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Tissue engineering by cell transplantation using degradable polymer substrates: determination of functional and phenotypic cell characteristic by in situ hybridization

Cell transplantation has been proposed as a strategy to achieve organ replacement or tissue repair for a variety of therapeutic needs. In cell transplantation, donor tissue is dissociated into individual cells or small groups of cells that may then be attached to, or encapsulated in, a support matrix, and transplanted into the patient to restore lost tissue function. In the past, our research group has centered on developing appropriate matrices for cartilage and liver cells as paradigms for the regeneration of metabolic and structural tissue. In our efforts to try to optimize culture conditions, as well as delivery substances, to achieve the best possible similarity of the in vitro created tissue transplant, one of the unsolved technical problems is how to characterize the metabolical/immunological activity of the cells, as well as the maintenance of the differentiation state. The aim of the present research was to examine methods for quantitative analysis of specific RNA transcripts in eukaryotic cells. As material, bioresorbable polymer fleeces of polyglycolic acid and polylactic acid were used as temporary cell carrier matrices to establish three-dimensional cultures of chondrocytes and hepatocytes. The cell-polymer tissue constructs were then placed into perfusion culture chambers to provide a constant supply of nutrients into the cultures. The cell-polymer tissue was harvested and cryopreserved for molecular biological analysis after different periods of time in culture. Labeled RNA antisense probes for enzymes, cytokines, collagens, and cytoskeleton were prepared by linearizing the cDNA probes with the appropriate restriction enzyme and transcribing. The cryostat sections were rehydrated and acetylated and then prehybridized for 10 min in 50% formamide in 2X SCC. The hybridization mixture was added and incubated overnight at 50° C. After washing, the slides were dehydrated in ethanol, allowed to dry and dipped in Kodak NTB-2 emulsion. They were placed in a lighttight box with dessicant at 4° C for 3–15 days. After developing the degree of hybridization, the relative amount of messenger RNA for a particular gene was estimated by computer-assisted image analysis. Both types of cells maintained a differentiated phenotype showing synthesis of specific products. The presence of mRNA for collagenase, stromelysin, and metalloproteinases was not detected, indicating an inactive state. Furthermore, gene expression for IL-1 and IL-6 was not achieved in hepatocytes or in chondrocytes, supporting the resting status of the cells. Hepatocytes showed a typical high presence of mRNA for lactic dehydrogenases. The presence of actin was detected in both types of cells. This technique can be used to hybridize specific messenger RNA transcripts in cell-polymer transplants. Furthermore, it allows the determination of relative amounts of mRNA for different kinds of proteins.