Continuos-Flow culture of Hepatocytes in Sugar-derivatized Poly (lactide-co-glycolide) Scaffolds Prepared by Gas-foaming/salt-leaching Method

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Abstract

Highly open porous polymer matrices are required for high density cell seeding, efficient nutrient, and oxygen supply to the cells cultured in the three dimensional matrices. However, there are severe problems of mass transfer limitations within the cell/scaffolds culture system. Thus we hypothesize that continuos-flow culture conditioning of cells with the scaffolds may improve the cell viability and the differentiated function. In this study, we fabricated porous PLGA scaffolds by using gas-foaming/salt-leaching method as previous described. Viscous PLGA gel paste contains ammonium bicarbonate particulates, acting as a gas-foaming agent as well as a salt-leaching porogen, were cast into Teflon mold and dried. Ammonium bicarbonate salt upon contact to an acidic aqueous solution evolves gaseous ammonia and carbon dioxide by itself. And we conjugated galactose moiety [AGA; N-(aminobutyl)-O-β-D-galactopyranosyl-(1→4)-D-glucoamide] to the terminal end group of a PLGA to increase the cell adhesion and matain the differentiated function of hepatocytes. Cell-seeded scaffolds were secured in a flow bioreactor chamber and exposed to continuous flow at 5 ml/min. As a result of our study, the high yield of hepatocytes attachment was accomplished by increasing the concentration of PLGA-AGA conjugate in polymer scaffolds and cells in the scaffolds under continuos flow condition maintained a high level of viability and albumin secretion rate of cultured hepatocytes showed a higher level that of control groups.

Introduction

Biodegradable porous polymer scaffolds have been used for temporal templates for tissue regeneration. For tissue engineering, well-interconnected open porous structures are needed to the scaffolds. Furthermore, high density cell seeding and improved mass transfer condition was required for efficient cell/tissue transplantation. Synthetic polymers can be designed to incorporate signals that affect cell regulation, function and reorganization. Furthermore selective

cell-polymer interaction can be introduced by polymer modification. As an example, conjugation of the tissue-specific ligands, such as RGDS or galactose to terminal end groups of biodegradable aliphatic polyesters caused the cell-polymer interaction and the identification of intracellular phenomena. To overcome the mass transfer limitation, continuous-flow perfusion in cell/scaffolds culture system has been used in other studies[1]. the perfusion culture can improve the oxygen diffusion limitation on cells in scaffolds and maintain the viability and differentiated function of the hepatocytes until they are transplanted to the patient.

As a part of development of implantable liver devices, we investigate the interaction between the sugar-deravetized hepatocyte-specific polymer surface to enhance the cell adhesion and differentiated function. The hepatocyte adhesion to a surface is induced by interactions between the galactose moieties conjugated to the terminal end groups of the PLGA and the asialoglycoprotein receptor. As a result of the galactose conjugation, hepatocyte attachment on the polymer surface was enhanced and albumin secretion rate of cultured hepatocytes was maintained at higher level than that of control groups. Cell viability within the scaffolds was increased under continuos-flow bioreactor condition.

Materials and Methods

Conjugation of galactose moiety to PLGA

The uncapped hydrophilic PLGA was activated by adding DCC and NHS. To conjugate AGA to PLGA, the activated PLGA and AGA were dissolved in anhydrous DMSO (1:1 molar ratio) under stirring. AGA-PLGA conjugate was purified by precipitation into anhydrous diethyl ether and vacuum drying (30 in Hg).

Polymer film and scaffold fabrication

galactose conjugate PLGA was blend with PLGA at weight ratio of 0.05, 0.5, 1 % in methylene chloride. The polymer blend solution was cast and dried. Galactose-derivatized PLGA scaffolds were fabricated by gas foaming process with ammonium bicarbonate as porogen additives[2]. Briefly described, cold ethanol as a non-solvent to precipitate and concentrate the polymer, was added to the polymer blend solution in chloroform. Ammonium bicarbonate salt particulates were mixed with the precipitated PLGA gel. The polymer gel paste was cast into a disc shaped Teflon mold (10 mm diameter and 1 mm thickness). After chloroform was partially evaporated under atmospheric pressure, the

semi-solidified samples were immersed into an excess amount of acidic aqueous solution at room temperature. Afterwards, the samples were then freeze-dried.

Galactose activity on polymer surface

Galactose-specific lectin (RCA120) was used for surface galactose quantification. Lectin solution was added to the polymer film and the surface binding lectin was dissociated by adding the supersaturated galactose solution. Resulting free lectin solution was assessed by colloidal gold to protein quantification.

Hepatocyte isolation and culture

Rat Hepatocytes were isolated from SD Rat by 2-step collagenase perfusion method. Hepatocytes ($3x10^4$ cells/cm2) were cultured on PLGA film. PLGA scaffolds contained 28 x 10^4 cells were incubated in static culture or perfusion culture at 37 ℃, 5 %-CO_2.

Viability and function of hepatocytes

MTT assay was utilized to determine cell viability. The albumin secretion was measured by ELISA.

Results and Discussion

A Bioactive galactose moiety on polymer surface serves as a cell adhesion ligand of asialogylcoprotein receptor on hepatocytes. In this study, we assessed the surface exposed galactose on galactosylated PLGA film by utilizing the binding assay of galactose-specific lectin (RCA120). As shown in Figure 1, the blend ratio of AGA-PLGA conjugate with PLGA was important for the high yield of galactose-specific lectin binding. But AGL, conjugate as glucose ligands, showed no effect on aell adhesion. As the blend ratio of AGA-PLGA in PLGA was increased, the amount of binding lectin was saturated at around 0.5 % (w/w) blend ratio.

The cell viability within the scaffolds was decreased as culture periods progressed. Although cell viabilities were decreased, cells in bioreactor system showed slow decreasing curve (Figure 3). It means that continuos-flow of culture media may improved the oxygen diffusion limitation. The albumin synthesis of cultured hepatocytes in Figure 4, was influenced by galactose-moieties on the polymer surface. Furthermore, the cells seeded onto galactose-derivatized PLGA scaffolds displayed an aggregated form. Therefore, galactose-derivatization promotes the cell-polymer interaction as well as the cell-cell interaction, and enhances the differentiated functions of hepatocytes.

Summary

Galactose moieties were conjugated to PLGA as a ligand for asialoglycoprotein receptor on the hepatocytes. As a result of AGA conjugation, Cell adhesion and

differentiation were enhanced. Rat hepatocytes were seeded into the porous scaffolds prepared by gas-foaming/salt-leaching method and cultured under continuos-flow bioreactor condition. Thus, cell viability was improved and albumin secretion was maintained at about 20 pg/cell/day during culture periods.

References

1. Pollok JM, et al., "Formation of spheroidal aggregates of hepatocytes on biodegradable polymers under continuous-flow bioreactor conditions"(1998), *Eur J Pediatr Surg*, Vol 8 (4), 195-199

2. Nam, YS, et al., "A novel fabrication method of macroporous biodegradable polymer scaffolds using gas foaming salt as a porogen additive" (2000), J Biomed Mater Res (Appl Biomater), Vol 53, 1-7

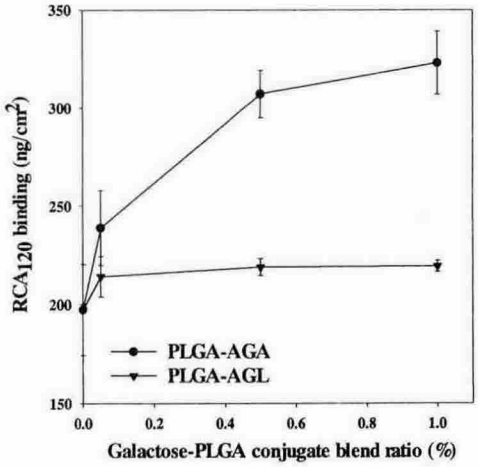

Figure 1. Galacotose-specific lectin binding on sugar-derivatized PLGA film surface.

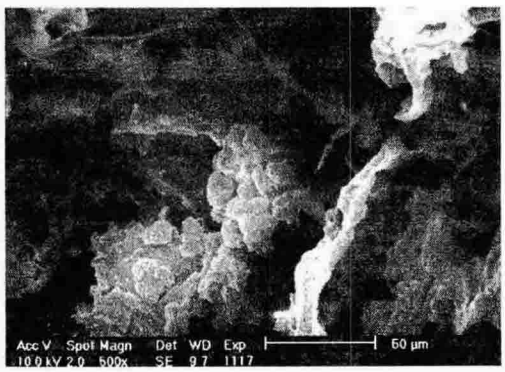

Figure 2. SEM images of hepatocyte aggregates in the scaffolds cultured in perfusion bioreactor.

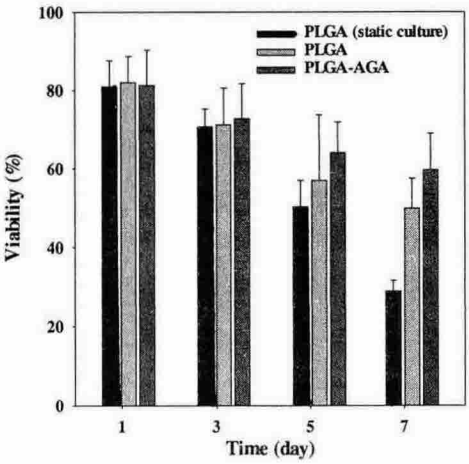

Figure 3. Cell viability within the PLGA scaffolds under perfusion culture condition.

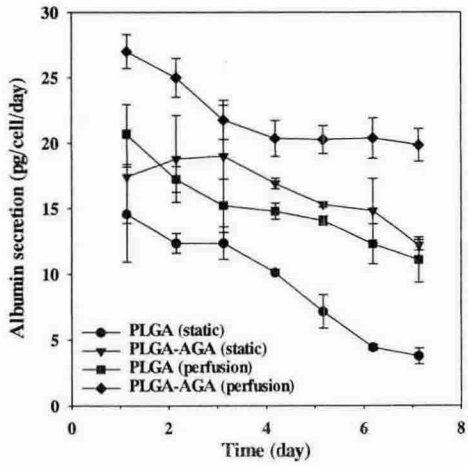

Figure 4. Albumin secretion of hepatocytes cultured on sugar-derivatized PLGA scaffolds.