191g Characteristics of Btc Tet Cells for Use in the Cryopreservation of a Model Tissue Engineered Pancreatic Substitute

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The use of encapsulated insulin-secreting cells constitutes a promising approach towards the treatment of insulin-dependent diabetes. However, long-term storage for off-the-shelf capsule availability still remains an issue. This can be effectively addressed by cryopreservation. The cryopreservation process is augmented through the use of cryoprotective agents (CPAs), which generally need to be added at appropriate concentrations and exposure times due to cytotoxic effects, as well as via proper loading and unloading procedures due to osmotic considerations. The optimization of such parameters for a specific system can enhance its preservation effectiveness. For this, cell membrane permeability properties and osmotic tolerances need to be determined and used in computer simulations to predict volumetric cell changes during stepwise loading and unloading of CPAs for ice-free preservation (vitrification). The steps of CPA addition and removal to reduce volume excursions to within their tolerable osmotic limits need to be evaluated. In addition, cytotoxicity studies should be performed to determine acceptable ranges of CPA concentrations and exposure times evaluated at different temperatures. These processes were investigated using mouse bTC tet insulinoma cells in alginate/ poly-L-lysine/ alginate (APA) beads as a model tissue engineered pancreatic substitute. Results helped define a domain of parameters with which to cryopreserve the encapsulated cell system with improved cell viability and functional outcome. Cryopreservation of constructs using an optimized vitrification protocol over a conventional freezing method resulted in improved cell viability and retention of the structure and function of the extracellular matrix components of the construct. Microscopic observations revealed holes and/or tears within beads subjected to freezing, whereas no such abnormalities were detected in the vitrified samples. The importance of developing cryopreservation protocols that retain not only cellular viability and function but also structure and function of the material constituents of three-dimensional tissue substitutes will be discussed.