

# Parathyroid Allotransplant for Persistent Hypocalcaemia: A New Technique Involving Short-Term Culture

Erhan Aysan,<sup>1</sup> Ulkan Kilic,<sup>2</sup> Ozlem Gok,<sup>2</sup> Burcugul Altug,<sup>3</sup> Cilem Ercan,<sup>2</sup> Cemile Kesgin Toka,<sup>1</sup> Ufuk Oguz Idiz,<sup>1</sup> Mahmut Muslumanoglu<sup>1</sup>

## Abstract

**Objectives:** To develop a new parathyroid allotransplant method for the treatment of permanent hypoparathyroidism.

**Materials and Methods:** Parathyroid cells  $50 \times 10^6$  derived from a parathyroid hyperplasia patient were transferred to a 61-year-old patient who had thyroidectomy 17 years earlier, allowing to papillary thyroid cancer; he was admitted to our outpatient clinic with symptomatic chronic hypocalcemia. Cell isolation, cryopreservation, and culturing were conducted according to a new protocol.

**Results:** During a follow-up of 5 months, the patient had no complications that could indicate rejection, and clinical symptoms completely resolved without requiring any drug supplementation.

**Conclusions:** Here, we report a new method, enabling fast and cost-effective parathyroid allotransplant with maintained tissue viability sufficient to treat persistent hypocalcemia.

## Introduction

Hypoparathyroidism persisting for more than 6 months<sup>1,2</sup> is termed *permanent hypoparathyroidism*. Extended or complicated thyroidectomy operations are common causes of permanent hypoparathyroidism.<sup>3,4</sup> Nephropathy, cataract, muscle dysfunction, myositis, fasciitis, basal ganglia, and/or cerebellar calcifications, and teeth malformations are complications of untreated permanent hypoparathyroidism.<sup>2,3</sup> Medical treatment of permanent hypoparathyroidism includes calcium and vitamin D replacement, which, however, may not be beneficial or

remains insufficient in many patients. Parathyroid allotransplant is a relatively new and alternative approach for the treatment of permanent hypoparathyroidism. Moreover, several parathyroid allotransplant methods have been described, such as human leukocyte antigen selection, cellular irradiation, and tissue culture manipulation to reduce immunogenicity and increase success rates.<sup>5</sup>

Here, we report a new parathyroid allotransplant method without long-term immunosuppression performed on a patient who underwent complete thyroidectomy 17 years earlier only to papillary thyroid cancer. Parathyroid allotransplant was preferred for this patient following an unsuccessful symptomatic treatment of hypocalcemia by standard clinical attempts.

## Materials and Methods

A 61-year-old female patient, who underwent thyroidectomy 17 years earlier owing to papillary thyroid cancer, was admitted to our outpatient clinic with chronic symptomatic hypocalcaemia. Hypoparathyroidism had emerged after the operation, and was aggravated by radioactive iodine ablation therapy. On admission to our outpatient clinic, she reported the consumption of oral calcium 9 g (ECZACIBASI Holding Co., İstanbul, Turkey) and calcitriol 1.5 mg (Roche Pharma AG, Grenzach-Wyhlen, Germany) daily for 17 years. She was admitted with the symptoms of severe weakness, severe bilateral leg numbness, inability to walk, digital paresthesia, and clinical tetany manifested at the wrists. Her total plasma calcium and parathormone levels measured 1.8 mmol/L (normal range, 2.05-2.55 mmol/L) and 0.01 ng/L (normal range, 10-65 ng/L). Electrocardiogram analysis revealed a prolonged QT interval.

In Turkey, obtaining approval from the Organ and Tissue Transplantation and Dialysis Board, a

From the <sup>1</sup>Departments of General Surgery; <sup>2</sup>Medical Biology; and <sup>3</sup>Biology, Bezmialem Vakif University, İstanbul, Turkey

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**Corresponding author:** Erhan Aysan, ATA-2 Sitesi Akasya Cad. No: 25 Cengelkoy, Uskudar İstanbul, Turkey 80700

**Phone:** +90 216 486 3059 **E-mail:** erhanaysan@hotmail.com

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subcommittee of the Turkish Ministry of Health, is a prerequisite for all organ and tissue transplants. We applied to the national board for the approval of PA in October 2012. The approval was granted in May 2013, and Bezmialem University was assigned as the first PA center in Turkey.

A 28-year-old woman was admitted to our outpatient clinic with chronic renal failure and secondary hyperparathyroidism; her calcium and parathormone levels measured 2.8 mmol/L and 1203 ng/L. After conducting routine preoperative evaluation and obtaining ethics approval and informed consent, a subtotal parathyroidectomy was scheduled. Neither the transplant donor nor the recipient had any contraindications to tissue transplant; both patients were negative for hepatitis B virus, hepatitis C virus, human immunodeficiency virus, herpes simplex virus, and venereal disease reaction level. The donor and recipient blood groups were AB+ and B+.

Subtotal parathyroidectomy surgical procedure was performed. Half of the excised parathyroid tissue was sent to the pathology department for histopathologic evaluation, and the other half, to the parathyroid transplant laboratory. The tissue was maintained at 4°C in 4 mL of AmnioMAX II Complete Medium (catalog No. 11269-016, Gibco, UK) supplemented with 20% inactivated fetal bovine serum (catalog No. 10500-064, Gibco, South America) and 1% penicillin-streptomycin (catalog No. 15140-122, Gibco, USA) for subsequent culture. After parathyroid hyperplasia was confirmed from the pathological examination, a novel protocol for cell culture and preparation was conducted. The main parts of the resected tissue were washed with culture medium 5 times, and the tissue was isolated from blood vessels, gland capsule, connective tissues, and fatty tissues. The parathyroid tissue was mechanically disintegrated in 1X phosphate-buffered saline (catalog No. AM9624, Ambion, Foster City, CA, USA) supplemented with 5% inactivated fetal bovine serum using a sterile filter in a biohazard safety cabinet (catalog No. L.02131262, Mars Safety Class 2, (ScanLaf A/S, Lynge, Denmark) under sterile conditions. The suspension was filtered into a 15-mL tube using a sterile cell strainer (100 µm, catalog No. 352360, BD Biosciences, San Jose, CA, USA), and treated with 600 µL of deoxyribonuclease I (DNase I from bovine pancreas, catalog No. A3778-0010, AppliChem, Darmstadt, Germany).

After centrifugation at 1600 rpm for 5 minutes at room temperature, the supernatant was removed, and the pellet was rapidly suspended in 1 mL of culture medium. The cell viability was assessed using Muse Cell Analyzer (catalog No. 0500-3115, Millipore, France), and found to be 85.79%. Approximately 50% of the suspended parathyroid cells ( $320 \times 10^6$  cells) were used for culture. The cells were cultured in 2 flasks (catalog Nos. sc-200262, UltraCruz Tissue Culture 25 cm<sup>2</sup> Flask, USA), each containing 10 mL of the culture media, and maintained in an incubator (CCL-170B-8, ESCO, Singapore) at 37°C in 5% CO<sub>2</sub>-containing humidified atmosphere for 24 hours. The culture supernatant from 1 of the flasks was transferred into a 15 mL tube, and the cell monolayer was washed with 4 mL of phosphate-buffered saline. After this step, 300 µL of trypsin (catalog No. 25300-054, Gibco, UK) was added to the flask and transferred to the incubator for 5 minutes to allow detachment of the cells from the flask. The cell suspension was then transferred into a tube, and centrifuged at 1600 rpm for 5 minutes. The supernatant was removed, and the pellet was suspended in 1 mL of cell culture medium. The cell concentration was determined to be  $145 \times 10^6$  live parathyroid cells per 1 mL of cell suspension. To obtain a final cell number of  $50 \times 10^6$ , 690 µL of the cell suspension was transferred into a microcentrifuge tube and centrifuged at 1600 rpm for 5 minutes; the pellet was then suspended in 1 mL of the recipient's blood serum for cell transplant. The remaining cells were cryogenically stored in culture media mixed with 5% dimethylsulfoxide (catalog No. sc-202581, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) using a controlled-rate freezer.

Cell transplant was performed via intramuscular injection at the left arm deltoid muscle. Prednisolone (Prednol 250 mg Ampul, Mustafa Nevzat İlaç Sanayii A.Ş, Istanbul, Turkey's) was administered for 5 consecutive days, with an initial dose of 500 mg and the dosage reduced by half each day. Plasma levels of circulating parathormone (pg/mL), calcium (mg/dL), and phosphorus (mg/dL) were measured every week for the first month and at monthly intervals subsequently.

## Results

The donor patient was discharged on postoperative day 2 with normal blood parathormone, calcium,

and phosphorus levels. The recipient patient was observed for 5 days in the outpatient clinic. No complications were observed in the donor or recipient. When the recipient's blood parathormone level increased after 2 weeks (Table 1), all calcium and vitamin D drug supplements were ceased by stages within 5 days. Symptoms, such as leg numbness and paresthesia resolved and the patient completely gained the ability to walk following the first month. The maximum blood parathormone level was 6.9 ng/L in posttransplant week 3. The last measured parathormone level was ng/L in post-transplant month 6 (Table 1).

During the follow-up period of 5 months, the patient had no complications that could indicate the total resolution of the rejection and clinical symptoms, without requiring any drug supplementation.

**Table 1.** Plasma Levels of Circulating Parathyroid Hormone, Calcium, and Phosphorus

	Parathyroid Hormone (ng/L)	Calcium (mmol/L)	Phosphorus (mmol/L)	Calcium Support (g/d)
Before transplant				
*Drug suppl. (+)	0.01	2.25	0.71	9
Posttrans. wk 1				
*Drug suppl. (+)	0.01	2.2	0.81	5
Posttrans. wk 2				
*Drug suppl. (+)	0.01	2.23	0.78	2
Posttrans. wk 3				
*Drug suppl. (-)	6.9	2.0	1.52	0
Posttrans. wk 4				
*Drug suppl. (-)	6.5	2.0	1.49	0
Posttrans. mo 2				
*Drug suppl. (-)	6.0	1.88	1.78	0
Posttrans. mo 3				
*Drug suppl. (-)	5.8	1.88	1.74	0
Posttrans. mo 3				
*Drug suppl. (-)	4.2	1.93	1.74	0
Posttrans. mo 4				
*Drug suppl. (-)	4.8	2.05	1.81	0
Posttrans. mo 6				
*Drug suppl. (-)	6.7	2.0	1.68	0

\*Drug suppl.: Oral calcium and vitamin D supplementations

## Discussion

Postoperative hypocalcemia has been observed in up to 16% of patients undergoing extensive thyroidectomy due to thyroid cancer or in cases of repetitive thyroidectomy.<sup>6</sup> Most of these patients recover after receiving calcium and parathormone supplements over a shorter period. However, hypocalcemia may persist in a minority of these patients and long-term calcium/parathormone supplementation may lead to several complications, such as multiorgan calcinosis and gastritis, affecting

the quality of life of these patients.<sup>5</sup> Thus, parathyroid transplant is emerging as an alternative treatment for persistent hypocalcemia.

Parathyroid transplant has been performed for more than 30 years and is gaining popularity. However, a limited viability of transplanted cells and subsequent rejection of implanted tissue still constitute major challenges. Several methods have been described to overcome problems related to the maintenance of the cell viability under in vitro conditions and protect implanted tissue from the host immune system in the absence of immunosuppression therapy.

Several in vitro techniques have been described to reduce immune responses to parathyroid tissue. Immune system-related cells, such as macrophages accompanying the parathyroid tissue carry HLA class 1 and 2 antigens on their surfaces.<sup>7</sup> Digestion by collagenase and filtering through a nylon mesh, culturing with HLA antibody-coated microspheres, temporary transfer of irradiated human parathyroid cells to a murine "interim host" by placing them under the mouse kidney capsule for several weeks prior to clinical transfer, and encapsulating parathyroid cells in engineered microcapsules of degassed 2% sodium-alginate are all examples of in vitro techniques described to reduce immunogenicity and prolong tissue survival.<sup>8</sup> In addition, immunosuppression is not desirable to those patients due to its potential side effects.<sup>9</sup>

In this study, we describe a novel cell culture method based on a modification of a method previously described.<sup>9</sup> Cell viability is inversely proportional to the length of storage time and approximately 70% of the tissue was viable after 24 hours.<sup>8</sup> We obtained 80% viability after 48 hours in vitro. In contrast to the method described by Nawrot and associates, one of the advantages of our method is its markedly low cost. The specific costs of the processing and culturing of cells described above amounted to only approximately \$40, making it a highly cost-effective method. Another advantage of our method is the reduced cultivation and processing time that makes it comparable with direct tissue transplant techniques. Furthermore, the success rate of direct tissue transplant techniques have been reported as low, while our method offers a prolonged survival of implanted cells. Hence, our novel technique promotes a short cultivation and processing time, with prolonged survival of implanted cells.

ABO blood group system compatibility and human leukocyte antigen matching was previously of primary concern to reduce rejection risk.<sup>5</sup> Barrier techniques, such as microencapsulation, offer the advantage of omitting the requirement for discriminating between ABO groups. Moreover, Nawrot and associates found that ABO compatibility was not required.<sup>9</sup> Similarly, in the present study, blood groups of donor and recipient were different and did not cause a subsequent rejection over the follow-up.

Implanted tissue viability was determined by resolving the symptoms and restoring the parathormone and calcium levels. Clinical symptoms had completely resolved after 1 month and the patient experienced no problems during the follow-up, although the parathyroid hormone's levels remained below 10 ng/L, which is the lower limit of the normal range. Nevertheless, no patients have yet reached up to normal plasma parathormone levels after a parathyroid allotransplant.<sup>5</sup> In our case, the patient's posttransplant calcium and parathormone levels were low. However, symptoms compatible with hypocalcemia were not observed in the patient. This could possibly be explained by the observations by Schmitt and associates who specified that calcium receptor sensitivity may not be stable. The authors hypothesized that calcium receptor sensitivity of hypocalcemic patients may be up-regulated.<sup>10</sup> Therefore, minimal changes in parathormone and calcium levels may be effective. Moreover, calcium levels before transplant were under the influence of oral calcium supplementation while posttransplant calcium levels were not.

We made the injection into the deltoid muscle but the injection can be made into the fore arm instead of

deltoid muscle. In this way we can observe the cell culture is functioning or not by taking simultaneous blood samples from both cephalic veins.

In conclusion, we report a new technique that enables a fast and cost-effective parathyroid allotransplant with sufficiently maintained tissue viability to treat persistent hypocalcemia. However, this study also has limitations and larger patient groups with longer follow-ups are mandatory to improve and clarify the outcomes of the application of this new parathyroid allotransplant method.

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