

T. Bohrer<sup>1,2</sup>, K. Stelter<sup>2</sup>, I.-S. Stelter<sup>2</sup>,  
C. Dietrich<sup>2</sup>, H. Sitter<sup>3</sup>, Ch. Hasse<sup>2</sup>

## Microencapsulation of Allogeneic Parathyroid Tissue Succeeds by Delaying Immunization of the Host, not by Protecting the Graft Against the Host's Activated Immune System\*

**Background:** To examine in an experimental animal study whether alginate microencapsulation of allogeneic parathyroid tissue succeeds by protecting the graft against the host's activated immune system or by delaying immunization of the host.

**Methods:** Lewis and Dark Agouti rats (n = 200 of each) were used. Parathyroidectomy (PTX) of the hosts was followed by allogeneic transplantation of microencapsulated parathyroid tissue (PT) to the following 3 groups of 5 animals each *without* immunosuppression: group I, early onset immunoreaction, group II, late onset immunoreaction, and group III, immunization. In group I, an allogeneic skin graft from the same donor was transplanted 3 weeks *after* microencapsulated PT transplantation and the rejection time determined to assess the host's immunoreaction. In group II, allogeneic skin graft transplantation was carried out under the identical conditions 8 weeks *after* microencapsulated PT transplantation. And in group III, allogeneic skin graft transplantation was performed as an immunostimulant 3 weeks *before* transplantation of microencapsulated PT. Each of the 3 study groups I to III was compared with a corresponding control group I to III receiving *native* PT allotransplants. Once a week, the titers of cytotoxic antibodies were analyzed and graft function was monitored based on total serum calcium levels [Ca<sup>++</sup>]. In group IV, 2 allogeneic skin grafts within 2 weeks were given after PTX (study animals), or without prior PTX (controls).

**Results:** Four out of 5 animals in group I, and 3 out of 5 animals in group II lost graft function within 11 weeks after transplantation of microencapsulated PT. In the respective control groups, graft function was lost within 6 weeks. Surprisingly, none of the immunostimulated animals in group III experienced any transplant success, all allogeneic skin grafts being rejected within 11.6 days. There was no significant (p > 0.05) difference in skin graft rejection between study and control groups III. Low cytotoxic antibodies were detected in both study animals and controls of all 4 groups one week after PT allotransplantation (p > 0.05 study and control groups), whereas cytotoxic antibodies were elevated to peak levels in all animals immediately after skin graft transplantation.

**Conclusion:** Earlier successes in microencapsulation technology are not due to the protection offered to the graft by alginate microencapsulation, but rather by the delayed immunization of the host.

### Key words:

cytotoxic antibodies, graft rejection, microencapsulation, parathyroid allotransplantation

<sup>1</sup> Department of Cardiothoracic Surgery, Julius-Maximilians University, Würzburg

<sup>2</sup> Department of Visceral, Thoracic and General Surgery, Philipps-University, Marburg

<sup>3</sup> Institute of Theoretical Surgery, Philipps-University, Marburg

Bohrer T, Stelter K, Stelter I-S, Dietrich C, Sitter H, Hasse Ch (2006) Microencapsulation of Allogeneic Parathyroid Tissue Succeeds by Delaying Immunization of the Host, not by Protecting the Graft Against the Host's Activated Immune System. *Tx Med* 18: 36-42

\* This study was supported by a grant from the German Research foundation (Ha 2036/3-4).

## **Kann die Mikroenkapsulierung allogene Gewebetransplantate der Parathyreoidea vor dem aktivierten Immunsystem in vivo schützen und verhindert sie die Immunisierung des Empfängers?**

**Einleitung:** Diese Studie klärt die wesentliche immunologische Frage, ob die Schutzfunktion von Mikrokapseln durch die Immunisierung des enkapsulierten Nebenschilddrüsengewebes gewährleistet wird oder die immunologische Barriere der Alginatkapsel ausreichend Schutz vor dem aktivierten Immunsystem in vivo bieten kann.

**Methoden:** Für die tierexperimentelle Studie wurden Lewis- und Dark-Agouti-Ratten ( $n = 200$  jeweils) verwendet. Nach Parathyreoidektomie (PTX) der Empfängertiere wurde eine Allotransplantation von mikroenkapsulierten Nebenschilddrüsenpartikeln bei folgenden 3 Testgruppen ( $n = 5$  Tiere jeweils) ohne Immunsuppression durchgeführt: Testgruppe I, frühe Immunreaktion, Testgruppe II, verzögerte Immunreaktion, und Testgruppe III, Immunisierung. In Testgruppe I wurde 3 Wochen nach Allotransplantation von mikroenkapsuliertem Nebenschilddrüsengewebe ein allogenes Hauttransplantat von demselben Spender transplantiert und der Aktivitätsgrad des Empfängerimmunsystems anhand der Abstoßungszeit ermittelt. In Testgruppe II wurde die allogene Hauttransplantation unter identischen Bedingungen 8 Wochen nach Allotransplantation von mikroenkapsuliertem Nebenschilddrüsengewebe vorgenommen. In Testgruppe III wurde die allogene Hauttransplantation 3 Wochen vor der Allotransplantation von mikroenkapsuliertem Nebenschilddrüsengewebe zur Immunstimulation vorgenommen. Jede der Testgruppen I - III wurde mit einer entsprechenden Kontrollgruppe I - III ( $n = 5$  Tiere jeweils), die mit nativen Nebenschilddrüsengewebe allotransplantiert wurden, verglichen. Die zytotoxischen Antikörpertiter und die Transplantatfunktion (Messung des Serumkalziums) wurden einmal wöchentlich bestimmt. In der Referenzgruppe IV wurden innerhalb von 2 Wochen zwei aufeinander folgende allogene Hauttransplantationen nach PTX (Testgruppe) und ohne vorherige PTX (Kontrollgruppe) vorgenommen.

**Ergebnisse:** 4 von 5 Tiere der Testgruppe I und 3 von 5 Tiere der Gruppe II entwickelten ein Transplantatversagen innerhalb von 11 Wochen nach Allotransplantation von mikroenkapsuliertem Nebenschilddrüsengewebe. In allen entsprechenden Kontrollgruppen (nativen Nebenschilddrüsentransplantate) kam es innerhalb von 6 Wochen zum Transplantatversagen. Überraschenderweise wiesen alle Tiere der immunisierten Gruppe III keinerlei Transplantationserfolg auf, die allogenen Hauttransplantate wurden innerhalb von 11,6 d abgestoßen. Es lag bezüglich der Hauttransplantatabstoßung kein signifikanter Unterschied zwischen der Testgruppe III mit mikroenkapsuliertem Nebenschilddrüsengewebe und der Kontrollgruppe mit nativen Allotransplantaten vor ( $p > 0.05$ ). Niedrige zytotoxische Antikörper konnten in allen 4 Gruppen bereits eine Woche nach Allotransplantation von Nebenschilddrüsengewebe nachgewiesen werden (Test- und Kontrollgruppen  $p > 0.05$ ), wohingegen ein sofortiger Anstieg der zytotoxischen Antikörper auf maximale Werte nach allogenen Hauttransplantationen bei allen Empfängertieren dokumentiert werden konnte.

**Schlussfolgerung:** Die bisherigen Erfolge in der Mikroenkapsulierungstechnologie sind nicht durch den Schutz der Mikrokapsel vor dem aktivierten Immunsystem zu erklären, sondern dürften durch eine verzögerte Immunisierung des Empfängers bedingt sein.

### **Schlüsselwörter:**

zytotoxische Antikörper, Transplantatabstoßung, Mikroenkapsulierung, Allotransplantation der Nebenschilddrüse

## **Conflict of Interest**

There were not any financial and personal relationships with other people or organisations that did inappropriately influence (bias) our work.

## **Abbreviations**

Allo Tx	allotransplantation
Ca <sup>++</sup>	Calcium
DA rats	Dark-Agouti rats
LEW rats	Lewis rats
PT	parathyroid tissue
PTH	parathyroid hormone
PTX	parathyroidectomy

## **Introduction**

Hypoparathyroidism arises in the majority of cases after subtotal or total thyroidectomy (1,2). The incidence of postoperative hypoparathyroidism has been put at 1% for partial and 10% for total thyroidectomies (3,4). In Germany alone the annual rate of 80000 partial and 4000 total thyroidectomies would mean at least 1200 patients contracting this disease every year. The actual rate should be even higher since these figures refer to only 47% of all hospitals in Germany (5).

Although permanent postoperative hypoparathyroidism is one of the most difficult to treat of all the endocrine dysfunctions, it is rarely life-threatening. Thus, while the need for causal therapy with transgeneic transplantation is beyond dispute, systemic immunosuppression posttransplantation is not justified. Solinger et al. (6) described a method for reducing antigen expression by allogeneic grafts using preoperative in-vitro cultivation. Though designed to prevent transplant rejection over the long term, this method did not obtain reproducible long-term success beyond 4 weeks.

Applying the results of studies on Langerhans' islet cell transplantation, we modified a technique for immunoisolation of grafts by encapsulating the transplanted tissue in a semipermeable membrane consisting of naturally occurring alginate, a technique known as microencapsulation. After extensive and successful attempts in vitro, we applied this technology in combination with a specific tissue culture for the first time in patients with permanent,

symptomatic hypoparathyroidism. Twelve weeks after transplantation, the patients' total serum calcium levels [ $\text{Ca}^{++}$ ] and parathyroid hormone [PTH] levels were in the normal range without any substitution or immunosuppressive therapy (7). They reported extraordinary improvements in their subjective well-being and even the symptoms of hypocalcemia disappeared. Nevertheless, transplant function was lost 3 months after allotransplantation.

The loss of function may have been due to a breakdown in the stability of the capsule membranes, as we have analyzed before (8). Probably it is also because the immunogenic rejection reaction has not been investigated thoroughly. While it is still not clear how rejection of transplanted PT can be prevented, 2 hypotheses currently exist. One claims the microcapsule prevents leakage of antigens from the transplant and immunization of the host, the other that the capsule protects the tissue transplant from the immunoreaction of the sensitized host.

Without exact knowledge of the immunological protective function of the alginate microcapsule, this technology cannot ethically be applied to patients. We therefore performed an experimental animal study to examine the precise mechanism underlying the ability of alginate microencapsulation to prolong graft function without immunosuppression.

## Materials and Methods

A total of 400 male animals of two different inbreeding strains (200 Lewis [LEW] rats [LEW/HAN, strain n° 86150M] and 200 Dark-Agouti [DA] rats [DA/OLA/HSD, strain n° 9205M], Harlan-Winkelmann, Netherlands) weighing 200g each were used in this study. The animals were assigned to 4 study groups, each with its own control group. Study groups I and II were used to explore whether microencapsulation of allogeneic PT offers protection against host sensitization and, in case of host immunization, whether the host organism has an early or late onset immunization reaction. Group III served to investigate whether microencapsulation protects the implanted allogeneic PT against the host's specifically activated immune system. Group IV was used to determine the maximum skin

graft rejection time after prior immunostimulation. The animal study was officially approved by the Regional Board of the City of Giessen, Germany. The immersed PT particles were microencapsulated using a special filter nozzle at a constant oxygen flow of 6.5 l/min to produce homogeneous particle sizes (inner diameter of the capsule = 1.6 mm). The filter nozzle and oxygen pressure on the alginate were adjusted according to the method of Ennis (9). The microcapsules were stabilised by brief incubation in barium chloride (20 mmol  $\text{BaCl}_2$ ) followed by several washings in sodium chloride (0.9% NaCl). The PT particles were then cultured for 3 days in RPMI solution (RPMI 1640 with L-Glutamin [no. 21875-034 Gibco, Germany], 10 % FCS [no. S0113, Seromed, Germany], 2.4 mmol/l Calcium and 10.000  $\mu\text{g}/\text{ml}$  penicillin/streptomycin [Biochrom KG, Berlin, Germany]) and kept in culture constantly at 37°C (95%  $\text{O}_2$  and 5%  $\text{CO}_2$  atmosphere) until microencapsulation or implantation. A commercial amitogenic alginate from *laminaria pallida* was used [lot no. MR-A01, Cellmed, Germany]. Total serum calcium levels [ $\text{Ca}^{++}$ ] were determined by a standard photometric colour alteration test (with chromogen solution; Roche Diagnostics; Cat. no. 1730240), the quantity of specific cytotoxic antibodies was checked using the microlymphocytotoxic test (trypan blue two-phases test).

*Study group I (30 DA and 30 LE rats): Does microencapsulation prevent early host immunization?*

To achieve the optimum 10 : 1 ratio of transplanted to physiologically present PT (Hasse, 1994), PTX was performed on 5 rats designated to be hosts for microencapsulated PT. Fourteen days later, PTX was performed on 50 donor rats. Thus for each 5 host animals 50 donors were parathyroidectomized. After explantation, the PT was subjected to the aforementioned tissue culture passage for 3 days and then microencapsulated. On the following day, the microencapsulated PT was transplanted into 5 host animals. To determine whether microencapsulation prevented immunization of the host, a 2 x 2 cm skin graft was taken 20 days later from the lateral thoracic wall region of the same donors as the parathyroids and

transplanted into 5 additional hosts. If early immunization had occurred, the skin graft would very likely be quickly rejected. During the test series, total [ $\text{Ca}^{++}$ ] and the cytotoxic antibodies were analysed once a week. After the skin transplantation, photo documentation of the transplanted allogeneic skin grafts was made daily.

The setup for group I was used in all of the groups described below with the noted differences. All procedures were performed under general anaesthesia with 0.8 ml ketamine/rompun (ratio 2 : 0.1) injected intramuscularly.

*Group I controls (30 DA and 30 LE rats)*

Group I controls underwent the same protocol as the study animals except that they received allotransplants of *native* PT. It was anticipated that the host animals would develop rapid skin graft rejection due to early immunization.

*Study group II (30 DA- and 30 LE rats): Does microencapsulation prevent late host immunization?*

The experimental setup was the same as for study group I except that allogeneic skin transplantation was performed after 8 weeks after transplantation of microencapsulated PT.

*Group II controls (30 DA- and 30 LE rats)*

The setup for group II controls was the same as for study group II except that *native* PT was transplanted, followed 8 weeks later by the skin graft allotransplantation.

*Study group III (30 DA and 30 LE rats): Does microencapsulation protect allogeneic transplants from the host's immune response after specific immunostimulation?*

PTX and subsequent allogeneic skin transplantation were performed on 5 hosts. Twenty-one days later, PTX was carried out on 50 donor rats. After explantation, the PT was cultivated for 3 days, microencapsulated as described for study groups I and II, then implanted in the 5 stimulated hosts on the following day.

*Group III controls (30 DA and 30 LE rats)*

The setup for group III controls was the same as for study group III except that *native* PT was transplanted.

*Study group IV (10 DA and 10 LE rats): What is the maximum skin graft rejection time after prior immunostimulation?*

To analyse the maximum rejection time of the second skin graft in the stimulated immune system, PTX was performed on 10 host rats, followed 20 days later by the first allogene skin transplantation. After an additional 14 days, a second skin transplantation for the same donor was carried out on these 10 parathyroidectomized hosts.

*Group IV controls (10 DA and 10 LE rats): Does PTX itself exert influence on rejection?*

In contrast to study group IV, the hosts in control group IV were not parathyroidectomized so that we could document what influence in any PTX and the ensuing hypocalcemia had on the rejection reaction.

#### Statistics

To obtain statistically utilizable results (significance =  $\alpha \leq 0.05$ , random beta error =  $\beta \leq 0.2$ ) according to Fuhrberg's formula for comparing different groups a minimum of 5 hosts per group was needed. The basic variable was the antibody titers, hereby the test serum was diluted to the higher degrees and the value of the highest dilution to still exhibit an antibody reaction documented. The dilution sequence was done logarithmically (1:1, 1:2, 1:4, 1:8 and 1:16) so as to avoid linear data. Antibody titers were analysed using the non-parametric test of Mann-Whitney. The descriptive auxiliary variable for assessment of immune status and graft condition was the skin transplant rejection time (in days) and  $[Ca^{++}]$  levels. A skin transplant rejection time less than 9 days indicated a specifically prior stimulated system in accordance with the literature (10). A skin transplant rejection time more than 11 days pointed to a non-stimulated immune system. Complete loss of parathyroid graft function was defined as permanent lowering of

total  $[Ca^{++}] < 1.9$  mmol/l ([11]; normal range of total  $[Ca^{++}] = 2.1 - 2.6$  mmol/l). Successful and complete PTX of all host animals was assessed by 2 independent determinations of  $[Ca^{++}] < 1.9$  mmol/l over a period of 2 weeks.

## Results

*Study group I: Does microencapsulation prevent an early host immunization?*

Early immunization is caused by donor antigen-bearing dendritic cells identifying and phagocytizing host cells and then presenting the antigens to the host's own lymphocytes (12). If microcapsular immunoisolation is complete, no antibodies against the donor nor late ( $> 10$  d) rejection of the skin graft are to be expected. In this group, antibodies were found in 4 out of 5 animals after 2 weeks. After skin graft transplantation, antibody titers rose abruptly, attaining peak levels after 4 weeks. In all 5 animals,  $[Ca^{++}]$  levels increased after parathyroid graft transplantation, attaining normal ranges ( $[Ca^{++}] > 1.9$  mmol/l) within approximately 8 weeks. The average skin graft rejection time of 11 days had a variance of  $v = 6.5$  days. The results are shown in figure 1.

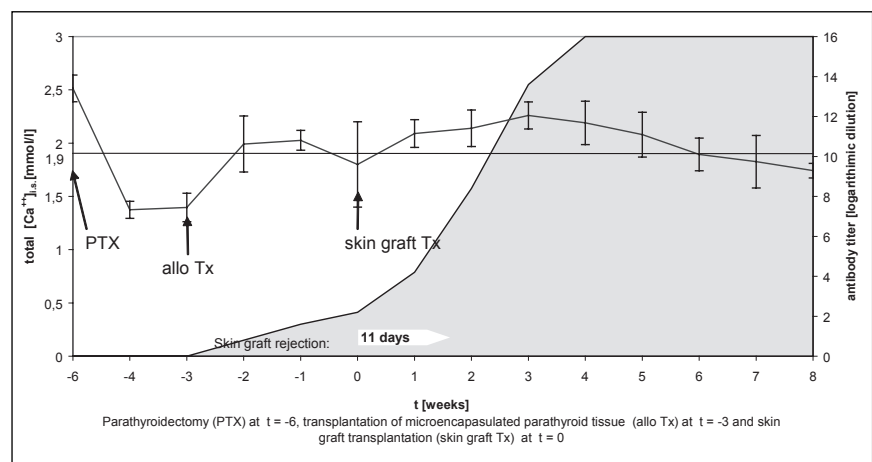
*Group I controls*

As expected, antibody titers of all 5 control animals rose clearly faster than in the study group. Two of the 5 animals attained maximum antibody levels even before the skin transplantation, all 5 animals had reached peak levels by 2 weeks after skin transplantation.  $[Ca^{++}]$

rose momentarily after allotransplantation of *native* PT, but only for a short time. After 3 weeks, all 5 animals had a permanent hypocalcemia of less than 1.9 mmol as a sign of total loss of graft function. Despite the high antibody levels and the hypocalcemia, the average skin graft rejection period for all 5 animals was 12 days, but with a high variance of  $v = 5.5$  days.

*Study group II: Does microencapsulation prevent late host immunization?*

Delayed immunization is mediated by presentation of donor specific antigens via the host's dendritic cells. These antigen-presenting cells must recognize and phagocytize parts of the donor cells first in order so as to present the antigens to the own T lymphocytes. This is termed an indirect immunization pathway and peak immunoreaction takes twice as long to reach via this pathway (12). If microcapsular immunoisolation is complete, no or very low antibodies against the donor and late ( $> 10$  d) rejection of the skin graft are likely. All 5 animals in study group II also exhibited an increase in specific cytotoxic antibodies against the allogeneic PT. In the sixth week after transplantation of microencapsulated PT, 3 of the 5 animals showed a rapid increase in antibody titers. At the time of skin transplantation, 2 of the 5 animals had already attained peak antibody levels, 3 weeks later all 5 animals had. In 3 of the 5 animals,  $[Ca^{++}]$  levels dropped 3 weeks after allotransplantation of PT  $< 1.9$  mmol/l ( $v = 0.04$  mmol/l). After skin graft transplantation, the rate of normo-



*Fig. 1: Skin graft rejection,  $[Ca^{++}]$  and cytotoxic antibody titers of study group I (n = 5)*

calcemia increased for a period of 1 week. Despite the significantly elevated antibody titers, skin graft rejection took an average of 11.6 days ( $v = 1.8$  d).

#### Group II controls

In the controls, antibodies titers increased much faster than in the study group II, but the data had a wide statistical spread. For example, one animal attained peak antibody titers the first week after transplantation of PT, while 2 of the 5 animals did not reach peak antibody titers of 1:16 until the first week following skin transplantation.  $[Ca^{++}]$  showed a short-term increase immediately after transplantation of native PT. All 5 animals were permanently hypocalcemic 4 weeks after allotransplantation of PT particles ( $[Ca^{++}] < 1.9$  mmol/l). Despite the high antibody titers and the distinct hypocalcemia, the skin grafts were not rejected on average until after 12.4 days ( $v = 4.3$  d). This did not accord with the expectation that successful microcapsular immunoisolation would lead to more rapid skin rejection in controls than in study animals.

#### Study group III: Does microencapsulation protect allogeneic transplants from the host's immune response after specific immunostimulation?

As expected, peak antibody titers were measured in all study group III animals within 2 weeks after skin transplantation. Consequently, the immune systems of all animals were highly stimulated at the time of allotransplantation of microencapsulated PT. The skin grafts were rejected after an average of 11.6 days ( $v = 0.3$  d). Surprisingly, despite the postulated protection provided by the alginate membrane, none of the animals exhibited normocalcemia ( $[Ca^{++}] = 2.1 - 2.6$  mmol/l) after transplantation of microencapsulated PT. Only a slight increase in  $[Ca^{++}]$  was noted in the first week after transplantation. Figure 2 clearly shows these interesting results.

#### Group III controls

Once again,  $[Ca^{++}]$  levels in the control group exhibited the characteristic short increase immediately after allotransplantation of native PT. Two weeks later, all 5 animals had permanent

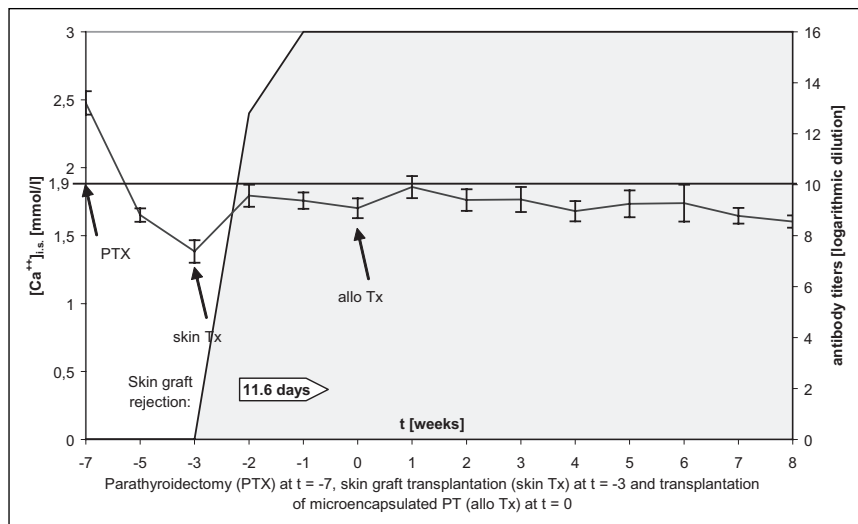


Fig. 2: Skin graft rejection,  $[Ca^{++}]$  and cytotoxic antibody titers of study group III ( $n = 5$ )

hypocalcemia ( $[Ca^{++}] = 1.73 \pm 0.06$  mmol/l).

#### Study group IV: What is the maximum skin graft rejection time after prior immunostimulation?

For both study animals and controls, the average rejection time for the first skin graft was 11.8 days ( $v = 1.1$  d), for the second 6 days ( $v = 0.93$  d), representing a 50% drop in the rejection time for the maximally stimulated immune system. There was very little spread of the data, a testimony to the reliability of this method. Two weeks after the second skin transplantation, all 5 animals showed maximum antibody titers.

#### Group IV controls: Does PTX itself influence rejection?

PTX and the ensuing hypocalcemia had no influence on rejection of the skin transplants or on the immunocompetence of either study animals or controls.

## Discussion

In the present study, we applied the parathyroid allotransplantation model in vivo to investigate for the first time whether alginate microcapsules can ensure immunoisolation of the graft against strong immunogenic stimuli. Parathyroid allotransplantation is not yet a standard clinical procedure. In our own first clinical study of allogeneic transplantation of microencapsulated

PT, the loss of endocrine graft function occurred after 12 weeks (7). Further own yet unpublished studies regarding histological analysis revealed infiltration of macrophages along hairline cracks of the alginate membrane as a sign of mechanical instability and immunological rejection. The aim of the present study was to investigate the immunoreaction to the allogeneic transplantation of microencapsulated PT in vivo. The results of this study could be the basis for further clinical applications. Although our present study was designed for parathyroid transplantation, the design can be applied to other endocrine deficiency syndromes. Based on our previous results with clinical autogenous parathyroid transplantation (13) and on our experience, in the present study we applied a 3-day tissue culture using RPMI 1640 and 10% host serum aimed at eliminating the immunogenic components of the allogeneic transplant responsible for rejection (14, 15). During the 3 day culturing period, microscopy revealed no structural changes or destruction of the cells. The following 3 factors must be considered when choosing a site for the microencapsulated transplant: 1) the supply of oxygen and nutrients, 2) optimal release of produced hormones as exocrine function and 3) immunological protection. Transplantation into the host musculature is a well-established practice (16). In our clinical study, we achieved clinically significant results following allotransplantation of microencapsulated PT into the brachioradial muscle of the non-dominant arm

(7). This site has the advantages of easy detection of the graft in the event of malfunction or hyperfunction and the ability to monitor graft function by e.g. the Ellis-Howard test. As in a previous studie, we obtained clinically significant results using a optimum transplant ratio of 10 : 1 (donor : host) for the transplantation of microencapsulated PT particles (17).

In the present study, we used a highly purified amitogenic alginate from *laminiaria pallida* algae. Own yet unpublished data showed that transplanted allogeneic encapsulated PT survived in 2 rats for 2 years with that type of alginate (transplant ratio of 10 : 1, donor : host).  $[Ca^{++}]$  levels in these animals remained within the normal range during the 2 years and vital encapsulated PT was found (H&E stain) following explantation. In contrast for host sensitisation, we employed a *strong* immunostimulus – the allogeneic skin transplant, an otherwise *non-physiologic* stimulus. Such allogeneic skin grafts are rejected much faster by an activated immune system than are less immunologically potent transplants (18) and were therefore suitable for examining the immunological topics of this study. Because of the known direct reciprocal relationship between  $[Ca^{++}]$  and [PTH] secretion, determination of  $[Ca^{++}]$  levels alone suffices as a parameter for graft monitoring (19). We therefore did not monitor [PTH] levels in this study. Since serum  $[Ca^{++}]$  levels also depend on the intestinal calcium uptake, we used a standardized low calcium diet and minimized the synthesis of vitamin D3 by neon light without UVA and UVB fractions.

In study group I, loss of graft function did not occur until 8 weeks after transplantation of microencapsulated PT, whereas in group I controls receiving *native* PT transplants graft function was permanently lost after only 3 weeks ( $[Ca^{++}] < 1.9$  mmol/l). If immunoisolation of the alginate microcapsules was complete, no antibodies were to be expected. Our data on antibody titers, however, showed that immunoisolation was not complete, although the formation of antibodies had been delayed by the microcapsules. In the study group, the average antibody titer was 1 : 2.2 after 3 weeks, in controls 1 : 10. The incomplete immunoisolation may have been caused by small cracks in the capsules resulting from colloidosmotic

pressure-induced swelling of the alginate capsules (20). Besides cytotoxic antibodies that damage the skin transplant directly, other factors such as MHC I and II antigens could also have contributed to the rejection reaction (21). The nearly identical long skin graft rejection times of 3 weeks for both *native* and microencapsulated PT appears to confirm the incomplete immunoisolation by the alginate microcapsules.

In late immunization, which does not occur until 6-8 weeks after transplantation, donor antigen-bearing cells must identify and phagocytize parts of host cells in order to present the antigens to the host's own lymphocytes (12). To determine whether microencapsulation of PT could prevent late immunization of the host, we carried out allogeneic skin graft transplantation in group II under conditions identical to those of group I but 8 weeks *after* transplantation. Microencapsulation postponed graft failure to 5 weeks postimplantation (with a rapid initial increase in antibody titers and simultaneous drop in  $[Ca^{++}] < 1.9$  mmol/l). Graft function in group II controls was equivalent to that in control group I. Also without the immunological stimulus of the skin transplant, total transplant failure followed after 3 weeks. In both study and control animals,  $[Ca^{++}]$  rose temporarily after the skin transplant. This effect, which we had observed in a previous study (11), was apparently not associated with the graft function but may have been caused by the surgical procedure itself or by the anaesthesia.

Immunization of the host animals produced surprising results in group III: The skin grafts were rejected as anticipated after 12 days, and the antibody titers rose to peak levels within 3 – 4 weeks. In control group III, the *native* PT was immediately recognized as foreign and rejected by the stimulated immune system. Remarkably, the microencapsulated PT allotransplants showed no function at all, with constant  $[Ca^{++}] < 1.9$  mmol/l. Study and control animals did not differ significantly with respect to  $[Ca^{++}]$  levels ( $p < 0.05$ ). At peak levels of the stimulated immune system, the alginate microcapsules could not prevent graft rejection. A possible explanation for this is that cytotoxic antibodies with a molecular weight  $< 150k$  Dalton, IgG<sup>3</sup> for example (10), could have penetrated the mi-

crocapsules and destroyed the graft cells together with the complement. This is possible because alginates possess pores that can be penetrated by molecules of this weight (22).

The maximum rejection time and antibody formation in all animals of study and control groups IV show that hypocalcemia after PTX had no influence on the immunoreaction. All animals in both groups reacted to the strong stimulus of the skin graft with the rapid formation of cytotoxic antibodies and an intense immunological sensitisation, which were the main reason for the typical acute phase rejection of the second transplant after just 6 days (12).

## Conclusion

Microencapsulation of endocrine allotransplants appears to be able to significantly prolong graft function without immunosuppression. However, graft survival in the present study was limited to 6 - 8 weeks. This can be explained not only by the limited supply of nutrients and oxygen to the graft, but also by the immunological rejection reaction of the host. Previous successes with microencapsulation technology are not therefore to be explained by its ability to protect the graft against the host's activated immune system, but rather by late immunization of the host. Immunological components, such as cytotoxic antibodies, seem to be capable of passing the barrier of the capsular membrane and causing rapid destruction of the microencapsulated transplants. It is clear, therefore, that different methods of immunoisolation and immunomodulation are required to ensure sufficient function of transgeneic parathyroid tissue transplants without long-term immunosuppression. Future studies should aim at the effective combination of these different methods and optimisation of the alginate microcapsule properties applying the present immunological findings. Other highly purified alginate lots could be applied, for example, immunosuppressive substances could be used in the capsular membrane, or additional stabilizing covers added to the microcapsules (23, 24).

## References

1. Harris SC (1992) Thyroid and parathyroid surgical complications. *Am J of Surg* 163: 476-488
2. Wax MK, Briant TD (1992) Completion thyroidectomy in the management of well-differentiated thyroid carcinoma. *Otolaryng. Head and Neck Surg* 107: 63-71
3. Gann DS, Paone JF (1979) Delayed hypocalcemia after thyroidectomy for Graves' disease is prevented by parathyroid autotransplantation. *Ann Surg* 190: 508-513
4. Demeester-Mirkine N, Hooghe L, Van Geertruyden J, De Maertelaer J (1992) Hypocalcemia after thyroidectomy. *Arch Surg* 127: 854-862
5. Klauber J, Robra BP, Schellschmidt H (2004) Krankenhausreport 2004 (S. 315-324). Stuttgart: Schattauer
6. Sollinger HW, Mack E, Cook K, Belzer FO (1983) Allograft transplantation of human parathyroid tissue without immunosuppression. *Transplantation* 36: 599-602
7. Hasse C, Klöck G, Schlosser A, Zimmermann U, Rothmund M (1997) Parathyroid allotransplantation without immunosuppression. *The Lancet* 350: 1296-97
8. Bohrer T, Hanz J, Barth P, Zimmermann U, Rothmund M, Hasse C (2001) Optimierung der Sauerstoffversorgung von Mikrokapseln zur transgenen Transplantation der Parathyreoidea durch Perfluorokarbon (FC 43). *Deutsche Gesellschaft für Chirurgie, Forumband* 30: 503-506
9. Ennis WB, James DT (1950) A simple apparatus for producing droplets of uniform size from small volumes of liquids. *Science* 112: 434
10. Kuby J (1996) General organization and inheritance of the MHC. In: Kuby J (Ed.) *Immunology* (3rd ed., pp. 224-227). New York: Freeman
11. Hasse C, Klöck G, Zielke A et al. (1996) Transplantation of parathyroid tissue in experimental hypoparathyroidism: In vitro and in vivo function of parathyroid tissue microencapsulated with a novel amphoteric alginate. *Int J Artificial Organs* 19: 1-7
12. Wonigeit K, Pichlmayr R (1997) Organtransplantation. In: Gemsa D, Kalden JR, Resch K (Hrsg.) *Immunologie: Grundlagen - Klinik - Praxis* (4th ed., S. 636-660)
13. Rothmund M, Wagner PK (1987) Epithelkörperchentransplantation. *Endokrine Chirurgie* (S. 77-95). Stuttgart: Thieme Publishers
14. Lafferty KJ, Cooley MA, Woolnough J, Walker KZ (1975) Thyroid immunogenicity is reduced after a period in organ culture. *Science* 188: 259-261
15. Sollinger HW, Burkholder PM, Rasmus WR, Bach FH (1977) Prolonged survival of xenografts after organ culture. *Surgery* 81: 74-79
16. Naji A, Barker CF (1976) The influence of histocompatibility on transplant site on parathyroid allograft survival. *J Surg Res* 20: 261-267
17. Hasse C, Schrezenmeier J, Stinner B et al. (1994) Successful allotransplantation of microencapsulated parathyroids in rats. *World J Surg* 18: 630-634
18. Streilein JW, Bergstresser PR (1980) Ia antigens and epidermal Langerhans cells. *Transplantation* 30: 319-323
19. Ziegler R (1987) Kalziumstoffwechsel und seine Störungen. In: Ziegler R (Hrsg.) *Hormon- und Stoffwechselbedingte Erkrankungen in der Praxis*. Weinheim: edition medizin, VCH Verlagsgesellschaft
20. Hillgärtner M, Zimmermann H, Mimietz S, Jork A, Thürmer F, Schneider H, Nöth U, Hasse C (1999) Immunoisolation of transplants by entrapment in 19F-labelled alginate gels: production, biocompatibility, stability and long-term monitoring of functional integrity. *Mat-wiss u Werkstofftech* 30: 783-792
21. Timm S, Hamelmann W, Otto C et al. (2001) Influence of donor MHC class I antigen expression on graft survival after parathyroid allotransplantation. *Langenbecks Arch Surg* 386 (6): 430-433
22. Gäserod O, Sannes A, Skjåk-Bræk G (1999) Microcapsules of alginate-chitosan. A study of capsule stability and permeability. *Biomaterials* 20: 773-783
23. Coradin T, Merecey E, Lisnard L, Livage J (2001) Design of silica-coated microcapsules for bioencapsulation. *Chem Commun* 23: 2496-2497
24. Schneider S, Feilen PJ, Slotty V et al. (2001) Multilayer capsules: a promising microencapsulation system for transplantation of pancreatic islets. *Biomaterials* 22: 1961-1970

Dr. Thomas Bohrer  
Department of Cardiothoracic Surgery  
Julius-Maximilians-University  
Oberduerrbacherstr. 6  
97080 Würzburg  
E-Mail:  
bohrer\_t.htc@klinik.uni-wuerzburg.de

G. H. Willital, E. Kiely, A. M. Gohary, D. K. Gupta, M. Li, Y. Tsuchida (Eds.)

## ATLAS of CHILDREN'S SURGERY

### Atlas for Surgeons

Latest edition on surgical procedures in children: for all surgeons operating children

### 1163 Illustrations

Surgical techniques are explained by 1163 illustrations, step by step.

### New: Safety Instructions for Surgeons

Points to be observed during surgery. How to avoid complications. Based on the European Databanc of Complications in Pediatric Surgery (EDBEC)

### Internet Updated Version

An updated version in the internet for purchaser of this book will be presented free of charge initially:  
[www.surgerychildrenatlas.com](http://www.surgerychildrenatlas.com)

### New: OP-Digital Videofilms - OP-Digital Pictures

To the most frequent procedures digital video teaching films are available on demand as well as intraoperative digital pictures.

For all further informations: [www.surgerychildrenatlas.com](http://www.surgerychildrenatlas.com)

528 pages, Hardcover, ISBN 3-89967-126-0, Price: 82,- Euro / 98,- US\$

PABST SCIENCE PUBLISHERS

Eichengrund 28, 49525 Lengerich, Tel. ++ 49 (0) 5484-308, Fax ++ 49 (0) 5484-550, E-Mail: [pabst.publishers@t-online.de](mailto:pabst.publishers@t-online.de)  
Internet: [www.pabst-publishers.com](http://www.pabst-publishers.com)