AirJet and JetCutter technology were less sensitive to higher viscosities. High frequency Scanning Acoustic Microscopy is used for mechanical characterization of the microspheres as well as for investigation of surface properties.

**Keywords:** alginate; biocompatibility; microencapsulation; scanning acoustic microscopy

## Introduction

Microencapsulation is a procedure where materials, such as enzymes, bacteria, yeast, or eucaryotic cells are enclosed within microscopic, semipermeable containers. Microencapsulation of mammalian cells is a novel and versatile tool of delivering therapeutically important natural or recombinant molecules *in vivo*. It can also have numerous applications as a platform for gene therapy of metabolic or neurological disorders and cancer.[1,2] Most of the previous work done on encapsulation of transgenic mammalian cells was concentrated on protection against the immune response of the host and on minimizing local inflammatory reactions generated by the microcapsules.[3,4] However, encapsulation techniques additionally are limited by physical factors, e. g. viscosity of the biopolymer solution, size distribution, or mechanical strength of the produced microcapsules. With the prevalent methods for bead formation, such as vibrating nozzles, gas jet droplet generators or laminar jet break-up, rather low concentrated alginate solutions can be employed.[5] In addition, beads with a small diameter (approximately 300 μm) are more difficult to produce than those

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with a larger diameter (> 500 μm) although smaller microcapsules may have several advantages, such as better oxygenation of encapsulated cells, smaller implant volume, and easier application to organs *in vivo*.[6,7]

A serious disadvantage of low viscosity alginate solutions is the lack of mechanical stability of the alginate hydrogel which is formed by cross-linking of the alginate molecules with polyvalent cations.[8] Therefore, covering solid beads with outer alginate layers and protective polycationic shells resulting in alginate-poly(L-lysine)-alginate (APA) microcapsules, or the use of $Ba^{2+}$- instead of $Ca^{2+}$- alginate has been suggested for improving the mechanical stability of hollow core microcapsules.[9,10]

The purpose of this study is to evaluate the physical properties of alginate microcapsules produced by three different methods, laminar gas flow (AirJet), vibrating nozzle, and JetCutter with the aim of optimizing the production of small size (approximately 350 μm) microcapsules suitable for biomedical applications in humans, and to investigate the proliferation of normal, neoplastic, or transgenic cells in these granules.

High-frequency Scanning Acoustic Microscopy (SAM) was used for investigation of mechanical properties in terms of acoustic impedance and 3D-surface topography of full alginate microspheres. Mean surface impedance was measured with SAM at 900 MHz with a spatial resolution of 1.5 μm. The sensitivity and reproducibility of SAM had to be increased considerably to receive and quantify signals in the very low impedance region. A multilayer analysis method was developed to get quantitative data with SAM at a microscopic level. The mechanical stiffness $c_{11}$ was obtained from mass density and longitudinal ultrasound velocity, measured with a pulse echo method at 6 MHz.

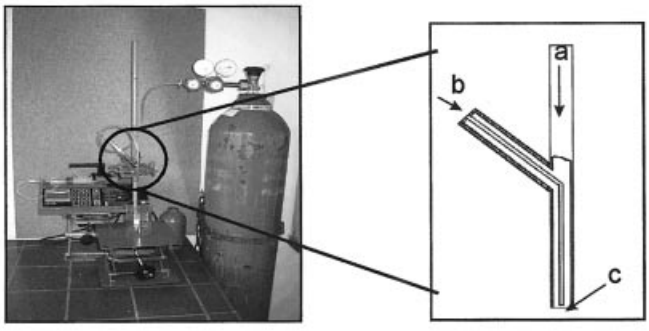


Figure 1. AirJet device used in our laboratory

## Materials and methods

Sodium alginate powder was obtained from Inotech AG (Dottikon, Switzerland) and from Fluka (Buchs, Switzerland). All alginates as well as a 0.1 % (w/v) poly-L-lysine solution (PLL; Mw 25.700 g/mol; Sigma) were sterilized by filtration through a 0.22 µm filter (Merck), and stored at 4°C. The Ca solution consisted of 100 mM Calcium chloride, 10 mM MOPS (3-[N-Morpholino]propanesulfonic acid, ICN Biomedicals, Eschwege, Germany) and 0.85 % (w/v) NaCl. The solutions were adjusted to pH 7.4, sterilized by autoclaving, and stored at room temperature.

The murine fibroblast cell line GLI 328 [11,12] (from Dr. E. Otto, GTI Inc., Gaithersburg, MD) was maintained in DMEM with 1 g/L glucose (Biochrom KG, Berlin, Germany) with addition of 10 % (w/v) donor calf serum (CS; Gibco BRL Life Technologies, Karlsruhe, Germany) and 1 % penicillin/streptomycin (Gibco BRL) at 37°C in humid atmosphere containing 5 % (v/v) $CO_2$.

For encapsulation the AirJet apparatus[13-15] (self-made, Figure 1), the vibrating nozzle apparatus[16] from Inotech AG (Dottikon, Switzerland) and the JetCutter system[17] from geniaLab GmbH (Braunschweig, Germany) were used.

Microspheres from alginate type Fluka 71238 were produced by the AirJet and the JetCutter method with a concentration of 1.5% (w/v). For the vibrating nozzle method 1.5 % (w/v) sodium alginate from Inotech was used. 8 ml of a suspension with $2.0 \times 10^6$ GLI 328 cells/ml were added to all alginate solutions. The JetCutter and the AirJet systems were placed in a class two clean bench to allow bead production under semi sterile conditions. The vibrating nozzle system was operated under sterile GMP conditions. The droplets were collected in 200 ml Ca solution, formed beads were separated from the bath by sieving. Subsequent formation of outer layers was carried out according to a simplified alginate-poly-L-lysine-alginate (APA) protocol.[18]

The acoustic microscope SAM 2000 (Kraemer Scientific Instruments, Herborn, Germany) with a broadband lens (0.8 - 1.3 GHz, 100° aperture angle) was operated at 900 MHz. It works in burst mode.[19] All measurements were performed in a temperature controlled water tank at $25.0 \pm 0.1$°C.

## Results

The size distribution of the produced microcapsules by AirJet is shown in Figure 2. The particle diameter and the arising size distribution depend on the volume flow of alginate and gas, the viscosity of the alginate, and the outer diameter of the capillary.[20] A monodisperse

distribution can only be obtained if the capsule size is smaller then the diameter of the capillary.

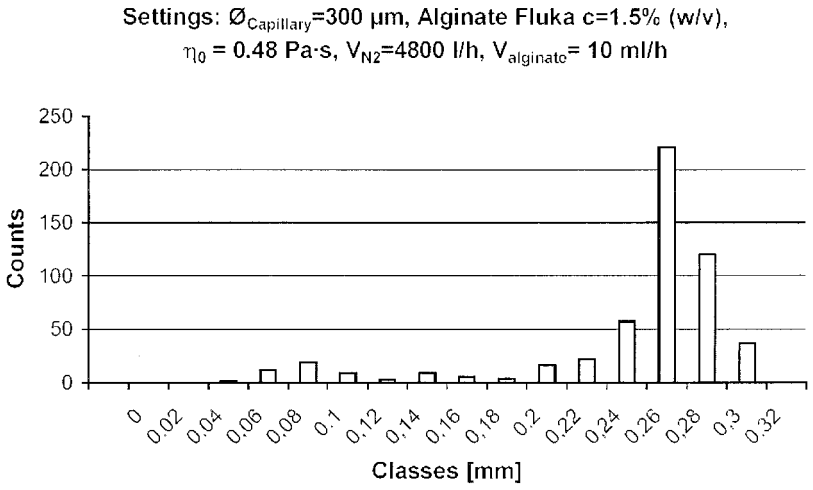


Figure 2. Size distribution with optimal settings of the AirJet apparatus.

After encapsulation of the cells by the different strategies, the cell growth shows a similar behavior. After one day, the cells formed a compact aggregate in the middle of the capsule. After approximately one week a significant cell growth can be seen and after four weeks the cells fill the whole capsule, as shown in Figure 3.

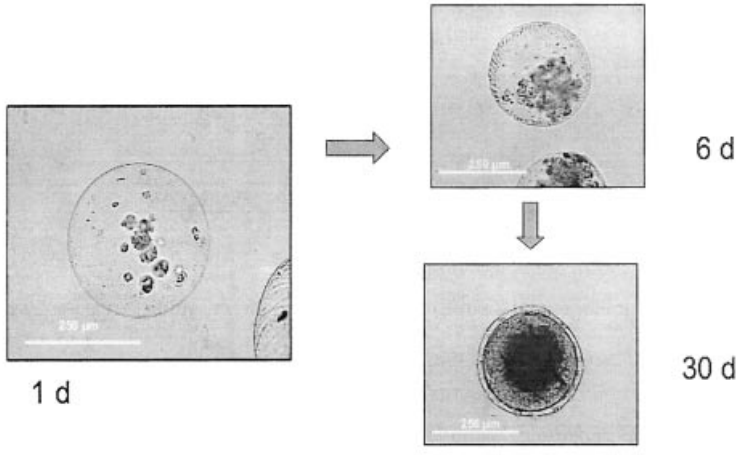


Figure 3. Cell growth (GLI 328) in a period of 30 days after encapsulation.

The results demonstrate that a custom made encapsulation AirJet device is well suitable for alginate encapsulation of mammalian cells and that it is comparable with a commercially available vibrating nozzle device (IEM-40, Inotech AG). The corresponding size distribution of the produced microcapsules is shown in Figure 4. Both encapsulation methods show the feasibility of generating uniform alginate microbeads for APA microcapsules.

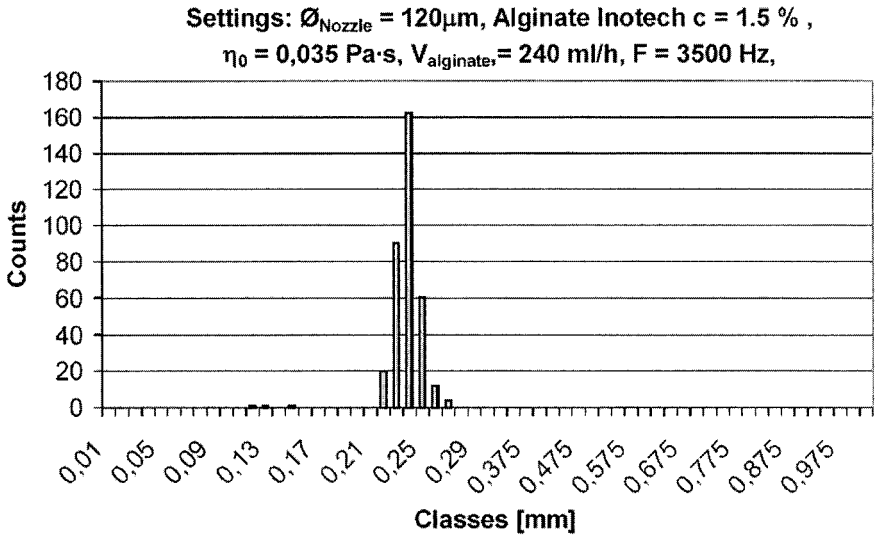


Figure 4. Size distribution with optimal settings of the vibrating nozzle apparatus.

The vibrating nozzle method is one of the most frequently used methods for large scale production of microbeads. It requires low viscosity of the biopolymer, however. Extrusion by coaxial gas flow (AirJet) is less sensitive against high viscosity polymers, but has a low throughput. It is therefore suitable for experimental purposes, but not for large scale production of microcapsules.

The JetCutter technology also seems to be appropriate for alginate encapsulation of living mammalian cells.[21] Small alginate beads (320 µm) containing viable cells could be produced at a very high throughput. The encapsulated murine fibroblasts formed colonies and proliferated at a considerable rate, which indicates that the mechanical stress during the encapsulation procedure is well tolerated and does not irreversibly damage the cells.

Sterilization or autoclaving of the parts of the JetCutter being in contact with the alginate-cell suspension, the use of sterile working solutions, and placement of the whole set-up in a clean bench may prevent contamination of the cultured beads and allow for microcapsule mass

production under GMP conditions. Another important aspect is the mechanical stability of the beads. One possible and rather straightforward approach is to increase the polymer content of the hydrogel, e. g. to use higher concentrated alginate solutions (2 to 5 %). At present, the JetCutter is the only technology that is able to process alginate at such concentrations.

Scanning Acoustic Microscopy is a suitable and sensitive tool for measuring elasto-mechanical properties of alginate specimens in terms of quantitative acoustic impedance.[22] The alginate spheres are investigated in their presumed environment, i.e. water. There is no further preparation required that could possibly change mechanical properties, as for electron microscopic methods. The topography of the surface as well as detailed structural information with a resolution of 1.5 µm can be obtained (see Figure 5).

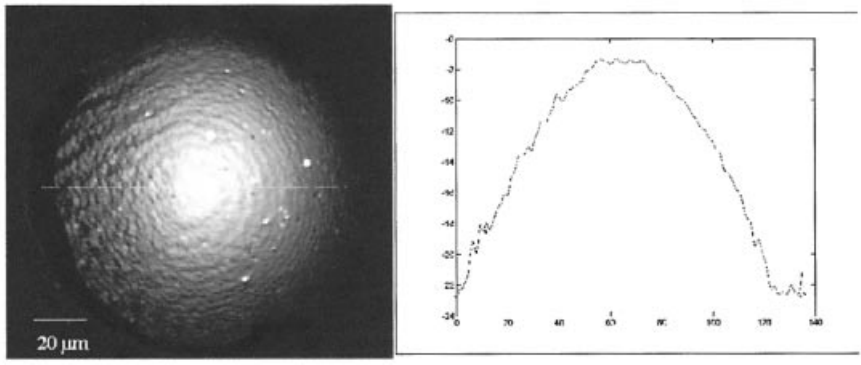


Figure 5. 2D-picture of an alginate sphere and the topography profile along the dotted hoizontal line.

The latter can be helpful for detailed studies of the surface inhomogeneities caused by material and by topography as well as for the optimization of the production process of the spheres. Bulk mechanical stiffness is estimated with low frequency ultrasound.

However, SAM is not only able to provide information on surface impedance of full microspheres but it also has the potential of investigating the elastic properties of capsule membranes. Because the SAM can be applied with both low- and high-frequency ultrasound, there are measurements at different stages of spatial resolution possible, beginning from bulk up to high resolution surface measurements.

## Conclusion

All three methods under investigation may be used for production of homogeneous beads with a diameter of approximately 350 µm. The vibrating nozzle technique was limited to an alginate viscosity of 0.2 Pa·s or less. With the JetCutter technology alginate beads could be produced at a very high throughput. High frequency Scanning Acoustic Microscopy is used for mechanical characterization of the microspheres as well as for investigation of surface properties.

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