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Ex situ bioengineering of the rat thyroid using as a scaffold the three-dimensional (3D) decellularized matrix of the glandular lobe: clues to the organomorphic principle

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Recently, we designed a bioreactor system for bioengineering ex situ (i.e. on the laboratory bench) a bioartificial thyroid gland suitable for transplantation. It is based on the organomorphic principle, i.e. the bioreactor mimics the macro-microscopic architecture of the thyroid stromal-vascular scaffold (SVS). To prove the reliability of this approach, we have initiated a pilot study using as a model the rat thyroid, and its natural decellularized 3D matrix to be eventually recellularized up to formation of a viable 3D thyroid lobe ex situ. Sprague-Dawley male rats (220-240 g) were used as thyroid donors. After penthobarbital anesthesia, rats were thyroidectomised and thyroid matrixes obtained by decellularization of the native SVS. Initially, we applied a sequence of liquid N2 freezing at - 80°C / thawing at 4°C for a total of 72 h, various washings with 0.02% trypsin / 0.05% EDTA for 1 h at 37°C, 3% Triton X-100 for 72 h at 4°C, and 4% deoxycholic acid for 24 h at 4°C, followed by sterilization with 0.1% peracetic acid, and 1% penicillin / streptomycin / fungizone for 24 h. Test matrixes were made electrondense with uranium / bismuth / lead counterstaining, and analyzed by microtomography (microTC). Primary thyroid cultures were prepared using enzymatic breaking of the native thyroid tissue. Cells were seeded at 19.000 / cm ^{2}, and grown 72 h in low-glucose DMEM supplemented with 10% FBS / 5% FCS. Following trypsinization, 450.000 cells were harvested to coat the inner surface of the matrix. After 7 and 14 days, colonized matrixes were fixed in aldheydes and processed for light (LM), transmission (TEM) and scanning electron (SEM) microscopy. Culture supernatants were collected every 48 h, and thyroid hormones assessed with chemiluminescent immunoassays. Complete decellularization and maintenance of the 3D architecture of the thyroid SVS were achieved. Thyroid-derived cells were found to aggregate, link and give rise to intracytoplasmic cavities up to follicular coating, whereas secretory de-differentiation occurred. These results show that the 3D matrix of the rat thyroid can be used as a natural scaffold to recellularize the thyroid lobe with progenitor-like elements, supporting the validity of the organomorphic principle for ex situ bioengineering of a bioartificial thyroid gland.

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