Scaffold Modification for Animal Cell Expansion in a Fibrous Bed Bioreactor

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Introduction

Recently, tissue engineering has been the most prospective alternative for organ loss and disease treatment. It has opened the window as the key to bioartificial organ transplantation and cell based therapy for a wide variety of diseases such as Parkinson, Alzheimer, and diabetes mellitus. The accomplishment of artificial organs could not have been succeeded without the appropriate design of the affecting cell-scaffold-based matrices. Consequently, the development of three dimensional matrices in the area of tissue engineering is also rapidly growing.

In vivo cells are organized in a three dimensional structure that enables those certain cells or organs to function properly. Evidences have demonstrated that proper spatial organization of cells can affect the metabolism of those cells such as cell proliferation, migration, differentiation, and functions[1]. Besides, it is manifestly shown that cells grow better and faster in a three dimensional arrangement.

A novel bioreactor, fibrous bed bioreactor, is a kind of perfusion bioreactors that uses non-woven fibrous as the scaffolding materials for cell immobilization. The cells are seeded in the matrix and the matrix is fixed in the bioreactor. Growth medium is then continuously fed into the bioreactor. When cell concentration inside the matrices is high, cell harvesting becomes an issue. Extra cellular matrix (ECM) rupturing by trypsin is one of the methods that can be used for harvesting the cells. However, besides its detrimental effects on cells, trypsinization is labor and time consuming.

Another method for harvesting the cells will be coating the matrices with biodegradable materials. In this experiment, two types of biomaterials will be examined, poly(lactic acid-co-glycolic acid) (PLGA) and chitosan, as the coating biomaterials. PLGA has been extensively studied and is one of the most popular biodegradable polymers due to its biocompatibility. Besides, it can be easily modified to obtain the desired degradation and mechanical properties. However, its application in drug delivery has been limited by its bulk degradation properties[2]. The chemical structure of PLGA is shown on figure 1. A natural polymer, chitosan, is a linear polysaccharide found in the exoskeletons of anthropods. It is a deacetylated derivative of chitin with N-acetyl group. Chitosan possesses the properties of fiber that is chemically similar to cellulose[3]. Figure 2 illustrates the chemical structure of chitosan.

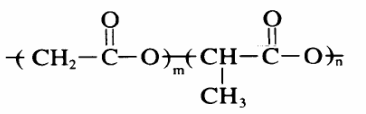

Figure 1. Chemical structure of PLGA

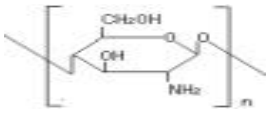

Figure 2. Chemical structure of chitosan

Experimental

Materials

PLGA 4a (50/50) were supplied by Alkermes (Medisorb®, Cincinati, OH). Acetonitrile was supplied by Fisher. Chitosan, poly-D-glucosamine, was obtained from Vanson HaloSource, Inc (Washington). The chitosan used has a viscosity of 723 mPas and is 82.8% deacetylated. Glacial acetic acid was supplied by Fisher.

Cell culture

Cancer cells HT-29 was obtained from ATCC. The culture medium used was DMEM with 10% FBS. DMEM and FBS were supplied by Gibco. All the experiments were done in Falcon multi wells. The scaffolds used were PET-based non-woven fibers. The cells were counted using hemacytometer.

Scaffold modification

Liquid porosimetry method was used to determine the pore size distribution. The procedure was as described before by Miller and Tyomkin[4]. The contact angle between water, air and fiber surface was chosen to be 60°. The air-liquid interfacial tension is 72 dyne/cm. Different concentration solution of PLGA and chitosan were prepared by dissolving PLGA and chitosan into acetonitrile and acetic acid, respectively. Both PLGA and chitosan were coated to PET fiber surface using solvent-casting method. The PET fibers were prewetted with DI water prior to the coating to enhance the coating efficiency. Biochemical detector from Yellow Spring Inc. was used to detect the lactic acid concentration released by the degraded PLGA. For degradation kinetics, the lactic acid concentrations of PLGA coated fibers were detected every 7 days. The solubilizations of chitosan from the PET matrices were done by soaking the matrices into different acetic acid concentration. All the samples are weighted before and after the coating.

Result

Solvent casting method was proven to be effective in altering the porosity of three dimensional scaffolds. The effective pore sizes of the PET-based scaffolds can be decreased by coating the fibers with other biomaterials using a solvent casting method. After the degradation and solubilization, the effective pore sizes increase as the coated biomaterials degrades. As the surfaces of the fibers vanish, the attachment of the cells to the fibers is loosening. This might be a facilitative method for harvesting the cells from the three dimensional matrices.

References

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