

Day 3. All medium was aspirated and replaced with DMEM complete medium.

Days 4-7. All medium was aspirated and replaced with 100-200 µg/ml G418 in DMEM complete medium for 48 hr on day 4 and again on day 6 to negatively select for the infected cells.

Day 8. All medium was aspirated and replaced with DMEM complete medium.

7.1.3. CULTURING OF RETROVIRALLY-INFECTED CELLS

Liver stromal cells that were transduced with the LNL-SLXβgal vector were grown in monolayer culture for 30 days. Acidophilic liver cells that were transfected with the LNL-SLXβgal vector were either cultured for 2-3 weeks in plastic flasks or were inoculated upon a three dimensional framework containing a pre-established growth of normal non-transfected liver stromal cells. The latter were cultured for 30 days. Expression of β galactosidase activity was visualized by the transformation of 5-bromo-4-chloro-3-indoyl β-D-galactoside (xgal) into a blue-colored Compound.

7.2. RESULTS

When the liver stromal cells were infected with the virus, they were efficiently transduced with retroviral vectors and expressed β galactosidase activity throughout the 30 day period of observation in vitro. Acidophilic PC were very sensitive to G418 selection. When they were inoculated into plastic flasks after transfection, they grew at a slower rate than usual but became detached from the plastic surfaces as they matured and developed into type I and type II PC. Approximately, 50-60% of these cells expressed β galactosidase after 3 weeks in culture. In addition, transfected acidophilic cells retained their expression of β galactosidase when cultured upon normal hepatic stromal cells on three-dimensional framework for 30 days.

The present invention is not to be limited in scope by the exemplified embodiments, which are intended as illustrations of individual aspects of the invention. Indeed, various modifications of the invention in addition to those shown

and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications cited herein are incorporated by reference in their entirety.

What is claimed is:

1. A method for transplantation or implantation of liver cells which comprises implanting in vivo the liver cells cultured on a living stromal tissue prepared in vitro, comprising stromal cells and connective tissue proteins naturally secreted by the stromal cells attached to and substantially enveloping a framework composed of a biocompatible, non-living material formed into a three-dimensional structure having interstitial spaces bridged by the stromal cells so that a tissue equivalent is formed.

2. The method according to claim 1 in which the stromal cells are fibroblasts.

3. The method according to claim 1 in which the stromal cells are a combination of fibroblasts and endothelial cells, pericytes, macrophages, monocytes, leukocytes, plasma cells, mast cells or adipocytes.

4. The method according to claim 1 in which the framework is composed of a biodegradable material.

5. The method according to claim 4 in which the biodegradable material is cotton, polyglycolic acid, cat gut sutures, cellulose, gelatin, or dextran.

6. The method according to claim 1 in which the framework is composed of a non-biodegradable material.

7. The method according to claim 6 in which the non-biodegradable material is a polyamide, a polyester, a polystyrene, a polypropylene, a polyacrylate, a polyvinyl, a polycarbonate, a polytetrafluorethylene, or a nitrocellulose compound.

8. The method according to claim 4, 5, 6 or 7 in which the framework is pre-coated with collagen.

9. The method according to claim 1, 2, 3, 4, 5, 6, or 7 in which the framework is a mesh.

10. The method according to claim 8 in which the framework is a mesh.

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