Research Article

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Fabrication and characterization of thermoresponsive composite carriers: PNIPAAm-grafted glass spheres

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Abstract: Processing capacity and product yield of three-dimensional (3D) smart responsive carriers are markedly superior to those of two-dimensional substrates with the same compositions due to the special structure; therefore, more attempts have been made to develop the 3D intelligent systems in recent decades. A novel preparation strategy of thermoresponsive glass sphere-based composite carriers was reported in this study. First, PNIPAAm copolymers were synthesized by free-radical polymerization of *N*-isopropylacrylamide (NIPAAm), hydroxypropyl methacrylate (HPM), and 3-trimethoxysilypropyl methacrylate (TMSPM). Then, the copolymer solution was sprayed on the surfaces of glass spheres using a self-made bottom-spray fluidized bed reactor, and the bonding between copolymers and glass spheres was fabricated by thermal annealing to form PNIPAAm copolymer/glass sphere composite carriers. The coating effects of PNIPAAm copolymers on sphere surfaces were investigated, including characteristic functional groups, surface microstructure, grafting density, equilibrium swelling, as well as biocompatibility and potential application for cell culture. The results show that the temperature-responsive PNIPAAm copolymers can be linked to the surfaces of glass spheres by bottom-spray coating technology, and the copolymer layers can be formed on the sphere surfaces. The composite carriers have excellent thermosensitivity and favorable biocompatibility, and they are available for effective cell

adhesion and spontaneous cell detachment by the use of smart responsiveness.

Keywords: composite carrier, smart responsiveness, poly (*N*-isopropylacrylamide), fluidized bed reactor, bottom-spray coating technology

1 Introduction

Poly(*N*-isopropylacrylamide) (PNIPAAm) contains hydrophilic amide bonds and hydrophobic isopropyl groups, and the polymer chains can change significantly between hydration and dehydration with temperature changes in aqueous solutions, thus displaying the temperature responsiveness. It is widely applied in cell culture (1,2), drug-controlled release (3,4), biosensors (5,6), and other biomedical fields. The preparation technology of two-dimensional (2D) smart substrates with PNIPAAm as main materials has been fully developed. However, 2D temperature-sensitive substrates have limitations in applications, including small processing capacity per unit of time, low yield of target products, and so on. Three-dimensional (3D) thermoresponsive carriers can break through these limits, owing to a larger surface-to-volume ratio. Furthermore, the functional carriers are often applied in a dynamic workflow, which is easy to form a continuous mass production system of target products. Therefore, more attempts have been made to introduce PNIPAAm and derivatives into carriers, scaffolds, hollow fiber membranes, and other stereoscopic systems, and accordingly to produce 3D thermosensitive platforms for a wide range of applications. However, the current studies on the formation of smart layers on the surfaces of 3D supports are still at an initial stage, and the key issue is how to set up the linkage between polymers and substrates.

The “grafting to” and “grafting from” are considered as common techniques for polymer fixing on solid substrates (7,8). In the former methods, smart polymers with

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functional end-groups are first synthesized, the complementary reactive groups are grafted on the surfaces of 3D supports as well, and then, the covalent linkages are formed between polymers and supports because of chemical reactions (9–12). This method is simple and the resultant polymers can be identified before being grafted on the surfaces, but the steric hindrance caused by intermolecular polymer chains will lead to a lower grafting density. In the latter methods, the activation sites are first created on the surfaces of 3D substrates, where smart monomers are then continuously aggregated (13,14). Higher grafting density can be obtained in this method without steric hindrance, but it is difficult to control the chain length and uniformity. The smart polymer chains can be grown on 3D supports via the above two schemes; however, the chain segments are usually divergent and disconnected from each other. If the coatings with a uniform network structure and controllable film thickness are formed on the surfaces of 3D carriers, the physical and chemical properties of the composite carriers will be more stable, which are conducive to further expanding the potentials of 3D intelligent supports in numerous fields.

This study provides a useful route for thermoresponsive composite carriers to facilitate new technological applications. The preparation scheme of PNIPAAm copolymers grafted glass sphere is designed according to our previous production mode of temperature-sensitive copolymer films formed on plain glass coverslips or silicon wafers (15). However, the size and shape of glass spheres are different from those of 2D substrates; so, the conventional preparation technology for planar membranes is no longer suitable for controlling the grafting uniformity and film thickness. The fluidized bed technology has been widely applied in particle coating. This principle has evolved in various configurations, based on top spray, bottom spray, and tangential spray. It is confirmed that the bottom-spray coating process provides the most uniform and smoothest final product among different forms of fluidized bed coaters (16,17). In a typical bottom-spray fluidized bed reactor, a spray nozzle is placed at the bottom of the chamber, beneath a draft tube is located at the center of the chamber, spraying concurrently the coating solution upward into the spray zone to the particles. The traveling path of atomized droplets is short enough to prevent premature evaporation, and particles pursue a predetermined circulation pattern in the coating system, which enable the bottom-spray fluidized bed to excellently provide homogeneous and dense films of the coating on the particles (17,18). There-

fore, a self-made bottom-spray fluidized bed reactor is introduced in this study, by taking advantage of distribution uniformity and ease of control to achieve the uniform coating and thickness accumulation on 3D carriers. There are few studies on the preparation of smart responsive composite carriers using a fluidized bed reactor. This strategy is expected to accomplish a predictable and cost-effective scaling up of intelligent composite carriers, which is conducive to industrial application and popularization.

Herein, PNIPAAm copolymers in response to temperature were first synthesized by free-radical polymerization, and then, the copolymer solution was evenly coated on the surfaces of glass spheres by using bottom-spray coating technology, as contrasted with the composite carriers formed by only soaking in copolymer solution. The properties of PNIPAAm copolymer/glass spheres composite carriers were preliminarily determined. Fourier transform infrared spectroscopy (FTIR) was used to detect the presence/disappearance of characteristic functional groups. The surface morphology of the coating layers was measured by scanning electron microscope (SEM). The grafting density and temperature-dependent equilibrium swelling were analyzed gravimetrically. The biocompatibility and temperature responsiveness of the composite carriers were also investigated by cell attachment and detachment experiments.

2 Materials and methods

2.1 Materials

For copolymer synthesis and formation of composite carriers, *N*-isopropylacrylamide (NIPAAm), hydroxypropyl methacrylate (HPM), 3-trimethoxysilylpropyl methacrylate (TMSPM), and 2-azobisisobutyronitrile (AIBN) were supplied by Sigma. NIPAAm and AIBN were fully recrystallized from *n*-hexane and ethanol, respectively, and then lyophilized by a freeze dryer. The others were used as received. Solid high-precision glass spheres with diameters of 2.5 mm were provided by Tiancheng Products (Jiangsu, China).

For cell culture, low glucose-Dulbecco's modified Eagle's medium (LG-DMEM, Sigma) supplemented with 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin/streptomycin (P/S, Sigma) was used as the basic culture medium.

2.2 Synthesis and identification of PNIPAAm copolymers

The synthesis and identification of PNIPAAm copolymers bearing siloxane and hydroxyl groups were performed in accordance with our previous studies (19,20). Briefly, PNIPAAm copolymers were synthesized by free-radical polymerization of NIPAAm, HPM, and TMSPM with the feed molar ratios of 20:1:1, using AIBN (1% of total mole for all reactants) as initiator at 60°C for 12 h under nitrogen protection, then precipitated using acetone as a solvent and *n*-hexane as a nonsolvent, freeze-dried under vacuum for 24 h, and finally kept in a refrigerator at 4°C for further use.

The functional groups of PNIPAAm copolymers were identified by Nicolet Magna 750 FTIR spectrometer (Nicolet Instrument Corporation, Madison, WI, USA) in the wavenumber range of 4,000–500 cm⁻¹. The molecular composition of the resulting copolymers was analyzed through Bruker AV 400 MHz NMR spectrometer (Bruker Corporation, Fallanden, Switzerland). The molecular weight distribution and polymerization degree of the copolymers were determined via GPC (PL-GPC-50, Varian Inc., Palo Alto, CA, USA). The thermoresponsive behavior of PNIPAAm copolymers was measured by Nano ZS 90 dynamic light scatterometer (Malvern Instruments Ltd., UK).

2.3 Structure design and process flow of bottom-spray fluidized bed reactor

2.3.1 Structure design of bottom-spray fluidized bed reactor

A self-made bottom-spray fluidized bed reactor was designed and constructed. The toughened glass was applied as the main body of the fluidized bed reactor. The connection and fixing brackets were made of 304 stainless steel. The main body of the reactor was inverted conical with the upper bottom diameter of 13 cm, lower bottom diameter of 8 cm, height of 78 cm, and cone angle of 10°. The reactor was equipped with ultrafine mesh at the top and an air distribution plate at the bottom. The pores of the distribution plate were evenly distributed with an aperture of 5 mm and a spacing of 8 mm. The high-pressure atomizing nozzle was placed in the center of the distribution plate. The experimental rig of the bottom-spray fluidized bed reactor system was shown in Figure 1a.

2.3.2 Process flow and operation parameters of a reactor system

Figure 1b illustrated the flow diagram of the bottom-spray fluidized bed reactor system. The air was transported from fan ① with the outlet pressure of 10–15 kPa, flowed through the gas control valve and anemometer ②, evenly dispersed by air distribution plate ⑦, and eventually entered into the fluidized bed reactor ③, thereby providing effective power for fluidization of inner glass spheres. The fluidized glass spheres highly contacted with the atomized droplets ejected from the atomizing nozzle ⑥ after the fluid flowed through the diaphragm metering pump ④, liquid control valve, and volumetric flowmeter ⑤. After a while, the liquid and air were exhausted from the top of the reactor, and the coated glass spheres were collected by ultrafine mesh. The operation parameters of the reactor system were described as followed: temperature of 20 ± 2°C, fluidizing air flowrate of 240 ± 5 m³/h, liquid spray rate of 1.7 ± 0.1 L/h, and atomization pressure of 0.2 MPa.

2.4 Preparation and characterization of thermoresponsive glass sphere-based composite carriers

2.4.1 Fabrication of thermoresponsive composite carriers

PNIPAAm copolymers were dissolved in anhydrous ethanol at a concentration of 10 mg mL⁻¹, stirred at room temperature for 12 h, and then purified with Millipore filter (0.2 µm) to remove any impurities. To compare the experimental results, the glass spheres were treated in two different ways:

I The copolymer solution was poured into the reservoir, and appropriate glass spheres were placed in the bottom-spray fluidized bed reactor and kept steady suspension by blowing. The atomizer was turned on immediately and turned off after 30 s, and blowing was continued to accelerate the evaporation of anhydrous ethanol on the sphere surfaces. The coated glass spheres were put in a vacuum oven and annealed at 125°C for 3 h. After that, any unconnected copolymers were extracted by soaking and washing the modified glass spheres in absolute ethanol and deionized water thoroughly. The mixture was then filtered and dried under a vacuum at room temperature. These were known as sprayed composite carriers.

Figure 1: Bottom-spray fluidized bed reactor system: (a) experimental rig; (b) flow diagram: ① fan, ② anemometer, ③ bottom-spray fluidized bed reactor, ④ diaphragm metering pump, ⑤ volume flowmeter, ⑥ atomizing nozzle, ⑦ air distribution plate, ⑧ glass spheres, ⑨ superfine mesh; (c) schematic diagram for the bottom-spray coating process.

II The glass spheres were immersed into the copolymer solution directly and soaked for 3 h. The coated carriers were collected through filtration and drying and then annealed at 125°C for 3 h under vacuum. The subsequent treatment was the same as that of the first way. These were termed as immersed composite carriers.

2.4.2 Composition analysis and structural characterization

For composition analysis, the sprayed and immersed composite carriers were ground into tiny particles with agate and mixed evenly with KBr powder. The specimens were determined with Nicolet Magna 750 FTIR spectrometer in the wavenumber range of 4,000–500 cm^{-1} , with a 32 scan per sample cycle.

For structure characterization, general view, and surface microstructure of bare glass spheres, the sprayed and immersed composite carriers were observed through a cold-field emission scanning electron microscope (SEM, su8000, Hitachi, Japan). The samples were sputtered with a thin layer of gold before imaging and measured under standard vacuum conditions at an acceleration voltage of 15 kV.

2.4.3 Grafting density and equilibrium swelling

The grafting density of the sprayed and immersed composite carriers was determined gravimetrically and

calculated based on the mass difference before and after the respective functionalization per outer surface area of glass sphere:

$$\text{Grafting density} = \frac{W_2 - W_1}{A} = \frac{W_{\text{dry}}}{A} \quad (1)$$

where W_1 is the dry weight of 100 randomly selected bare glass spheres, W_2 is the dry weight involving 100 sprayed or immersed composite carriers extracted at random, and A is the outer surface areas of 100 glass spheres with a diameter of 2.5 mm ($A \approx 19.635 \text{ cm}^2$). The results were reported along with the error bars representing the standard deviation of triplicate samples.

The temperature dependency of the sprayed and immersed composite carriers was investigated in triplicate by measuring the equilibrium swelling ratio using a classical gravimetric method at 20°C and 40°C. The total grafting amount (W_{dry}) of 100 dry sprayed or immersed composite carriers was initially calculated from the mass difference ($W_2 - W_1$). These 100 composite carriers were then submerged and kept in deionized water at 20°C (below LCST) for at least 24 h. The equilibrium swelling behavior was then determined by removing the modified glass spheres from the bath through a fine mesh screen, wiping off the water on the surfaces with moistened filter paper and immediately measuring the weight (W_3 , $W_{\text{wet}, 20^\circ\text{C}} = W_3 - W_1$). After that, the samples were immersed in fresh deionized water thoroughly and returned to the vacuum oven at room temperature for 24 h to obtain the dried composite carriers again (21). Then, the composite carriers were placed in deionized water at 40°C (above LCST). The following

steps were the same as described above to get the wet weight of composite carriers at 40°C ($W_{\text{wet},40^\circ\text{C}}$). The equilibrium swelling ratio (ESR) was defined as follows (22,23):

$$\text{ESR} = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \quad (2)$$

2.4.4 Biocompatibility and smart responsiveness

After autoclaved, the bare glass spheres and sprayed composite carriers were immersed into a basic culture medium for complete swelling and then placed into the silanized culture plates. Bone marrow mesenchymal stem cells (BMMSCs) were incubated at a cell density of 1×10^5 cells/mL⁻¹ onto the bare and modified glass spheres. BMMSCs were cultured at 37°C with 5% CO₂. After 24 h, the used culture medium was replaced with an equal amount of fresh culture medium. The OD value was measured with Cell Counting Kit-8 (cck-8, Dojindo, Japan) to determine the number of cells adhered to the bare and modified glass spheres. In the other groups, the culture plates were kept in a sterile environment at 20°C, followed by replacing the medium with the cold and fresh culture medium without FBS and pipetting gently; 10 min later, the procedure for cck-8 testing was repeated to analyze the number of cells detached from the glass spheres and composite carriers. The experimental results were averaged from six repeated runs with a standard deviation.

3 Results and discussion

3.1 Synthesis and identification of thermoresponsive PNIPAAm copolymers

NIPAAm monomers are commonly used as reactants and can be polymerized through initiator, heating, and irradiation to form polymer chains or gels. However, the inherent performance of PNIPAAm homopolymers makes them difficult to expand the applications. Therefore, the final products with different response speeds and transition temperatures are prepared by changing reaction conditions or adding functional groups according to various requirements (13). In this study, PNIPAAm copolymers were synthesized by free-radical polymerization

Figure 2: Molecular structure of PNIPAAm copolymer and covalent linkage between glass spheres and copolymers during the preparation of composite carriers.

with NIPAAm, HPM, and TMSPM as reactants. The copolymer molecules consisted of functional groups and reactive groups, as shown in Figure 2. Of all the reactants, NIPAAm was the main monomer, and amide linkages and isopropyl bonds located in the copolymers were functional groups, making the final products to have temperature sensitivity. TMSPM was a cross-linker that was stable during polymeric synthesis but that was effective at forming cross-linking bonds with any materials bearing hydroxyl groups (24). HPM can provide useful hydroxyl groups for the bonding between molecular chains of copolymers. Both siloxane bonds of TMSPM and hydroxyl groups of HPM were employed as the reactive groups of PNIPAAm copolymers. The condensation reaction between hydroxyl and methoxyl groups resulted in the release of methanol, and the newly formed bonds provided integration within the film and coupling with a surface bearing residual hydroxyl groups.

The synthetic PNIPAAm copolymers were important precursors of thermoresponsive glass sphere-based composite carriers. Although the copolymer chains would be changed into the cross-linking structure during heating treatment, the basic performance of the copolymer segments within PNIPAAm copolymer films did not change significantly; therefore, the comprehensive properties of the copolymers directly affected the application potentials of

thermoresponsive carriers. The FTIR spectrum confirmed that NIPAAm, HPM, and TMSPM were involved in the resultant copolymers, and the copolymers had useful functional groups of amide linkages and isopropyl groups, as well as valuable reactive groups of siloxane bonds and hydroxyl groups. The ^1H -NMR spectrum indicated that the molar ratio of NIPAAm, HPM, and TMSPM in the final product was in strong agreement with the feeding ratio of 20:1:1. The average weight molecular weight of the composites was $24,061\text{ g mol}^{-1}$, the average number molecular weight was $15,182\text{ g mol}^{-1}$ with the polydispersity index of 1.584, and the degree of polymerization was about 6. The resultant copolymers had remarkable thermosensitivity with the lower critical solution temperature (LCST) of around 26°C . All the identification results were summarized in Figure 3. Therefore, PNIPAAm copolymers can be used for the preparation of thermoresponsive glass sphere-based composite carriers.

3.2 Preparation of thermoresponsive glass sphere-based composite carriers

In our previous studies, siloxane and hydroxyl groups were introduced into the polymer backbone; thus, PNIPAAm copolymers were successfully grafted onto the surfaces of glass coverslips and silicon wafers. The

temperature-sensitive copolymer films with different film thicknesses and surface wettability were obtained through adjusting the concentration of copolymer solution, speed of spin or dip coating, as well as operating time. The prepared PNIPAAm copolymer films showed favorable thickness controllability, structural stability, rapid response, reusability, and biocompatibility based on the characterization results, which can be applied in the cultivation and nonenzymatic recovery of various adherent cells (15,19). The development of temperature-sensitive materials should not be limited to 2D surfaces. It is urgent to develop 3D thermoresponsive substrates to meet a wide range of requirements. However, the above-mentioned spin and dip coating can only be used for the preparation of planar films, but they are helpless to get the uniform coating on the surfaces of 3D substrates. Hence, it is necessary to introduce available coating techniques.

This study provides a novel preparation strategy for thermoresponsive glass sphere-based composite carriers on the strength of bottom-spray fluidized bed reactor: PNIPAAm copolymers are first synthesized, then the copolymer solution is evenly coated on the surfaces of glass spheres via bottom-spray coating technology, and finally, the thermoresponsive composite carriers are available fabricated by thermal annealing. In the typical bottom-spray coating process, the droplets produced by copolymer solution are sprayed from the

Figure 3: Identification results of PNIPAAm copolymers: (a) FTIR spectrum, (b) ^1H -NMR spectrum, and (c) DLS plots.

The disappearance of the characteristic absorption peak at $1,079\text{ cm}^{-1}$ arising from siloxane bonds of PNIPAAm copolymers in the FTIR spectrum of the sprayed composite carriers had demonstrated that the siloxane bonds had lost methoxy structure owing to methanol removal. Hence, it could be confirmed that the temperature-responsive PNIPAAm copolymers had been energetically grafted onto the surfaces of glass spheres by bottom-spray coating technology.

3.3.2 Surface topography

The SEM analysis of bare glass spheres, sprayed and immersed composite carriers, showed significant morphological changes on account of the formation of interactive PNIPAAm copolymer chains during heating treatment, as shown in Figure 5. The commercial glass spheres were spherical in shape with an average diameter of 2.5 mm. The surface of the bare glass sphere looked smooth with the primary amplification of 50, whereas some scratches caused by mutual friction could be observed with a much greater magnification of 500. After coating and annealing treatment, the surfaces of both modified glass spheres became rough. Some tiny particles appeared on the surface of the immersed composite carrier with smaller magnification, and several particles deposited on the partial glass sphere were visible with larger magnification, but they are not much and not crosslinked. It also can be seen that the surface of the sprayed composite carrier was covered with particles under lower magnification, while the film layer with obvious thickness had formed underneath the interspersed

particles with an increase in magnification. The results showed that only a few copolymer particles could be connected and distributed on the surfaces of glass spheres by simply immersing into the copolymer solution, whereas PNIPAAm copolymers could not only be grafted to the glass spheres by bottom-spray fluidized bed reactor but also formed a coating layer on the sphere surfaces, thus developing the PNIPAAm copolymer/glass sphere composite carriers.

3.3.3 Grafting density and equilibrium swelling

The grafting density of the sprayed and immersed composite carriers was summarized in Table 1. There was virtually a slight increase in the weight of the immersed composite carriers with smaller grafting density in comparison to bare glass spheres. However, higher grafting density had shown in the group of the sprayed composite carriers. This result reflected the advantages of the bottom-spray fluidized bed used for the coating of sphere surfaces. In the spraying process of copolymer solution, the atomized droplets constantly converge on and quickly dry on the surface of the glass sphere, thus forming a continuously accumulated and superposed copolymer coating on the sphere surface, as shown in Figure 1c. The superiority of bottom-spray coating technology is that the probability of the droplets touching the spherical surface in different directions is almost equal, so a uniform and dense copolymer film can be formed on the sphere surface through high temperature under vacuum. In the immersed method, only partial copolymers can be adsorbed onto the surface of the glass

Figure 5: Surface morphology of glass spheres (a and d), immersed composite carriers (b and e), and sprayed composite carriers (c and f).

Table 1: Grafting density and equilibrium swelling ratio of sprayed and immersed composite carriers

Group	Weight					
	W_{dry} (g)	Grafting density ^a ($\mu\text{g}/\text{cm}^2$)	$(W_{\text{wet}, 20^\circ\text{C}} - W_{\text{dry}})$ (g)	ESR _{20°C}	$(W_{\text{wet}, 40^\circ\text{C}} - W_{\text{dry}})$ (g)	ESR _{40°C}
Sprayed composite carriers ^b	0.0176 ± 0.0012	896.36 ± 61.12	0.0790 ± 0.0045	4.489	0.0268 ± 0.0028	1.523
Immersed composite carriers ^b	0.0031 ± 0.0002	157.88 ± 10.19	0.0087 ± 0.0015	2.806	0.0035 ± 0.0006	1.129

^a $A \approx 19.635 \text{ cm}^2$. ^b 100 randomly selected samples.

sphere, and the homogeneity of copolymer adsorption cannot be supported due to acting forces.

The equilibrium swelling behaviors of the sprayed and immersed composite carriers were measured at 20°C and 40°C. Because the molecular chains of PNIPAAm copolymers required some time to accept appropriate conformation at a fixed temperature (26), the composite carriers to be measured were given adequate response time to approach the equilibrium swelling state. Table 1 also displayed that the PNIPAAm mediated composite carriers in both cases absorbed significantly more water at lower temperatures and become dehydrated at the temperature above LCST. As expected, the swelling ratio of the sprayed composite carriers decreased from 4.489 at a lower temperature to approximately 1.523 at 40°C with an excluded water content of 66.07 wt%. A similar temperature-dependent tendency was also found in the immersed composite carriers but with a low swelling ratio of 2.806 at 20°C and a lower swelling ratio of 1.129 at a higher temperature. This result further proved that more PNIPAAm copolymers could be grafted onto glass spheres via the self-made bottom-spray fluidized bed reactor when compared with a simple soaking treatment. The weight reduction confirmed that both sprayed and immersed composite carriers showed thermoresponsive character via phase transition of PNIPAAm side chains. Upon immersion in deionized water at 20°C, greater swelling of the composite carriers displayed under the synergistic action of hydrated hydrogen bonds consisting of water molecules and hydrophilic amide bonds, hydroxyl groups, and siloxane bonds. Strong syneresis would be activated by heating to 40°C owing to strengthening hydrophobic interaction and nonhydrous hydrogen bonds within PNIPAAm copolymer chains and expelling off the excess water from the interpenetrating copolymer networks (27). This temperature-dependent swelling ratio was important for the application of thermoresponsive substrates in cell culture and harvest. The above results not only indicated that PNIPAAm copolymers grafted on the glass spheres

were still sensitive to temperature but also confirmed that much more copolymers could be connected to the glass surfaces by bottom-spray coating technology.

3.3.4 Cell attachment and detachment

There are great achievements in the studies on the PNIPAAm-mediated 2D temperature-responsive culture substrates in recent years. However, some limitations should also be considered. It is difficult to simulate the natural growth of cells, and material preparation and cell culture are time-consuming and tedious with lower productive rate and purity. The 3D cell culture systems can not only retain the material and structural basis of the microenvironment *in vivo* but also reflect the intuition and controllability during cell culture *in vitro* and, furthermore, facilitate the combination of cell culture techniques and tissue engineering applications. Therefore, researchers attempt to introduce PNIPAAm and its derivatives into stereoscopic systems to form 3D temperature-sensitive substrates (28–31), which could be applied in large-scale amplification and nonenzymatic harvest of adherent cells as well as repair or reconstruction of tissues and organs. It is preferred to culture cells with 3D thermosensitive carriers or scaffolds. This mode is conducive to intercellular signal transduction and cytokine interaction, to maintain the original physiological characteristics of cells. The goal of large-scale amplification and nonenzymatic harvest of cells can be achieved in a short time and a small space, owing to an increase in the cell growth area and the introduction of temperature-sensitive PNIPAAm.

As mentioned earlier, HPM and TMSPM introduced during copolymer synthesis can be coupled with hydroxyl-containing materials of any shape and size and can also form linkages between molecular segments of PNIPAAm copolymers. The copolymer solution was evenly sprayed on the surfaces of glass spheres via a bottom-spray fluidized bed reactor, and the connection

Figure 6: Attachment and detachment of BMSCs on the glass spheres and sprayed composite carriers (** $p < 0.05$).

between copolymers and substrates was made by heating and annealing, to form a stable copolymer film layer on the sphere surfaces. BMSCs were then cultured on bare glass spheres and sprayed composite carriers. Figure 6 showed the adhesion and deadhesion of BMSCs after 24 h culture. Due to adaptations to new growth environments, there was no obvious BMSCs multiplication after 24 h short-term culture. The number of cells adhering to the surfaces of the sprayed composite carriers was slightly less than that of the control group, but there was no significant difference between the two groups, indicating that BMSCs showed good growth status in both carriers. The results proved that both carriers had good biocompatibility, providing effective growth space for cell adhesion and growth. When the temperature dropped to 20°C, the number of cells attached to the glass spheres only displayed a slight decline, owing to pipetting treatment, while few BMSCs adhered on the sprayed composite carriers, showing a statistically significant difference between the two groups ($p < 0.05$). This demonstrated that an available culture mode of effective adhesion and spontaneous desorption of cells can be designed using thermoresponsive composite carriers, offering an effective nonenzymatic hydrolysis harvesting method for the cultivation of adherent cells *in vitro*.

4 Conclusions and outlook

In this study, combined with the preparation scheme of smart responsive planar films and bottom-spray coating technology, the synthesized copolymers were evenly

coated on the surfaces of glass spheres to form thermoresponsive composite carriers, and the properties of the prepared carriers were characterized. PNIPAAm copolymers with temperature response were first synthesized by free-radical polymerization. The copolymer solution was uniformly coated on the surfaces of glass spheres by spray coating, and the PNIPAAm copolymer-glass sphere composite carriers were then obtained by heating and annealing. The FTIR spectra showed the characteristic functional groups of the composite carriers and confirmed the successful grafting of PNIPAAm copolymers on the glass spheres through a bottom-spray fluidized bed reactor. The SEM analysis displayed that the copolymers can not only be grafted on the glass spheres but also form a film layer on the glass surfaces, to obtain the smart responsive composite carriers. The results of grafting density and equilibrium swelling behaviors indicated that PNIPAAm copolymers grafted on the glass spheres were still sensitive to temperature and further confirmed that much more copolymers could be connected to the glass surfaces by bottom-spray coating technology. Cell attachment and detachment experiments demonstrated that the thermoresponsive glass sphere-based composite carriers had excellent biocompatibility, and readily cell adhesion and spontaneous cell removal could be easily controllable by temperature change.

The thermoresponsive composite carriers fabricated in this study can be used in laboratory studies and clinical trials through several paths. First, PNIPAAm-grafted glass spheres can be employed as functional substrates for *in vitro* culture and nonenzymatic recovery of adherent cells. If combined with bioreactor technology, it is expected to form an attractive culture system of large-scale cell expansion *in vitro*. Second, the core glass of the thermoresponsive composite carriers can be etched by template method to form copolymer capsules with temperature responses. The loading and controlled release of targeted drugs is available by changing temperature in the thermoresponsive capsules. Hence, it is necessary to investigate more deeply on the performance parameters and preparation technology of the thermosensitive composite carriers.

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