Cell therapies in hypoparathyroidism

Anette Jork[1]
Herma Glöckner[1]
Frank Thürmer[1]
Frank Ulrich[2]
Maria Luisa Brandi[3]

[1]CellMed AG, Alzenau, Germany, [2]Charité, Virchow Clinical Centre, Department of General, Visceral and Transplantation Surgery, Berlin, Germany and [3]Department of Internal Medicine, University of Florence, Medical School, Florence, Italy

Address for correspondence:
Maria Luisa Brandi, M.D., Ph.D.
Department of Internal Medicine
University of Florence
Viale Pieraccini 6, 50139 Florence, Italy
Ph. +39 055 4296586
Fax +39 055 2337867
E-mail: m.brandi@dmi.unifi.it

Summary

Identification of a biocompatible immunoprotective membrane to prevent graft rejection remained elusive until the development of microcapsules formulated in alginate. We report *in vitro* parathyroid hormone secretion from encapsulated human parathyroid tissue using a highly biocompatible alginate produced under GMP conditions. The *in vitro* function of human parathyroid tissue fragments was maintained after 8 days of culture of alginate microcapsules in serum-free conditions. The structural features of parathyroid tissue was maintained in these culture conditions. Using a biopolymer of clinical grade may be a crucial step toward a clinical use of this technique in parathyroid allotransplantion without immunosuppression.

KEY WORDS: parathyroid tissue, parathyroid cells, hypoparathyroidism, alginate, cell therapies.

Introduction

Attempts to transplant parathyroid glands began almost a century ago, only two decades after the organ had been discovered, with the demonstration by histologic means that canine parathyroid autografts survived (1). Graft function was assessed only fifty years later, with the demonstration that parathyroid transplanted tissue in rodents functions sufficiently to maintain the host in a normocalcemic state (2). However, parathyroid allografts were successful only in immunosuppressed canine and human recipients (3,4).
Indications for parathyroid allotransplantation are rare, as hypoparathyroid patients who cannot be controlled on a medical regimen are also unusual. In these subjects the symptoms and associated complications of hypoparathyroidism cannot be controlled sufficiently with oral calcium and vitamin D. Due to these problems, alternate treatment modalities, involving more physiological and longer-acting systems for parathyroid hormone (PTH) delivery, needs to be pursued.
Various methods of immunomanipulation have been proposed to overcome allograft rejection, which include immunosuppression, immunomodulation and immunoisolation. By using the immunoisolation concept the transplant is enclosed in a biocompatible material to immobilize the transplant and at the same time preventing an immune response of the recipient. In 1964, T.M.S. Chang proposed the idea of using ultrathin polymer membrane microcapsules for the immunoprotection of transplanted tissues and cells (5). When implanted into rats, the microencapsulated pancreatic islets corrected the diabetic state for several weeks (6). Since then, bioencapsulation has provided a range of promising therapeutic treatments for diabetes, hemophilia, cancer and renal failure (7-10). In addition, the applicability of cell encapsulation in humans has also been reported in several clinical trials (11,12). Indeed, cell encapsulation in biocompatible and semipermeable polymeric membranes has been an effective method for immunoprotection, regardless of the type of recipient (13), as encapsulated cells can maintain their viability while allowing for the secretion of desired therapeutic agents, either continuously or in response to specific physiologic stimulations.
Also for the treatment of refractory hypoparathyroidism, immunoisolated allogeneic transplantation of parathyroid tissue is regarded as a promising therapy concept (14), which has been successfully applied in iso-, allo-, and xeno-transplantations in animals by using purified alginates (15-17). Alternatively, isolated parathyroid cells are under investigation (18,19).
Up to now, a substantial challenge has been the lack of clinical grade polymers and the demanding task is to accomplish specific material requirements of high quality standards. By using homogeneous raw material and a standardized purification protocol a new class of clinical grade biopolymers was developed which establish the basis for the clinical application of encapsulation technology (20). The batches of the purified alginate have to be standardized as for the endotoxin, protein and phenolic content and also for the physical properties such as the distribution of the molar mass and the viscosity.
In the present study, we report *in vitro* data for the use of clinically suitable alginate-encapsulated human parathyroid tissue microspheres as a novel allo- and xeno-transplantation method for the delivery of PTH.

Materials and methods

Quality control of the biopolymer and cytotoxicity testing

Three different batches (a-c) of biocompatible alginate isolated from freshly collected *Laminaria pallida* (20) were tested in eight replicates, using three different cell lines (mouse, ape, human): L-929 (DSMZ # ACC 2), VERO-B4 (DSMZ ACC # 33) and NHDF (CellSystems, St. Katharinen, Germany). Experiments were repeated five times.
For cytotoxicity tests 100 µl of cell suspensions were plated at a density of $2x10^5$ cells/ml in 96-well plates. Cells were incubated overnight in an incubator at 37°C in 5% CO_2/95% air hu-

midified atmosphere. A 0.1% (w/v) suspension of the biopolymer was prepared in cell culture medium (RPMI 1640, supplemented with 2 mM L-glutamine and 10% fetal calf serum). Culture medium was used as negative control and $CuSO_4$ (50 ng/ml) in culture medium was used as positive control. Biopolymer suspension and control media were mixed overnight on an overhead shaker at room temperature. Cultured cells were checked for confluency and morphology after 24-hr incubation, when cell culture medium was removed and replaced by 100 µL of the biopolymer suspension. Positive and negative controls were tested in every dish. Cells were incubated for three days in the incubator. At day three 25 µL of Alamar-Blue solution (Biozol, Echingen, Germany) were added to each well and incubated. Fluorescence (excitation wavelength 535 nm, emission wavelength 590 nm) was measured after three hours. Metabolic activity of the cells was calculated versus baseline (negative controls) and expressed as percent difference.

Culture and encapsulation of human parathyroid tissue

Fresh human parathyroid tissue was obtained from six different patients undergoing surgery for parathyroid hyperplasia due to secondary hyperparathyroidism. Parathyroid tissue was transported to the laboratory under sterile conditions and then cut into fragments of about 2 mm^3. Tissue particles were then kept either in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10% AB-serum (17) or in serum-free culture medium in the incubator for three days with daily changes of culture medium. Thereafter, tissue particles were microencapsulated using the CellBeads® Biopolymer, produced according to GMP requirements (CellMed AG, Alzenau, Germany), through suspension in the biopolymer solution. The solution was passed through a spray nozzle and the resulting microcapsules were transferred into culture medium and further cultured for 10 days.

In vitro *release of PTH from human encapsulated parathyroid tissue*

Intact PTH release was daily measured during a period varying from seven to eight days. For the measurement of PTH release five microcapsules were transferred into 5 mL of fresh serum-free medium and incubated for two hours, when 500 µl of the supernatant were removed and immediately frozen at –20°C. After thawing human PTH 1-84 concentrations were measured by an ELISA method (DSL, Webster, USA) and the median PTH release of one capsule was derived.

Hematoxylin-eosin staining

At day seven encapsulated tissue particles were fixed in 4% formalin, Hematoxylin-Eosin (H&E) stained and histologically analyzed.

Results

Quality control of the biopolymer and cytotoxicity testing

The biopolymer used in this investigation expressed a whole set of quality control measures as it is produced under GMP conditions. The biopolymer was highly biocompatible, as demonstrated *in vitro* and *in vivo* (20). For each lot a whole set of quality controls was routinely performed, such as cytotoxicity testing in three different batches of the biopolymer using three different cell lines of diverse origin. No cytotoxic effect was observed for three different batches of the biopolymer using the three different cell lines (Fig. 1).

In vitro *release of PTH from human encapsulated parathyroid tissue*

The median intact PTH release per microcapsule was calculated over two hour period of incubation in serum-free medium using parathyroid tissue obtained from three different patients. At day one PTH release was below 500 pg/mL for all tissues with a constant increase in the following days (Fig. 2).

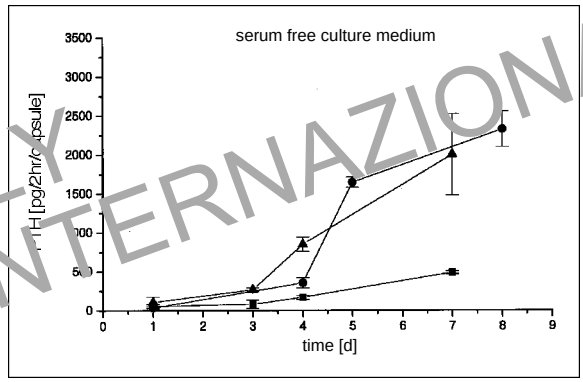

Figure 2 - PTH release from encapsulated human parathyroid tissue fragments obtained from three different donors. Intact PTH release over 2 hours of incubation in serum-free medium was evaluated for a period up to 8 days. The mean intact PTH release was elaborated from the release from 10 microcapsules and expressed as pg/2hr/capsule.

Histological examination

Microencapsulated human parathyroid tissue showed an excellent viability over 7/8 days in culture (Fig. 3).

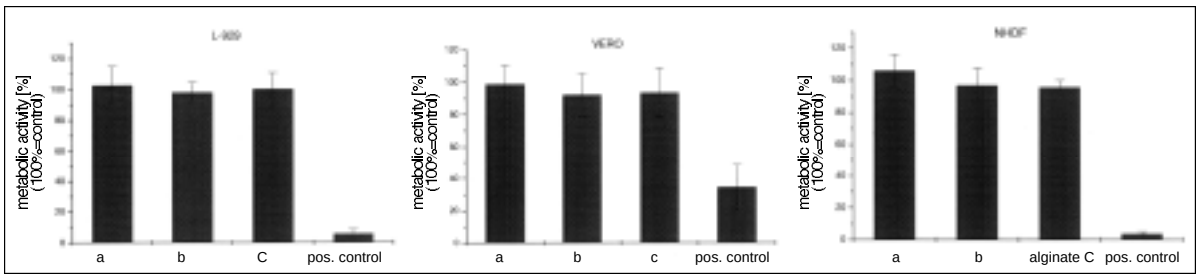

Figure 1. Cytotoxicity testing was performed for three biopolymer solutions (a-c) using three different cell lines (L-929, VERO, and NHDF). The metabolic activity of the cell lines is expressed as percentage on negative controls. The positive control (pos. control) is represented by RPMI 1640 culture medium containing 50 ng/mL $CuSO_4$.

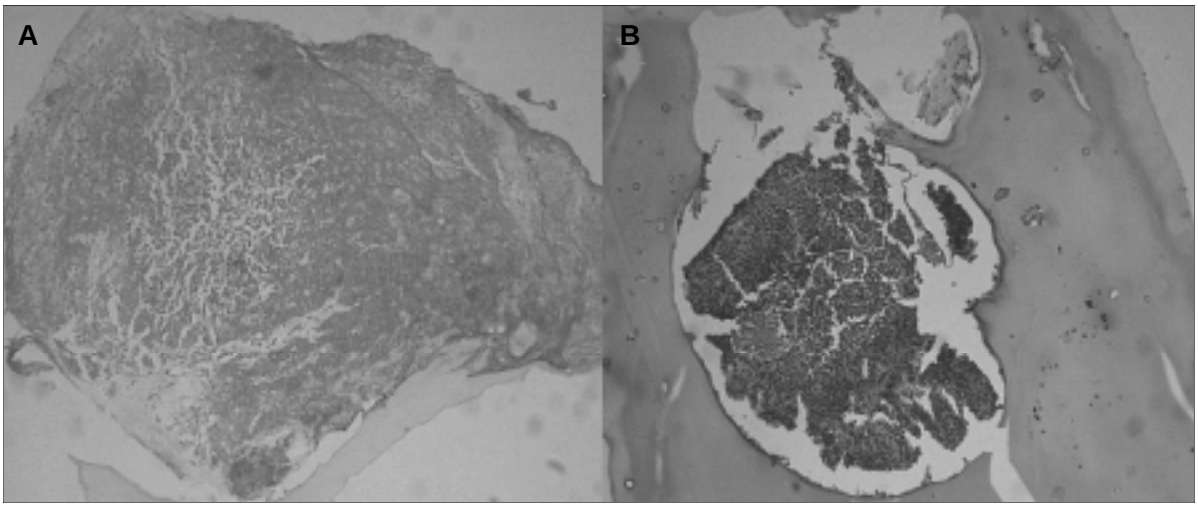

Figure 3 - Histological sections of encapsulated human parathyroid fragments. H&E staining showed a conserved morphology after 7 days in culture with serum-free culture medium.

Discussion

Secondary hypoparathyroidism with permanent hypocalcemia is a well recognized complication after thyroid surgery, reaching up to 30% after total thyroidectomy (21, 22). More rarely, hypoparathyroidism can occur as a congenital primary disorder, characterized by genetic heterogeneity (23,24). Permanent hypoparathyroidism is one of the most difficult of all endocrine disorders to treat medically. This is particularly true for subjects who do not respond to medical therapy with calcium and vitamin D3 (refractory hypoparathyroidism) (25). Parathyroid transplantation is an option for permanent organ dysfunction, the need for parathyroid allotransplantation could be justified. Because hypoparathyroidism rarely is vital threat to the patient, systemic post-transplant immunosuppression is not justified. The applicability of biomicroencapsulation to immunoisolate parathyroid tissue or cells has provided a range of encouraging results in *in vitro* and *in vivo* experimental models (14-19).

While a large number of synthetic, semi-synthetic and natural water soluble polymers have been evaluated as potential materials for cell and tissue encapsulation (26, 27), the most promising approach are hydrogel- and particularly alginate-based microcapsules, as these materials meet best the requirements for long-term immunoisolation and simultaneous maintenance of transplant function (28). However, a consistent and standardized quality of the material which is a pre-requisite for biomedical applications has not been provided so far. Other challenges involve the production of uniform capsules through automated machines, the suitability of cell or tissue types for immobilization, and the transplantation site. For all these reasons allotransplantation of parathyroid tissue is still in its experimental stage, with the exception of isolated attempts in humans (15, 29). Moreover, the variability of the materials used in different investigations accounts for the poor comparability of results.

In a stepwise analysis of the essential obstacles, we have developed a clinical grade biomaterial. Indeed, the purified biopolymer used in this investigation was produced under GMP conditions, encompassing several quality control measures, such as cytotoxicity testing. This material made possible the *in vitro* maintenance of both morphological and functional features of human parathyroid tissue, as shown through histological examination and measurement of PTH release. In addition, the lack of need for fetal calf serum in the culture medium is another interesting characteristic of our microencapsulation model because potential sources of disease transmission, such as additives of animal or human origin, could be avoided. The results of this study, together with those of previous *in vivo* studies using immunogenic alginate (16) provided the rationale needed for the clinical application of encapsulation technology in the treatment of hypoparathyroid patients refractory to medical therapy. The use of parathyroid cell suspension may provide an additional opportunity to further improve this therapy option.

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