Surface Immobilization of Galactose onto Aliphatic Biodegradable Polymers for Hepatocyte Culture

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Abstract: A novel surface modification method of biodegradable polymers was investigated for inducing the attachment of specific cells onto the polymer surface via ligand-receptor interactions. Galactose, a targeting ligand specific to asialoglycoprotein receptors present on cell membrane of hepatocytes, was introduced on the surface of poly(D,L-lactic-co-glycolic acid) (PLGA) films. A terminal end group of carboxylic acid in PLGA was activated by dicyclohexylcarbodiimide and *N*-hydroxysuccinimide for the direct conjugation of lactose by reductive amination reaction. Di-block copolymers of PLGA-b-poly(ethylene glycol) (PEG) having a free terminal amine group were also synthesized and used for the conjugation of galactose for the introduction of a PEG spacer between PLGA and galactose. The presence of galactose moieties on the blend film surface was characterized by measuring water contact angle and X-ray photon spectroscopy, and the amount of galactose was indirectly determined by a specific lectin-binding assay. With increasing the galactose concentration on the blend film surface, the initial attachment as well as the cell viability of hepatocyates concomitantly increased. The introduction of PEG spacer reduced the cell attachment and viability. Albumin secretion rate from hepatocytes was enhanced for galactose modified surfaces, whereas it was reduced for the surfaces not having galactose moieties. © 2002 Wiley Periodicals, Inc. *Biotechnol Bioeng* **78**: 1–10, 2002; DOI 10.1002/bit.10239

Keywords: hepatocytes; biodegradable polymer; surface modification; galactose

INTRODUCTION

Aliphatic biodegradable polyesters such as poly(L-lactic acid) (PLA) and its various copolymers with D-lactic acid and glycolic acid have been extensively studied as cell adhesion substrates for tissue regeneration. When they are used as polymer substrates for cell adhesion, surface characteristics would be dictated by their bulk properties (Black et al., 1999; Drumheller and Hubbell, 1995). It was reported that chemical composition as well as surface morphology in various PLA and poly(D,L-lactic-co-glycolic acid) (PLGA) films affected cell adhesion behaviors differently. Cell adhesion and functional studies on these surfaces so far have been limited mainly to the effect of polymer composition and lactic/glycolic acid ratio (Cima et al., 1991). Unfortunately, all the PLA and PLGA polymers have no available functional groups in the backbone to conjugate specific cell-recognizable ligands to promote cell adhesion. To introduce functional groups in the polymer backbone, poly(L-lactide-co-lysine) was synthesized to provide reactive amine groups in the PLA backbone for the conjugation of a specific cell adhesion peptide ligand such as arginine-glycine-aspartic acid (Barrera et al., 1993; Cook et al., 1997). Synthesis of new functional biodegradable polymers, although aiming at chemical modification of the PLA surface for controlled ligand immobilization, changes their bulk properties as well. Physical and chemical surface modifications of biodegradable polymer surface recently were attempted to enhance the cell attachment (Ramsey et al., 1984; Rasmussen et al., 1977). Partial surface hydrolysis by chemical reagents increased surface hydration, resulting in the improved cell adhesion (Gao et al., 1998; Nam et al., 1999). More recently, di-block copolymer of PLGA-PEG having a terminal ligand in the PEG end was used to modify the surface (Hrkach et al., 1997; Otsuka et al., 2000).

In this work, galactosylated PLGA surface was fabricated for the improvement of polymer-hepatocytes interaction. A galactose moiety was directly conjugated to a dicyclohexylcarbodiimide/*N*-hydroxysuccinimide (DCC/NHS)-activated terminal carboxylic acid end group of PLGA by using lactose via a reductive amination reaction. Additionally, PLGA-PEG di-block copolymers having a terminal free primary amine group were synthesized and used for the conjugation of galactose in the PEG end. Two PEG chains with different molecular weights were introduced as a spacer between a galactose moiety and a PLGA chain. It was hypothesized that a hydrophilic ligand moiety terminally conjugated to PLGA was oriented outside in an aqueous

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medium whereas the relatively hydrophobic PLGA polymer backbone was anchored into the PLGA bulk phase, thereby providing specific interaction between surface-exposed ligands and cell receptors. The extent of surface galactose immobilization was confirmed by water contact angle and X-ray photon spectroscopy (XPS), and the surface amount of galactose was indirectly determined by a lectin-binding assay. Rat hepatocytes were cultured onto the surface of various blend films consisting of different amounts of galactosylated PLGA and PLGA. Cell attachment, viability, and function (albumin secretion rate) were investigated by changing the surface amount of galactose as well as by varying PEG chain lengths.

MATERIALS AND METHODS

Materials

PGLA (lactide:glycolide ratio 50:50, RG504H, Mw 45,000) was purchased from Boehringer Ingelheim (Ingelheim, Germany). This polymer has an uncapped free carboxylic acid group in their terminal end. PLGA 75:25 (Medisorb®, Mw 120,000) were purchased from Alkermes (Cincinnati, OH). This polymer was end capped with lauryl alcohol. All polymers were used without further purification. Jeffamine® ED-2,000 (Texaco, Huntsman Co., Austin, TX) and PEG diamine 8,000 (Sigma Chemical Co., St. Louis, MO) were used as received. *N*-(aminobutyl)-O-α-D-glucopyranosyl-(1→4)-D-gluco-amide (AGL) and *N*-(aminobutyl)-O-β-D-galactopyranosyl-(1→4)-D-gluco amide (AGA) were prepared as described previously (Kobayashi and Akaike, 1986). NHS, DCC, and lactobionic acid were purchased from Aldrich (St. Louis, MO). Galactose-specific lectin (RCA_{120}) was obtained from Sigma. Dulbeccos's modified Eagle's medium (DMEM) with 4.5 g/L glucose and insulin was purchased from Gibco (Gaithersburg, MD); epidermal growth factor (EGF) from Collaborative Research (Bedford, CA); and glucagon, hydrocortisone, and 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) from Sigma. Perfusion buffer used to isolate hepatocytes consisted of 154 m*M* NaCl, 5.6 m*M* KCl, 5.5 m*M* glucose, 35 m*M* $NaHCO_3$, and 20 m*M* *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES) (pH 7.4). Hanks' balanced salt solution (HBSS) consisted of 138 m*M* NaCl, 5.4 m*M* KCl, 0.33 m*M* NaH_2PO_4, 0.33 m*M* K_2HPO_4, 0.8 m*M* $MgSO_4$, and 5.6 m*M* glucose (pH 7.4). All other chemicals were of analytical grade.

Preparation of Galactose-Conjugated PLGA

Six grams (0.4 mmol) of uncapped hydrophilic PLGA 50:50 (RG504H) was dissolved in 50 mL of methylene chloride. DCC (165 mg, 0.8 mmol) and NHS (300 mg, 0.8 mmol) were added into the polymer solution under magnetic stirring. The activation reaction of the carboxylic acid end group in PLGA was accomplished for 12 h at room temperature. Insoluble dicyclohexylurea was removed by filtration (Millipore 0.45 μm, HVLP 04700) and the polymer was isolated by precipitation into anhydrous diethyl ether. The activated PLGA (4.3 g, 0.287 mmol) was dissolved in 50 mL of anhydrous DMSO and reacted with AGA (or AGL) (123 mg, 0.288 mmol) for 12 h at room temperature under magnetic stirring. PLGA-AGA (AGL) conjugate was purified by precipitation into anhydrous diethyl ether and vacuum drying.

Preparation of Galactosylated PLGA-PEG Di-block Copolymers

Amine-Terminated PEG-PLGA Di-block Copolymers

PLGA-PEG terminated a primary amine group was synthesized via a coupling reaction between PEG diamine and NHS-activated PLGA. An excess amount of PEG diamine was used to prevent the formation of PLGA-PEG-PLGA tri-block copolymers. The coupling reaction was performed in methylene chloride under magnetic stirring for 9 h at room temperature. PLGA-PEG di-block copolymer was precipitated by slowly dropping into cold ethanol. The collected gel mass was washed with an excess amount of ethanol and dried under vacuum. Molecular weight distributions of synthesized PEG-PLGA di-block copolymers were determined by gel permeation chromatography relative to polystyrene standards. ^{1}H-Nuclear magnetic resonance and differential scanning calorimetry were used to confirm the synthesis of di-block copolymers.

Galactosylation of Aminated PEG-PLGA Di-block Copolymers

A series of PLGA-PEG di-block copolymers (500 mg, 0.01 mmol based on the average molecular weight) and lactobionic acid (35.8 mg, 0.1 mmol) were dissolved in anhydrous DMSO (30 mL). To the solution were added NHS (23.0 mg, 0.2 mmol) and DCC (41.3 mg, 0.2 mmol). The reaction mixture was magnetically stirred for 7 h at room temperature. After an insoluble fraction was eliminated by filtration, the product solution was dispersed in deionized distilled water (200 mL) and freeze-dried.

Preparation and Characterization of Sugar-Derivatized Biodegradable Polymer Surfaces

A total of 3 g of PLGA 75/25 and galactosylated PLGA 50/50 blended in different weight ratios was dissolved in 24 mL of methylene chloride and cast into a glass petri dish (ϕ = 35 mm) then successively dried under a laminar flow at room temperature for 3 days and 24 h

under vacuum. The surface hydration of galactose-derivatized PLGA blend films was characterized by the measurement of water contact angle (n = 5 for each sample) at room temperature. An optical bench-type goniometer (Model 100-0, Rame-Hart, Inc.) was used. Surface atomic compositions of the galactose-derivatized PLGA blend films were evaluated with XPS (ESCALAB MK II, V.G. Scientific Co., UK) which was equipped with an Al K radiation source at 1487 eV and 300 watt at the anode. A survey scan and C_{1s} core-level spectra were taken for analysis.

Galactose-Specific Binding Activity of RCA_{120} on Sugar-Derivatized Biodegradable Polymer Surface

Galactose-specific binding lectin (RCA_{120}) was used for the semi-quantitation of surface galactose (Hatakeyama et al., 1996). Briefly describing, dried PLGA blend films were cut to fit the size of a 96-well tissue culture plate and placed into the wells. Each well was twice rinsed with 10 m*M* Tris-HCl buffer containing 0.15 *M* NaCl (pH 7.5; TBS). One hundred μL of RCA_{120} dissolved in TBS (1 μg/mL; w/v) was added to the wells and incubated for 1 h at 37°C. The unbound lectin fraction was removed and the wells were washed with cold TBS. Surface bound lectin was dissociated by adding 100 μL of supersaturated galactose solution to the wells and incubated for 1 h at 37°C. Afterwards, the solution was transferred to other wells and 200 μL of 2% (w/v) colloidal gold solution was added in each well (Stoscheck, 1987). After a 30-min incubation, absorbance change at 630 nm was measured using a microplate reader (Bio-Rad M550, Hercules, CA). Different amounts of lectin were incubated with a known amount of colloidal gold particles to construct a standard calibration curve.

Isolation of Rat Hepatocytes

Rat hepatocytes were isolated from 40-week-old male Sprague–Dawley rats, weighing ca. 125 g, by a modified procedure of Seglen (1976). Detailed procedures were previously described by Dunn et al. (1991). Briefly, the liver of ether-anesthetized rat was first perfused through portal vein in situ with 200 mL of perfusion buffer containing 1 m*M* EDTA at a flow rate of 25 mL/min. Subsequently, the liver was perfused with 100 mL of 500 μg/mL collagenase in perfusion buffer with 5 m*M* $CaCl_2$. All perfusion buffer was saturated with 95% O_2 and 5% CO_2 by oxygenator during the perfusion. Temperature was controlled at 37°C by heat exchanger during liver perfusion. After perfusion, the swollen liver was transferred to a 100-mm petri-dish containing 15 mL of ice-cold perfusion buffer. Single cells of hepatocytes were prepared by teasing the liver capsule with smooth cell-scraper, and the resulting cell suspension was filtered through nylon mesh of 125-μm grid size (Small Parts, Miami, FL). Cells were washed twice with perfusion buffer by centrifugation at 50 g for 5 min. Viable hepatocytes was separated with Percoll solution containing HBSS. Routinely, about 200 million cells were isolated with ca. 90% viability as judged by trypan blue dye exclusion. Nonparenchymal cells, as judged by their size (less than 10 μm in diameter) and morphology (nonpolygonal or stellate), were less than 1%.

Hepatocyte Culture

PLGA blend films cast in a 35-mm glass petri dish were sterilized by dipping them in 0.22-μm-filtered 70% ethanol solution for 24 h at 4°C. After removing ethanol from the dish, PLGA films were rinsed with culture medium five times. Isolated rat hepatocytes were inoculated to PLGA films on a petri dish. One million viable hepatocytes per dish were seeded evenly with gentle shaking. The culture medium consisted of DMEM supplemented with a hormone mixture of 0.5 units/mL insulin, 7 μg/mL glucagon, 20 ng/mL epidermal growth factor, 7.5 μg/mL hydrocortisone, 200 units/mL penicillin, and 200 μg/mL streptomycin. Two milliliters of culture medium per dish was added and the dishes were incubated at 37°C under 5% CO_2 condition. Collagen-coated polystyrene tissue culture dishes were used as a control group. The dishes were coated with 1 mL of collagen solution (1 mg/mL). Each culture experiment was performed in triplicate.

Attachment Efficiency of the Hepatocytes on the Polymer Surface

After seeding hepatocytes into the dish containing PLGA blend films, suspended cells were harvested at specified time intervals, and cell numbers were estimated by trypan blue dye exclusion. Attached cell number was determined by subtracting the suspended cell number from the initial seeding number. Cell attachment efficiency was then calculated.

Analytical Measurement

Rat serum albumin was measured by enzyme-linked immunosorbent assay (ELISA). Rat albumin standards and anti-rat albumin antibodies were purchased from Cappel Laboratories (Cochranville, PA). The bound antibodies were detected by the conversion of *o*-phenylenediamine, and the absorbance was measured with a microplate reader (Bio-Rad M550, Hercules, CA). Results were reported in picogram albumin per cell per day, and the values were calculated from an average of duplicate ELISA experiments. For each experiment, media were collected from triplicate cultures of hepatocytes. Reagents and detailed procedures of the ELISAs for albumin are described elsewhere. To

measure cell viability, mitochondrial dehydrogenase activity of hepatocytes was determined by using water-soluble enzyme substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma), which was coverted to blue water-insoluble product formazan accumulated in the cytoplasm of viable cells (Mosmann, 1983). In brief, 200 μL of MTT solution (5 mg/mL) was freshly added to culture dish containing 2 mL of fresh medium and incubated at 37°C and 5% CO_2 for 4 h. The intracellular formazan was solubilized using 2 mL of lysing buffer containing 45% dimethyl formamide and 10% sodium dodecyl sulfate. The absorbance of formazan produced was measured at 590 nm with a Bio-Rad microplate reader. Viable cell number was determined using a linear correlation between absorbance and hepatocytes concentration.

RESULTS AND DISCUSSION

Cellular recognition to specific ligands on polymer surface has received much attention in tissue-engineering applications. Surface immobilization techniques of specific ligands for receptor-mediated cell adhesion become very important for molecular design of synthetic biomaterials (Drumheller and Hubbell, 1995). The introduction of cell-recognizable moieties on the polymer surface leads to the facilitation of cell adhesion as well as the enhancement of cell viability and differentiation functions. For hepatocytes, carbohydrate-derivatized polystyrenes, which induced specific interactions between galactose moieties exposed on the polymer surface and asialoglycoprotein receptors on hepatocytes, were used to enhance the adhesion of hepatocytes on the solid surface and subsequently to improve differentiated functions such as albumin secretion (Gutsche et al., 1996b; Kobayashi et al., 1994; Takei et al., 1997). Although extensive studies have been reported regarding sugar-derivatized polymer surfaces for the culture of hepatocytes, most of them were non-degradable polymers (Gutsche et al., 1996b; Kobayashi et al., 1986, 1994; Liang and Akaike, 1998). Thus, it is of interest to investigate galactose-immobilized biodegradable polymer surface for the cultivation of hepatocytes.

Figure 1 shows a synthetic scheme of galactose-derivatized PLGA polymers. Uncapped PLGA has two functional groups, a hydroxyl group and a carboxylic acid group, at its terminal ends. In the present study, the carboxylic acid terminal end of PLGA was first activated with NHS by using DCC and then conjugated to a primary amine group of AGA and AGL, respectively. The conjugation of AGA and AGL to PLGA resulted in the formation of galactose and glucose moiety in the PLGA terminal end, respectively. PLGA-AGL containing a glucose moiety was used as a negative control. Figure 2 shows a synthetic scheme of galatose-derivatized PLGA-PEG di-block copolymers (PLGA-PEG-gal). PLGA-PEG di-block copolymers terminated with a primary amino group (PLGA-PEG-NH_2) were prepared via coupling reaction between PEG diamine derivatives (Jeffamine® ED-2,000 and PEG diamine 8,000) and DCC/NHS-activated PLGA. Excess amount of the

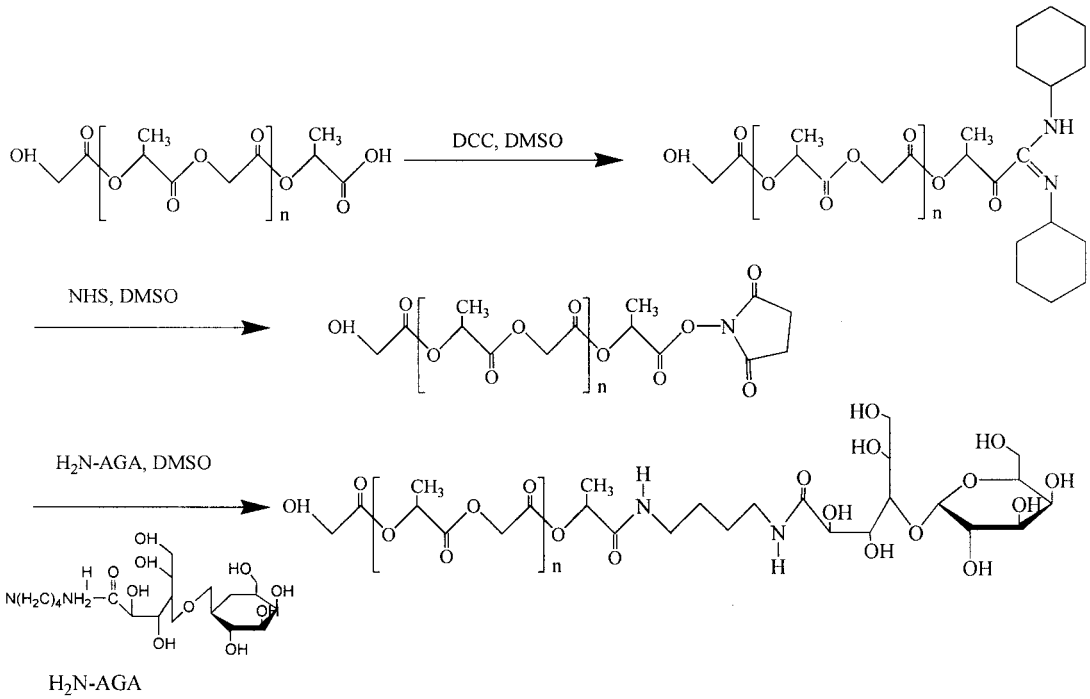

Figure 1. Synthetic scheme of PLGA-AGA.

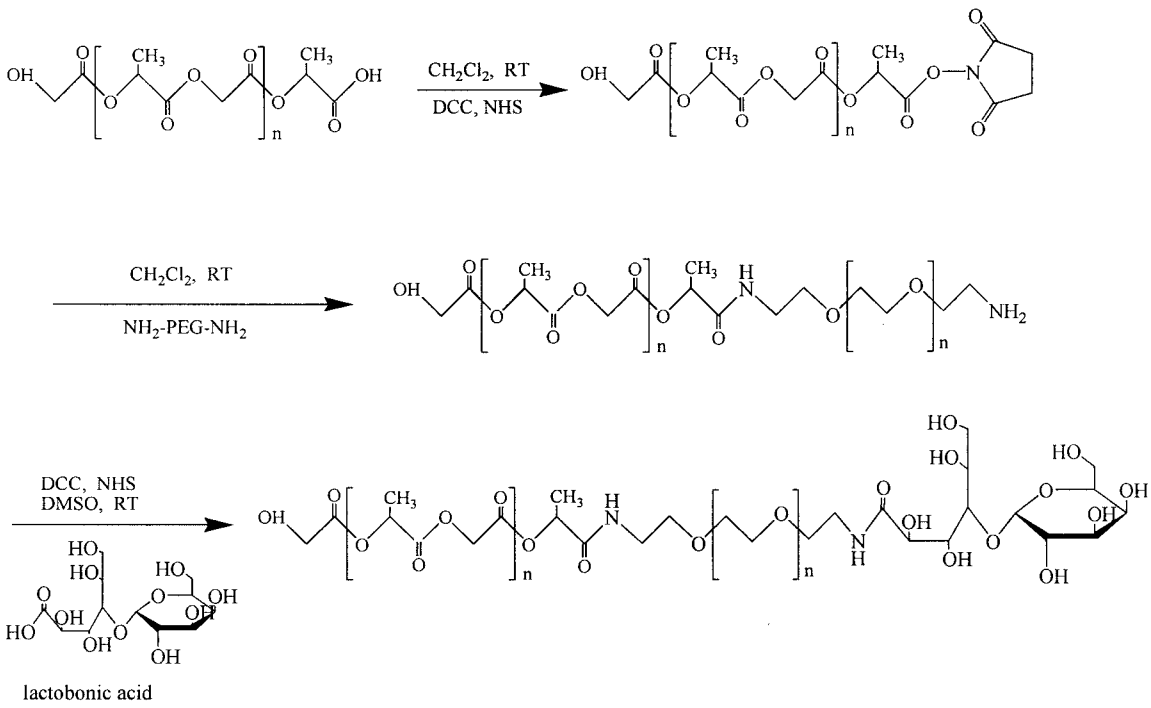

Figure 2. Synthetic scheme of PLGA-PEG-gal.

PEG diamine derivatives was used to suppress the formation of PLGA-PEG-PLGA tri-block copolymers. Gel permeation chromatography and nuclear magnetic resonance analysis demonstrated that the tri-block copolymers were not formed (data not shown). A primary amine group in PLGA-PEG-NH_2 was coupled in anhydrous DMSO with lactobionic acid, of which a carboxylic acid group was pre-activated by using NHS and DCC. PLGA-PEG-gal was dialyzed to remove unreacted reactants and then freeze dried.

A series of polymer blend films for the culture of hepatocytes were prepared by a solvent casting process. They were prepared by blending PLGA-AGA with PLGA in different weight ratios. Because the blending ratio was kept low up to 1% (w/w), there was no apparent change in bulk mechanical properties. The surface characteristics of the blend films were examined by water contact angle and XPS. Water contact angle and XPS results of PLGA-AGA blend films were represented in Table I. Figure 3 shows XPS spectra. It can be seen that the water contact angle value decreases with increasing the amount of PLGA-AGA, suggesting that hydrophilic sugar moieties were exposed onto the surface region of polymer substrates. XPS results additionally indicate that the ratio of ether –C–O (286.6 eV) to alkyl C–H_x (284.6 eV) increases with increasing the amount of AGA-PLGA in the polymer film. The amount of surface exposed galactose on the blend films was semi-quantitatively analyzed by using a specific binding activity of lectin with galactose according to the previous report (Hatakeyama et al., 1996; Kobayashi et al., 1997). As shown in Figure 4, as the blend ratio of PLGA-AGA or PLGA-PEG-gal was increased, the extent of galactose-specific lectin binding was concomitantly increased. The lectin-binding capacity onto galactosylated PLGA surface tended to level-off as the PLGA-AGA blend ratio approached 1%, indicating the saturation of lectin binding on the polymer surface. The blend film containing the PLGA-AGL that had a glucose moiety showed insignificant lectin binding, as expected. The introduction of PEG as a spacer between PLGA and a galactose moiety reduced the extent of lection binding, and the higher molecular weight PEG exhibited more reduced lectin binding. This can be attributed to the facts that PEG chains present onto the surface suppressed the lectin-binding capacity because of their role in repelling protein adsorption and that galactose moieties were sterically buried within them with reduced effective surface concentration.

Table I. XPS results and water contact angle data of PLGA blend films as a function of PLGA-AGA content.

PLGA-AGA content (%)	C–O/C–H ratio	Contact angle (degree)
0	0.33	86.3 ± 5.8
1	0.40 ± 0.01	72.8 ± 3.9
5	0.44 ± 0.03	71.8 ± 3.2

The initial cell attachment behavior of hepatocytes onto the surface of galactose-derivatized blend films was studied as shown in Figure 5. The cell attachment was determined at 3 h after cell seeding. The cell attachment was markedly enhanced with increasing the blend ratio of PLGA-AGA in the PLGA film. Particularly, the cell

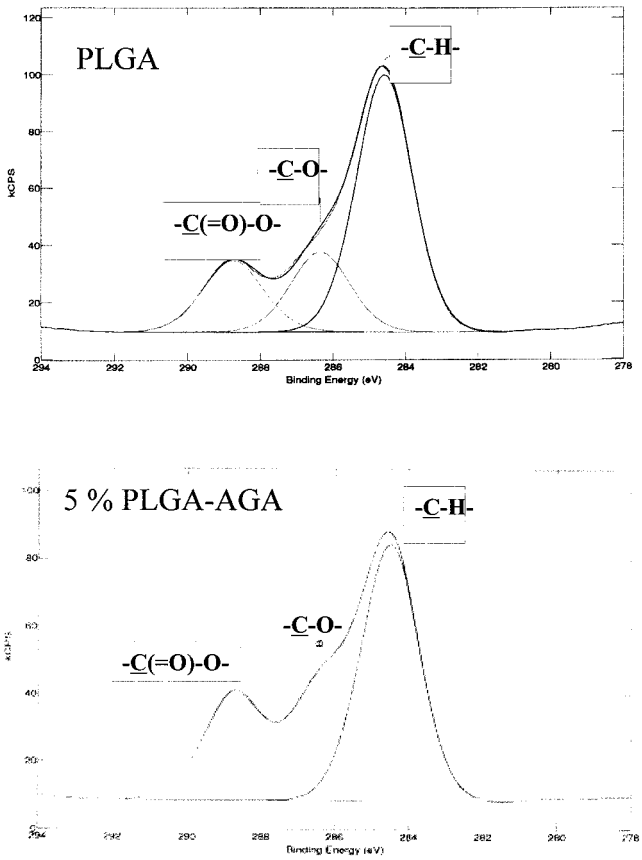

Figure 3. C_{1s} XPS results of PLGA film (top) and PLGA-AGA blend film (bottom).

attachment efficiency reaches about 80% for the blend film having as low as 0.05% of PLGA-AGA blending ratio. In the studies of sugar derivatized non-degradable

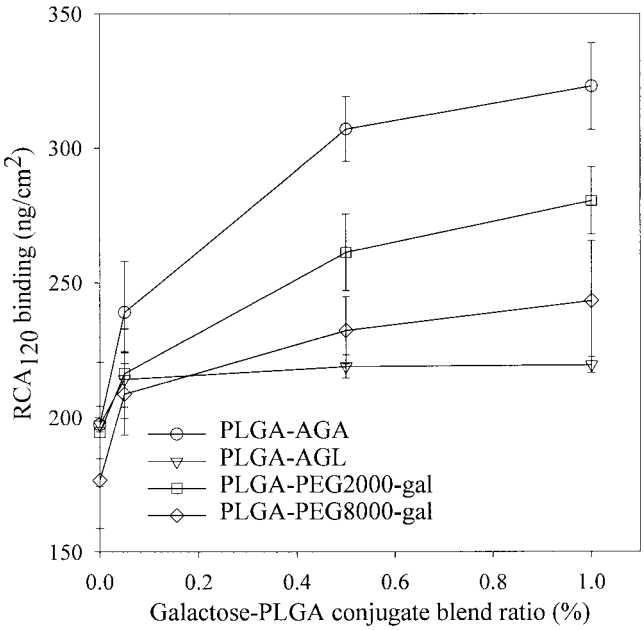

Figure 4. Effect of galactosylated PLGA blend amount on galactose-specific lectin (RCA_{120}) binding to the PLGA blend films.

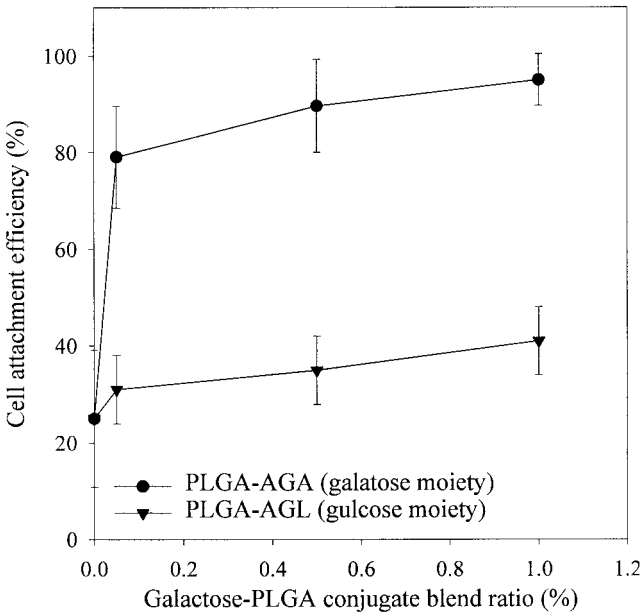

Figure 5. Attachment of hepatocytes on the surface of PLGA blend films having different galactosylated PLGA amounts. Cell number was estimated at 3 h after seeding.

polymer surfaces, it was reported that the concentration of surface exposed galactose on the polymer substrates controlled the adhesion extent of hepatocytes on the surface, and the cell adhesion showed a saturation level above the critical concentration (Blackburn and Schnaar, 1983; Guarnaccia and Schnaar, 1982). In this point of view, the galactosylated PLGA surfaces have a similar saturation effect of surface exposed galactose on the attachment of hepatocytes. In contrast, there were no significant improvements of the cell adhesion onto the PLGA-AGL blend polymer film that was used as a negative control, confirming that glucose moieties exposed on PLGA-AGL blend film did not affect the adhesion of hepatocytes. This experiment directly shows that surface-immobilized galactose onto polymer blend films was indeed responsible for the interaction of galatcose with asialoglycoprotein receptors on cell membrane of hepatocytes. The results agree well with the previous findings that galactose-derivatized non-degradable polymer surfaces enhance the attachment of hepatocytes (Kobayashi and Akaike, 1986; Lopina et al., 1996; Weigel et al., 1979; Weisz and Schnaar, 1991). In vitro rat hepatocyte behaviors were investigated by using four different biodegradable polymers as substrates: PLGA, galactose-derivatized PLGA (PLGA-AGA), and galactose-derivatized di-block copolymers (PLGA-PEG-gal) having different PEG chain lengths (Mw 2,000 and 8,000). Galactose-conjugated PLGA polymers were blended with PLGA at 1% of weight blend ratio. Figure 6 shows the galactose-specific cell adhesion onto PLGA-PEG-gal blend films. As the PEG chain length was increased, the percent of cell adhesion decreased as discussed earlier (Dai and Saltzman, 1996; Han and

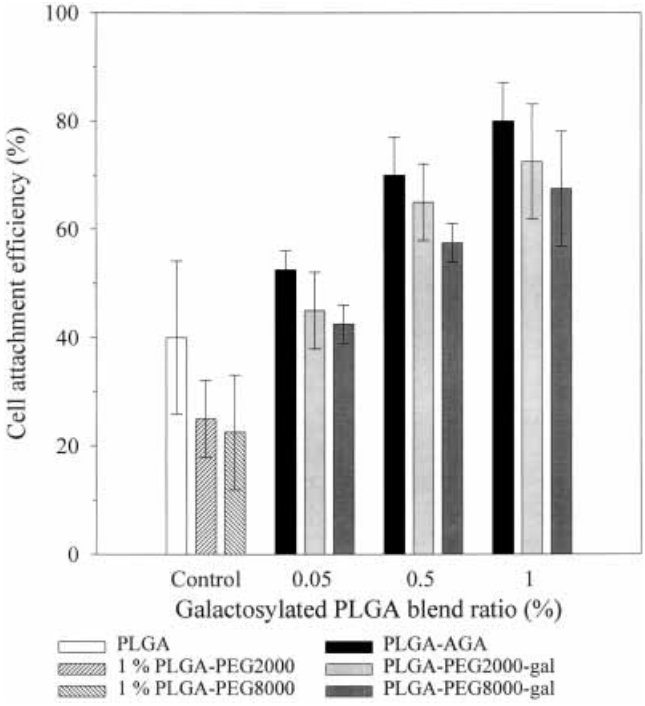

Figure 6. Attachment efficiency of rat hepatocytes cultured on different galactosylated PLGA blend films at 4 h after seeding.

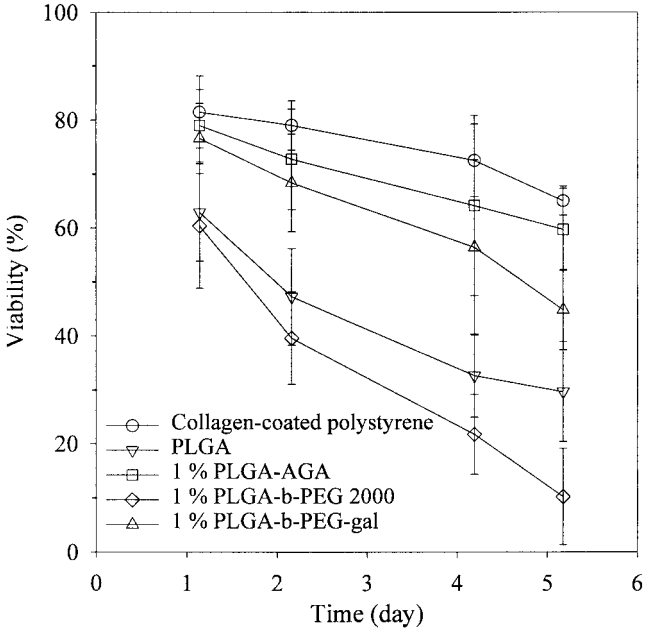

Figure 7. Cell viability of rat hepatocytes cultured on different galactose-derivatized PLGA blend films.

Hubbell, 1997). Although PEG chains exposed on polymer surface reduced cell adhesion, the galactose moieties conjugated to the terminal end groups of PLGA-PEG still induced specific interaction between cells and polymer surface. The extent of cell adhesion was dependent on the presence of PEG spacer and its chain length.

Figure 7 shows cell viabilities of hepatocytes attached on various galactose-derivatized PLGA films. Cell viability of collagen-coated polystyrene surface was maintained at about 70–80% of initial viable cell number. The collagen-coated polysyrene was used as a control because cell adhesive ligands such as arginine-glycine-aspartic acid are abundantly present in collagen. Viabilities of cells cultured onto the galactose-derivatized PLGA surface could be maintained to a much greater extent than those cultured onto the polymer films containing either PLGA or PLGA-PEG. Furthermore, cell viability depends on the blending amount of galactose-derivatized PLGA in the polymer blends as shown in Figure 8. With increasing the galactose-derivatized PLGA amount in the polymer blends, cell viability increased as a function of culture time.

Albumin secretion rates of hepatocytes cultured onto various polymer surfaces were measured (Fig. 9). The albumin secretion rate for the films of collagen-coated polystyrene, PLGA, and PLGA-PEG was progressively reduced as a function of time because of de-differentiation of hepatocytes as a result of cell proliferation as reported previously (Cima et al., 1991; Elsdale and Bard, 1972). The PLGA-PEG 2000 blend film shows a lower albumin secretion rate than the PLGA film because of the PEG spacer effect on cell attachment and viability as described previously (Dai and Saltzman, 1996). On the other hand, galactose-derivatized PLGA blend films exhibit an increasing albumin secretion rate with culture time up to 4 days. This was likely to be caused by the formation of cell aggregates cultured onto galactose-derivatized PLGA surface. It was reported that galactose immobilized polymer surface induced the

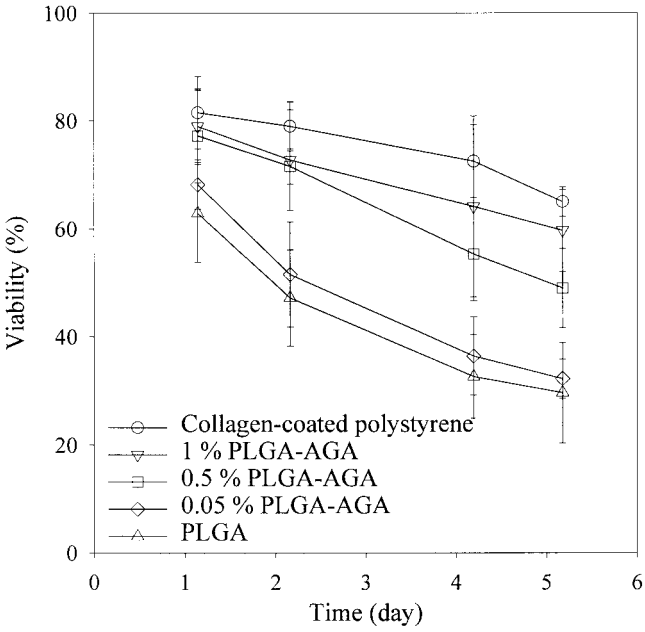

Figure 8. Cell viability of rat hepatocytes culutred on galactosylated PLGA with different galactose-PLGA conjugate blend ratios.

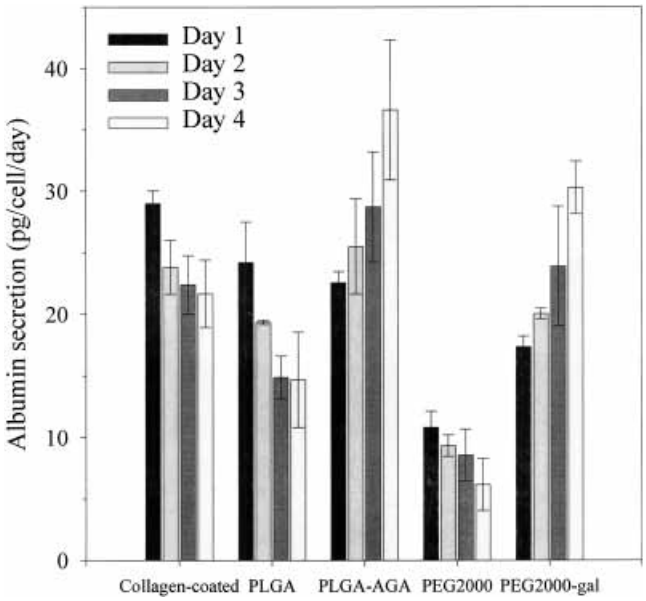

Figure 9. Albumin secretion rate of rat hepatocytes cultured on galactosylated PLGA blend films.

formation of spheroidal aggregates of hepatocytes (Gutsche et al., 1996a, 1996b; Kobayashi and Akaike, 1986; Murata et al., 1997). Figure 10 demonstrates an aggregated form of hepatocytes cultured on the galactose-derivatized polymer surface. The formation of hepatocyte aggregates has been also reported by many investigators (Koide et al., 1989; Landary et al., 1985). The aggregates, spheroids, which were often observed on the surface of sugar-derivatized polystyrene and within porous non-degradable polymer scaffolds, showed an increased albumin secretion rate (Gutsche et al., 1996a; Yagi et al., 1997). The culture of hepatocytes onto collagen-coated polymer surfaces showed spread cell morphologies with decreased albumin secretion rates. Different albumin secretion rates of hepatocytes were attributed to the changes in cytoskeletal structure of the cells cultured on collagen and sugar-derivatized polystyrene (Gutsche et al., 1996b; Oka and Weigel, 1986; Powers and Griffith-Cima, 1996). From this standpoint of view, the galactose-derivatized PLGA surface similarly induced specific cellular recognitions to immobilized ligands on the polymer surface. Therefore, galactose derivatization promoted cell-polymer interaction as well as the cell-cell interaction and enhanced the differentiated functions of hepatocytes in spheroidal aggregates. It should be noted that the albumin secretion rates observed in this study were relatively low compared to those in vivo (~140 pg/cell/day) or other culture systems (Cima et al., 1991; Glicklis et al., 2000; Gutsche et al., 1996a, 1996b; Kaufmann et al., 1997; Peters and Peters, 1972;). A major goal of the current study was to demonstrate the enhanced cell attachment and function onto the surface-modified PLGA films based on the conjugates of PLGA and a cell-specific

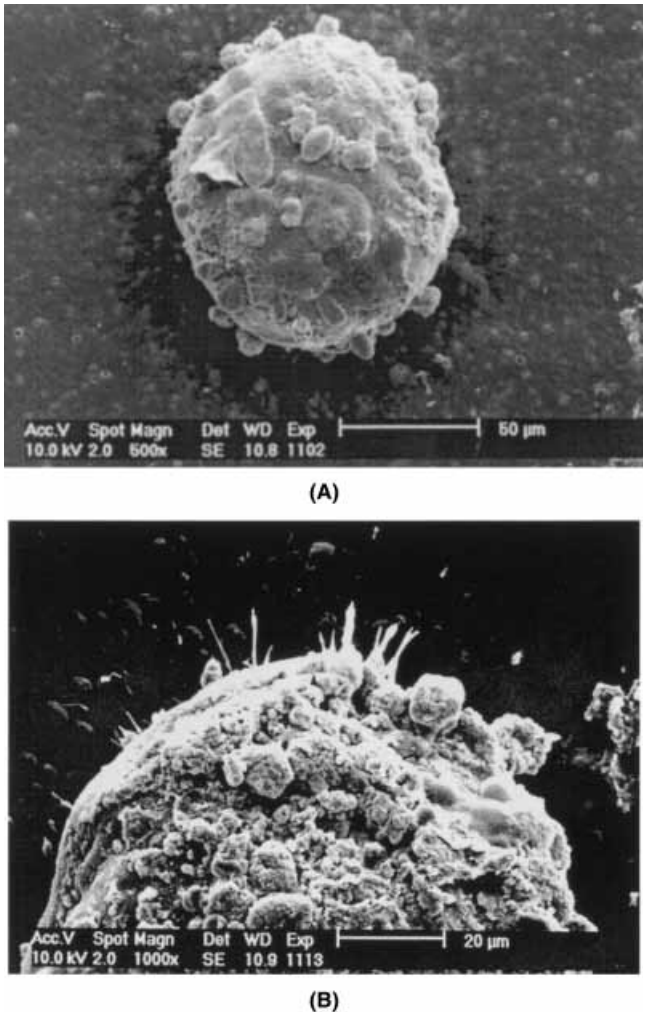

Figure 10. Scanning electron microscopic images of rat hepatocytes cultured on galactosylated PLGA film after 5-day culture periods. (A) spheroid on 1% (w/w) PLGA-AGA blended PLGA film and (B) magnified image of the spheroid.

ligand in a two-dimensional culture system, not to compare the functional activity of hepatocytes with other recently reported three-dimenisonal culture systems. Quite recently, surface-modified macroporous PLGA scaffolds were fabricated according to the gas foaming/salt leaching method (Nam et al, 2000; Park, 2002) for cultivating hepatocytes in a perfusion reactor under optimized conditions. The preliminary results showed that the albumin secretion rate was maintained over 80 pg/cell/day over a 1-week period, suggesting that the functional activity of hepatocytes can be improved dramatically by combining the surface modification strategy with an optimized three-dimensional culture system.

In conclusion, it was demonstrated that targeting moieties against receptors of a specific cell type could be introduced onto biodegradable polymer surface by directly conjugating the targeting ligand to the terminal end of PLGA. By blending it with unconjugated PLGA, sufficient surface orientation of the hydrophilic ligands

into the aqueous medium could be achieved for inducing specific cell-polymer interaction. The surface modification approach using a di-block copolymer of PLGA-PEG-ligand was not as effective as that using a PLGA-ligand conjugate. It would be necessary that the introduction of PEG between the ligand and PLGA should be carefully designed by controlling the chain length of PEG to optimize the cell attachment. The use of PLGA-PEG di-block copolymer as a blend additive to PLGA may be a useful way for inducing the selective cell adhesion of a specific cell type on the polymer surface when co-cultured with other cells. It can be expected that various hydrophilic cell receptor recognizing ligands such as small oligopeptides can be conjugated to PLGA in a similar way to create surface immobilized bioactive scaffolds for tissue regeneration. Furthermore, this method can be potentially applied for the surface modification of internal pores within three-dimensional biodegradable scaffolds for efficient cell adhesion and function.

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