Bio-Medical

Tissue Engineering Using Transfected Growth-Factor Genes

Cells, matrices, and bioreactors are tailored to promote functional tissue engineering of cartilage.

Lyndon B. Johnson Space Center, Houston, Texas

A method of growing bioengineered tissues includes, as a major component, the use of mammalian cells that have been transfected with genes for secretion of regulator and growth-factor substances. In a typical application, one either seeds the cells onto an artificial matrix made of a synthetic or natural biocompatible material, or else one cultures the cells until they secrete a desired amount of an extracellular matrix. If such a bioengineered tissue construct is to be used for surgical replacement of injured tissue, then the cells should preferably be the patient's own cells or, if not, at least cells matched to the patient's cells according to a human-leucocyte-antigen (HLA) test. The bioengineered tissue construct is typically implanted in the patient's injured natural tissue, wherein the growth-factor genes enhance metabolic functions that promote the *in vitro* development of functional tissue constructs and their integration with native tissues. If the matrix is biodegradable, then one of the results of metabolism could be absorption of the matrix and replacement of the matrix with tissue formed at least partly by the transfected cells.

The method was developed for articular chondrocytes but can (at least in principle) be extended to a variety of cell types and biocompatible matrix materials, including ones that have been exploited in prior tissue-engineering methods. Examples of cell types include chondrocytes, hepatocytes, islet cells, nerve cells, muscle cells, other organ cells, bone- and cartilage-forming cells, epithelial and endothelial cells, connective-tissue stem cells, mesodermal stem cells, and cells of the liver and the pancreas. Cells can be obtained from cell-line cultures, biopsies, and tissue banks. Genes, molecules, or nucleic acids that secrete factors that influence the growth of cells, the production of extracellular matrix material, and other cell functions can be inserted in cells by any of a variety of standard transfection techniques.

The method was developed for polyglycolic acid scaffolds, but can (at least in principle) be extended to other biodegradable matrix materials, which include collagen, fibrin, and poly(lactic acid) [PLA], poly(glycolic acid) [PGA], and PLA/PGA copolymers. Nonbiodegradable matrix materials include polystyrene, polyesters, polypropylene, and numerous other polymers. Preferably, the matrix for a given therapeutic application should be fabricated so as to have a microstructure similar to that of the extracellular matrix to be replaced. Mechanical loads imposed on the matrix by the surrounding tissue influence the cells on and in the matrix in such a manner as to promote the regeneration of an extracellular matrix that has the proper microstructure. The cross-link density of the matrix can be tailored in fabrication in order to tailor the mechanical properties of the matrix and, in the case of a biodegradable matrix, to tailor the rate of its biodegradation. The shape and size of the matrix and the implant made from it should, of course, be chosen to suit the implant site and tissue type. The matrix material can be coated with materials that promote specific adhesion and metabolic behavior of both transfected cells and native cells.

Another important consideration in the design of a matrix is porosity. Pores must be large enough that cells can reside within them and that nutrients can migrate to the cells and waste products can diffuse away from the cells. Typical pore sizes range from 50 to 300 μm; the size or range of sizes can be chosen to obtain the cell behavior and matrix properties desired for a given application. Moreover, the range of pore sizes for a given application can be chosen to promote a specific timetable and amount of vascular ingrowth from the surrounding tissue as well as migration of native cells.

This work was done by Henning Madry, Robert S. Langer, Lisa E. Freed, Stephen Trippel, and Gordana Vunjak-Novakovic of Massachusetts Institute of Technology for **Johnson Space Center**.

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Massachusetts Institute of Technology
Five Cambridge Center, Kendall Square
Room NE25-230
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Phone: (617) 253-6966
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Automation of Vapor-Diffusion Growth of Protein Crystals

High-throughput experiments are accelerated through automation of routine operations.

Marshall Space Flight Center, Alabama

Some improvements have been made in a system of laboratory equipment developed previously for studying the crystallization of proteins from solution by use of dynamically controlled flows of dry gas. The improvements involve mainly (1) automation of dispensing of liquids for starting experiments, (2) automatic control of drying of protein solutions during the experiments, and (3) provision for automated acquisition of video images for monitoring experiments in progress and for post-experiment analysis.

The automation of dispensing of liquids was effected by adding an automated liquid-handling robot that can aspirate source solutions and dispense them in either a hanging-drop or a sitting-drop configuration, whichever is specified, in each of 48 experiment chambers. A video camera of approximately the size and shape of a lipstick dispenser was added to a mobile stage that is part of the robot, in order to enable automated acquisition of images in each experiment chamber. The experiment chambers were redesigned to enable the use of sitting drops, enable backlighting of each specimen, and facilitate automation.

The evaporation of water from the protein solution in each chamber can be controlled independently of the evaporation in the other chambers. Hence, a total of 48 unique evaporation-rate-versus-time profiles can be tested simultaneously. Interface software was written for use in controlling all aspects of operation of the system. The software also enables the user to specify the evaporation profile for each chamber and provides for automatic acquisition of the images from each experiment chamber and the storage of the images for later analysis.

This work was done by David T. Hamrick of Diversified Scientific, Inc., and Terry L. Bray of the University of Alabama at Birmingham for **Marshall Space Flight Center**. *For further information, access http://www.dsitech.com/.*

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