

## Original Article

# Parathyroid allotransplantation in rabbits without cultivation

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**Abstract:** Permanent hypoparathyroidism is a serious clinical situation. Allotransplantation of the parathyroid cells is relatively new approach to treatment. Non-cultivated allotransplantation in rabbits is not tried before. In this research parathyroidectomy was performed in six female New Zealand white rabbits. After division of surgically removed tissues into two, cryopreservation after cell isolation was done. Non-cultivated cross allotransplantation was performed under immunosuppression. Serum calcium and phosphorus levels were observed 15 days and histopathological analyses of the transplanted parathyroid tissues were studied. Significant changes in serum calcium and phosphorus levels during the experiment were observed ( $p=0.001$  for both). Calcium levels which were significantly dropped to  $6.66\pm 0.7$  mg/dL after parathyroidectomy and progressively increased up to  $15.98\pm 1.25$  mg/dL at the end of the experiment ( $p=0.004$ ). Phosphorus levels which were increased to  $9.38\pm 0.63$  mg/dL after parathyroidectomy and stabilized to  $4.46\pm 1.06$  mg/dL at the end of the experiment ( $p=0.007$ ). All allotransplanted parathyroid tissues showed normal tissue architecture without evidence of cellular rejection. In conclusion allotransplantation of the parathyroid tissues without cultivation may be considered as an alternative and safe approach for the treatment of permanent hypoparathyroidism.

**Keywords:** Parathyroid, transplantation, allotransplantation, cultivation, immunosuppression, rabbit

### Introduction

Permanent hypoparathyroidism (PH) is a serious clinical situation [1]. Thyroid surgery is the most common etiologic factor [2]. PH occurs in 3-5% of the patients with total thyroidectomy, 6-7% after total thyroidectomy with central or unilateral lymph node dissection, and 16% after total thyroidectomy with bilateral lymph node dissection [3]. The most important result of PH is hypocalcemia because complications of PH like weakness, fatigue, tremor, loss of workforce, and myocardial dysfunction are directly related to chronic hypocalcemia [1]. At present, supplementations of calcium and vitamin D3 are the only established modalities to palliation. However, besides being difficult and expensive way of treatment, such supplementations are frequently associated with over- or under-dosing of vitamin D3 and calcium products due to the absence of negative feedback

mechanisms leading to complications associated with hypo- or hypercalcemia [1, 4, 5].

Auto or allotransplantations of parathyroid tissue have been tried as a new therapeutic options [6, 7]. Microencapsulation, cultivation and direct transplantation are most commonly used parathyroid allotransplantation techniques [6]. In this research, we present the results of allotransplantation of the parathyroid cells after cryopreservation without cultivation as a technique applied for the first time in rabbits.

### Materials and methods

The methodology was approved by the local ethics committee and the research was performed at the Experimental Animal Production and Research Laboratory of Bezmialem Vakif University. All protocols were in accordance

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**Table 1.** Resected parathyroid cells and viabilities

	Viable cells	Viability	Total cells
Group I	$1.5 \times 10^6$	57.9%	$2.5 \times 10^6$
Group II	$3.28 \times 10^6$	54.7%	$6 \times 10^6$

with the regulations governing the care and use of laboratory animals of the declaration of Helsinki. Six female New Zealand white rabbits (mean weight,  $2800 \pm 410$  g; mean age, 4 months) without bred production were used. The rabbits were sheltered at one per cage in standard cages, whose top and bottom parts were made of stainless metal, and sides of woven wire. The floors of the cages were covered with wood shavings, which were changed daily. Rabbits were kept at room temperature and with adequate ventilation. Water and feeding containers were made of standard plastic, with sideways entrances. Animals were fed specially produced pellet feeds for small laboratory animals. Before surgical intervention, calcium and phosphorus levels were sampled (day 0). Following one night without food, the rabbits were anesthetized with Ketamine (Ketalar®, Parke Davis Co.) 40 mg/kg body weight and Xylazine (Rompun®, Bayer Co.) 5 mg/kg body weight. After shaving and antiseptics provided with povidone iodine (Batticon®, 10 g Povidon-iyot, Adeka Ilac) anterior neck area explored with midline longitudinal incision. Thyroid and the perithyroidal tissues were visualized completely and parathyroidectomy performed with microsurgical technique. Postoperative early complication did not occur but one rabbit (case-3) was died in the postoperative day 1. Cases monitored postoperatively. Serum calcium and phosphorus levels were sampled at the postoperative 3<sup>rd</sup> and 10<sup>th</sup> days. Excised tissues were cryopreserved according the technique described below.

### *Cryopreservation and cell preparation*

Surgically removed tissues from six rabbits were randomly divided into two groups: Cases 1 and 2 were in the first group, cases 4, 5, and 6 were in the second group. Parathyroid tissues pooled in each group and cryopreserved in three main steps; cell isolation, viability and cell counting and finally storing in liquid nitrogen tank. Tissues were taken immediately to an ice chilled RPMI 1640 media after removal, and

the cells were isolated. Whole protocol was carried out in sterile condition under a sterile hood. All solutions and instruments were sterilized by autoclaving, filtering or exposing UV radiation. The tissues were gently placed in a steel filter and rinsed into PBS+5% FCS (medium I). The tissues were then smashed with help of a piston taken from a syringe until the cells from whole tissues were split apart. The cells, floating in the medium I, were infiltrated through the cell strainer. During these steps some cells may explode and let their DNA content float in the solution freely. Those DNA causes cell aggregation which can be prevented by addition of DNase. Once whole tissues were disassociated and infiltrated through cell strainer. The cell number and cell viability were measured (**Table 1**).

Viability was determined with Vi-Cell (Beckman Coulter) and viable cells are counted with trypan blue staining method. Viable cells prepared for cryopreservation as in following steps: 500  $\mu$ l FBS 20% DMSO (400  $\mu$ l FBS+100  $\mu$ l DMSO) solution is prepared and gently dropped into 500  $\mu$ l FBS solution containing  $0.25 \times 10^6$  cells on ice. The temperature of solution is gradually decreased and stored in liquid nitrogen tank.

### *Transplantation*

Postoperative 10<sup>th</sup> day 100 mg/kg prednisolone (Prednol-L 40 mg Ampul®, Mustafa Nevzat Co.) were used to cases subcutaneously as an induction dose and continued 10 mg/kg/day subcutaneously in 15 days. The cell solutions were thawed by rinsing into 37°C water bath. The second group cells were divided into two sections equally and injected intramuscularly into the superior part of the right back extremity of the cases 1 and 2. The first group cells were divided into three sections equally and injected into the same areas of the cases 4, 5 and 6. Calcium and phosphorus levels were sampled in post-transplantation days 2, 10, and 15. In day 15, rabbits were sacrificed, and operative fields including previous parathyroidectomy (the thyroid, the trachea, anterior neck muscles) and transplantation area (superior part of muscular tissue found at the right back extremity) were resected for histopathologic evaluation. Primary evaluation parameters were calcium and phosphorus levels and complications related to allotransplantation were the secondary parameters of this research.

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**Table 2.** Changes of mean serum levels of calcium and phosphorus during the experiment

	Day 0	Postop Day 3	Postop Day 10	After Trans Day 2	After Trans Day 10	After Trans Day 15
Calcium (mg/dL)	15.82±1.02	6.58±1.06	6.66±0.7	15.10±0.34	16.54±0.68	15.98±1.25
Phosphorus (mg/dL)	6.62±0.57	8.66±1.33	9.38±0.63	2.64±0.74	4.96±0.59	4.46±1.06

Postop: Post-parathyroidectomy operation, After Trans: After parathyroid cell allo-transplantation.

**Table 3.** Comparison of serum calcium levels during the experiment

Pre-transplantation Period	Post-transplantation period	p
Day 0	After Trans Day 0	0.022
Day 0	After Trans Day 2	0.499
Day 0	After Trans Day 10	0.447
Day 0	After Trans Day 15	0.554
Postop Day 10	After Trans Day 2	0.108
Postop Day 10	After Trans Day 10	0.002
Postop Day 10	After Trans Day 15	0.004

Postop: Post-parathyroidectomy operation, After Trans: After parathyroid cell allo-transplantation.

**Table 4.** Comparison of serum phosphorus levels during the experiment

Pre-transplantation period	Post-transplantation period	p
Day 0	After Trans Day 0	0.176
Day 0	After Trans Day 2	0.011
Day 0	After Trans Day 10	0.237
Day 0	After Trans Day 15	0.176
Postop Day 10	After Trans Day 2	0.002
Postop Day 10	After Trans Day 10	0.011
Postop Day 10	After Trans Day 15	0.007

Postop: Post-parathyroidectomy operation, After Trans: After parathyroid cell allo-transplantation.

### Statistics

All statistics were performed using SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD). Significance of the measurements of serum calcium and phosphorus levels were evaluated by the Friedman test. Dunn's pairwise comparisons were also performed for the analysis of the significance between each of two different measurements during the experiment. The differences were considered statistically significant if the *p* value was less than 0.05.

### Results

Mean serum calcium and phosphorus levels are presented in **Table 2**. Changes for both electrolyte levels were statistically significant ( $p=0.001$  for both).

**Serum calcium levels:** Mean serum calcium level of all subjects were 15.82±1.02 mg/dL in the beginning (day 0). Postoperative 10<sup>th</sup> day mean serum calcium levels were significantly dropped to 6.66±0.7 mg/dL ( $p=0.022$ ) but after the allotransplantation progressively increased up to 16.54±0.68 mg/dL ( $p=0.002$ ) and 15.98±1.25 mg/dL ( $p=0.004$ , **Table 3**).

**Serum phosphorus levels:** Mean serum phosphorus level of all subjects were 6.62±0.57

mg/dL in the beginning (day 0). Postoperative 10<sup>th</sup> day mean serum phosphorus levels were significantly increased to 9.38±0.63 mg/dL ( $p=0.176$ ) but after the allotransplantation progressively dropped to 2.64±0.74 mg/dL ( $p=0.002$ ), and 4.46±1.06 mg/dL ( $p=0.007$ , **Table 3**). When mean serum calcium and phosphorus levels were compared in the first and last days of the research no statistically significant differences were found ( $p>0.05$  and  $p=0.176$  respectively) (**Table 4**).

No complication and side effect were seen during and after the procedure. There was only one mortality case which was seen just after the parathyroidectomy. All allotransplanted parathyroid cells revealed normal tissue architecture without evidence of cellular rejection including lymphocyte infiltration and necrosis.

### Discussion

Transplantation of allogeneic parathyroid tissues has been performed for the treatment of surgically induced hypoparathyroidism. However, the success of the procedure was limited due to the several immunologic mechanisms like rejection or graft nonfunctioning [8]. Therefore, preventive measures including allotransplantation of parathyroid tissues into immunologically privileged sites such as the kidney capsule or the cerebral ventricle, the

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short-term or long-term immunosuppression, immune alteration by organ culture, preoperative depletion of passenger leukocytes, x-ray irradiation, and immune protection by microcapsules were investigated [8-10].

It is generally accepted that the long-term immunosuppression is not justified for PH due to the developing of infections or malignancies [9, 11]. In several studies, the short-term systemic or topical immunosuppression has been shown to prolong survival of the transplanted parathyroid grafts which would be a feasible preventive measure in selected cases [8, 9]. But long-term graft function cannot be reached by these approaches even there is a longer maintenance of normocalcemia [9]. Substitution of parathyroid hormone and implantation of parathyroid cells after special tissue culture are the only alternatives to the transplantation of cultivated and microencapsulated parathyroid tissue without immunosuppression [11].

Graft rejection or nonfunctioning is one of the main problems of allotransplantation [8, 12]. Long-term survival and function of the transplanted organs and tissues is possible when the transplant is devoid of antigen-presenting cells which strongly express human leukocyte antigen (HLA) class II [6, 13-16]. It has been shown that parathyroid cells usually negative for HLA class II antigens expression and also weak positive for HLA class I antigens.

In experimental studies of allotransplantation of parathyroid grafts, several different species including dogs, cats or rats have been used with variable success [9, 10, 12]. This is the first study in which rabbits have been used for allotransplantation of parathyroid tissues without cultivation.

We evaluated function of the allotransplanted grafts by biochemical analyses including serum calcium and phosphorus levels. Statistical analyses revealed that total parathyroidectomy caused significant hypocalcemia which was relieved by allotransplantation ( $p=0.001$ ). Similar changes were also observed for serum phosphorus levels ( $p=0.001$ ). However, serum phosphorus decreased to  $2.64\pm 0.74$  mg/dL which was lower than the baseline at the second day after the transplantation, and could not reach to the baseline values until the end of the experiment. After induction of hypocalce-

mia caused by total parathyroidectomy, allotransplantation caused serum calcium levels to return to the baseline values at the second day after the transplantation. Although length of the survival for the allotransplanted grafts was beyond for purpose of the study, they were still functioning at the 15<sup>th</sup> day after transplantation, as shown by normocalcemia without any supplementation.

In this research we revealed first that parathyroid allotransplantation without cultivation is feasible in rabbits as a delicate and sensitive alive. These results support to consideration of non-cultured parathyroid cells transplantation as an alternative and safe approach for human.

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### Disclosure of conflict of interest

None.

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