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Cell proliferation and thermally induced cell detachment of galactosylated thermo-responsive hydrogels

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ABSTRACT

A novel thermo-responsive and biocompatible copolymer hydrogel based on *N*-isopropylacrylamide (NIPAAm) and galactosylated acrylate (GAC) was synthesized successfully by the free radical copolymerization. The copolymer hydrogel poly(NIPAAm-*co*-GAC) exhibits reversible temperature-response at ~30 °C in both water and cell culture medium. Compared with conventional PNIPAAm hydrogel, the adhesion and proliferation of HepG2 and L929 cells were higher on surfaces of hydrogels, suggesting that the incorporation of GAC could stimulate cell adsorption and growth. In addition, both HepG2 and L929 cells could spontaneously detach from the surface of the poly(NIPAAm-*co*-GAC) hydrogels without treating with any enzymes, and the detached cells at 20 °C showed higher viability than those detached from that on PNIPAAm hydrogel.

1. Introduction

Today, rational design of biomaterials for cell culture and reconstruction of tissues and organs have received much attention in tissue engineering fields. Among them, polymer hydrogels with stimuli-sensitivity (e.g., poly(*N*-isopropylacrylamide) (PNIPAAm)) hydrogels in particular which exhibit a clear coil-*to*-globule transition at its lower critical solution temperature (LCST = 32 °C) have attracted considerable attention for use in biomedical and tissue engineering, such as artificial insulin control systems, efficient bioseparation devices, carriers of immobilized enzyme, drug delivery system, and culture dishes for cell-sheet engineering (Cromton et al., 2007; Gil & Hudson, 2004; Lorenzo et al., 2005; Plunkett, Berkowski, & Moore, 2005; Prabaharan & Mano, 2006; Yamato & Okano, 2004).

Using a thermo-reversible polymer, PNIPAAm chemically grafted on tissue culture polystyrene (TCPS) dishes, Okano's group developed a novel technique of cell-sheet engineering for tissue reconstructions (Isenberg et al., 2008; Mizutani, Kikuchi, Yamato, Kanazawa, & Okano, 2008). PNIPAAm is hydrophobic at 37 °C and hydrophilic at 20 °C; thus the cultured cells can be harvested as a continuous cell sheet after incubation at 20 °C. The harvested cell sheets have been used for various tissue reconstructions, including ocular surfaces, periodontal ligaments, cardiac patches, and bladder augmentations (Yang et al., 2007).

However, PNIPAAm shows poor biocompatibility which restricts the cell attachment application. Therefore, some polymers with good biocompatibility were introduced to improve biocompatibility of PNIPAAm. Uludaga et al. had found that the tripeptide arginine–glycine–aspartic acid (RGD)-grafted thermo-reversible polymers facilitated attachment of BMP-2 responsive C2C12 cells (Smith, Yang, McGann, Sebald, & Uludaga, 2005). Okano et al. showed that cell adhesion and proliferation could be modulated by appropriate design of the thermo-responsive surfaces with immobilized insulin, and the cultured cells can be easily recovered by lowering incubation temperature (Hatakeyama, Kikuchi, Yamato, & Okano, 2005).

Much attention has been paid in recent years to synthesizing polymers having sugar residues for cell attachment since such polymers could offer a good surface for cell attachment (Bahulekar et al., 1998). In our previous work, chitosan (CS) was introduced into PNIPAAm to prepare poly(NIPAAm-*co*-CSA) hydrogel. It was found that the incorporation of CS could enhance the attachment of L929 cells. When the temperature decreased, the poly(NIPAAm-*co*-CSA) hydrogel showed hydrophilic and the cells spontaneously detached along with their deposited extracellular matrix (Wang, Chen, Zhao, Guo, & Zhang, 2009).

Galactosylated substrate is an attractive alternative to collagen because of the specific interaction of cell surface asialoglycoprotein receptor (ASGPR) with the galactose moiety (Geffen & Spiess, 1992). Akaike et al. have found that lactose-carrying polystyrene

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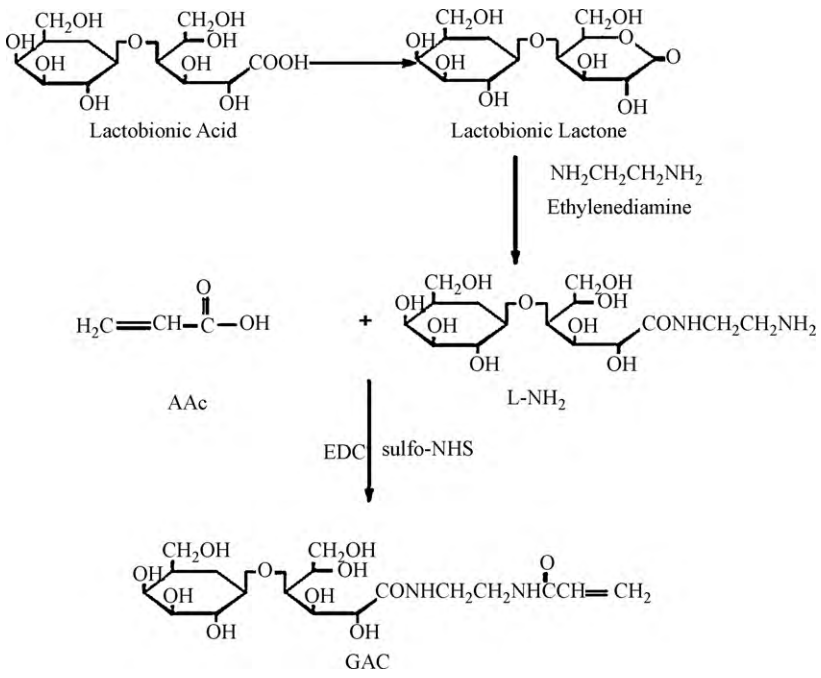

Fig. 1. Reaction scheme of galactosylated acrylate GAC.

(PVLA) substrates with induction of selective adhesion of hepatocytes exhibited aggregated morphology and maintained good level of cellular functions (Kobayashi et al., 1992; Tobe, Takei, Kobayashi, & Akaike, 1992). Gutsche, Lo, Zurlo, Yager, and Leong (1996) showed similar results using a galactose derived polystyrene porous network. In a hydrogel configuration, Griffth et al. also showed that a galactose-conjugated poly(ethylene oxide) hydrogel could promote hepatocyte attachment and functional maintenance (Griffth & Lopina, 1998; Lopina, Wu, Merrill, & Griffth, 1996). In addition, galactose moiety has an excellent cell affinity due to containing sugar groups.

In this paper, due to its excellent cell affinity and specific adhesive ligand to the ASGPR of hepatocyte, galactose moiety was introduced to PNIPAAm gel to improve its biocompatibility as well as to promote the cell attachment. The hydrogel (poly(NIPAAm-*co*-GAC)) was prepared via a copolymerization of monomer galactosylated acrylate (GAC) and NIPAAm in aqueous solutions. The swelling behavior and temperature-sensitivity of poly(NIPAAm-*co*-GAC) hydrogel were studied by both gravimetric method and DSC. Adhesion, proliferation and detachment behaviors of HepG2 and L929 cells on poly(NIPAAm-*co*-GAC) hydrogels were investigated by photomicrography and MTT method.

2. Experimental

2.1. Materials and methods

NIPAAm was purchased from Tokyo Chemical Industry Co., Ltd., Japan, and used after recrystallization from *n*-hexane. Lactobionic acid was obtained from Shanghai Huishen Chemical Co., Ltd. TCPS, trypsin–EDTA solution, streptomycin, penicillin and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. All other reagents, including 1-ethyl-3-(3-dimethylaminopropyl)carboiimide (EDC), ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetra-methylenediamine (TEMED), *N*,*N*′-methylenebis (acrylamide) (MBAA), *N*-hydroxysulfosuccinimide sodium salt (Sulfo-NHS), ethanol and dimethyl sulfoxide (DMSO) were of analytic grade and used as received.

2.2. Synthesis of galactosylated acrylate (GAC)

Firstly, primary amine was introduced into lactobionic acid. Briefly, lactobionic lactone prepared by dehydration of lactobionic acid was refluxed with 5-fold excess ethylenediamine and anhydrous dimethyl sulfoxide (DMSO) as reactive media at 70 °C for 2 h. The monoamine terminated lactobionic lactone (L-NH_2) was precipitated with chloroform and vacuum-dried. Then, 2 mmol L-NH_2 and 5 mmol acrylic acid were dissolved in 100 mL DMSO and carried out for 24 h at room temperature with EDC and co-reactant Sulfo-NHS as the activation agents. The obtained GAC monomer was precipitated with chloroform and vacuum-dried. The reaction scheme is shown in Fig. 1.

2.3. Synthesis of poly(NIPAAm-co-GAC) hydrogels

Poly(NIPAAm-*co*-GAC) hydrogels were prepared by the free radical copolymerization of NIPAAm and GAC in distilled water with APS and TEMED severed as oxidation–reduction initiator. Specifically, GAC and NIPAAm were dissolved according to a weight ratio *r*, in the feed (GAC/GAC + NIPAAm) of 0.2 and 0.5, respectively. MBAA (2 wt.%), APS (0.2 wt.%) and TEMED (0.5 wt.%) were then added under nitrogen atmosphere and stirring. The reaction was performed at room temperature for 24 h. As a comparison, pure PNIPAAm hydrogel was prepared in the presence of MBAA (2 wt.%). The obtained hydrogels were cut into thin disks of 12 mm in diameter and 2 mm in thickness and then immersed in distilled water for 1 week to remove the unreacted monomer.

2.4. Measurements

The chemical structure of L-NH_2, GAC and poly(NIPAAm-*co*-GAC) hydrogels were investigated by infrared spectroscopy (FTIR, TENSOR37, Germany) and nuclear magnetic resonance spectroscopy (NMR, AVANCE AV 300 MHz, Germany) using D_2O as solvent.

The thermal behaviors of equilibrium swollen hydrogels with a weight ratio of $r = 0.5$ in distilled water and cell culture medium were measured on a differential scanning calorimeter (DSC7,

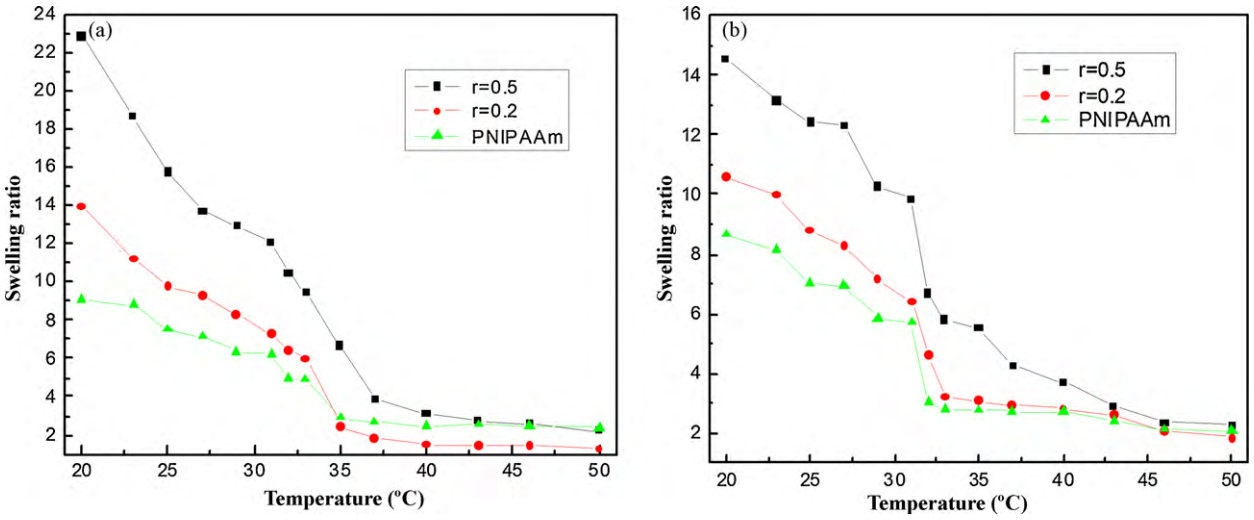

Fig. 2. Temperature dependence of the swelling ratio of poly(NIPAAm-*co*-GAC) hydrogels with $r = 0.5$ (■), $r = 0.2$ (●), and PNIPAAm hydrogel (▲) in (a) distilled water and (b) cell culture medium.

PerkinElmer Co., USA) at a heating rate of 2 °C/min from 20 to 50 °C under nitrogen protection.

The equilibrated swelling ratio (SR) was measured after the hydrogels were immersed in distilled water (or cell culture media) for 24 h over a temperature range from 20 to 50 °C. SR was defined as W_s/W_d, where W_s and W_d were the weights of the swollen and dry gels, respectively.

2.5. Cell culture on hydrogels

Two types of cells, human hepatoma cells (HepG2) and mouse fibroblast cell (L929) (both from Shanghai Queen & King Biochem Co., Ltd.), were used to study cell cultivation on the surfaces of poly(NIPAAm-*co*-GAC) and PNIPAAm gels. A cell bank was first created by expanding the cells on 25 cm^2 flasks in a humidified environment of 95%/5% air/CO_2, and the cells were frozen at 7.0×10^4 cells/mL (1 mL) in cryogenic vials. Cell freezing was achieved at a rate of 1 °C/min and the cells were stored in liquid N_2 until use.

For each experiment, a vial of frozen cells was thawed and seeded on 25 cm^2 flasks. The cells were cultured in DMEM with 10% FBS and 1% penicillin/streptomycin until they reached about 80% confluence and trypsinized once more for seeding onto polymer surfaces. Then, HepG2 and L929 in each medium were inoculated at a density of 1×10^4 and 1.5×10^4, respectively, on the hydrogel surfaces. All cells were incubated in 5% CO_2–95% air at 37 °C for 1 week, and the medium was replaced every 2 days.

Cell adhesion and proliferation were studied by photomicrography using a phase-contrast optical microscope (XDS-1B). The number of cells adhered to a specific surface was quantified using a hemacytometer. For this method, the excess culture media was drained and 0.5 mL of trypsin–EDTA was added to each well. After about 5 min, 1 mL of fresh culture medium was added, cells were counted manually from five different fields and averaged, giving the number of cells/mL. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to quantify metabolic activity in each well. At the desired time point, the culture medium was drained and re-supplied with 1 mL of fresh culture medium. MTT dissolved in HBSS (5 mg/mL) was added to each well. After incubating at 37 °C for 4 h, excess medium was removed. The cells were then dissolved in 1.2 mL of DMSO and the OD value of each well was determined by Auto Microplate Reader (Σ960, Metertech Co., America) while the wavelength was selected at 570 nm.

2.6. Cell-sheet detachment

The detachment of HepG2 and L929 cell sheets was performed by decreasing the temperature to 20 °C after HepG2 and L929 were cultured almost to confluence on the hydrogel surface for 4 days. The detached cell sheet was firstly bubbled to single cells with a pipette and then was counted by hemacytometer to quantify the detached cell number.

3. Results and discussion

3.1. Synthesis of GAC and poly(NIPAAm-co-GAC) hydrogel

GAC was prepared by the similar method as reported in the literature (Yang, Gotob, Ise, Cho, & Akaike, 2002). The reaction scheme was shown in Fig. 1. The structures of L-NH_2 and GAC were determined by IR and ^{1}H NMR. For IR measurements, lactobionic acid was found to exhibit characteristic absorptions at 1749 cm^{-1}, which was attributed to the carbonyl stretching (C=O) of carboxylic groups. In the IR spectrum of L-NH_2, the characteristic peaks at

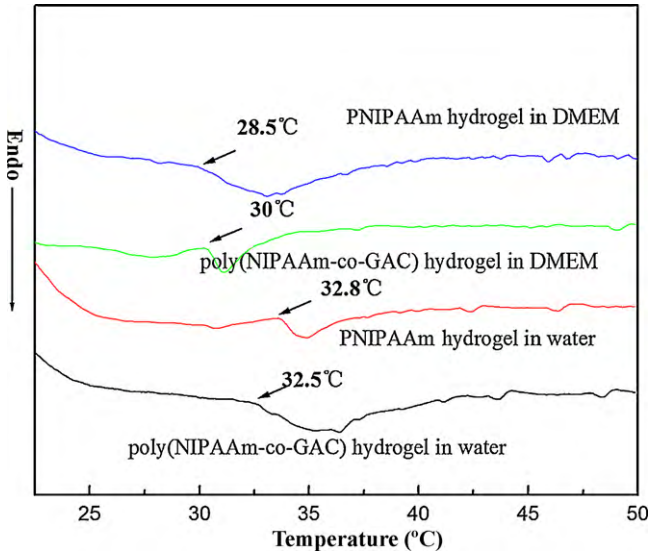

Fig. 3. DSC thermograms of poly(NIPAAm-*co*-GAC) ($r = 0.5$) and PNIPAAm hydrogels in distilled water and cell culture medium.

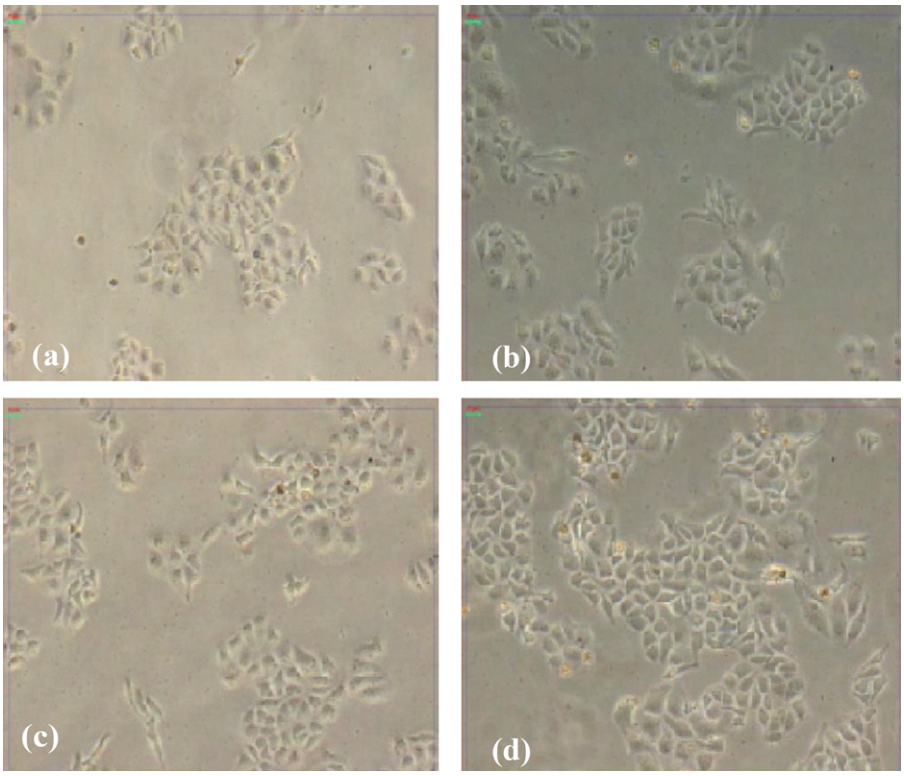

Fig. 4. Phase-contrast photomicrographs (100×) of HepG2 cell cultured on the surfaces of different hydrogels after 4 days. (a) PNIPAAm, (b) poly(NIPAAm-*co*-GAC) ($r = 0.2$), (c) poly(NIPAAm-*co*-GAC) ($r = 0.5$), and (d)TCPS.

1644, 1587 and 1490 cm^{-1} came from amides I, II and III, respectively, but the characteristic absorptions at 1749 cm^{-1} disappeared due to the amide bond formation between carboxylic groups of lactobionic acid and amine groups of ethylenediamine. For AAc, the absorption bands with peak positions at 1702 cm^{-1} were due to the carbonyl group, and the absorption bands with peak positions at 980 and 920 cm^{-1} were due to the double bond of alkene. In the IR spectrum of GAC, 1702 cm^{-1} disappeared but 980 and 920 cm^{-1} existed, and the characteristic peaks at 1644, 1587 and 1490 cm^{-1} came from L-NH_2.

^{1}H NMR measurement (D_2O): for L-NH_2, the integrated signals of 2.55 and 4.28–3.11 ppm were assigned to anomeric and remaining protons of ethylenediamine and LA, respectively. For GAC, the peaks at 4.28–3.11 and 2.55 ppm were from L-NH_2, and 6.55–5.94 ppm can be assigned to C=CH_2 from acrylic acid. No peak was found at 11 ppm which could be assigned to –COOH of acrylic acid. Accordingly, it is reasonable to assume that GAC was synthesized successfully.

As for poly(NIPAAm-*co*-GAC) hydrogels, the characteristic peaks at 1644, 1587 and 1490 cm^{-1} from GAC were observed, while the absorption of –C=C– from NIPAAm and GAC at 920–980 cm^{-1} disappeared, indicating a successful copolymerization of GAC and NIPAAm in the present of cross-linker MBAA.

3.2. Thermo-responsive properties of hydrogels

Fig. 2 shows the swelling behaviors of poly(NIPAAm-*co*-GAC) hydrogels in water (a) and cell culture medium (b) at various temperatures. It could be seen that the SR of all hydrogels decreased with increasing temperature, suggesting that poly(NIPAAm-*co*-GAC) hydrogels were temperature-sensitive. It could be ascribed to the coil-*to*-globule transition of macromolecular chain, and the PNIPAAm component played a dominant role for the volume change of the hydrogels in response to the temperature. However, the SR value in cell culture media was lower than that in water. It is probably because the electrostatic interaction between hydrogels and the ions (e.g., OH^-\$HCO_3^-$\$Na^+$\$Cl^-$) occurred in cell culture media. In addition, since GAC is hydrophilic, the SR of poly(NIPAAm-*co*-GAC) hydrogels is higher than that of pure PNIPAAm hydrogel and increases with increasing GAC content.

The LCSTs of PNIPAAm and poly(NIPAAm-*co*-GAC) hydrogel were defined as the onset temperature of the endotherms. As shown in Fig. 3, The LCSTs of PNIPAAm and poly(NIPAAm-*co*-GAC) hydrogel ($r = 0.5$) were 32.8 and 32.5 °C in distilled water, respectively. However, these two values slightly decrease to 30.0 and 28.5 °C in DMEM, respectively. The decrease of the LCST might be associated with the weakening of the hydrogen bonds due to the presence of ions in cell culture medium. The DSC result indicated that poly(NIPAAm-*co*-GAC) hydrogel is favorable for the transition of adhesion and detachment of cell by controlling temperature since the LCST is between room temperature and normal body temperature.

3.3. HepG2 cell cultivation on PNIPAAm and poly(NIPAAm-co-GAC) hydrogels

The cell compatibility of the poly(NIPAAm-*co*-GAC) hydrogels was examined by morphology and assessing the cellular adhesion. Fig. 4a–d shows phase-contrast micrographs of HepG2 after culturing for 4 days on the surfaces of poly(NIPAAm-*co*-GAC) ($r = 0.2$, $r = 0.5$) and PNIPAAm hydrogels, respectively. The images clearly showed the cell adhered well and began to grow on the surface of hydrogels, indicating good cell compatibility of hydrogels.

Fig. 5 shows that the difference in cell proliferation was also observed by cell numbers (by Coulter) and cellular activity (by MTT assay) on hydrogel quantificationally. It was found that cell number has little change in 2 days, but abruptly increased after 2 days and then saturated for all hydrogels and TCPS. However,

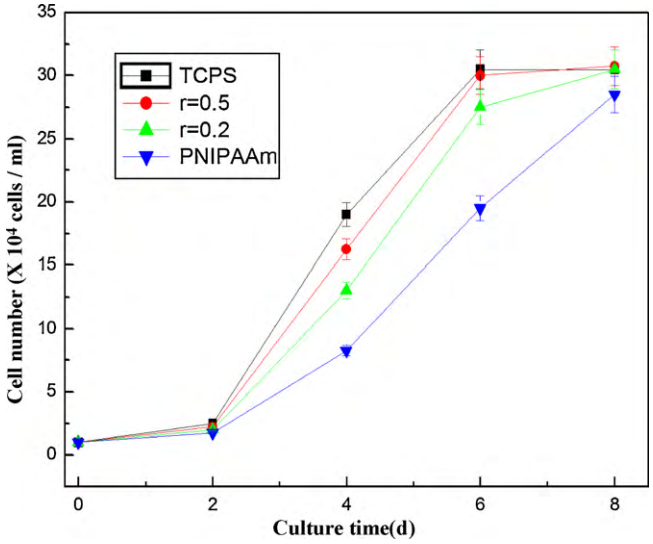

Fig. 5. Proliferation curves of HepG2 cell on poly(NIPAAm-*co*-GAC) hydrogels with $r = 0.5$ (●), $r = 0.2$ (▲), PNIPAAm hydrogel (▼) and TCPS (■).

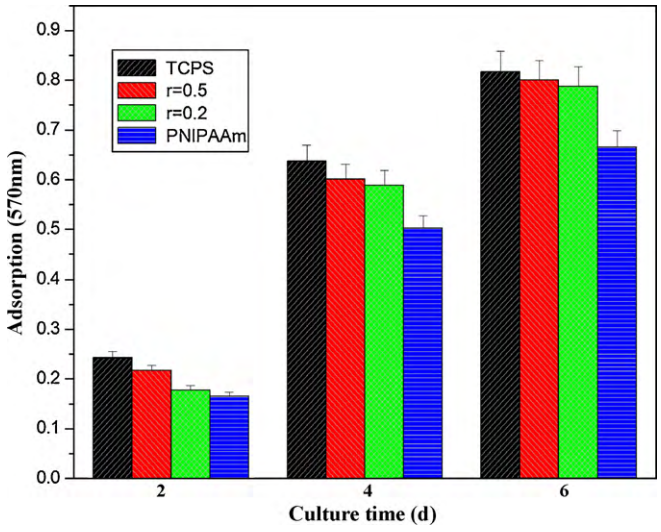

Fig. 6. Growth of HepG2 cell on poly(NIPAAm-*co*-GAC) hydrogels, PNIPAAm hydrogel and TCPS using MTT assay.

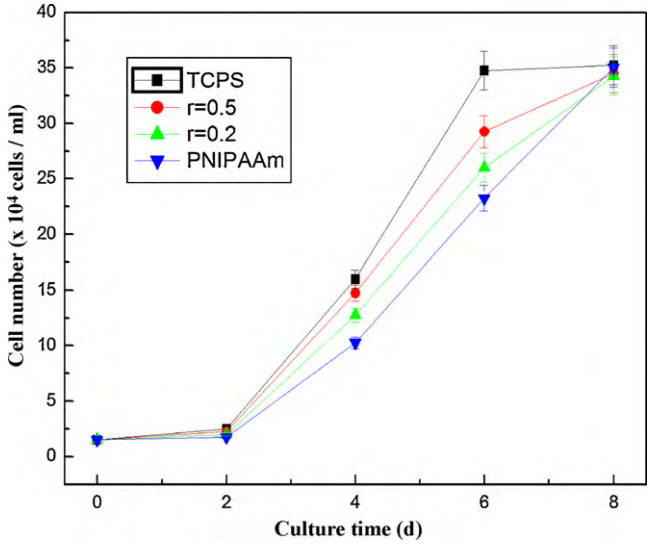

Fig. 7. Proliferation curves of L929 cell on poly(NIPAAm-*co*-GAC) hydrogels with $r = 0.5$ (●), $r = 0.2$ (▲), PNIPAAm hydrogel (▼) and TCPS (■).

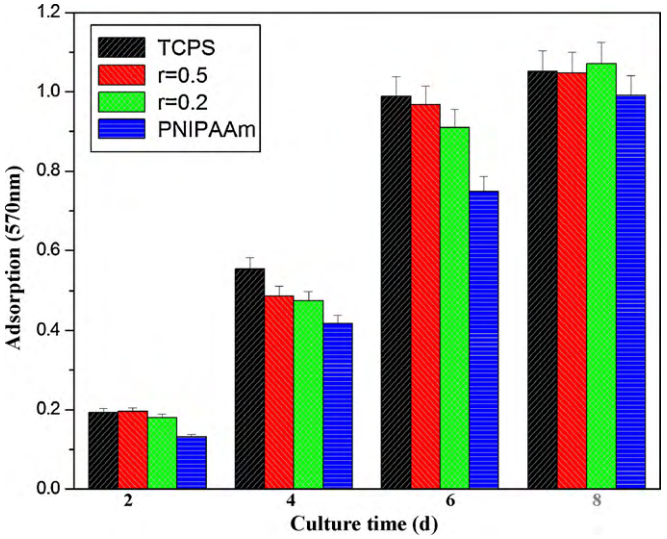

Fig. 8. Growth of L929 cell on poly(NIPAAm-*co*-GAC) hydrogels PNIPAAm hydrogel and TCPS using MTT assay.

the increased speed was different. The cell on the surface of poly(NIPAAm-*co*-GAC) hydrogels grew more rapidly than that on PNIPAAm hydrogel. Especially the surface of poly(NIPAAm-*co*-GAC) hydrogel with $r = 0.5$ supported higher numbers of cells than that of other hydrogels. This result confirmed that the cell compatibility of poly(NIPAAm-*co*-GAC) hydrogels enhances due to the introduction of galactose moiety and increased with the addition of GAC content, suggesting that galactosyl could stimulate cell adsorption and growth.

A similar result was obtained by the total metabolic activity (Fig. 6). As shown in Fig. 6, at all time points measured, the cell adhesion to poly(NIPAAm-*co*-GAC) surface was higher than the PNIPAAm surface, but lower than the TCPS control surface.

3.4. L929 cell cultivation on PNIPAAm and poly(NIPAAm-co-GAC) hydrogels

Another cell, L929, was used to test the generality of the cell cultivation behavior on poly(NIPAAm-*co*-GAC) hydrogels. It was clearly observed by phase-contrast micrographs that L929 cell became attached to and began spreading and spreading continued to develop for 4 days on all hydrogels. Almost all surfaces complete coverage by cells within a 6-day culture period (figures were not given). Fig. 7 showed the proliferation curves of L929 cell on the surfaces of poly(NIPAAm-*co*-GAC) hydrogels, PNIPAAm hydrogel and TCPS. Also in a manner similar to HepG2, the cell number abruptly increased in 2–6 days and then saturated for hydrogels and TCPS. At all time points measured, the cell adhesion to poly(NIPAAm-*co*-GAC) surface was higher than the PNIPAAm surface, but lower than the TCPS control surface. And the cell on the surface of poly(NIPAAm-*co*-GAC) hydrogel ($r = 0.5$) grew more rapidly than that on poly(NIPAAm-*co*-GAC) ($r = 0.2$).

The metabolic activity of each sample was also investigated (Fig. 8). It was found that L929 can be cultured satisfactorily for 8 days for poly(NIPAAm-*co*-GAC) hydrogel with $r = 0.2$ and 0.5, and that more L929 cells adhered to or proliferated on their surfaces. In contrast, less adhesion and proliferation of L929 were tested on the surface of PNIPAAm gels throughout 8-day culture period. Thus, it is reasonable to conclude that poly(NIPAAm-*co*-GAC) hydrogels were capable of culturing cells,

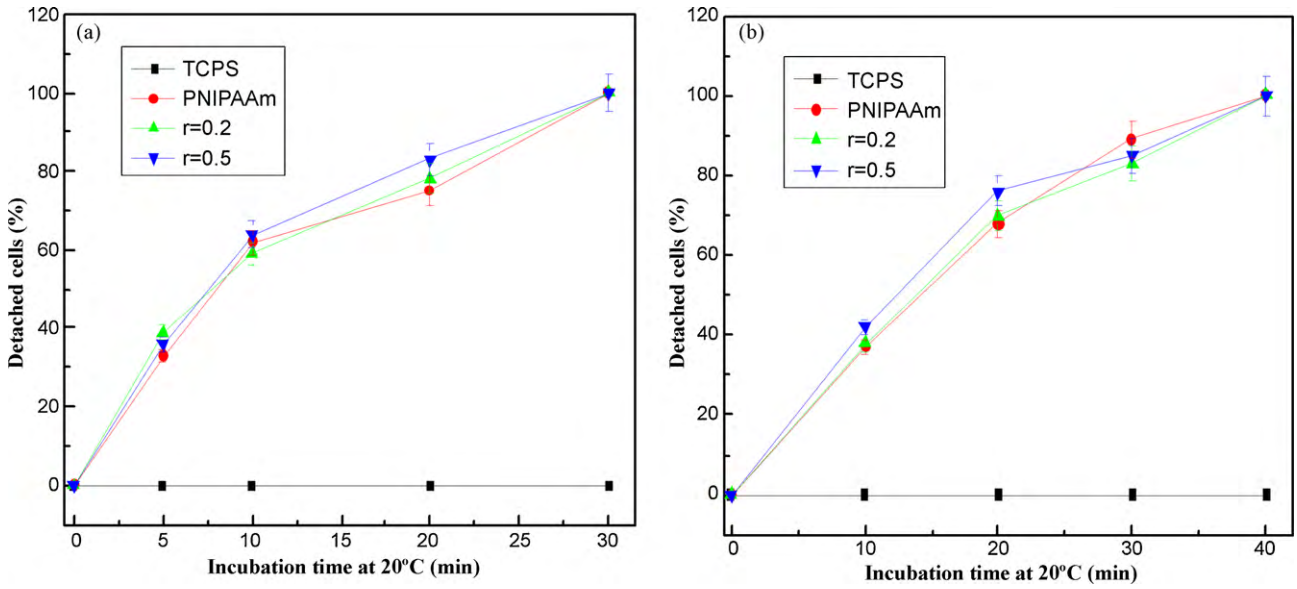

Fig. 9. Percentage of detached cell sheets from poly(NIPAAm-*co*-GAC) hydrogels with $r = 0.2$ (▲) and $r = 0.5$ (▼), PNIPAAm (●) and TCPS (■) as a function of incubation time in culture medium at 20 °C. (a) HepG2 cell and (b) L929 cell.

and the incorporation of galactosyl could stimulate cell adsorption and growth.

3.5. Detachment of cell sheets without trypsin treatment

Since poly(NIPAAm-*co*-GAC) hydrogels exhibit well-defined temperature-sensitivity, it is expected that cells cultured on poly(NIPAAm-*co*-GAC) hydrogels could be detached simply by decreasing the temperature from 37 °C (hydrophobic) to 20 °C (hydrophilic) without treatment by trypsin.

Fig. 9a and b shows changes in HepG2 and L929 cell sheets detached on PNIPAAm hydrogel, poly(NIPAAm-*co*-GAC) hydrogels ($r = 0.2$, $r = 0.5$) and TCPS as a function of reduced temperature treatment time. The detachment of both cell sheets occurs nearly the same on the surfaces of all hydrogels. There were no cells detached from TCPS because of no surface property alteration, while the cells could detach from these thermo-sensitive hydrogels by reducing the temperature. The cell sheets of HepG2 were recovered from the substratum within 30 min of starting the decrease in temperature, and L929 within 40 min. It is known that hydrogel has porous conformation, and just this porous conformation could accelerate the hydration of the material. When the temperature decreased from 37 to 20 °C, reswelling of thermo-sensitive hydrogels occurred due to the hydration of PNIPAAm. The reswelling of hydrogel supplied water molecules not only from the periphery of each cell but also through pores underneath the adherent cells. Thus, the resulting cell sheets detached from poly(NIPAAm-*co*-GAC) gel surfaces may contain extracellular matrix (ECM).

3.6. Cell transshipment

To investigate whether the cells detached from poly(NIPAAm-*co*-GAC) hydrogels by reducing temperature still retain their ability to re-attach, the floating detached cells were again placed on TCPS dishes. It has found that the detached cells began to re-attach to TCPS dish surfaces shortly after seeding and attached cells appeared to exhibit normal morphology (data not shown). The activity of the detached cells from thermo-sensitive hydrogels was examined by

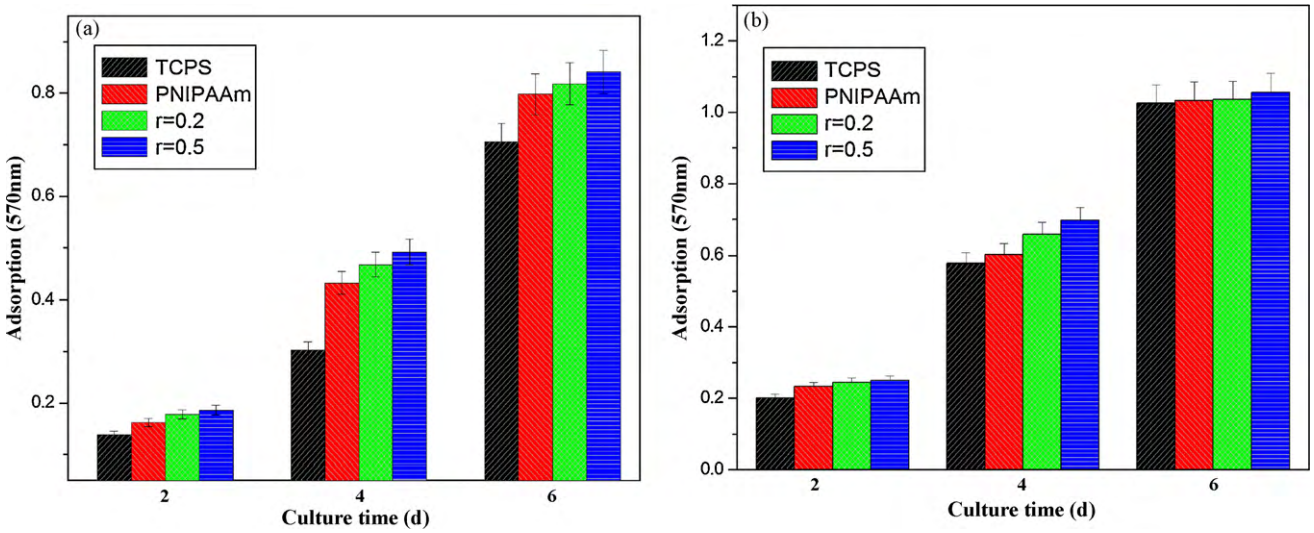

Fig. 10. Cell (HepG2 (a) and L929 (b)) transshipment of poly(NIPAAm-*co*-GAC) hydrogels with $r = 0.2$, $r = 0.5$, and PNIPAAm. Here they were compared with cells digested from TCPS.

the total metabolic activity. The HepG2 and L929 cells detached from poly(NIPAAm-*co*-GAC) and PNIPAAm hydrogels and digested from TCPS were seeded, respectively, onto dishes at a cell density of 2×10^4 cells/cm^2.

Fig. 10a and b shows the proliferation of detached HepG2 and L929 cells when cultured at 37 °C, respectively. It could be seen that the cell number also increased in 2–6 days and then saturated when cultured renewedly. This indicates that the detached cells at 20 °C retain alive and characteristics similar to attached condition at 37 °C. At all time points measured, the activity of cells detached from hydrogels was higher than digested from TCPS control surface. It should be noted that the activity of cells detached from poly(NIPAAm-*co*-GAC) surface was higher than the PNIPAAm surface, and the cell detached from the surface of poly(NIPAAm-*co*-GAC) hydrogel with $r = 0.5$ grows more rapidly than that from poly(NIPAAm-*co*-GAC) with $r = 0.2$. These results imply that the incorporation of GAC was not only capable of enhancing the attachment of cells, but also capable of retaining the activity of the detached cells.

4. Conclusions

In this work, we proposed a new cell-adhesive technique using a thermo-responsive substrate. Galactosylated acrylate (GAC) and a series of poly(NIPAAm-*co*-GAC) hydrogels were successfully synthesized. It was found that poly(NIPAAm-*co*-GAC) hydrogels with weight ratio from 0.2 to 0.5 still exhibit good temperature-sensitivity and better swelling properties compared with pure PNIPAAm hydrogel. Furthermore, HepG2 and L929 cells could be cultured on the surfaces of both poly(NIPAAm-*co*-GAC) and PNIPAAm, but the adhesion and proliferation of HepG2 and L929 were better on surfaces of poly(NIPAAm-*co*-GAC) hydrogels than on PNIPAAm hydrogel. Lowering temperature from cultivation temperature (37 °C) to any temperature below PNIPAAm LCST (e.g., 10–20 °C) resulted in the cells to detach from the surfaces of hydrogels without trypsin treatment. The activity of cells detached from poly(NIPAAm-*co*-GAC) surface was higher than that from the PNIPAAm surface, and the more the GAC content, the higher the activity of cells detached. These results indicated that the incorporation of GAC was not only capable of enhancing the attachment of cells, but also capable of retaining the activity of the detached cells.

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References

Bahulekar, R., Tokiwa, T., Kano, J., Matsumura, T., Kojima, I., & Kodama, M. (1998). Polyacrylamide containing sugar residues: Synthesis, characterization and cell compatibility studies. *Carbohydrate Polymers*, *37*, 71–78.

Cromton, K. E., Gond, J. D., Bellamkonda, R. V., Gengenbach, T. R., Finkelstein, D. I., Horne, M. K., et al. (2007). Polylysine-functionalised thermoresponsive chitosan hydrogel for neural tissue engineering. *Biomaterials*, *28*, 441–449.

Geffen, I., & Spiess, M. (1992). Asialoglycoprotein receptor. *International Review of Cytology*, *137B*, 181–219.

Gil, E. S., & Hudson, S. M. (2004). Stimuli-responsive polymers and their bioconjugates. *Progress in Polymer Science*, *29*, 1173–1222.

Griffth, L. G., & Lopina, S. (1998). Microdistribution of substratum-bound ligands affects cell function: Hepatocyte spreading on PEO-tethered galactose. *Biomaterials*, *19*, 979–986.

Gutsche, A. T., Lo, H., Zurlo, J., Yager, J., & Leong, K. W. (1996). Engineering of a sugar-derivatized porous network for hepatocyte culture. *Biomaterials*, *17*, 387–393.

Hatakeyama, H., Kikuchi, A., Yamato, M., & Okano, T. (2005). Influence of insulin immobilization to thermoresponsive culture surfaces on cell proliferation and thermally induced cell detachment. *Biomaterials*, *26*, 5167–5176.

Isenberg, B. C., Tsuda, Y., Williams, C., Shimizu, T., Yamato, M., Okano, T., et al. (2008). Physiological strains remodel extracellular matrix and cell–cell adhesion in osteoblastic cells cultured on alumina-coated titanium alloy. *Biomaterials*, *29*, 2565–2575.

Kobayashi, A., Goto, M., Sekine, T., Masumoto, A., Yamamoto, N., Kobayashi, K., et al. (1992). Regulation of differentiation and proliferation of rat hepatocytes by lactose-carrying polystyrene. *Artificial Organs*, *16*, 564–567.

Lopina, S. T., Wu, G., Merrill, E. W., & Griffth, C. L. (1996). Hepatocyte culture on carbohydrate- modified star polyethylene oxide hydrogels. *Biomaterials*, *17*, 559–569.

Lorenzo, C. A., Concheiro, A., Dubovik, A. S., Grinberg, N. V., Burova, T. V., & Grinberg, V. Y. (2005). Temperature-sensitive chitosan-poly(*N*-isopropylacrylamide) interpenetrated networks with enhanced loading capacity and controlled release properties. *Journal of Controlled Release*, *102*, 629–641.

Mizutani, A., Kikuchi, A., Yamato, M., Kanazawa, H., & Okano, T. (2008). Preparation of thermoresponsive polymer brush surfaces and their interaction with cells. *Biomaterials*, *29*, 2073–2081.

Plunkett, K. N., Berkowski, K. L., & Moore, J. S. (2005). Preparation of methacrylamide containing peptides. *Biomacromolecules*, *6*, 632–637.

Prabaharan, M., & Mano, J. F. (2006). Chitosan derivatives bearing cyclodextrin cavitiesas novel adsorbent matrices. *Carbohydrate Polymers*, *63*, 153–166.

Smith, E., Yang, J., McGann, L., Sebald, W., & Uludaga, H. (2005). RGD-grafted thermoreversible polymers to facilitate attachment of BMP-2 responsive C2C12 cells. *Biomaterials*, *26*, 7329–7338.

Tobe, S., Takei, Y., Kobayashi, K., & Akaike, T. (1992). Tissue reconstruction in primary cultured rat hepatocytes on asialoglycoprotein model polymer. *Artificial Organs*, *16*, 526–532.

Wang, J., Chen, L., Zhao, Y., Guo, G., & Zhang, R. (2009). Cell adhesion and accelerated detachment on the surface of temperature-sensitive chitosan and poly(*N*-isopropylacrylamide) hydrogels. *Journal of Materials Science: Materials in Medicine*, *20*, 583–590.

Yamato, M., & Okano, T. (2004). Cell sheet engineering. *Materials Today*, *7*, 42–47.

Yang, J., Gotob, M., Ise, H., Cho, C. S., & Akaike, T. (2002). Galactosylated alginate as a scaffold for hepatocytes entrapment. *Biomaterials*, *23*, 471–479.

Yang, J., Yamato, M., Shimizu, T., Sekine, H., Ohashi, K., Kanzahi, M., et al. (2007). Reconstruction of functional tissues with cell sheet engineering. *Biomaterials*, *28*, 5033–5043.