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Poly-L-lactic acid and poly-ε-caprolactone as biomaterials for ex-situ bioengineering of the rat thyroid tissue

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We are currently developing innovative methodologies for rapid prototyping of three-dimensional (3D) replicas of the vascular matrix of soft and hard tissue organs, primarily the thyroid and bones, with the intent of obtaining organomorphic scaffolds for their ex situ reconstruction (1, 2). To identify biocompatible materials suitable for this application, we have studied the effect of poly-L-lactic acid (PLLA) and poly-ε-caprolactone (PCL) on survival, adhesion and hormonal secretory activity of primary rat thyroid cells. Sprague-Dawley male rats (225-250 g) were used as thyroid donors. After penthobarbital anesthesia rats were thyroidectomised, thyroids surgically excised, and primary cells prepared using enzymatic breaking. After 72 hs in standard monolayer culture, cells were trypsinized and seeded (20 x103 / cm2) onto microporous scaffold sheets. Biomaterial scaffolds were prepared using either 3.5-5% PLLA (Lacea H100E, Mistui Chemicals) in anydrous dichloromethane / amylene (Sigma) or 3% PCL (Aldrich) in anydrous tetrahydrofurane / BHT (Aldrich). Solutions were dropped onto a wet glass support, and let polymerize as a thin layer at 25°C in the absence of forced air flow. Pattern of polymerization was assessed with thermogravimetry. Cells were let grow on biomaterials up to 8 days. Single biomaterial sheets without cells, and standard multiwell cultures were used as controls. Every 2 days, percentage of viable cells was assessed using a count chamber and Trypan blue exclusion, whereas morphology of growing cells was analyzed using a scanning electron microscopy (SEM) technique without critical point drying. Culture supernatants were collected every 48 hs, and free forms of thyroid hormone (FT3 and FT4) assessed with chemiluminescent immunoassays. PLLA and PCL scaffold sheets exhibited a quite homogeneous thickness (10-12 μm), a repetitive and regular pore geometry, and a crystalline pattern of polymerization. Both biomaterials promoted survival, adhesion and proliferation of primary thyroid elements. Secretion of FT3 and FT4 was variably maintained during the culture period, and resulted statistically higher with respect to monolayer cultures on standard multiwells. Our results indicate that PLLA and PCL are suitable for growth and differentiation of adult rat thyroid cells in culture, and suggests that they might be exploited as bioerodible materials for rapid prototyping of vascular-like, organomorphic scaffolds.

References

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