

19 Microencapsulation-Based Cell Therapy

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1 Introduction

Many diseases are closely tied with deficient or subnormal metabolic and secretory cell functions. Diabetes mellitus, Parkinson's disease, hemophilia, hypoparathyroidism, chronic pain, and hepatic failure are only a few examples for this kind of degenerative and disabling disorders. Milder forms of these diseases can be managed by a variety of treatments. However, very frequently it is extremely difficult or even impossible to imitate the "moment-to-moment" fine regulation and the complex roles of the hormone, factor, or enzyme that is not produced by the body (KÜH-TREIBER et al., 1999). For example, patients who suffer from the insulin-dependent diabetes mellitus (IDDM) must take daily insulin injections. While such treatment can restore the average blood glucose level, true glucose homeostasis is not achieved. This failure leads to serious secondary side effects (such as micro- and macroangiopathy, diabetic neuropathy, nephropathy, and retinopathy), associated with a great reduction in life quality and expectancy. Also, the health care costs are staggering. Similarly, patients with chronic hypoparathyroidism show increased neuromuscular excitability (tetanic convulsions) resulting from a deficiency of the parathyroid hormone (parathormone; PTH) that regulates serum calcium. Patients with hypoparathyroidism are usually treated with oral calcium and vitamin D (calcitriol) when the symptoms of this disorder do not disappear and normocalcemia in the serum is not achieved. However, calcitriol lacks the complete renal calcium-retaining ability of parathormone. Accordingly, such patients have an increased risk of nephrolithiasis, nephrocalcinosis, and subsequent impairment of renal function (HASSE et al., 1999 and literature quoted there).

These two examples demonstrate the long-term failure and the high (partly unrealistic) costs of current therapies and the urgent need for alternative therapeutic strategies. Immunisolated transplantation (i.e., encapsulated-cell therapy) is one of the most promising approaches to overcoming the limitations of the current treatment protocols (LIM and SUN, 1980; GEISEN et al., 1990; LANZA et al., 1996).

Instead of drug administration or of engineering the patient's own cells (somatic-gene therapy), non-autologous standard laboratory cell lines, allogeneic (intraspecific), and xenogeneic (interspecific) cells/tissues are used that release the therapeutic substances that the body of the patient cannot itself produce – the only causal therapy. To avoid a life-time of immunosuppression therapy while excluding an immune response in the host, the transplants must be enclosed in immunoprotective capsules or devices (COLTON and AVGOUSTINIATOS, 1991).

Studies with macrocapsules (e.g., hollow fibers, diffusion chambers) made up of different materials have shown a number of drawbacks that stand in the way of their clinical use (LANZA et al., 1996; KÜH-TREIBER et al., 1999). Aside from surgery and retrieval problems, non-specific fibrotic overgrowth, necrosis of the encapsulated cells due to unfavorable (disk and tube) geometries, and thus diffusion limitations, breakage and other problems resulted in the early failure of the grafts. In contrast, microcapsules that are produced from hydrogels offer potential solutions to the problems of macrocapsules.

First, because of their spherical configuration and their small size, microcapsules have much better surface-to-volume ratios than macrocapsules. Second, microcapsules allow precise tailoring of their permeability to allow diffusion of anabolic compounds (oxygen, glucose, etc.) and of cell-derived products (carbon dioxide, lactate, hormones, etc.) while, simultaneously excluding immunoglobulins. Third, microcapsules minimize the overall risk of immunoprotection failure by using thousands of them instead of a single large macrocapsule. Fourth, they can be injected directly or transplanted with minimal-invasive surgery into the muscle, peritoneal cavity, liver, or elsewhere.

Over the past two decades, a number of microcapsules made up of different hydrogels (e.g., alginate, agar, agarose, gellan gum, chitosan, synthetic polymers) have been developed and tested (DULIEU et al., 1999). This research has shown the feasibility of alginate-based microcapsules for transplantation of laboratory cell lines as well as of allo- and xenogeneic tissue. Numerous technical accomplishments of this immunoisolation method have recently

made possible the successful therapeutic transplantation of allogeneic parathyroid tissue into patients with hypoparathyroidism (HASSE et al., 1997a). Other clinical applications (e.g., treatment of diabetes mellitus with encapsulated porcine islets) are currently under development as well (KÜHTREIBER et al., 1999).

Progress towards a routine clinical application of encapsulated-cell therapy requires continued rigorous investigation and elucidation of the processes underlying the interactions (and therefore the long-term risks and efficacy) of the tissue transplant and its immunoprotecting barrier with the immune system of the host. Thus, this review article does not pretend to give a complete overview of the enormous bulk of literature published to date on alginate-based microcapsules and related fields (see, e.g., KÜHTREIBER et al., 1999; ZIMMERMANN et al., 1999; HILLGÄRTNER et al., 1999). Rather, this article focuses on the exploitation of factors that have received limited attention in the past, but are important for the formulation of an alginate-based immunoisolation system that can gain medical approval.

2 Bioencapsulation Techniques

Although the concept of encapsulated-cell therapy is rather appealing, in practice a great deal of technology and know-how is needed for the production of long-term functional alginate-based transplants. Alginate microcapsules can be formulated by many different methods (MATTIASSON, 1982; KÜHTREIBER et al., 1999). The features of the capsules depend – among other things – on the medical application. Therefore, when discussing the optimum characteristics of alginate beads for encapsulation, we will focus mainly on immunoisolation of pancreatic islets, parathyroid tissue, and dopamine-secreting continuous cell lines for treatment of diabetes mellitus, hypoparathyroidism, and Parkinson's disease, respectively.

Alginate is one of the most abundant naturally occurring polymers; thus availability of the raw material imposes no problem for clinical

application. Alginate constitutes a family of unbranched anionic polysaccharides, mainly extracted from brown algae. It is composed of 1,4-linked α -L-guluronic acid (G) and β -D-mannuronic acid (M). The monomers are sequenced in homopolymeric G–G and M–M blocks. These are interspersed with alternating or nearly random sequences containing M–G blocks (MCHUGH, 1987; KING, 1994). Both homopolymeric sequences are found together, although to a different extent, in all alginates independent of their origin.

Gelation is induced by cross-linking the alginate by oppositely charged, di- or trivalent counterions (HARTMEIER, 1986; BRODELIUS and VANDAMME, 1987). For immunoisolated transplantation, Ba^{2+} and Ca^{2+} are usually used for cross-linking of the polymeric chains (LIM and SUN, 1980; GEISEN et al., 1990; KÜHTREIBER et al., 1999). Fe^{3+} can also be employed, but the gels are not translucent. Very stable capsules can also be obtained (see Sect. 6.2) when high-viscosity alginate is cross-linked with FCSIII (HyClone Laboratories, Utah, USA) or when putrescine or other oligoamines are added to the alginate before bead formation (for further details about the features of oligoamines, see SULTZBAUGH and SPEAKER, 1996; MESSIAEN et al., 1997).

Ca^{2+} cross-linked beads have the disadvantage that they are sensitive to chelators such as citrate, phosphate, and lactate (PILWAT et al., 1980; SCHNABL and ZIMMERMANN, 1989). Thus, long-term survival of solid Ca^{2+} cross-linked alginate microcapsules is limited, but this can be advantageous when autologous (i.e., the patient's own) cells are transplanted, e.g., chondrocytes and osteoblasts for the restoration of cartilage and bone (JORK et al., 2000). Ca^{2+} in combination with chelators is also often used to produce liquid-core capsules (DULIEU et al., 1999). This encapsulation procedure involves forming alginate beads (usually in the range of 0.3–2 mm; GENTILE et al., 1995), coating them with poly-L-lysine or other polyelectrolytes (e.g., polyethylene imine, polybrene, polyethylene glycol) and then performing core reliquefaction (LIM and SUN, 1980; GOOSEN, 1993; KLÖCK et al., 1993; see also KÜHTREIBER et al., 1999). Frequently, a second alginate coat is applied by suspending the coated capsules in alginate solution (O'SHEA et al., 1984). The

major shortcomings of alginate-poly-L-lysine capsules are their vulnerability (including the cells) to the reliquefaction process; their relatively weak stability (i.e., their high colloid osmotic pressure due to the dramatic reduction in water activity by the liquid polymers; see also Sect. 6), and the potential for an inflammatory response to capsule fragments (i.e., polyelectrolyte-alginate residues) upon breakage during transplantation (PLUMB and BRIDGMAN, 1972; FRITSCHY et al., 1991; ZIMMERMANN et al., 1994; DE VOS et al., 1996, 1997a; VAN SCHILF-GAARDE and DE VOS, 1999; GÅSERØD et al., 1999). Moreover, poly-L-lysine is stringently cytotoxic and is used as an antineoplastic agent (ARNOLD et al., 1983).

The *in vitro* and *in vivo* long-term integrity of the capsules is greatly improved when Ba^{2+} is used (TANAKA and IRIE, 1988; SCHNABL and ZIMMERMANN, 1989), but this divalent cation is an inhibitor of the K^+ channels present in cell membranes. Thus, careful control of the bead manufacturing process and the subsequent removal of excessive Ba^{2+} after gelation (see Sect. 6) are important to maintain high cell viability.

For bead formation, cells (or tissue pieces) are first suspended in an iso-osmolar, saline-sodium alginate solution. The cell-alginate suspension is then forced (by using a syringe or a motor-driven piston) through a nozzle, which forms droplets of cell-containing alginate. These droplets fall into an iso-osmolar NaCl solution containing 20 mM Ba^{2+} (or Ca^{2+}) that complex with the alginate, resulting in formation of spherical beads. The pH of the dropping solutions must be adjusted to pH 7 by using histidine or other buffering biomolecules of very low molecular weight (JORK et al., 2000). Organic buffers (such as HEPES and MOPS) used by many authors in the past (e.g., KLÖCK et al., 1997; DE VOS et al., 1999; ZEKORN and BRETZEL, 1999) should be avoided because these buffers can be cytotoxic when released from the capsule during transplantation.

Drop formation is greatly improved by application of a coaxial air jet (GRÖHN et al., 1994), i.e., by using a two-channel bead generator (Fig. 1A). Studies have shown (JORK et al., 2000) that viscosity and concentration of the alginate, air flow rate, and the geometric prop-

erties of the channels and the nozzle are crucial for obtaining microcapsules that are (nearly) spherical, small in diameter, and with a uniform size distribution (see Sect. 6).

Optimum drop formation is also obtained by application of a high electrostatic potential between the nozzle and a stainless-steel ring placed between the nozzle and the bath solution (Fig. 1C; see also KRESTOW et al., 1991; COCHRUM et al., 1995; GOOSEN, 1999). When an axisymmetric and sinusoidal disturbance of a frequency of about 500 to 7,000 Hz is additionally imposed on the laminar jet flow, small droplets, with a very narrow size distribution, are formed (PLÜSS et al., 1997; BRANDENBERGER and WIDMER, 1998; HEINZEN, 1999).

There are several other (commercial) dropping techniques or modifications of the above devices (for an excellent overview the reader is referred to a recent review article of DULIEU et al., 1999). For transplantation, the most important one is the three-channel, air-jet bead generator (Fig. 1B; see also JORK et al., 2000). This device allows the one-step formation of microcapsules of homogeneous as well as of spatially heterogeneous composition. These include solid beads with a liquid core (e.g., oil or other hydrophobic fluids; see Sect. 6) or solid beads composed of a core of low alginate concentration (containing the cells) that is surrounded by a layer of higher alginate concentration ("layered solid microcapsules").

Drop formation under coaxial air flow and under electrostatic potential have been widely used in bioencapsulation (KÜHTREIBER et al., 1999). For medical applications the "coaxial-air-flow" technique is the method of choice because it allows – in contrast to the "electrostatic-potential" method – the use of highly viscous alginate, i.e., alginate of high molecular mass. Compared to low-viscosity alginate beads, microcapsules made up of high-viscosity alginate provide features that are advantageous for long-term transplantation (see Sect. 6). A disadvantage of the technique may be that tiny air bubbles can be included in the beads during the gelation process (if the procedure is not performed carefully). Such bubbles may lead to diffusion limitations and/or to long-term adverse side effects on the bead integrity.

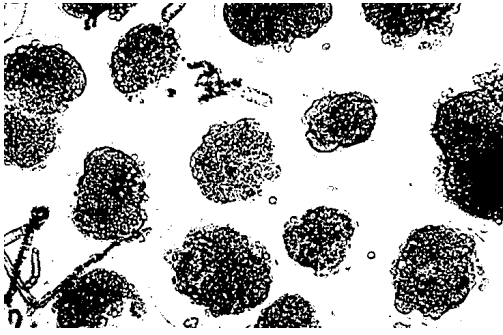


Fig. 1. Schematic diagrams of alginate capsule generators; **(A)** two-channel-, **(B)** three-channel coaxial air-jet bead generator, and **(C)** capsule formulation under high electrostatic potential, V. Channel 1 is fed with the alginate/cell suspension, channel 2 serves for air flow supply, and channel 3 is fed with an alginate solution, usually of higher concentration than that of channel 1 (for the formation of “layered beads”). The size of the beads is controlled by the speed of the air and alginate flow (**A, B**), or by the electrostatic potential (**C**). For further details, see Sect. 2.

3 Production of Transplantation-Grade Alginates

The demands on the alginate material to be used for transplantation are stringent. It should be produced with reproducible characteristics according to medical approval standards, and it should not elicit any inflammatory or fibrotic response from the host, i.e., it should not engender any cytotoxicity, and should be biocompatible for both the host and the cells it encloses.

Due to the harvesting and extraction process commercial alginates contain a fairly high number of impurities (ZIMMERMANN et al., 1992). Common contaminants are proteins, complex carbohydrates, fatty acids, phospholipids, lipopolysaccharides, toxins, and polyphenols (SKJÅK-BRÆK et al., 1989; DE VOS et

al., 1993; SUN et al., 1996). These mitogenic and inflammation-provoking impurities engender ultimately fibrotic overgrowth (see, e.g., OTTERLEI et al., 1991; MAZAHARI et al., 1991; WIJSMAN et al., 1992; COLE et al., 1992; CLAYTON et al., 1993; DE VOS et al., 1993; KLÖCK et al., 1994) with the result that transport of nutrients and oxygen to the encapsulated cells is greatly impeded leading ultimately to cell necrosis. Removal of the impurities from the commercial alginate by free-flow electrophoresis or by chemical means (ZIMMERMANN et al., 1992; KLÖCK et al., 1994, 1997; DE VOS et al., 1997a; VAN SCHILFGAARDE and DE VOS, 1999), and subsequent implantation of the empty alginate gels into rodents did not evoke any significant foreign body reaction, even when alginate was implanted in diabetes-prone BB rats that exhibit elevated macrophage activity (ROTHE et al., 1990; GOTFREDSEN et al., 1990; WIJSMAN et al., 1992). Extensive research with purified high-M and high-G

alginates gave further clear-cut evidence (see ZIMMERMANN et al., 1999) that neither the M–M nor the G–G blocks of alginate polymers of high molecular mass initiate an immunostimulatory response (cytokine production) as discussed very controversially in the literature (SOON-SHIONG et al., 1991; CLAYTON et al., 1991; OTTERLEI et al., 1991, 1993; ESPEVIK et al., 1993; JAHR et al., 1997; DE VOS et al., 1997a; KULSENG et al., 1999).

Purification of crude commercial alginate has the decisive disadvantage that many impurities have also to be removed which are not natural constituents of the brown algae, but rather present contaminants from the harvesting process (pollution by animal proteins, bacteria products, etc.). Treatment of the raw algal material by formaldehyde imposes further complications. Purification of alginate is not easy since high concentrations of alginates are difficult to work with because of the high viscosity of the solutions. Removal of mitogenic and inflammation-provoking contaminants requires, therefore, multiple-step and very time-consuming procedures. Because of the large number of operations the risk of further contamination is increased. As a final result, only small quantities of alginate of quite variable purity are obtained (KLÖCK et al., 1994).

Techniques of purification and monitoring have been recently improved sufficiently to allow the reproducible reduction of mitogenic and cytotoxic impurities to a negligible level. Progress was achieved by using clearly defined algal material for the production of highly purified alginate that fulfills the standards for medical application. Research in this direction has shown (HILLGÄRTNER et al., 1999; JORK et al., 2000) that fresh stipes of brown algae harvested directly from the sea or sporophytes of brown algae grown in bioreactors are ideal input sources. When using such material, the manufacturing process can be simplified considerably. Extraction and purification steps comprise (see flow chart in Fig. 2; for further information see JORK et al., 2000): extraction with 50 mM EDTA, removal of all visible aggregates by filtration in the presence of diatomaceous material, adjustment to 0.13 M KCl, precipitation with ethanol (37.5% v/v) under injection of air or nitrogen, manual sampling of the alginate layer accumulated at the sur-

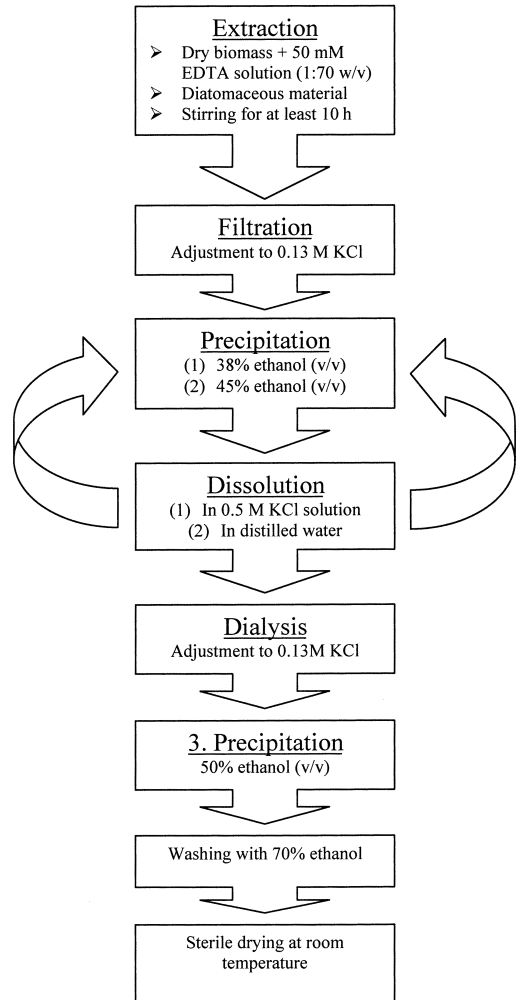


Fig. 2. Flow chart of the alginate purification process.

face of the liquid phase, redissolution in 0.5 M KCl under agitation, repetition of the precipitation (with an ethanol concentration of 45% v/v) and redissolution of the alginate in distilled water followed by dialysis, adjustment of the solution to 0.13 M KCl, precipitation (50% v/v ethanol), ethanol sterilization and drying of the snow-white alginate. All steps must be performed at room temperature because alginate solutions, particularly highly viscous ones, depolymerize when the temperature is raised (McHUGH, 1987).

If the parameters of the process are carefully controlled, the above extraction and purification protocol leads to a final product with reproducible characteristics. Depending on the origin of the alginate, alginates with a high viscosity and thus with a high molecular mass much larger than about 300 kDa can be obtained. Such alginates have optimum transplantation properties (see Sect. 6). The above protocol can be applied to alginates of different uronic acid composition, and is, additionally, amenable to large-scale production. Thus, medically approved alginate can be produced in sufficient quantities for routine clinical applications and also at reasonable costs.

4 Biocompatibility Assays for Medically Approved Alginate Gels

Clinical application of alginate microcapsules must be based on adequate preclinical data including assays for the continuous control of the purification process. For practical, but also ethical reasons, *in vitro* evaluation of the alginates is required before implantation into animals and, ultimately human trials can be contemplated. Current routine analytical tests of the purity of the alginate comprise measurements of endotoxin, protein, phenolic-like compounds, and other contaminants by using the limulus-lysate assay, the Bradford test, fluorescence- and NMR-spectroscopy (SKJÅK-BRÆK et al., 1989; JORK et al., 2000; SCHILLER, ARNOLD, CRAMER, THÜRMER, ZIMMERMANN, unpublished data). Measurements with partly purified alginates in combination with implantation studies showed (ZIMMERMANN et al., 1999) that the above analytical tests are necessary, but not sufficient to exclude immunological reactions to the alginate under implantation and transplantation conditions. The reason for this is that fucoidan and other related mitogenic compounds (ARFORS and LEY, 1993) are difficult to detect by fluorescence- and NMR-spectroscopy.

Our laboratory has recently developed a “cell culture” assay for highly sensitive screen-

ing of mitogenic impurities in alginates. The assay is based on the activation and proliferation of murine splenocytes by mitogenic alginates (“mixed-lymphocyte test”; ZIMMERMANN et al., 1992; KLÖCK et al., 1994, 1997). The proliferation is considerably increased when the splenocytes are simultaneously costimulated by lipopolysaccharides (LPS; ZIMMERMANN et al., 1999). Growth and viability of the activated lymphocytes can be measured colorimetrically by the so-called XTT assay (SCUDIERO et al., 1988). However, long-term experience of our laboratory has shown that the rate of formazan production is not always proportional to the number of activated cells and thus very variable. Cell clumping, debris, and the viscosity of the alginate (lowering the water activity) do apparently interfere with the dye reaction. The reproducibility and sensitivity of the “mixed lymphocyte test” can be greatly improved (KÜRSCHNER, JORK, ZIMMERMANN, unpublished data) if the number and the size of the proliferating cells are determined electronically 3 d after co-stimulation and culture. By use of an electronic cell analyzer it is possible to distinguish clearly between dead cells, debris, and clumped cells on the one hand and viable, activated lymphocytes on the other because of the large differences in the signal amplitude of these populations (FRIEDRICH et al., 1998). Typical electronic size distribution measurements of murine splenocytes co-stimulated by various crude and purified alginate samples together with LPS are shown in Fig. 3. It is obvious from this figure that the purification process of Fig. 2 almost completely removed the mitogenic impurities. Most interestingly, alginate extracted from stipes collected at the beach of southern Africa as well as from fresh algal material showed no significant mitogenic activity according to this assay. When these alginate samples were implanted beneath the kidney capsule of rats, a (reduced) fibrotic overgrowth was, however, provoked by the “beach alginate”. This clearly shows that assurance of biocompatibility still requires the implantation of empty microcapsules in rodents and that further efforts have to be made to develop efficient and simultaneously fast *in vitro* assays in order to replace the (sometimes very variable) animal models.

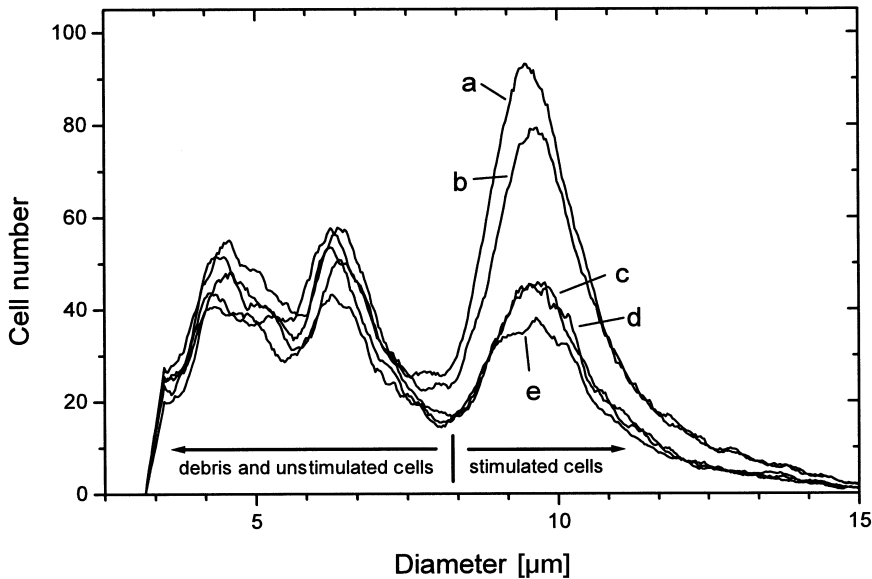


Fig. 3. *In vitro* bioassay for the detection of mitogenic impurities in alginates based on the activation and proliferation of murine splenocytes. Mixed lymphocytes prepared from 6- to 8-week-old male C3H/HEJ mice were co-stimulated with $10 \mu\text{g ml}^{-1}$ lipopolysaccharides and commercial, unpurified alginates (a, b) and with alginates purified from algae (c, d), respectively. After 3 d culture the ratio of dead cells/debris (diameter $< 8 \mu\text{m}$) to viable proliferating cells (diameter $> 9 \mu\text{m}$) was determined by using an electronic size analyzer. The starting algal material for the purification of the alginate according to the regime in Fig. 2 was: fresh algae (c) and algae collected at the beach of southern Africa (d). Curve e represents the control, i.e., the stimulation of the mixed lymphocytes with lipopolysaccharide in the absence of alginate.

Note that the purified alginate of curve c, but not that of curve d exhibits a foreign body reaction after implantation under the kidney capsule of BB rats (Fig. 4) indicating the limitations of the *in vitro* bioassay (KÜRSCHNER, JORK, and ZIMMERMANN, unpublished data).

The animal model has a major impact on the histological results of *in vivo* biocompatibility tests. Spontaneously diabetic BB rats are the most appropriate small animal models (MAZAHARI et al., 1991; PFEFFERMANN et al., 1996; ZIMMERMANN et al., 1999) even though the breeding of this strain is very man-power-intensive, time-consuming, and expensive. Many authors have used Lewis and other rats as well as mice (BALB/C) for biocompatibility tests (e.g., WEBER et al., 1993; COCHRUM et al., 1995). However, these animal models cannot be recommended because extensive studies in the laboratory of the authors have shown (ZIMMERMANN et al., 1999; KÜRSCHNER, JORK, ZIMMERMANN, unpublished data) that these

rodents even tolerate alginates that evoked not only a strong non-specific foreign body reaction in BB rats, but also exhibited mitogenic activities in the “electronic activation/proliferation” assay. Typical results of Ba^{2+} cross-linked alginate beads (Fig. 4A) retrieved 3 weeks after implantation beneath the kidney capsules of BB rats are depicted in Fig. 4B. In contrast to commercial, unpurified alginate (Fig. 4D) no significant fibrotic overgrowth can apparently be detected when alginate purified according to the flow chart in Fig. 2 is used.

Similar results were obtained, when corresponding alginate gels were implanted for four weeks in the muscle of baboons (Fig. 4C;

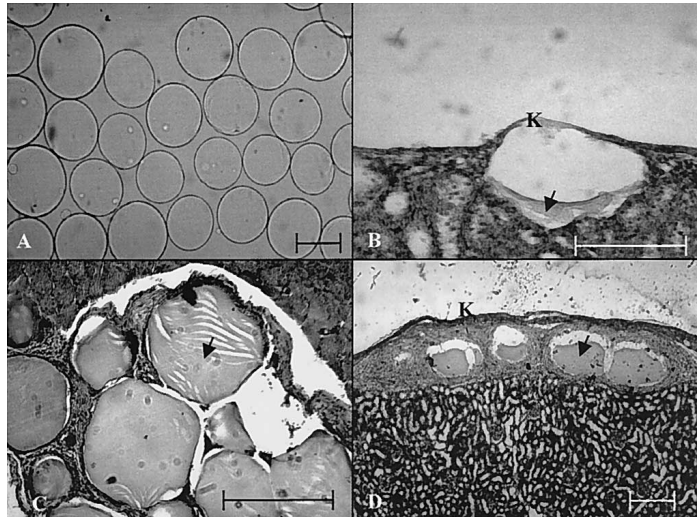


Fig. 4. *In vivo* bioassay for the detection of mitogenic impurities in alginates based on the induction of fibrotic overgrowth due to the immune response of the host. **(A)** Empty Ba^{2+} alginate beads (diameter 300–400 μm) were made from purified and highly viscous alginate (curve c in Fig. 3) by using the dropping device in Fig. 1A. **(B)** Capsules (arrow) retrieved 3 weeks after implantation beneath the kidney capsule (K) of a spontaneously diabetic BB rat and **(C)** capsules (arrow) retrieved 4 weeks after implantation in the muscle of a baboon. **(D)** Control capsules (arrow) made up of commercial, unpurified alginate (curve b in Fig. 3) and retrieved 3 weeks after implantation beneath the kidney capsule (K) of a BB rat (for fixation and staining of the tissue/beads see KLÖCK et al., 1997 and HILLGÄRTNER et al., 1999). Note that due to the fixation process the alginate beads may collapse **(B)** or may become deformed **(C)** (for further explanations, see text); Bars = 350 μm .

GEßNER, G. ZIMMERMANN, JORK, BOHRER, MELCHER, HASSE, ROTHMUND, ZIMMERMANN, unpublished data). In this case, a slight fibrotic reaction was observed. However, such a slight fibrotic overgrowth does not prevent nutrient and oxygen exchange between encapsulated cells and their environment. Rather, as will be demonstrated in the following section, such a reaction is advantageous to reduce capsule breakage and movement from the transplantation site.

5 Animal and Clinical Trials with Encapsulated Tissue

Immunoisolated-islet transplantation is an attractive therapy for insulin-dependent diabetes mellitus (IDDM) patients. Therefore, it is not surprising that extensive animal studies have been made with encapsulated rat, porcine, or human islets in the last two decades. In most of these experiments islets were entrapped in alginate-poly-L-lysine made up of commercial (non-purified), low-viscosity alginate. Intraperitoneal allo- and xenografts

could normalize blood glucose of (diabetic) mice and rats for about 100–150 d, occasionally some grafts functioned up to 1 year (O'SHEA and SUN, 1986; WEBER et al., 1999; WANG, 1999; VAN SCHILFGAARDE and DE VOS, 1999). Normalization of hyperglycemia by xenotransplantation of microencapsulated (porcine) islets has also been reported for spontaneously diabetic dogs and cynomolgus monkeys (WARNOCK and RAJOTTE, 1988; SOON-SHIONG et al., 1992; ZHOU et al., 1994; SUN et al., 1996; LANZA et al., 1999). However, as a rule, the results were very variable and the success was always of limited duration as expected in the light of the above considerations (see also below). Graft failure could occur even 2 weeks after transplantation due mainly to foreign body reactions (MAZAHERI et al., 1991; WISMAN et al., 1992) but sometimes in their absence (DE VOS et al., 1997b).

Ba²⁺ microcapsules made up of alginate purified from commercial alginates (KLÖCK et al., 1994) have also been successfully used for the encapsulation of rat and porcine islets of Langerhans (Fig. 5; ZEKORN et al., 1992a, b). In glucose perfusion challenges, evaluation of insulin secretion by encapsulated rat islets showed the typical biphasic insulin release pattern of non-encapsulated islets. During static glucose challenge, the insulin release ranged from 40% to 70% as compared to the controls.

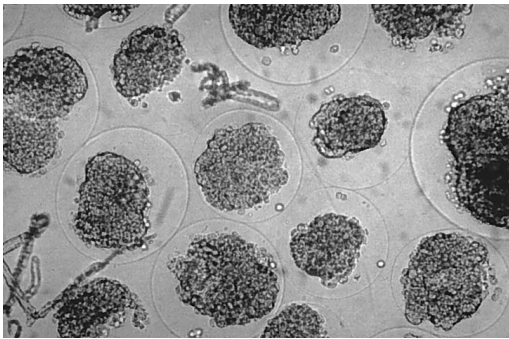


Fig. 5. Uniform preparation of porcine pancreatic islets immunoisolated by Ba²⁺ alginate capsules of small diameter. Encapsulation of the islets was performed by using the dropping device in Fig. 1A. Separation of empty beads (not shown) was achieved by discontinuous density gradient centrifugation according to the protocol of GRÖHN et al., 1994.

Accordingly, xenotransplantation of encapsulated rat and porcine islets in chemically induced diabetic mice demonstrated long-lasting graft function (up to 1 year) even though failure of some grafts was also observed (about 30%; ZEKORN et al., 1992a, b; SIEBERS et al., 1992, 1993). Histological examinations of long-term-functioning microcapsules demonstrated well preserved islets.

Despite the promising results of animal studies, there has been little success with the clinical allotransplantation of pancreatic islet cells into IDDM patients. This failure has generally been attributed to the inability to obtain large numbers of viable human pancreatic islets for grafting. About 1 million islets must be transplanted in order to cure diabetes (see below). However, the source of human organs is limited, thus only a small number of patients could benefit from the encapsulation method. Immunoisolation of porcine islets has the potential to fill the gap, but concerns remain about possible cross-species transmission of porcine endogenous retrovirus (PATIENCE et al., 1997; but see PARADIS et al., 1999; HUNKELER et al., 1999).

Animal and clinical data are available for allo- and xenotransplantation of encapsulated parathyroid glands. Parathyroid tissue excised from Lewis rats, encapsulated in Ba²⁺ alginate matrices and transplanted in parathyroid-ectomized Dark-Auita rats exhibited long-term function (HASSE et al., 1996, 1998). More than 6 months after allotransplantation (without systemic immunosuppression) nearly all animals that had received microcapsules made up of amitogenic alginate were normocalcemic. These results were independent of whether the alginate used was purified from commercial alginates according to the protocol of KLÖCK et al. (1994) or from fresh algal material (HASSE et al., unpublished data). Throughout the studies PTH and calcium concentrations were always concordant. Accordingly, histology of retrieved transplants revealed vital parathyroid tissue and intact microcapsules. The transplants were partly covered by a very thin fibrotic layer that apparently did not affect the function of the encapsulated tissue. Similar results were obtained for xenotransplantation of encapsulated human parathyroid tissue in rats with experimental hypoparathyroidism (HAS-

SE et al., 1997b). Evaluation of the Ca^{2+} level in the serum showed that about 100 d after transplantation 75% of the animals that received xenotransplanted human parathyroid tissue were still normocalcemic.

These and other results established the basis for pilot randomized clinical trials performed recently (HASSE et al., 1997a). The number of patients with clinical hypoparathyroidism is much lower than that of IDDM. Thus, there should be no shortage of donor human parathyroid tissue in the future. Cultured allogeneic parathyroid tissue immunoprotected by Ba^{2+} alginate was transplanted into the muscle of the right upper arm of two patients suffering from symptomatic persistent postoperative hypoparathyroidism (HASSE et al., 1997a). Shortly after allotransplantation, the two patients were normocalcemic and revealed normal levels of PTH without immunosuppression (Fig. 6). With ongoing transplantation

both patients reported an impressive improvement of symptoms and sequelae of hypoparathyroidism. After about 90 d graft failure occurred. Residues of the alginate capsules and of the allogeneic tissue could not be found when – with permission of one of the patients – the transplantation site was re-examined 10 months after surgery (HASSE et al., unpublished data). Studies in animal models gave evidence (BOHRER et al., unpublished data) that activated macrophages degrade encapsulated parathyroid tissue when capsule breaks occur. However, this cannot be the only explanation because one year after transplantation, re-examination of one of the patients showed nearly normocalcemia and absence of symptoms, requiring much less Ca^{2+} and vitamin D than prior to surgery. Appropriate tests (CASANOVA et al., 1991) revealed that the PTH was released from the transplantation arm (HASSE et al., unpublished data). The authors of this re-

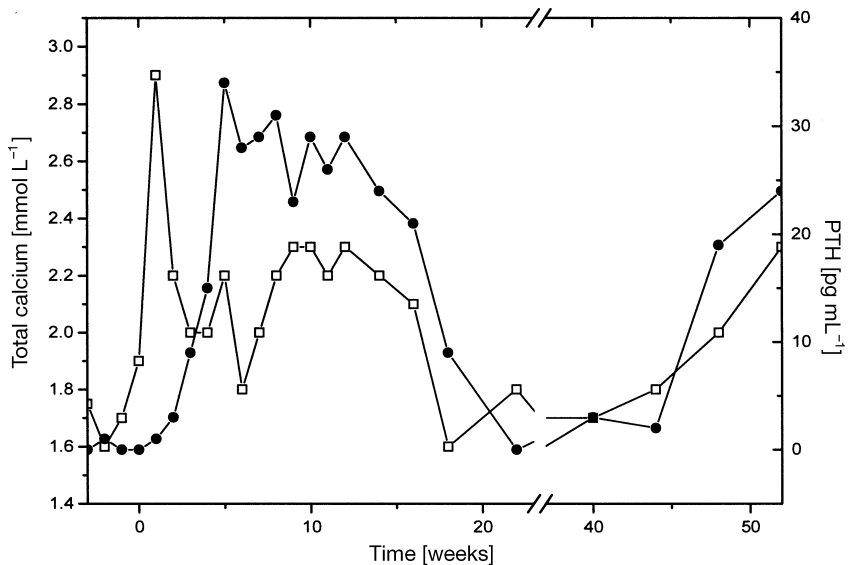


Fig. 6. Ca^{2+} (open squares) and parathormone (PTH; filled circles) levels in a patient with hypoparathyroidism after allotransplantation of parathyroid tissue into the muscle of the non-dominant forearm. The allogeneic tissue pieces were encapsulated by gelation of purified alginate (according to the protocol of KLÖCK et al., 1994) with Ba^{2+} .

Note that graft failure occurred after about 3 months, but that functional activity of the transplant was recorded again about 1 year after transplantation (for further details, see Sect. 5).

view believe that migration of the microcapsules from the transplantation site took place and/or that over a longer period of transplantation the alginate matrix was replaced by a fibrotic layer.

6 Conceptual Configuration of Microcapsules for Long-Term Transplantation

The above animal and, in particular, the first small-scale clinical studies are encouraging, but they have also clearly demonstrated that the duration of immunoprotection and/or function of the transplants were subjected to a large variability. There are strong indications (see also DE VOS et al., 1997b; ZIMMERMANN et al., 1999; ZEKORN and BRETZEL, 1999) that the biocompatibility of the beads is not only influenced by the chemical composition and purity of the material, but also by (1) physical and physicochemical imperfections of the capsule, (2) adverse properties of the precultured donor tissue, and even by (3) recipient- and operation-related factors.

The difficulty of designing a microcapsule system with optimum clinical properties is to adjust the numerous capsule parameters (such as size, permeability, mechanical strength, surface topography, swelling properties, etc.) independently. Therefore, seemingly minor modifications of one of the capsule parameters may have an important impact on other parameters of the cell-containing capsule, and thus ultimately on the outcome of the graft. Progress can only be expected when the underlying biophysical and immunological principles are thoroughly understood and documented. It is much more difficult and discouraging to troubleshoot problems that arise in cell therapy applications if one cannot fall back upon a solid foundation of first principles. This important issue was unfortunately ignored by many authors working with alginate-poly-L-lysine capsules (for notable exceptions,

see, e.g. VAN SCHILFGAARDE and DE VOS, 1999) with the result that there are numerous different encapsulation procedures that have led to the formulation of capsules with a broad, but rarely documented spectrum of properties.

In contrast to alginate-poly-L-lysine capsules, systematic, well documented improvements of the solid Ba^{2+} alginate microcapsules have been reported in the last couple of years (DE VOS et al., 1997a; ZIMMERMANN et al. 1999, 2000; NÖTH et al., 1999; HILLGÄRTNER et al., 1999). Because of recent promising animal studies with this new generation of Ba^{2+} alginate microcapsules, we will focus mainly on the optimum design of this type of capsules in the following. However, it should be noted that many solutions found for the Ba^{2+} alginate barrier (summarized in Fig. 7) can also be assigned to the alginate-poly-L-lysine capsules.

6.1 Size and Diffusion

Immunoisolated cells lack intimate vascular access, and must be supplied with oxygen and nutrients by diffusion from the nearest blood vessels into the capsule interior, i.e., over distances greater than those normally encountered. Therefore, if the ratio of the capsule material to encapsulated tissue (cells) is too unfavorable, graft failure can occur (ZEKORN et al., 1992b). This is not a problem when encapsulated parathyroid tissue sections are transplanted because they are fairly large (about 1 mm^3), but functional survival of islet transplants may be affected. Islets are about $100\text{--}150\text{ }\mu\text{m}$ in diameter and are commonly entrapped in capsules of $500\text{--}800\text{ }\mu\text{m}$ in diameter for animal studies. In such cases, the large stagnant fluid environment between the tissue and the microcapsule wall can create pronounced diffusion resistances (by analogy to macrocapsules, see above). This was demonstrated both theoretically (GOOSEN, 1999) and experimentally in model cellular systems (BEUNINK et al., 1989; BÜCHNER and ZIMMERMANN, 1982; HARTMEIER, 1986; SCHUBERT, SCHNEIDER, ZIMMERMANN, unpublished data; see also below). Diffusion restrictions generally result in a dramatic reduction of nutrient and, particularly, of oxygen transfer, leading ultimately to necrosis of the encapsulated tissue (FAN et al., 1990; DE