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# Long-term preservation of vitality of xenogenic thyrocytes in the recipient after their transplantation into the blood stream

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## ABSTRACT

**Purpose:** This research is aimed at studying the possibilities of the long-term preservation of thyrocytes xenogenic culture in the recipient without using immunosuppressive therapy.
**Material and Methods:** The research was carried out on the experimental model "rabbit-dog." Using sixteen dogs having undergone total thyroidectomy, a macroencapsulated culture of a rabbit's thyrocytes was transplanted into the lumen of their arterial blood stream.
**Results:** Morphologic and laboratory research carried out six months later showed the preservation of thyrocytes vitality, the formation of organized thyroid tissue in the vessel's lumen, and the compensation of experimental primary hypothyroidism. A radioisotopic study showed an active absorption of the isotope by the transplanted thyroid tissue.
**Conclusions:** The macroencapsulation and transplantation of thyrocytes into the blood stream is the most effective method of its long-term preservation.

**Key words:** thyrocytes, xenograft, hypothyroidism

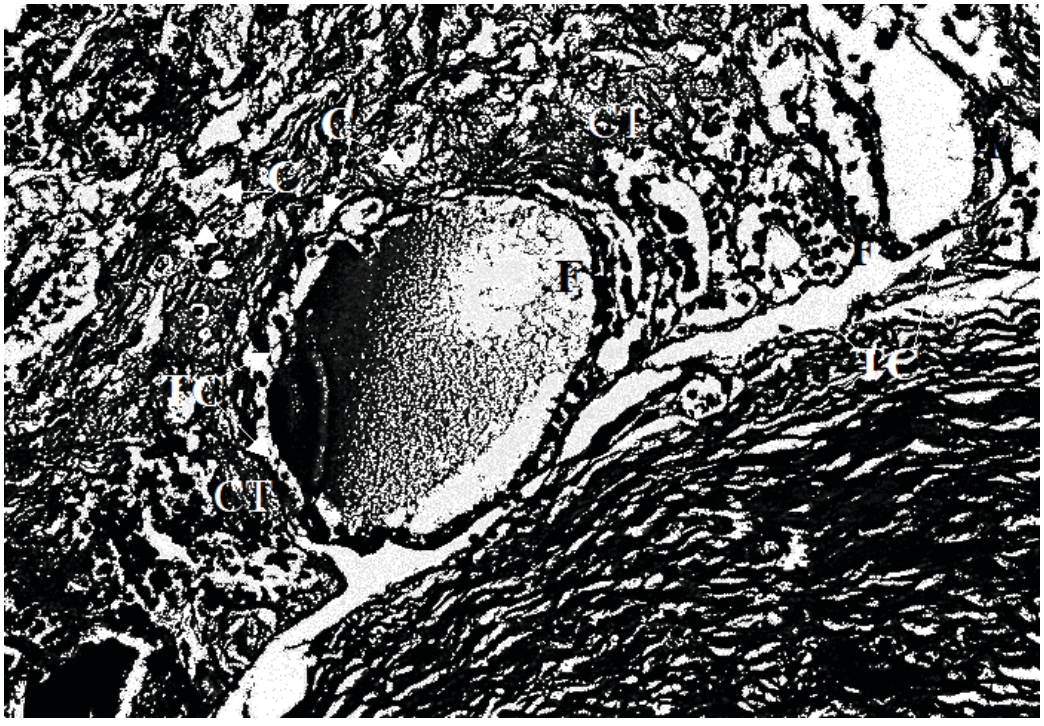
## INTRODUCTION

Following thyroidectomy, the restoration of normal metabolism by transplanting donor thyrocytes is a promising and physiologic approach in treating patients with hypothyroidism. However, graft rejection, the lack of allogenic donor material and the need for immunosuppressive therapy are still the main obstacles in the way of a successful cellular transplantation. Our previous research and studies carried out by other researchers on grafting pancreatic islets showed that it was possible to preserve the vitality of xenogenic tissue for a long time and maintain its functioning in the blood stream without using immunosupression, i.e. the blood stream is one of the immunologically privileged areas [1-3]. Therefore, we set as a goal of the present research to study the possibility of the long-term preservation and functioning of thyrocytes xenogenic culture in the recipient without using medicinal immunoprotection.

## MATERIALS AND METHODS

The research was carried out on the experimental model "rabbit-dog." All the studies were carried out following the permission of the ethics committee. The experimental model of the primary hypothyroidism was reproduced by total thyroidectomy. In 16 mongrel dogs, weighing 12-16 kg, there was performed a total resection of the thyroid under 1%-thiopentone intravenous anesthesia in dose 70.0±2.0 mg/kg. We carried out visualization of the thyroid bed by means of static thyroid scintigraphy in the γ-camera Dyna-camera Series 5 (Picker International, Hungary) in order to confirm the efficacy of thyroidectomy. We used a technetium-99m ($^{99m}$Tc) generator designed to obtain a isotopic solution of sodium pertechnetate $Na^{99m}TcO_4$ ("POLATOM", Poland). Sodium pertechnetate-$^{99m}$Tc was introduced intravenously just before the test (activity 25 MBq, $T_{1/2}$ – 6 hours, period of invading into the organ tested – 15-20 min.). Hypothyroidism

***Figure 1.*** **Thyroid xenograft 3 month after transplantation contained follicles (F) different in size and shape whose lining thyroid cells (TC) were more flattened than normal. Surrounding each follicle is a connective tissue (CT) and network of capillaries, a few of which are indicated (C).**




development was assessed based on clinical findings and by determining the amount of triiodothyronine ($T_3$), thyroxin ($T_4$) and free thyroxin ($T_{4f}$) in the blood.

Thyrocytes culture for transplantation was obtained from the rabbits' fetuses in the third trimester of pregnancy after the techniques of Korsgren et al. [4] and Blomkin et al. [5]. A warm ischemia time ranged between 15-30 min. Donor thyroid tissue was minced with scissors and washed in HEPES-buffered Hanks' solution at pH 7.4. The HEPES-buffered Hanks' solution was supplemented with bovine serum albumin (0.05%), penicillin (100 units/ml), and streptomycin (100 µg/ml). The minced tissue was then incubated while being shaken for 15 min in 1% collagenase solution at 27ºC and then with 1.25% pronase for 15 min at 37ºC and passed through a Millipore filter. Groups of approximately 150 000-350 000 thyroid cells were transferred to Petri dishes containing 1 ml of culture medium, and then incubated at 37ºC in an atmosphere of 95% $O_2$ and 5% $CO_2$ for the desired period of time. The culture medium was changed three times a week. Before transplantation, the thyrocytes suspension was placed into the synthetic microporous capsule ("Ecoflon", Russia) 0.5x3 cm in size with pores 1-2 mcm in diameter. In 11 animals the macrocapsule was implanted into the lumen of the abdominal aorta; in 5 dogs it was placed into the femoral artery using autovenous angioplasty for enlarging the vessel's lumen. After cross-clamping the abdominal aorta (femoral artery) with two vascular clamps and Satinsky's forceps, we made a longitudinal dissection 1.0-1.5 cm long of the front wall of the aorta, and it is through that dissection that we sewed the graft – a selectively permeable capsule with the cell culture with two fixed areas – to the internal surface with two U-shaped stitches Vicryl 7/0 (outside the dissection area).

Material sampling for morphologic investigation (macrocapsule with thyrocytes culture and a fragment of the aorta or femoral artery) was performed at 7, 14, 30 days, 3 months, and 6 months after transplantation. In all cases, animals were sacrificed under 1%-thiopentone intravenous anesthesia at the end of the procedure to remove the capsule and parts of the recipient's vessel. The preparations were washed in distilled water, dehydrated in ethanol, fixed in a 10% solution of neutral formalin and embedded in an Epon-araldite mixture followed by staining the 0.5-µm thick preparation with hematoxylin-eosin, and by Van Gison. Light microscopy was carried out by means of the programmed apparatus "Leika DMLS-Qwin" (Leika, Germany). Statistical analysis of the results was performed with the use of Student's paired *t* test. Statistical significance was assumed when $P < 0.05$.

## RESULTS

On the 7th day after thyroidectomy, all the animals developed clinical signs of primary hypothyroidism (bradycardia, loss of hair, reduced appetite, loss of weight, drowsiness). By the 7th day, the level of $T_4$ in the blood reduced to 9.0±1.96 nmol/l (15.0±1.82 nmol/l, $P<0.01$ before the operation),

***Figure 2.*** **Thyroid xenograft 6 month after transplantation contained more monomorphic follicles (F), which were surrounded by thin layers of connective tissue (CT) with blood vessels (BV), the epithelium (e) was cubic in shape.**

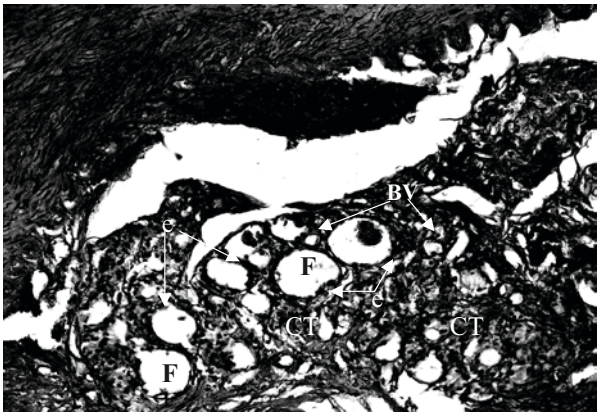



***Figure 3.*** **Thyroid xenograft 6 month after transplantation. This illustrates the position of the capsule (C) inside the vessel (V) and its tissue content (T).**


$T_{4f}$ – 6.03±1.66 pmol/l (11.51±1.72 pmol/l, P<0.01 before the operation) and $T_3$ – 0.89±0.08 nmol/l (1.46±0.09 nmol/l, p<0.05 before the operation). On scanning the thyroid bed area, there was revealed no deposit of radiotracer which excluded the presence of ectopic or residual thyroid tissue in the animal.

By the 14th day, after the xenotransplantation of thyrocytes, there was a noticable increase in the thyroid hormones level: $T_4$ – 11.5±2.39 nmol/l vs 9.0±1.96 nmol/l, P<0.01; $T_{4f}$ – 8.53±2.03 pmol/l vs 6.03±1.66 pmol/l, P<0.01; $T_3$ – 1.09±0.12 nmol/l vs 0.89±0.08 nmol/l, P<0.05. Findings close to those of euthyroid were obtained on the 21st day after transplantation ($T_4$ – 14.14±1.49 nmol/l vs 15.0±1.82 nmol/l, P<0.01; $T_{4f}$ – 11.1±1.57 pmol/l vs 11.51±1.72 pmol/l, P<0.05; $T_3$ – 1.31±0.1 nmol/l vs 1.46±0.09 nmol/l, P<0.05), and they were observed during a period of 6 months. There were no statistic differences revealed in hormonal indices after the transplantation of thyrocytes into the abdominal aorta and femoral artery.

On morphologic investigation 7 days after transplantation, there were noted different-size groups of thyrocytes with single follicles containing colloid between the single fibers of the young connective tissue in the macrocapsule. The follicles were different in size and shape, and they were lined with flat cubic epithelium. Fourteen days after transplantation, in the macrocapsule, there were noted numerous follicles and thyrocytes arranged in groups. Among thin collagenous fibres there was observed the formation of single capillaries. The fact that there was revealed no lymphocytic infiltration of the graft indicated sufficient immunoisolation. Thirty days after transplantation, the formation of the structure similar to the thyroid was observed, namely, a group of thyrocytes and an accumulation of follicles surrounded by a net of various-size capillaries and fibers of connective tissue. Three months after transplantation, groups of follicles of various size and shape with signs of disintegration in the preparations were observed; they contained a small amount of colloid and were separated by thin layers of connective tissue with blood vessels (*Fig.1*). Six months after transplantation, thyroid tissue formation was observed. The follicles were more monomorphic, the epithelium was cubic in shape, and they contained colloid and were surrounded by thin layers of connective tissue with blood vessels. The fact that foci of lymphocytic monocytic infiltration in the graft were not revealed confirmed the lack of acute or chronic graft rejection (*Fig.2, Fig.3*). Scanning carried out 3 and 6 months after transplantation showed an isolated area of intensive accumulation of $^{99m}$Tc in the graft projection.

## DISCUSSION

Our research showed that, during a period of 6 months after transplantation, the thyroid xenograft remained vital. The presence of cubic or cylindrical follicular cells and an increase in the level of thyroid hormones in the recipient's blood confirmed the graft's functional activity. The study we carried out confirmed the concept of the immunological privileges of the vascular bed, and showed that it is possible to preserve the xenogenic thyrocytes' vitality without immunosupression. The concept suggested is in accordance with the findings obtained by other researchers who proved it possible to considerably increase the terms of the islets functioning in the portal vein lumen compared to their intraperitoneal (omental pouch) and intrarenal (renal subcapsular space) introduction [2]. According to Prochorov AV, Tretjak SI et al., an unaffected intima is a powerful barrier that provides a nonstandard immune response to the grafted tissue, which eventually makes it possible for the foreign tissue to survive in the recipient's organism [1]. Ohzato and colleagues pointed out that the lack of the graft's cellular infiltration, humoral rejection and complications in grafting encapsulated xenogenic islets in the arterio-venous fistula (common iliac arteries and veins) [3]. The researchers explain that the reduction of the xenograft's functional activity on the

80th day was caused by the imperfection of the technique of the islets' preparation and by a considerable time of their warm ischemia, as well as by the capsule's construction defects.

As shown in our study, the capsule's wall adjacent to the artery contained a considerable number of capillary-type vessels, which are a possible source of the graft's vascularization. The arterial wall in the area of the capsule's fixation was moderately infiltrated by neutrophils and lymphocytes, which shows the presence of inflammatory reaction to the operative trauma. The formation of the perifollicular net of capillaries by the 14th day after transplantation is an important and necessary condition for the graft's nutrition and long-term preservation of functional vitality, which is consistent with the results of the tests carried out by Brauker et al. [6] and Stagner et al. [7]. In order to improve the neovascularization of the encapsulated tissue, the researchers indicated that it is important to use membranes with a high permeability (> $2x10^{-4}$ cm/s) and porosity (> 70%), as well as to add 20 ng/ml of endothelial cellular growth factor α (ECGF-α) into the cellular suspension in vitro and in vivo before transplantation. The prerequisites indicated make it possible to considerably reduce the recipient's immune (especially IgG-mediated) and inflammatory reaction to the graft, as well as to reduce the period of post-grafting ischemia.

The increase of the level of thyroid hormones to subnormal indices by the end of two weeks after grafting testifies to the beginning of xenograft functioning. There might be "a latent period" between thyroidectomy and the beginning of xenograft functioning, which may be necessary for the vascular supply of xenograft from the surrounding tissues. During this period a transient hypothyroidism can develop [8,9].

## CONCLUSIONS

The macroencapsulation and transplantation of thyrocytes into the blood stream makes it possible to overcome acute and chronic xenograft rejection without immunosuppressive therapy and makes up a bioartificial thyroid in the recipient and compensates primary hypothyroidism. At present, the transplantation of the xenogenic thyroid tissue into the vascular stream is the most effective method of its long-term preservation, which provides a basis and ensures hope for transplanting other endocrine tissues in order to treat a number of endocrinopathies.

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