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ORIGINAL ARTICLE

Preoperative evaluation of microencapsulated human parathyroid tissue aids selection of the optimal bioartificial graft for human parathyroid allotransplantation

Vasiliy Moskalenko,[1]* Karin Ulrichs,[1]* Andreas Kerscher,[1] Eberhard Blind,[2] Christoph Otto,[1] Wulf Hamelmann,[1] Yurij Demidchik[3] and Stephan Timm[1]

1 Department of Surgery I, Centre of Operative Medicine, University of Wuerzburg, Wuerzburg, Germany
2 Department of Endocrinology, Clinic of Internal Medicine, University of Wuerzburg, Wuerzburg, Germany
3 Department of Oncology and Thyroid Surgery, Medical University of Minsk, Minsk, Belarus

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Correspondence
Professor Dr Karin Ulrichs, Department of Surgery I, Centre of Operative Medicine, University of Wuerzburg, Oberduerrbacher Strasse 6, D-97080 Wuerzburg, Germany.
Tel.: +49 931 201 31710;
fax: +49 931 201 31719;
e-mail: ulrichs_k@chirurgie.uni-wuerzburg.de

*Vasiliy Moskalenko and Karin Ulrichs contributed equally to this paper.

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Summary

Allotransplantation of microencapsulated parathyroid tissue is a promising approach to the treatment of permanent hypoparathyroidism. Preoperative assessment of the quality of microencapsulated parathyroid tissue could facilitate selection of the optimal bioartifical graft for human parathyroid allotransplantation. Parathyroid tissue from patients with secondary hyperparathyroidism ($n = 15$) was processed mechanically or enzymatically (collagenase type II). Tissue particles and single cells/cell clusters were routinely microencapsulated with amitogenic Ba^{2+} alginate. Parathyroid secretion dynamics in response to stimulation of nonencapsulated and microencapsulated parathyroid tissue with Ca^{2+} were evaluated in a perifusion system. The stability of the different types of microcapsule was assessed using an osmotic pressure test. Mechanical cutting of parathyroid tissue led to peripheral necrosis of tissue particles and impaired their vitality. Collagenase digestion, in contrast, resulted in single cells and cell clusters without peripheral necrosis. The quality of microencapsulation of single cells/cell clusters was significantly better than that of tissue particles (deformed and imperfect capsules). Microencapsulation itself did not decrease cell vitality. Nonencapsulated and microencapsulated tissue particles and single cells/cell clusters from different donors maintained their own levels of response to stimulation with low Ca^{2+}. Microcapsules containing tissue particles showed poor stability compared with those containing single cells/cell clusters. Preoperative evaluation of microencapsulated parathyroid tissue can disclose differences in vitality and function and thus facilitate selection of the optimal bioartifical graft for human parathyroid allotransplantation.

Introduction

Permanent hypoparathyroidism occurs in 1–5% of patients following thyroid or parathyroid surgery. It is characterized by the combination of low calcium and low parathyroid hormone (PTH) levels in a patient's serum for longer than 6 months, with or without clinical symptoms [1]. The metabolic and biologic actions of PTH may explain why calcium and vitamin D substitution alone is often not sufficient treatment of permanent hypoparathyroidism [1]. Parathyroid allotransplantation, therefore, has been investigated as a possible curative treatment of hypoparathyroidism. To overcome organ rejection, a variety of approaches have been employed:

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short-term immunosuppression [2], and immunoalteration by depletion of passenger leukocytes or by preoperative organ culture [3]. All of these approaches resulted in prolonged but not indefinite graft function. Until today successful human parathyroid allotransplantation has only been possible in combined transplantation protocols, such as kidney-pancreas and parathyroid allotransplantation, under permanent immunosuppression [4].

Attempts to reduce transplant immunogenicity culminated in the technique of immunoisolation by microencapsulation, first described in 1980 by Lim and Sun [5]. Since then, the technique of microencapsulation has been extensively studied and developed not only in the field of transplantation, but also as a therapeutic treatment of hemophilia, cancer, renal failure and diabetes. However, despite very promising results from some of these approaches, long-term experimental results could not be reproduced in the clinic. Among the reasons for this failure as far as parathyroid allotransplantation is concerned appears to be the inability to reproduce data due to the wide variety of approaches used for allotransplantation of microencapsulated parathyroid tissue. More importantly, transplantation in most experimental studies is performed without any preoperative analysis of the quality of microencapsulation, making it impossible to differentiate between biotechnologic and immunologic reasons for subsequent graft nonfunction.

To determine whether preoperative assessment of the quality of microencapsulation of parathyroid tissue can aid in selection of the optimal graft for human parathyroid allotransplantation, we used an *in vitro* assay system to test the vitality and function of microencapsulated parathyroid tissue particles and parathyroid single cells/cell clusters. Cell clusters consisted of approximately 2–500 cells and were able to adhere to the bottom of the culture dish forming a monolayer of the parathyroid cells (later *single cells*).

Materials and methods

Parathyroid glands from 15 patients with secondary hyperparathyroidism (sHPT) removed in the course of therapeutic parathyroid surgery were used for this study (nine for measuring the secretory dynamics of parathormone and six for the stability tests). Diagnoses were based on standard clinical criteria for sHPT, surgical findings, and histologic examination. Glands were transported on ice in complete culture medium (RPMI 1640, 25 mM Hepes, stable L-glutamine, 1% penicillin-streptomycin, 10% fetal calf serum; CellConcepts, Umkirch, Germany). The cold ischemia time was less than 15 min. After removal of necrotic and fat tissue, parathyroid glands were processed either mechanically or enzymatically. All studies on the human parathyroid tissue were approved by the local ethics committee.

Mechanical preparation of the parathyroid tissue

Parathyroid tissues were cut routinely in complete culture medium under the dissection microscope (Helmut Hund GmbH, Wetzlar, Germany) to produce tissue particles of different size (<500 μm or >500 μm). Cell debris was removed by washing the tissue particles three times with medium. After standard testing for vitality using fluorescein-diacetate (FDA) and propidium iodide (PI) stain, tissue particles were incubated in complete medium at 37 °C and 5% CO_2. The vitality of the parathyroid tissue was calculated as per cent of vital tissue (per 100 tissue particles), or as the number of vital cells in a single cell/cell cluster preparation as ascertained using the BX50 microscope (Olympus, Hamburg, Germany), digital Camera ColorView12 (Olympus), and Software analySIS® (Soft Imaging System, Muenster, Germany).

Enzymatic preparation of the parathyroid tissue

Enzymatic digestion was performed as previously described [6] with some modifications. Briefly, the parathyroid glands in complete medium on ice were chopped into 3–4 mm particles. The tissue particles (100 mg parathyroid tissue per 10 ml of the digestion solution) were digested by incubation at 37 °C with 1.2 mg/ml collagenase type II (Sigma-Aldrich, Taufkirchen, Germany) dissolved in DMEM-HAM's F-12 medium (CellConcepts). In order to accelerate preparation, the tissue particles were accurately aspirated with a 30 ml pipette every 10 min. The quality of the digestion (the size of cell clusters) was checked microscopically. Digestion usually continued for 50–70 min. At the end of digestion, the resulting suspension was centrifuged at 1400 rpm^{-1}, 346 × g for 5 min and washed two times with complete RPMI 1640 culture medium.

Microencapsulation of the parathyroid tissue

Microcapsules for the *in vitro* studies were prepared as follows: parathyroid tissue particles (<500 μm and >500 μm) or *single cells* (100 μl cells per 1 ml alginate solution) were suspended in highly purified, nonmitogenic alginate (2% solution, Pronova UP MVG; NovaMatrix, Oslo, Norway). Alginate microcapsules were formed by pushing the resulting suspension through a 0.8 mm inner nozzle; the size of the microcapsules was regulated by changing the air flow in the outer nozzle. All microcapsules were gelled in 30 ml per 1 ml alginate of gelling solution (30 mM $BaCl_2$ for 4 min). Microcapsules were

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stored in complete RPMI 1640 culture medium in 5% CO_2 at 37 °C. The quality of the microencapsulation was assessed by phase contrast microscopy. The vitality of microencapsulated tissue was measured by FDA/PI staining. Only optimally microencapsulated tissue particles were hand-selected under the dissection microscope for subsequent *in vitro* studies.

In vitro testing of the nonencapsulated and microencapsulated parathyroid tissue

Before starting the *in vitro* studies, both nonencapsulated and microencapsulated tissue (tissue particles smaller than 500 μm, *single cells*) were cultured in complete RPMI 1640 culture medium supplemented with 1.3 mM Ca^{2+} and 5% CO_2 at 37 °C for 24 h to stabilize the rate of hormone secretion. Acute parathyroid secretory dynamics were tested in a perifusion system consisting of a stainless steel perifusion chamber (0.5 ml), a peristaltic pump, polyethylene connecting tubing and a perifusion medium (RPMI 1640 medium with three different concentrations of ionized calcium: 0.42, 1.6, 2.6 mM]. The medium was pumped at 0.3 ml/min (the prechamber volume was <0.5 ml, the postchamber volume was approximately 0.3 ml). The temperature inside the chambers was kept at 37 °C. Perifusion samples were collected at 10-min intervals and stored at −20 °C for later measurement of intact PTH (chemiluminescent immunometric assay; IMMULITE® 2000; DPC Bierman GmbH, Bad Nauheim, Germany). The tissue particles (<500 μm, 10 per chamber) from 10 donors (nine sHPT and one normal tissue) were used to study the secretional potentials of parathyroid tissue from different donors; the results were expressed as the mean ± the standard error per μg DNA. The DNA content was assessed as previously described [7]. Each experimental condition was tested in triplicate. The functional performance of microencapsulated parathyroid tissue particles and parathyroid *single cells* (capsule size approximately 800 μm) was compared with that of nonencapsulated tissue particles from the same donors. The vitality of the tissue was measured at the end of the functional studies.

Osmotic pressure test of microcapsule stability

We studied the stability of empty barium alginate microcapsules and microcapsules containing either parathyroid *single cells*, tissue particles >500 μm, or tissue particles <500 μm. Many parameters potentially influencing the stability were excluded by producing capsules of identical size and form (Fig. 1a). Only optimal spheroid microcapsules with tissue inside were hand-selected. Microcapsules containing tissue that protruded from the capsule's wall were excluded from the studies. The stability of the microcapsules was assessed by osmotic pressure test as described previously [8] with some modifications. The microcapsules (for each study triplicates of 70–100 capsules) were stained by a 1:1 solution of complete RPMI 1640 culture medium and trypan blue for 5 min. The microcapsules were washed twice in 0.9% NaCl and transferred into a

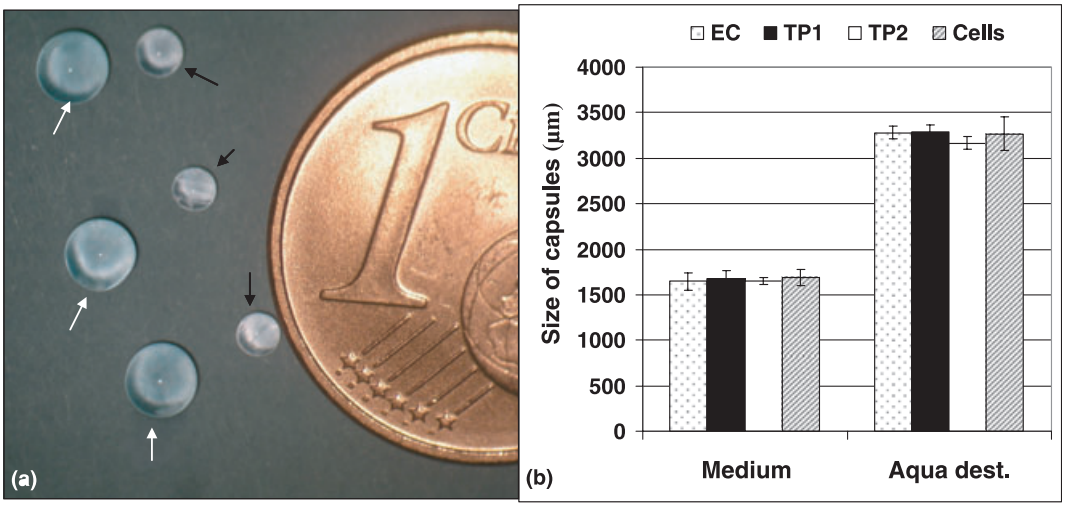

Figure 1 Osmotic pressure test. The different types of microcapsules were incubated in aqua destillata for 3 h: (a) Empty microcapsules incubated in complete RPMI 1640 culture medium (black arrows) and after osmotic swelling in hypotonic solution (aqua dest.; white arrows). (b) The different types of microcapsules (EC – empty capsules, TP1 – microcapsules containing larger tissue particles (644 ± 168 μm), TP2 – microcapsules containing smaller tissue particles (338 ± 72 μm), cells – microencapsulated parathyroid *single cells*) were of comparable size before and after osmotic swelling ($P > 0.05$).

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hypotonic solution (aqua dest.). After 3 h in hypotonic solution, the capsules had swollen dramatically, with no statistical difference between the different types of capsules (Fig. 1b). The number of intact microcapsules was counted and the results presented as per cent of intact capsules (mean ± SD). The significance of the results was calculated by a two-tailed student's *t*-test.

Results

Mechanical preparation of the parathyroid tissue versus enzymatic digestion

Mechanical cutting of the parathyroid tissue under the dissection microscope led to peripheral necrosis of the tissue particles (Fig. 2a) and thus to a loss of vitality of up to 71% ± 11.3% and a massive release of PTH from the necrotic tissue. Collagenase digestion, in contrast, resulted in single cells and cell clusters (*single cells*) without the peripheral necrosis (both 86% ± 5.9% vital) (Fig. 2b). The vitality of both tissue particles and *single cells* was improved by short-term culture (2–3 days) in complete RPMI 1640 culture medium.

Microencapsulation of parathyroid tissue particles versus microencapsulation of single parathyroid cells/cell clusters

During routine microencapsulation of the parathyroid tissue particles with barium alginate, 30–45% of the microcapsules were found to be defective (deformed microcapsules, microcapsules with partly 'naked' tissue, etc.). The number of completely microencapsulated tissue particles could be increased by increasing the size of microcapsules from 800 to 1500 µm (30–50% and 70–75% acceptable microcapsules accordantly). Due to the formation of empty capsules (approximately 40–50%), optimally encapsulated tissue particles had to be hand-selected in order to reduce the volume of the graft. Some of the completely encapsulated tissue particles were positioned eccentrically in the microcapsule, which could potentially cause mechanical instability of the microcapsule. The microencapsulation of *single cells* was of significantly better quality than that of tissue particles. All microcapsules of the former were of identical shape and size; the latter could be changed without impairing the quality of microencapsulation. Less than 4% of capsules were defective.

Influence of the microencapsulation procedure on the vitality of the encapsulated parathyroid tissue particles and encapsulated single cells/cell clusters

The microencapsulation procedure itself did not reduce the vitality of either the encapsulated *single cells* or tissue particles. The microencapsulated cells could be maintained in a sufficiently vital state during a 2-week culture.

PTH secretory dynamics of the nonencapsulated hyperplastic parathyroid tissue

Dynamic regulation of the secretion of PTH by changes in calcium levels was documented during perifusion of

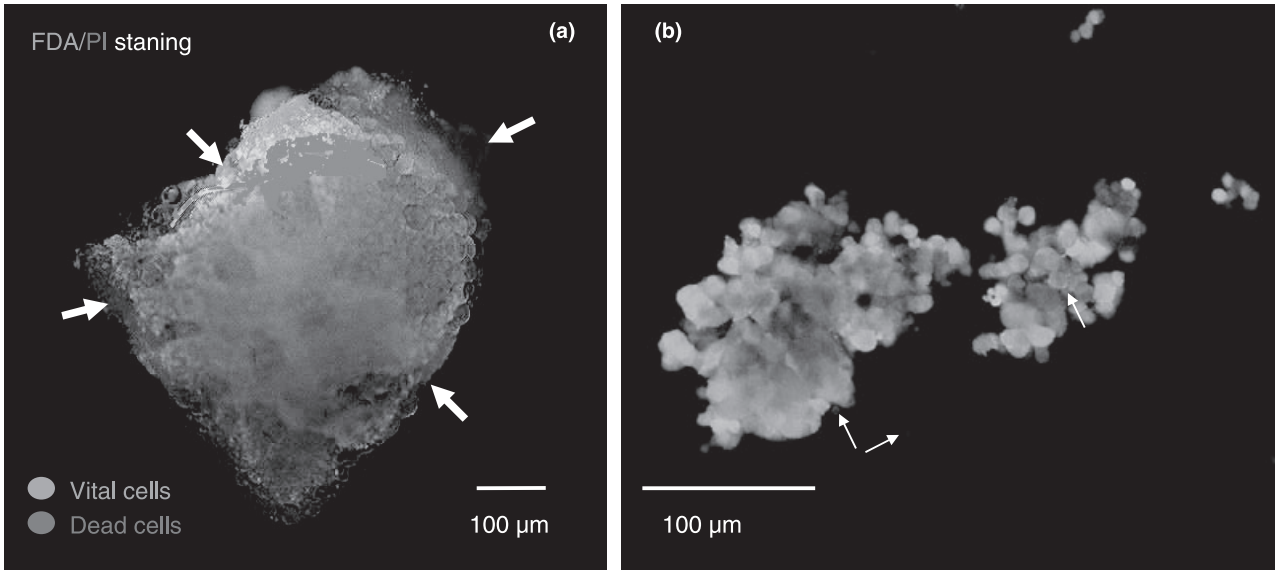

Figure 2 Vitality of the human parathyroid tissue (secondary hyperparathyroidism) after mechanical (a) and enzymatic (b) preparation (FDA/PI staining). The green fluorescence shows vital cells. Mechanical cutting (a) created a red halo of necrotic tissue (white arrows) surrounding the tissue particle. Collagenase digestion (b), in contrast, did not lead to peripheral necrosis of the cell clusters.

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the nonencapsulated hyperplastic parathyroid tissues from different donors (Fig. 3). Parathyroid tissues showed different maximal (0.42 mM Ca^{2+}, ranging from 90.7 ± 7.9 to 4.3 ± 1.2 pg/μg DNA) and basal (2.6 mM Ca^{2+}, ranging from 60 ± 6.3 to 1.6 ± 0.35 pg/μg DNA) secretion rates. No autonomous function of the hyperplastic parathyroid tissue was noted.

PTH secretory dynamics of the encapsulated hyperplastic parathyroid tissue

Continued perifusion of nonencapsulated tissue particles, microcapsules containing tissue particles, and *single cells* with 1.6 mM Ca^{2+} resulted in a stable baseline PTH secretion rate (Figs 4–6). Both microencapsulated parathyroid tissue particles and *single cells* maintained their good response to different calcium concentrations in the perifusion medium (Figs 5 and 6). Low Ca^{2+} perifusion (0.42 mM) led to a rapid and sustained release of PTH, which could be suppressed by perfusion with high Ca^{2+} (2.6 mM). Restimulation with 0.42 mM Ca^{2+} at the end of experiments resulted in a rapid increase of PTH secretion. The microencapsulation did not impair minute-to-minute regulation of the PTH secretion: the lag times (*t*; Figs 4–6) for the maximal response to low calcium by both nonencapsulated and microencapsulated tissue particles and *single cells* from the same donors were similar ($P > 0.05$). The peak levels occurred within 20–30 min after start of stimulation. The magnitude of response to low calcium ranged from 1.3- to 3.1-fold (mean 1.9 ± 0.75 SD) above basal levels for microencapsulated tissue particles. The maximal suppression with high calcium ranged from 1.5 to 4.8 times (mean 2.65 ± 1.88) the maximal stimulation of microencapsulated tissue particles.

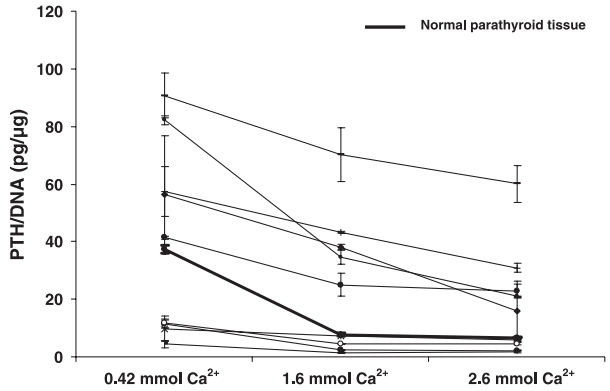

Figure 3 Secretory rates of human PTH by parathyroid tissues from 10 patients ($n = 9$ sHPT, $n = 1$ normal tissue) in response to different calcium concentrations in the perifusion medium. The normal parathyroid tissue was procured during a diagnostic biopsy of the parathyroid gland during a thyroid gland operation. Autonomous function of hyperplastic parathyroid tissue was not noticed, although parathyroid tissue from each donor had its own unique secretion of PTH.

Comparing the stability of different types of barium alginate microcapsules

Microcapsules containing the smaller (<500 μm) tissue particles had a higher percentage of stable capsules than those with the larger (>500 μm) particles (62% ± 5.6% vs. 28% ± 2.7%; $P < 0.05$). Microcapsules containing parathyroid *single cells* were significantly more stable than those containing tissue particles. Only very few empty and cell-containing microcapsules were broken at the end of the osmotic pressure test (Fig. 7).

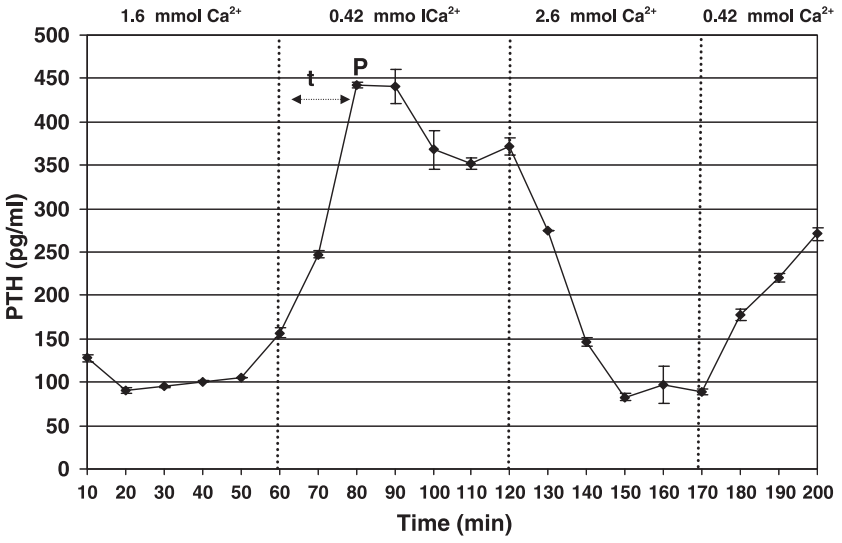

Figure 4 Parathyroid hormone (PTH) secretory dynamics of the nonencapsulated parathyroid tissue from a patient with secondary hyperparathyroidism (10 tissue particles, <500 μm per perfusion chamber). Well-regulated function of parathyroid tissue from different donors was noticed during perifusion with complete RPMI 1640 culture medium containing different calcium concentrations. At the end of perifusion, tissue particles retained their vitality (FDA/PI staining). P – peak PTH secretion, t – lag time of the maximal response to low calcium in the perifusion medium. Results of three parallel experiments are expressed as the mean values ± SD and are representative of three donors.

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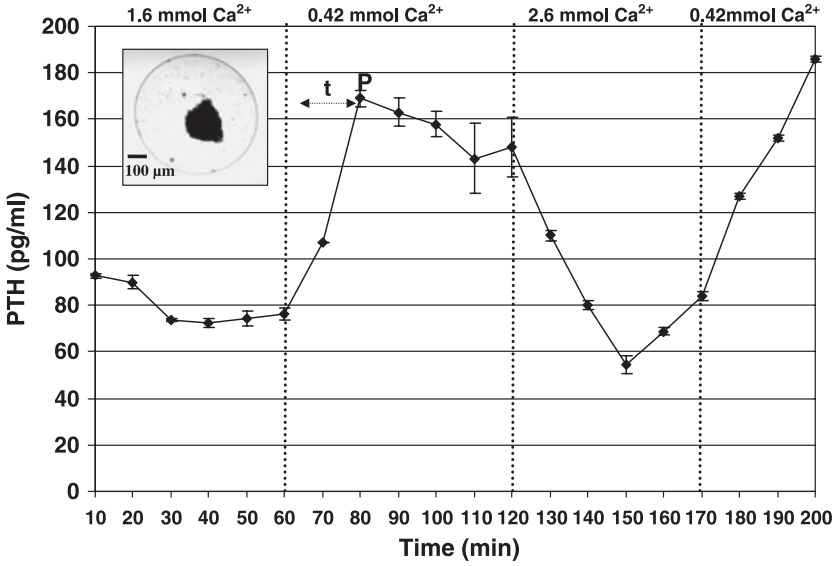

Figure 5 Acute parathyroid secretory dynamics of the microencapsulated parathyroid tissue particles (<500 µm, 10 microcapsules per perfusion chamber) from the same patients as in Fig. 3. The size of the microcapsules was 600 ± 180 µm (insertion). Microencapsulation did not impair minute-to-minute regulation of the PTH secretion, nor did it prolong the lag time (t) of maximal response (P) to low calcium.

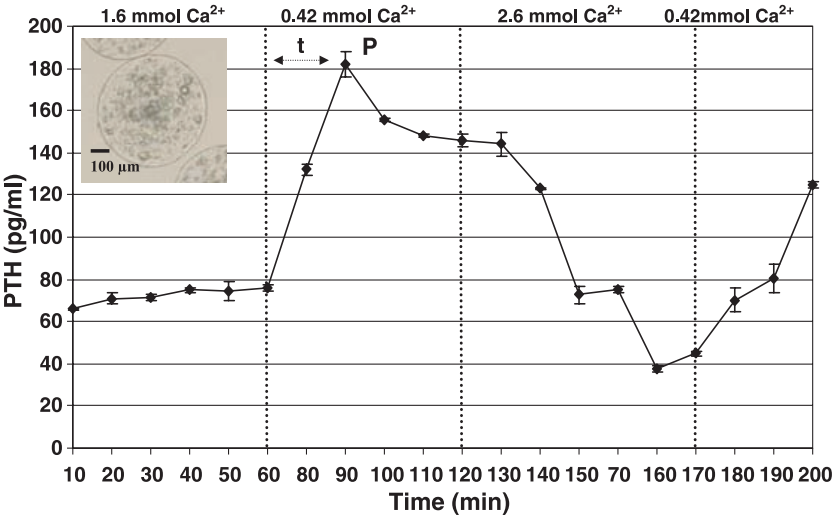

Figure 6 Time course in min of the response to different calcium concentrations of microencapsulated human parathyroid *single cells* (40 microcapsules per perifusion chamber) from the same patients as in Figs 3 and 4. The size of the microcapsules was 590 ± 170 µm (insertion). P – peak PTH secretion, t – lag time of the maximal response to low calcium in the perifusion medium.

Discussion

A variety of factors influence the function of an encapsulated parathyroid allograft *in vivo*. Apart from immunological factors, preparation of the tissue, the quality of microencapsulation, the viability and function of the encapsulated tissue are of major importance. Routine use of preoperative *in vitro* tests makes is possible to select the optimal bioartifical graft for human parathyroid allotransplantation.

Methods of parathyroid tissue preparation for microencapsulation

Although the recent literature describes two principal methods of parathyroid tissue preparation (mechanical cutting and enzymatic digestion), each method still represents a sort of 'in-house' procedure with its own modifications. The experience with parathyroid autotransplantation shows that autotransplantation of fresh parathyroid tissue has a success rate exceeding 80%, but only 17–83% of cryopreserved autografts remain functional [9]. This means that survival of the autotransplant depends on the vitality of the graft. Revascularization of nonencapsulated parathyroid tissue takes place after transplantation [10,11]; direct revascularization of the microencapsulated tissue, however, is not possible. The diffusion of oxygen through the microcapsule plays an important role in the survival of the bioartificial graft. Both central necroses of microencapsulated parathyroid tissue particles (size <500 µm) and of microencapsulated islets of Langerhans (size >100 µm) *in vivo* have

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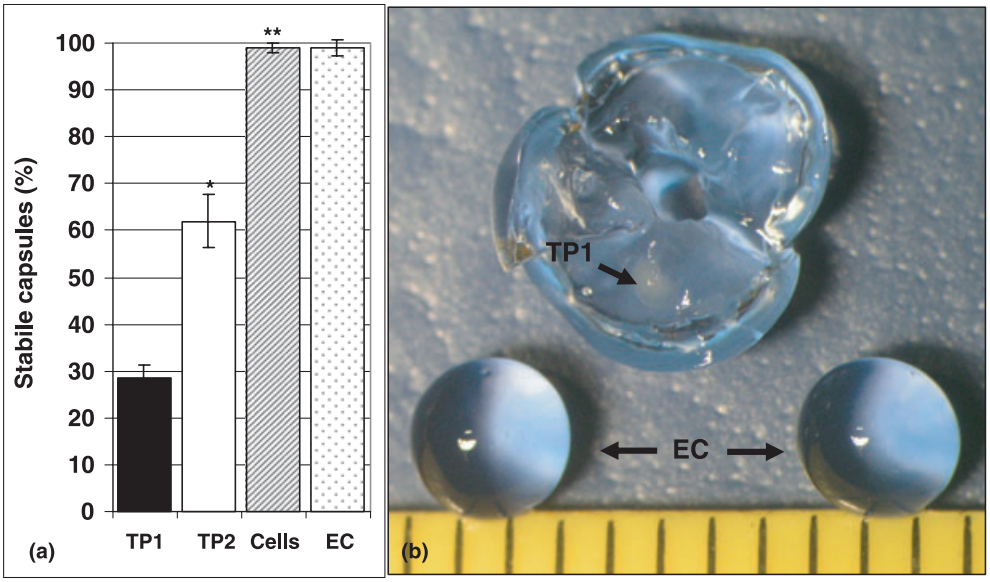

Figure 7 Comparison of the stability of barium alginate microcapsules using the osmotic pressure test. (a) Per cent of intact capsules of different types: microcapsules containing smaller tissue particles (338 ± 72 μm) showed better stability than those with lager particles (644 ± 168 μm, $P < 0.05$*). Most of the empty capsules (EC) and capsules containing *single cells* (cells) were stronger than microcapsules with tissue particles ($P < 0.05$**). (b) After 3 h incubation in aqua destillata the capsules were examined under the light microscope and the percent of intact capsules was calculated (mean ± SD): EC – intact empty microcapsules after 3 h incubation in aqua destillata, TP1 – broken microcapsule containing parathyroid tissue particles (>500 μm).

been documented as signs of limited graft nutrition [12,13].

The mechanical trauma during preparation leads to peripheral necrosis of the tissue and to the release of various proteins (we noticed massive release of PTH during *in vitro* perifusion) and cytokines. This has the consequence of immunizing the donor via the indirect pathway of allorecognition or of a nonspecific inflammation around the bioartificial graft, even if a nonmitogenic polymer is used for microencapsulation. Enzymatic digestion of the parathyroid tissue enabled us to prepare vital single cells and cell clusters of different sizes (<100 μm, which is better for graft survival).

Quality of the microencapsulation of the parathyroid tissue

The production of uniform capsules of high quality using a standardized procedure is essential for the clinical success of microencapsulation and the reproducibility of the results [14]. The imperfections of existing techniques forced us to try various methods of microencapsulation (Table 1), which unfortunately has not yet led to the elaboration of a unified protocol. Barium alginate microcapsules are known to sufficiently protect different types of allografts (parathyroid tissue, islets of Langerhans) from rejection [15], and barium alginate microcapsules possess sufficient mechanical stability. Concerning the microencapsulation of parathyroid tissue particles, hand-selection of optimal capsules is the method of choice in spite of optimized methods for microencapsulation. Imperfect capsules (i.e. capsules with partly naked tissue or eccentrically positioned tissue particles), and empty capsules (which unnecessarily increase the volume of the bioartificial graft) are the limitations to an up-scaling of the technology. Microencapsulation of single cells and small cell clusters (<100 μm), however, allowed us to produce uniform microcapsules of good quality with homogeneous distribution of the cells. FDA/PI staining makes it possible to routinely assess the vitality of the bioartificial graft, which is essential for transplantation or preoperative *in vitro* studies.

Hyperplastic parathyroid tissue for human parathyroid allotransplantation

Normal hyperplastic (sHPT) or adenomatous parathyroid tissue can be transplanted to potentially cure permanent parathyroid insufficiency. The use of hyperplastic tissue eliminates many logistic problems connected with harvesting, preservation and transportation of normal tissue. Despite these beneficial features, however, the functional properties of the pathologic tissue should be checked before transplantation. Indeed, pathologic parathyroid tissue has diminished expression of calcium receptors [16] and altered regulation of PTH secretion with a shift to the right and upwards of the

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Table 1. Parathyroid tissue can be prepared using one of two different approaches: mechanical cutting and enzymatic digestion (ED).

References	Method of tissue preparation	Vitality of the cells after preparation	Purpose of tissue preparation
Brandi *et al.* [19]	ED: collagenase type II (1.5 mg/ml) + DNAse (50 μg/ml), 180 min	>90% after 24 h culture	The first long-term *in vitro* culture of parathyroid cells (bovine tissue): 140 doublings
Wen Fu *et al.* [20]	ED: collagenase type I (2 mg/ml) + DNAase (40 μg/ml), 60 min	92%	The first allotransplantation of microencapsulated parathyroid cells (8-week function); alginate-poly-l-lysine microcapsules, 500–600 μm
Hasse *et al.* [12,15]	Mechanical cutting under the dissection microscope	Not indicated	The first long-term experimental iso-, allo- and xenotransplantation of microencapsulated parathyroid tissue; barium-alginate microcapsules, >1000 μm
Roussanne *et al.* [21]	ED: collagenase type II (0.1%), 90 min	Not indicated	The first long-term culture of human parathyroid cells (sHPT)
Kobayashi *et al.* [22]	ED: collagenase type I (2 mg/ml) + DNAase (40 μg/ml)	Not indicated	*In vitro* culture of microencapsulated parathyroid particles; polyelectrolyte microcapsules; capsule size not indicated
Picariello *et al.* [6]	ED: collagenase type II (1.2 mg/ml), 120 min	90% after 24 h culture	*In vitro* study of microencapsulated parathyroid cells (flask incubation); alginate-poly-l-lysine capsules, 2000 μm

Although there is no standard protocol for the enzymatic digestion of parathyroid tissue (type of enzyme, concentration, duration of digestion, etc.), the results (vitality of the cells) of the various methods described to date are comparable. There is also no standardized procedure for microencapsulation of parathyroid tissue (type of tissue, preparation, type of polymer, size of capsules).
sHPT, secondary hyperparathyroidism.

set-point. Tertiary hyperparathyroidism occurs in 7–30% of patients after autotransplantation of hyperplastic tissue [17]; it is caused mainly by mitogenic factors that stimulate proliferation of the parathyroid cells and induce an increase in the graft's volume. These factors include humoral parathyroid mitogenic factor in the blood of patients with multiple endocrine neoplasia type I hyperparathyroidism or increased levels of phosphates, and impaired metabolism of vitamin D in patients with renal insufficiency [18]. Proliferation of parathyroid cells, however, can be controlled by microencapsulation. Thus, employment of barium alginate microcapsules makes it possible to restrict proliferation of immortalized cells [8]. Although the signs of hyperparathyroidism were not described after xenotransplantation of relatively large volumes of microencapsulated hyperplastic tissue (sHPT) in a rodent model (transplant ratio 10:1 per rat), it can be explained by necrosis of the tissue particles resulting from insufficient nutrition and/or failure of the grafts due to broken capsules [12]. Therefore, it is advisable to know the level of basal PTH secretion for potential retrospective analysis of the graft function *in vivo*. In the present study, we show that hyperplastic parathyroid tissue from different patients with sHPT each has its own secretory dynamics with a unique basal secretion and modulation of the release of PTH in the presence of different calcium concentrations.

Functional performance of microencapsulated hyperplastic human parathyroid tissue

Employment of a polymer membrane to protect parathyroid cells from the immune system may limit perfusion and impair native regulation of hormone secretion and release from the bioartificial graft. It was important therefore to ensure that microencapsulation of both tissue particles and *single cells* with barium alginate did not change their normal minute-to-minute regulation of hormone secretion, nor impair the modulation of hormone release from the parathyroid tissue in response to different calcium concentrations. Our results in this regard correlate with those produced by flask incubation of hyperplastic parathyroid cells microencapsulated with poly-L-lysine-alginate (2000 μm) [6].

Stability of barium alginate microcapsules

Insufficient mechanical stability can lead to failure of a bioartificial graft due to destruction of the microcapsules *in vivo*. We studied the mechanical properties of barium alginate microcapsules approximately 1500 μm in diameter (comparable in size to the barium alginate microcapsules used for different *in vivo* experiments [12,15]) and found that microcapsule stability decreased with increased size of the encapsulated tissue particles. Microencapsula-

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tion of parathyroid *single cells* produced stable microcapsules without any additional modification of the capsule surface (e. g. poly-L-lysine; poly-L-ornithine).

Conclusions

Parathyroid *single cells* appear to be more suitable for transplantation of bioartificial parathyroid grafts than tissue particles. Enzymatic preparation does not lead to peripheral necrosis of the cell clusters, whose microencapsulation is of much better quality than that of tissue particles. Parathyroid tissues from different donors display different patterns of secretion that can be maintained after microencapsulation. Preoperative evaluation of graft function combined with vitality tests can facilitate selection of the optimal bioartifical graft for parathyroid allotransplantation, thus prolonging graft survival and improving retrospective analysis after transplantation.

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