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Polymer 47 (2006) 4348–4358
polymer
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Synthesis and characterization of a novel glycoconjugated macromolecule

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Received 28 September 2005; received in revised form 17 March 2006; accepted 2 April 2006
Available online 2 May 2006

Abstract

In this study, synthesis and characterization of a new galactosylated chitosan with high affinity to HepG2 (a liver cancer cell line) were reported. We designed the novel glycoconjugated macromolecule using chitosan to be grafted with branched galactose units. The branch-type of galactosylated chitosan was prepared by firstly introducing L-lysine spacer arms to chitosan, followed by covalent coupling of lactobionic acid with the lysine spacer to provide chitosan with multivalent galactose units. Essential properties of the branch-type of galactosylated chitosan measured from X-ray diffraction, mechanical and dynamic mechanical thermal analysis suggested that the introduction of branched galactosyl groups decreased the crystallinity and thermal stability of chitosan. Introducing galactosyl groups also resulted in a great enhancement of hydrophilicity, consequently increased the antibacterial capability of chitosan. The novel glycoconjugated chitosan possessed especially higher binding efficiency toward galectin-1 (Gal-1), a galactose-binding lectin, due to its multivalent galactose units. HepG2 cells were cultured onto the surface of the novel galactosylated chitosan films to examine the cell attachment and proliferation. Observation from the cell culture basically demonstrated that adjusting the length of spacer arms between the galactose units and macromolecular backbone led to a significant change in the HepG2 cells attachment and proliferation. The results suggest that the new chitosan derivative with branched galactose units may had a specific interaction with HepG2 cells via ligand–receptor recognition.

Keywords: Chitosan; Galactosyl group; Glycoconjugated macromolecule

1. Introduction

Macromolecules have been extensively applied in the biomedical field for drug-delivery purpose [1]. The macromolecule–drug incorporation could provide time-specific and site-specific drug delivery through the modification of polymers with stimuli-responsive functional groups or specific tissue-targeting ligands [2–7]. Glycotargeting was newly found to exploit the highly specific interactions of endogenous lectins with carbohydrates [8]. Covalently modification of macromolecules with β-D-galactose units resulted in significantly higher drug levels in the liver than those obtained without using the galactose–macromolecule conjugated drug delivery system [9–10].

Many types of the glycoconjugated macromolecules have been developed for targeting a drug to its proposed site of action [11–18]. Among them, the galactose–chitosan conjugate is one type of the glycoconjugated macromolecules attracting great attentions. Chitosan is a polysaccharide formed primarily of repeating units of β-(1-4)2-amino-D-glucose (glucosamine) and β-(1-4)2-acetamido-D-glucose (*N*-acetylglucosamine). Being a non-toxic, biocompatible and biodegradable polymer, chitosan has been investigated for pharmaceutical and biomedical applications [19–22]. Reductive amination has been used to prepare glycosylated chitosan. The product prepared by introducing a galactosyl unit to lactose, only by an acyclic modification, was poorly recognized by lectins [23]. Similarly, chitosan was also reacted with lactobionic acid to introduce galactosyl unit to chitosan [24–27]. The galactosylated chitosan was used as a gene carrier, allowed successful

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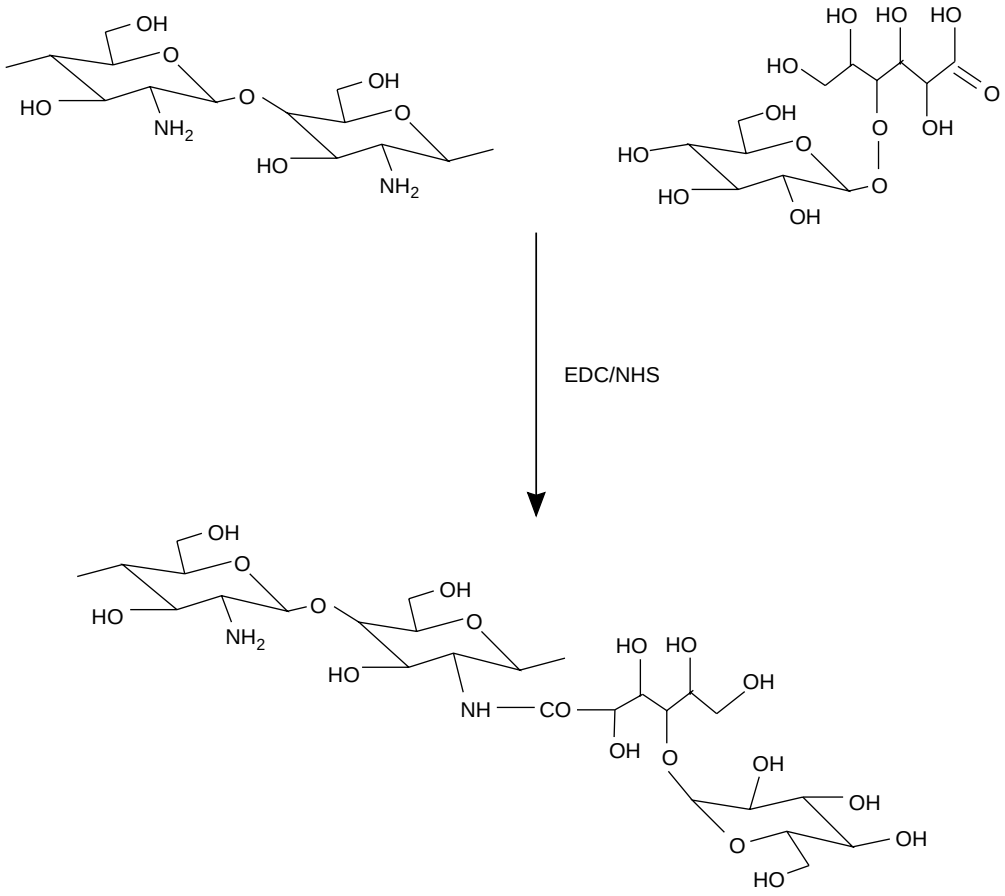
doi:10.1016/j.polymer.2006.04.005



Scheme 1. Synthesis route of gal-chit.

transfection into HepG2 liver cell lines [27]. Additionally, the galactosylated chitosan scaffold has been developed as a synthetic extracellular matrix for hepatic tissue-engineering application [28].

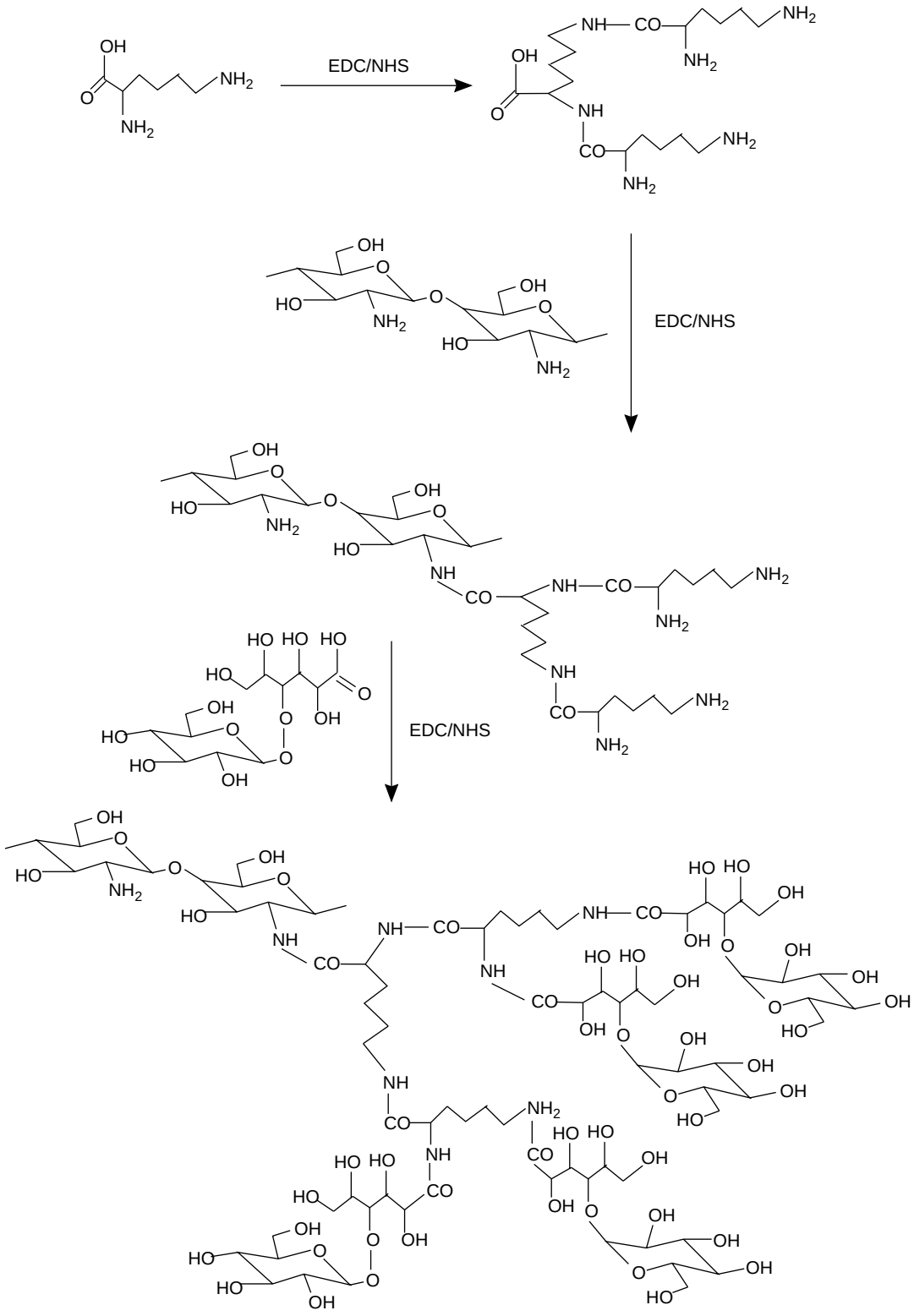
Asialoglycoprotein receptors (ASGPR) expressed by liver parenchymal cells showed strongly binding efficiency with galactose. The targeting of glycoconjugated macromolecules to hepatocyte was mediated by the specific galactose–ASGPR interactions between ASGR of the hepatocytes and galactose residues of the glycoconjugated macromolecules [29]. Gal-1 was a galactoside-binding lectin that can organize cell surface glycoproteins through multivalent binding and cross-linking of terminal or polymeric lactose or *N*-acetyllactosamine residues. Gal-1 and some other galactoside-binding lectins possess the ability to mediate cell adhesion, signaling, and apostasies [30–31]. As reported by some literatures, Gal-1 played multiple roles in cancer and was believed to be important for aggregating cancer cells into tumor emboli [32]. Synthetic multivalent ligands for Gal-1 were recently found to have the potential to act as cancer diagnostics and therapeutics [33]. Perhaps, an effective glycoconjugated macromolecule based on galactose–chitosan conjugation can be designed to deliver drugs to cancerous hepatoma cells.

Based on the knowledge that branched galactosyl units seems to exhibit the 'cluster effect' demonstrating especial higher binding strength with ASGPR [34], we describe the preparation of a novel galactosylated chitosan with branched galactosyl groups as targeting moieties to HepG2 cells (a liver cancer cell line). The branch-type of galactosylated chitosan was prepared by firstly introducing a branched lysine spacer to chitosan, followed by covalent coupling of lactobionic acid with the lysine spacer to providing chitosan with multivalent galactose units. Such a new type of galactosylated chitosan provides a template with branched galactosyl groups for the multivalent display of galactose. Essential properties of the branch-type of galactosylated chitosan, in a film type, were examined by FT-IR analysis, X-ray diffraction, swelling study, antibacterial test, mechanical and dynamic mechanical thermal analysis. The efficiency of Gal-1 binding to the chitosan films, after being grafted with multivalent galactosyl ligands, was examined by an immunostaining method. Furthermore, cancerous hepatoma cells were cultured onto the surface of the novel galactosylated chitosan films to examine the effect of grafted multivalent galactosyl ligands on the hepatcoyte attachment and proliferation.

2. Experimental

2.1. Materials

Chitosan ($M_W \sim 2.5 \times 10^5$) with a degree of deacetylation of approximately 85% were purchased from Fluka Chemical Co.



Scheme 2. Synthesis route of gal-lys-chit.

(Switzerland). Lactobionic acid was purchased from TCI (Tokyo, Japan). 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and *N*-hydroxylsuccinimide (NHS) were purchased from Sigma Chemical Co. (USA). All other reagents and solvents used were of reagent grade.

2.2. Synthesis of galactosylated chitosan and branch-type of galactosylated chitosan films

The synthesis of galactosylated chitosan and branch-type of galactosylated chitosan was performed as shown in

Schemes 1 and 2, respectively. A stock solution of chitosan in aqueous acetic acid (1.5% by w/v) was prepared by dissolving 1.5 g of chitosan in 100 ml aqueous acetic acid (0.5% by v/v) and stirring for 12 h at room temperature. The dissolved chitosan solutions were sonicated to remove the air bubbles and poured into shallow dishes and dried in air at room temperature for 3 days for the preparation of chitosan films (chit). The synthesis of branched lysine spacer was performed before introducing galactosyl units to chitosan films. L-lysine with protected amino groups (Lys_p) was dissolved in deionized water and was reacted with an equivalent molar ratio of EDC/NHS for 24 h to activate the carboxylic acid groups on L-lysine. In this study, *t*-Boc (*tert*-butyloxycarbonyl group) was used for protecting the amino groups on L-lysine. Afterward, a half molar ratio of L-lysine with reactive amino groups (Lys_a) was added to the aforementioned Lys_p/EDC/NHS mixing solution for covalently coupling of the amino groups on Lys_a with the carboxylic acid groups on Lys_p. After 24 h of reaction, the branched lysine spacer was synthesized through the formation of amide bonding. The synthesis process was shown in Scheme 1.

Coupling of branched lysine spacer with the chitosan film was performed using EDC as a coupling agent. The protected amino groups on the branched lysine spacer were deprotected in acidic condition. The chitosan film was immersed in *N*-hydroxylsuccinimide (NHS) buffer solution (pH 4.7) with a mixture of EDC. Subsequently, the branched lysine spacer was added into the solution at an equivalent molar ratio to chitosan. The reaction was performed for 24 h at room temperature to obtain chitosan films with the branched lysine spacer (noted as lys-chit). The resulting product was extensively washed with PBS to remove residual reagents. Introduction of galactosyl unit to the branched lysine spacer on chitosan film was performed as aforementioned process. The chitosan film with branched lysine spacer was immersed in *N*-hydroxylsuccinimide (NHS) buffer solution with a mixture of EDC. Subsequently, lactobionic acid was added into the solution at an equivalent molar ratio to the amino groups on the branched lysine spacer. The reaction was performed for 24 h at room temperature to obtain a branch-type of galactosylated chitosan film (noted as gal-lys-chit). The resulting product was extensively washed with PBS and readied for the binding of Gal-1.

A traditional galactosylated chitosan derivate (noted as gal-chit) was prepared by directly coupling lactobionic acid with chitosan according to the method reported [28]. The synthesis process was shown in Scheme 2. The physical, chemical and biological properties of the aforementioned, branch-type of galactosylated chitosan (gal-lys-chit) film were compared with the traditional galactosylated chitosan (gal-chit) and chitosan (chit) films. The free amino groups of chit, gal-chit, lys-chit and gal-lys-chit were determined by ninhydrine assay using glucosamine standard for the estimation of substituted galactose contents [24].

2.3. FT-IR analysis

FT-IR analysis was conducted by, respectively, mixing the powder form of chitosan (chit), galactosylated chitosan (gal-chit) and branch-type of galactosylated chitosan (gal-lys-chit) with KBr (1:100). The mixed powder then was pressed into a disk and analyzed by a FT-IR spectrometer (Perkin–Elmer Spectrum RXI FT-IR System, Buckinghamshire, England).

2.4. SEM studies

The prepared chitosan (chit), galactosylated chitosan (gal-chit) and branch-type of galactosylated chitosan (gal-lys-chit) films were attached onto a double-sided adhesive tape and fixed to an aluminum stage, respectively. The films were sputter-coated with gold in a thickness of 500×10^{-8} cm using a Hitachi coating unit (IB-2 coater). Subsequently, the morphologies on the surface of the films were examined using a Hitachi S-2300 scanning electron microscopy.

2.5. X-ray diffraction

The diffraction patterns of the chitosan (chit), galactosylated chitosan (gal-chit) and branch-type of galactosylated chitosan (gal-lys-chit) films were recorded using a Shimadzu XD-5 diffractometer with Cu Kα radiation.

2.6. Swelling study

The swelling capacities of the chitosan (chit), galactosylated chitosan (gal-chit) and branch-type of galactosylated chitosan (gal-lys-chit) films were determined by soaking the films in PBS buffer solution at room temperature, respectively. At specific time intervals, the samples were removed from the swelling medium and were blotted with a piece of paper towel to absorb excess water on the surfaces. The swelling ratios of the chit, gal-chit and gal-lys-chit films were calculated from the formula

$$E_{sw} = \left[\frac{(W_e - W_o)}{W_o}\right]$$

where E_{sw} is the swelling ratio of the chit, gal-chit or gal-lys-chit film at equilibrium. W_e denotes the weight of the chit, gal-chit or gal-lys-chit film at equilibrium swelling ratio and W_o is the dry weight of the chit, gal-chit or gal-lys-chit film.

2.7. Mechanical test

In the mechanical property measurement, the chitosan (chit), galactosylated chitosan (gal-chit) and branch-type of galactosylated chitosan (gal-lys-chit) films were cut from each studied group using a razor blade. Stress–strain curve of a test film was determined by an Instron 4201 at a constant speed of 50 mm/min. The strain-at-fracture was taken as the percent strain at the point of fracture, while the

ultimate-tensile-strength was taken as the force at which fracture occurred divided by the initial cross-sectional area.

2.8. Dynamic mechanical thermal analysis

Dynamic mechanical thermal analysis (DMTA) was performed with a DMA-2980 (TA Instrument). Dynamic mechanical properties were measured from −100 up to 250 °C while heating the sample at 5 °C min^{-1}. Film stripes of about $15\times5\times0.1$ mm^3 were tested at a frequency of 5 Hz, with a displacement of 0.015 mm and an initial static pre-stress of about 2.0 MPa.

2.9. Binding of Gal-1 to galactosylated chitosan and branch-type of galactosylated chitosa films

To immobilize Gal-1 proteins onto the chitosan (chit), galactosylated chitosan (gal-chit) and branch-type of galactosylated chitosan (gal-lys-chit) films, the various films were incubated with recombinant Gal-1, respectively. The sterilized chit, gal-chit and gal-lys-chit films were incubated with phosphate buffer saline (PBS) containing Gal-1 for 24 h at 4 °C. Afterward, the films were extensively washed with PBS and readied for immunostaining assay.

2.10. Immunostaining of Gal-1 binding to galactosylated chitosan and branch-type of galactosylated chitosa films

The efficiency of Gal-1 binding to the surface of chitosan (chit), galactosylated chitosan (gal-chit) and branch-type of galactosylated chitosan (gal-lys-chit) films, were determined by using monoclonal Gal-1 antibodies. The Gal-1 binding to chit, gal-chit and gal-lys-chit films, were washed three times with PBS containing 0.05 Tween 20 (PBST) and reacted with PBS containing 5% skim milk for 1 h. After washing with PBST, the chit, gal-chit and gal-lys-chit films were reacted with Gal-1 monoclonal antibodies overnight at 4 °C. At the end of incubation, the chit, gal-chit and gal-lys-chit films were washed with PBST as described above and incubated with alkaline phosphatase-conjugated anti-mouse Ig (Kirkegarrd and Perry Laboratories Inc.,) at room temperature for 1 h. After extensively washing with PBST for 5 times, the chit, gal-chit and gal-lys-chit films were immersed in a substrate solution (bromochloroindoyl phosphate/nitro blue tetrazolium) for color development. To determine the color development of the films, whiteness of all films was measured in term of the whiteness index denoted by WI, as recommended by AATCC test method 110. A spectrophotometer, Dataflash®100, was employed in this experiment with illuminant D65 and a 10 [degrees] observer. WI of the white calibration standard 4786 was zero and was used as a control to determine the WI of the films. Higher values of WI indicate greater whiteness, in contrast, lower values of WI indicate greater darkness.

2.11. Antibacterial test

In the antibacterial assay, 16-mm-diameter test samples cut from the sterilized chitosan (chit), galactosylated chitosan (gal-chit) and branch-type of galactosylated chitosan (gal-lys-chit) films were placed on the bottoms of the wells in a 24-well plate (the diameter of each well is about 16 mm). Subsequently, 50 μl of bacterial broth culture was seeded onto the chit, gal-chit and gal-lys-chit films (10^6 CFU/ml), respectively. The bacterial broth cultured in the well without containing any films was used as control. The bacteria used for the test were *Escherichia coli* and *Staphylococcus aureus*. Subsequently, the films were put in the moisture incubator and incubated at 37 °C. After 4 h of incubation, each chit, gal-chit or gal-lys-chitn film was placed into test tubes containing 1 ml PBS and sonicated for 75 s in an ultrasonic washer (64 kHz). Subsequently, 50 μl of the incubated medium taken out from each tube was seeded on agar plates containing nutrient broth and incubated at 37 °C for 24 h. Finally, the units of colony formation in each agar plate were calculated to examine the antibacterial ability of each chit, gal-chit or gal-lys-chitn films.

2.12. HepG2 cells attachment and proliferation

HepG2 cells were seeded in 24-well plates, respectively, containing chit, gal-chit and gal-lys-chit films at a density of 1×10^5 cells/well with 1 ml of complete medium (DMEM containing 10% FBS, supplemented with 2 mm L-glutamate, 100 units/ml penicillin and 100 g/ml streptomycin). The HepG2 cells were cultured in a humidified atmosphere at 37 °C and 5% CO_2. After indicated incubation time, the plates were washed with PBS three times and the medium containing unattached HepG2 cells was collected. The number of unattached HepG2 cells was determined by a hemocytometer. The cell attachment efficiency was calculated from the number of attached cells on the surface and the seeding density. The morphology of attached cells on different films was observed under an inverted microscope with phase-contrast optics (Olympus CK30, Tokyo, Japan).

3. Results and discussion

It has been reported that normal cells containing markedly reduced levels of Gal-1 failed to grow on chitosan film. Gal-1 is overexpressed in cancer, and plays important role to increase malignant cell survival in circulation. Multivalent carbohydrate ligands for Gal-1 have the potential to act as cancer diagnostic and therapeutics. In this study, a branch-type of galactosylated chitosan was prepared and used as a multivalent carbohydrate ligand to bind with Gal-1. Afterward, we will examine the attachment and proliferation of HepG2 cells on the chitosan, traditional galactosylated chitosan and branch-type of galactosylated chitosan films for the purpose of targeting anticancer drugs to HepG2 cells, in future.

Schemes 1 and 2 schematically illustrated the procedures for the synthesis of a traditional galactosylated chitosan and a branch-type of galactosylated chitosan. The traditional

galactosylated chitosan derivate (gal-chit) was prepared by directly coupling lactobionic acid with chitosan. To prepare branch-type of galactosylated chitosan, branched lysine spacers were synthesized and were conjugated with chitosan to produce chitosan derivate with branched spacer arms. Afterward, lactobionic acid was introduced to the branched lysine spacer on chitosan to produce the branch-type of galactosylated chitosan (gal-lys-chit). Fig. 1 showed the FT-IR spectra of gal-chit and gal-lys-chit. The spectrum of chitosan displayed peaks around 905 and 1150 cm^{-1} of assigned saccharide structure, and an amide characteristic peak at 1650 cm^{-1} as well as an amino characteristic peak at around 1570 cm^{-1}. The spectrum of gal-chit showed strong peaks around 1150 cm^{-1}, suggesting the successful introduction of galactosyl unit to chitosan (Fig. 1(a)). The spectrum of the chitosan derivate with lysine spacer displayed strong peaks around 1650 (amide I) and 1590 cm^{-1} (amide II) of amide bond arising from the consideration of amine group of chitosan and carboxylic acid on lysine. Intensity of the peak at 1150 cm^{-1} assigned to saccharide structure increased after introducing galactosyl unit to the chitosan derivate with lysine spacer, suggesting successful synthesis of the novel galactosylated chitosan (Fig. 1(b)). By determining the free amino groups of chit, gal-chit, lys-chit and gal-lys-chit using ninhydrine assay, the galactose contents on chit, gal-chit, lys-chit and gal-lys-chit could be estimated. The substitution values of galactose coupled with gal-chit and gal-lys-chit films were 0.16 ± 0.034 galactose/glucosamine unit in gal-chit and 0.18 ± 0.047 galactose/glucosamine unit in gal-lys-chit, respectively.

Fig. 2 showed SEM micrographs of chit, gal-chit and gal-lys-chit films. It was found that the surfaces of chit, gal-chit and gal-lys-chit films are all very smooth. Fig. 3 showed XRD

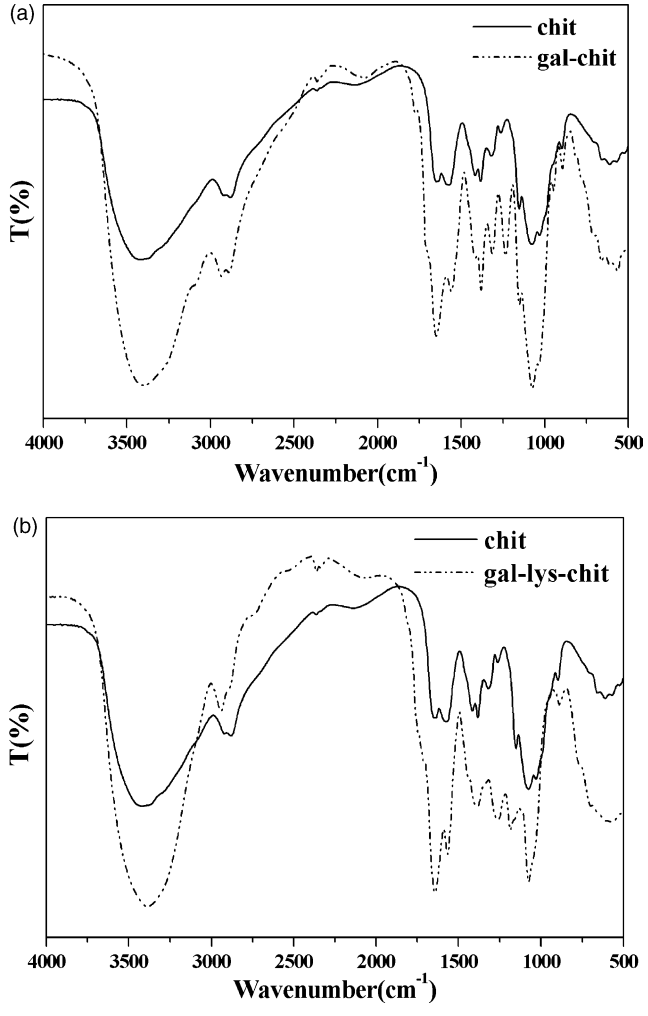


Fig. 1. FT-IR spectra of the chit, gal-chit and gal-lys-chit: (a) gal-chit films; (b) gal-lys-chit films.

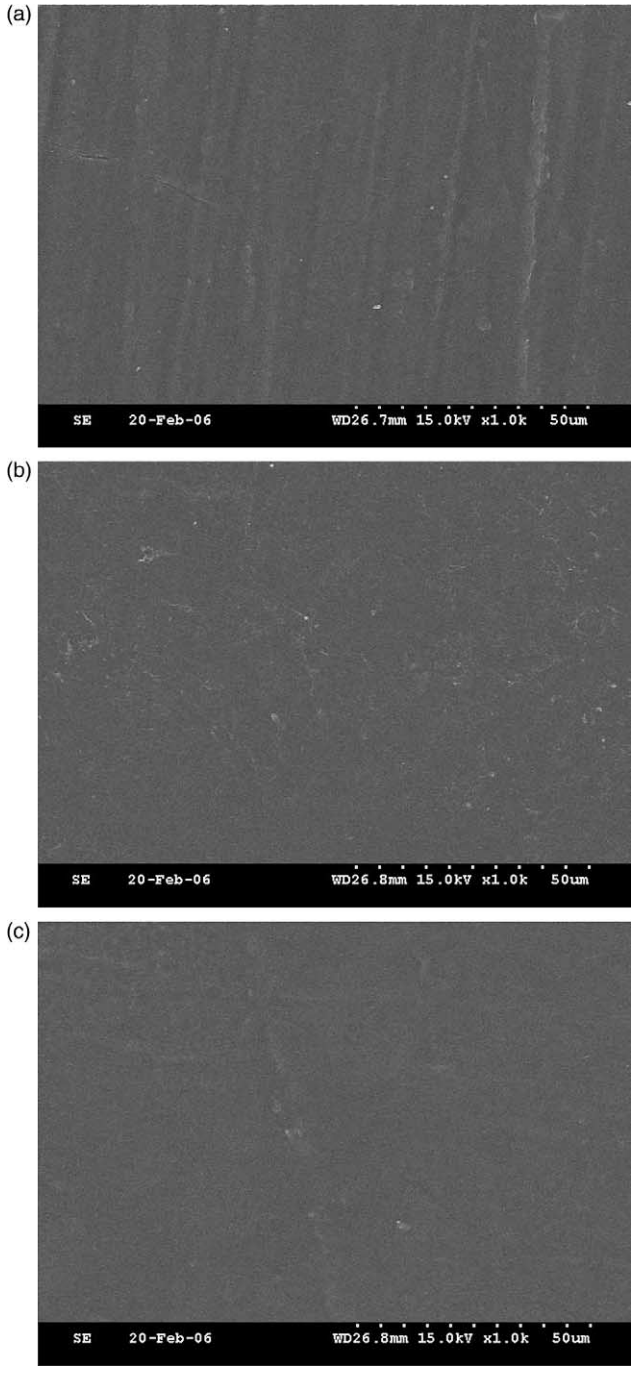


Fig. 2. SEM micrographs of chit, gal-chit and gal-lys-chit films.

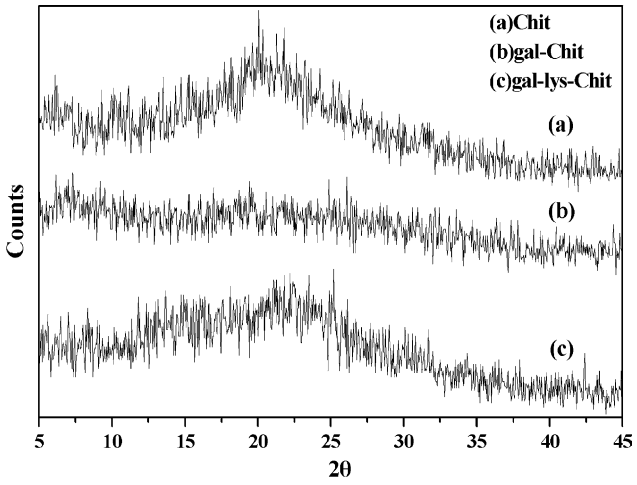


Fig. 3. XRD patterns of the chit, gal-chit and gal-lys-chit films.

patterns of chit, gal-chit and gal-lys-chit films. The peak found around 20° was assigned to chitosan chains aligned through intermolecular interactions. The peak became weaker in the galactosylated chitosan (gal-chit and gal-lys-chit) films. The weakening of the crystalline peaks induced by introducing galactosyl groups to chitosan films implies the disruption of chitosan crystal by the introduced galactosyl units. In chitosan crystal, chitosan molecular chains were packed in an antiparallel fashion to make a sheet structure. Introduction of a large subsidiary group, the galactosyl unit, on the backbone of chitosan led to decrease the crystallinity of chitosan macromolecule. Fig. 4 showed the swelling ratio of gal-chit and gal-lys-chit films. Chitosan film exhibited a low degree of swelling. However, the gal-chit and gal-lys-chit films significantly swelled, forming a soft gel easily broken. This result can be due to the reduced crystallinity of the gal-chit and gal-lys-chit films, as well as due to the introduction of the hydrophilic galactosyl groups.

The curves of tan δ of gal-chit and gal-lys-chit films were shown in Fig. 5. A damping peak was found for the chitosan films conjugated with lysine spacer at around 250 °C. Chitosan film was more heat-resistant and demonstrated the damping

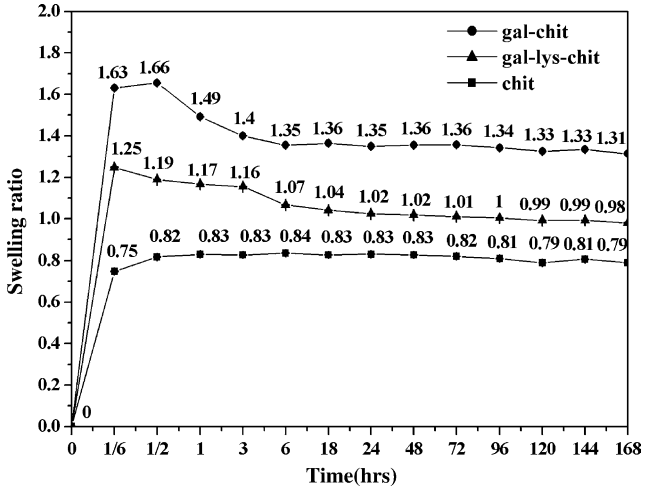


Fig. 4. Swelling ratios of the chit, gal-chit and gal-lys-chit films.

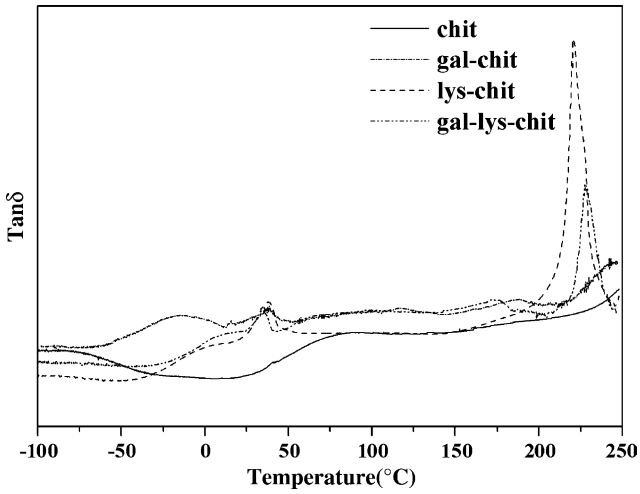


Fig. 5. Curves of tan δ of the chit, gal-chit and gal-lys-chit films.

peak higher than 250 °C. As compared with the chitosan film (chit), the damping peak for the film conjugated with branched lysine spacer (chit-lys) and branched galactosyl units (gal-lys-chit) appeared at the temperatures lower than 250 °C. This

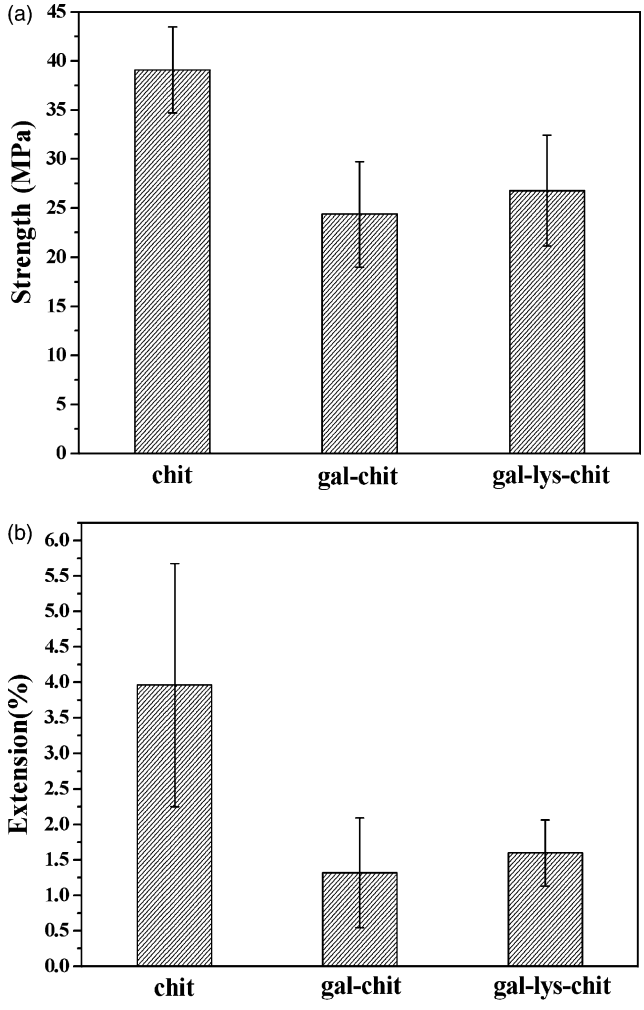


Fig. 6. Tensile strength and break extension of the chit, gal-chit and gal-lys-chit films.

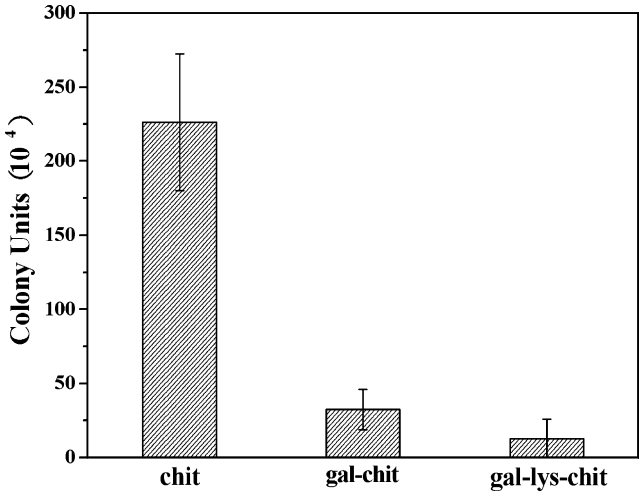


Fig. 7. Numbers of colony of *E. coli* on the chit, gal-chit and gal-lys-chit films.

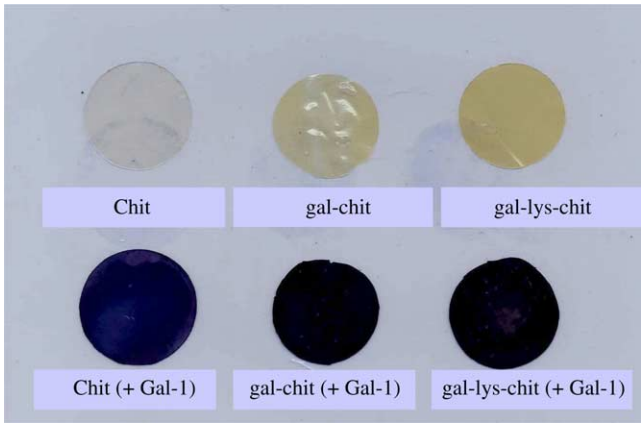


Fig. 8. Whiteness index (WI) of the chit, gal-chit and gal-lys-chit films binding with Gal-1.

suggested that the introduction of branched lysine spacer or branched galactosyl units decreased the thermal stability of the chitosan-based films. Fig. 6 showed the tensile strength of gal-chit and gal-lys-chit films. Chitosan films presented tensile strength of 38 MPa. It was worthnoting that the galactosylated chitosan (gal-chit and gal-lys-chit) films demonstrated lower tensile strength at break as compared with chitosan films. These results were again attributed to the reason that the introduction of galactose units decreased the crystallinity of chitosan.

In this study, *E. coli* were used as the test bacteria to examine the antibacterial properties of gal-chit and gal-lys-chit

Table 1
Whiteness index (WI) of chit, gal-chit and gal-lys-chit films before and after binding with Gal-1

Nomenclature	Chit	Gal-chit	Gal-lys-chit
Control	82.0	60.1	26.9
Binding Gal-1	32.7	−106.3	−187.0

Table 2
The attachment efficiency of HepG2 cells on different films analyzed at 3 h after cell seeding ($n=3$)

Nomenclature	Chit	Gal-chit	Gal-lys-chit
Efficiency (%)	41.0±4.2	67.2±6.5	75.6±5.1

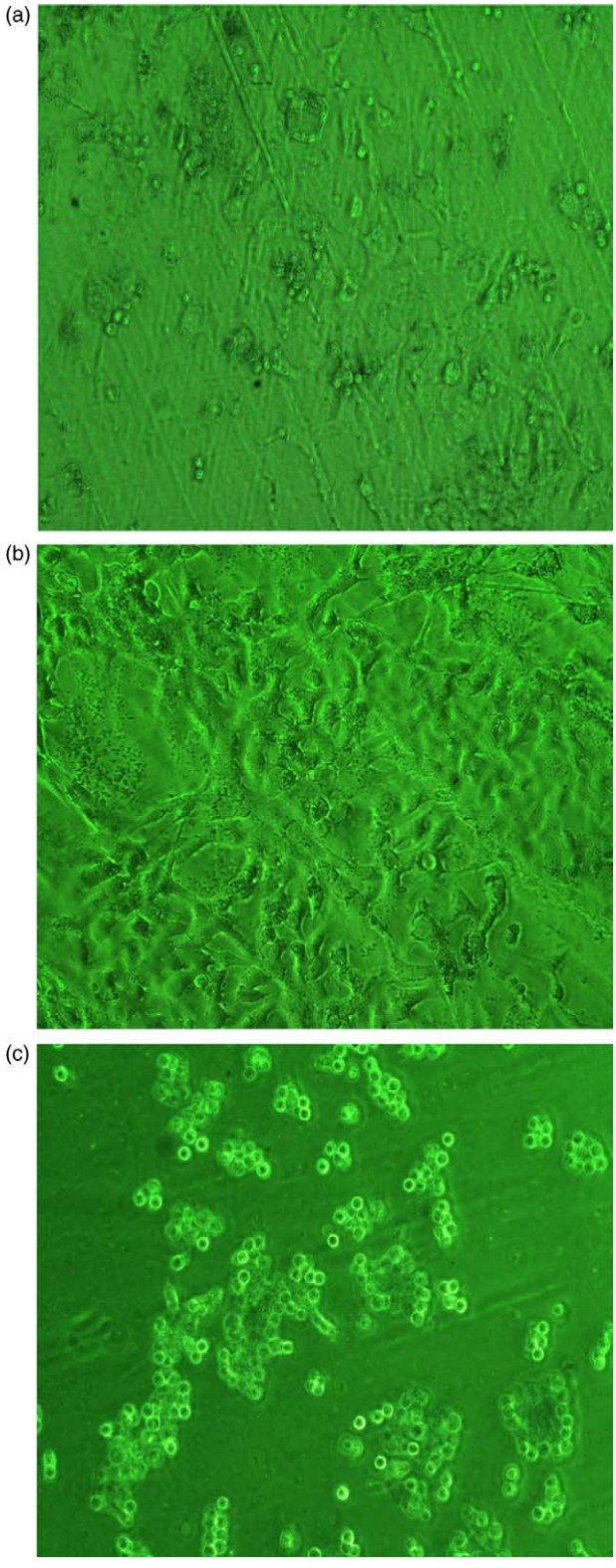


Fig. 9. Morphology of HepG2 cells on the chit, gal-chit and gal-lys-chit films: (a) chit films; (b) gal-chit films; (c) gal-lys-chit films.

films. The numbers of colony of the bacteria formed on the test films are shown in Fig. 7. It was found that the galactosylated chitosan (gal-chit and gal-lys-chit) films demonstrated more effective antimicrobial ability against *E. coli* as indicated by the lower colony unit. Several studies revealed that surface properties such as surface energy, wettability, and uniformity, significantly influenced the adherence of bacteria on a biomaterial. Modifying the surface to become more hydrophilic could reduce the adherence of some bacterial strains. The galactosylated chitosan (gal-chit and gal-lys-chit) films with hydrophilic galactosyl groups might be a potential biomaterial with antimicrobial ability.

Fig. 8 and Table 1 showed the binding efficiency of Gal-1 estimated by immunostaining. Gal-1, a beta galactoside-binding lectin, was involved in multiple biological functions, such as cell adhesion, apoptosis, and metastasis. It displayed greater specificity for multivalent carbohydrate ligands than monovalent ligands. In the present study, Gal-1 was bound

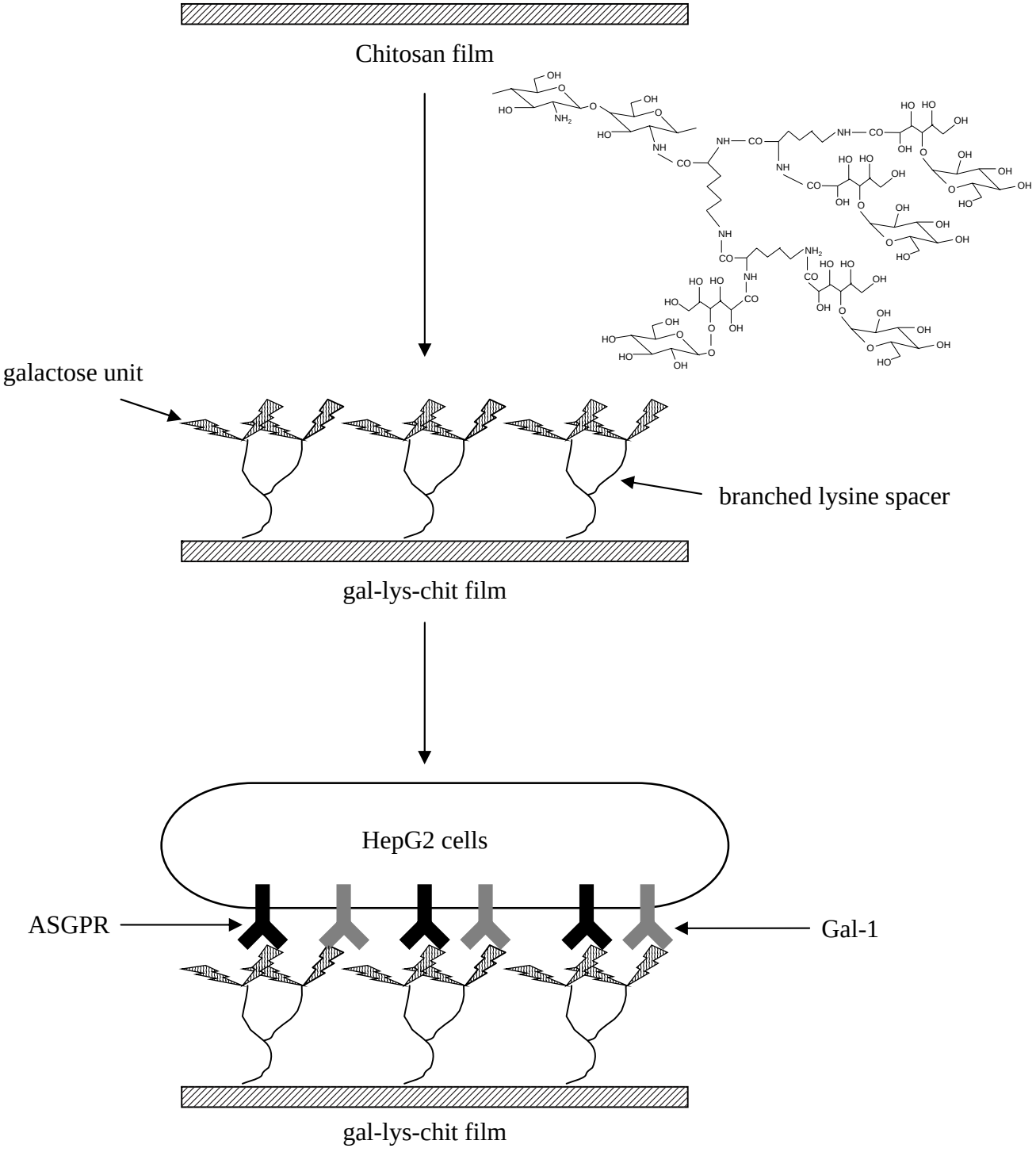


Fig. 10. Schematic surface modification of branched galactose units in affecting HepG2 cells attachment.

with the chitosan films as indicated by the appearance of blue color. The whiteness index (WI) of chitosan films binding with Gal-1 is 32.73, as compared with that of original chitosan film (WI was 81.97). The color of gal-chit film was deep blue and its WI decreased to be −106.3. The gal-lys-chit film demonstrated even more deep blue color (WI was −187.0). This suggested that the branch-type of galactosylated chitosan (gal-lys-chit) was a more effective multivalent carbohydrate ligand for binding with Gal-1, as compared with the traditional galactosylated chitosan.

In the study of HepG2 cells culture, we examined the cell attachment efficiencies and observed the morphology of HepG2 cells on different chitosan films. Table 2 shows the galactose-specific cell attachment onto chit, gal-chit and gal-lys-chit films. The cell attachment was determined at 3 h after cell seeding. The cell attachment was enhanced after introducing galactosyl units to chitosan film (chit). Particularly, the cell attachment efficiency reaches about 76% for the gal-lys-chit film having multivalent galactose units. From the observation by contrast-phase microscopy (Fig. 9), it was found that HepG2 cells on the gal-lys-chit film were aggregated to form multicellular lumps. In contrast, most HepG2 cells on the chit film remained as single cells and only a few cells began to form aggregates. HepG2 cells closely attached to the gal-chit film and were observed as a flat-like morphology. It is known that an increase in the spacer length between lectins and support has altered the accessibility and resulting affinity of immobilized lectins for D-galactosamine [35]. In comparison with the adhesion of hepatocytes on the gal-chit and gal-lys-chit films, it could be found that the HepG2 cells attached to those galactosylated chitosan films in different types. The result basically demonstrated that adjusting the length of spacer arms between the galactose units and macromolecular backbone led to a significant change in the HepG2 cells attachment.

Several studies have shown that polymer surfaces conjugated with galactose ligands could improve HepG2 cells attachment and retain most of the cellular functions [28,36–38]. Fig. 10 showed the schematic surface modification of branched galactose units in affecting HepG2 cells attachment. Stable attachment of HepG2 cells on gal-chit and gal-lys-chit films could be attributed to the high affinity of immobilized galactosyl ligands to ASGPR and Gal-1, which could modulate cell adhesion, apoptosis, and metastasis cell adhesion, apoptosis, and metastasis. HepG2 cells cultured on the gal-lys-chit film clearly showed superior functions in terms of cell attachment, proliferation and aggregation. Formation of the aggregates is desirable to maintain higher HepG2 cells functions. It suggests that the gal-lys-chit film with multivalent galactose ligands has not only higher affinity to HepG2 cells, but also a higher impact on cell functional maintenance compared with the chit and gal-chit films.

4. Conclusion

We prepared a novel galactosylated chitosan possessed the ability to effectively bind galectin-1 (Gal-1). The physical and some biological properties of the novel galactosylated chitosan were examined in this study. From the results of these studies, we found that the novel glycoconjugated macromolecules bearing branched galactose units demonstrated superior antibacterial capability and higher affinity to HepG2 cells. The branch-type of galactosylated chitosan was promising as a novel glycoconjugated macromolecule for specific liver-targeting drug delivery system.

Acknowledgements

Financial support for this research was provided by National Science Council of Taiwan, ROC for the (NSC94-2216-E-012-001). This work was supported partly by a grant from the Naval Foundation for Research and Development (ROC).

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