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| Original Article |

Successive Grafting of PHEMA and PIPAAm onto Cell Culture Surface Enables Rapid Cell Sheet Recovery

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Abstract : Fabrication of functional tissue constructs from designed three-dimensional structure of cells using layered method of cultured cell sheets could prove to be an attractive approach to tissue engineering. Rapid recovery of cell sheets is considered important to maintain the biological functions and viability of recovered cell sheets, as well as for practical assembly of tissue structures. To accelerate required culture substrate hydrophilic/hydrophobic structural changes in response to culture temperature alteration, different amounts of poly(2-hydroxyethyl methacrylate) (PHEMA) and poly(*N*-isopropylacrylamide) (PIPAAm) were grafted successively onto tissue culture polystyrene (TCPS) dishes by electron beam irradiation. Analysis by attenuated total reflection-Fourier transform infrared revealed that PHEMA and PIPAAm were successfully grafted to the surfaces of TCPS dishes. PIPAAm-PHEMA-grafted TCPS (PIPAAm-PHEMA-TCPS) dishes were compared with PIPAAm-grafted TCPS (PIPAAm-TCPS) dishes for cell sheet detachment experiments. Approximately 75 min was required to completely detach cell sheets from PIPAAm-TCPS dishes, compared to only 13 min to detach cell sheets from PIPAAm-PHEMA(40 wt%)-TCPS dishes, which is successively grafted with 40 wt% of HEMA and 60 wt% IPAAm solutions. PHEMA is a well-known as a highly hygroscopic polymer. In the case of PIPAAm-PHEMA-TCPS dish, PHEMA layer acted as a water pool to accelerate the hydration of PIPAAm layer due to the effective and simultaneous water supply to PIPAAm layer, resulting in rapid hydration of grafted PIPAAm molecules and detachment of cell sheet compare to PIPAAm-TCPS dishes.

Key words: *PHEMA, PIPAAm, cell sheet engineering, temperature-responsive polymer, tissue engineering, surface modification*

1. Introduction

Rapid detachment of intact cultured cell sheets is a very important recovery method to fabricate a functional tissue structure by stratification of recovered cell sheets. To recover intact cell sheets from culture surfaces, we have fabricated tissue culture polystyrene (TCPS) dishes grafted with poly(*N*-isopropylacrylamide) (PIPAAm) that show hydrophilic/hydrophobic surface property alterations in response to temperature changes.[1-6] Cultured cell sheets form on these surfaces, and we have successfully recovered intact, viable cell sheets to construct 3-dimensional tissue-like structures. The hydrophobic, collapsed PIPAAm-grafted surface above the LCST can be modulated to hydrophilic by decreasing culture temperature below the LCST.

We have already reported that cells cultured on TCPS surfaces grafted with PIPAAm can be recovered by reducing temperature without enzymatic treatment.[1-3] In previous reports, we have described the recovery of bovine aortic endothelial cell sheets from PIPAAm-grafted TCPS surfaces,[4] and also showed that cell sheets are recovered together with their deposited fibronectin matrix by low temperature treatment.[5] These recovered cell sheets easily attached to another surfaces and proliferated again probably due to the recovered extracellular matrices including

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fibronectin component accompanied with detached cell sheets.

However, cell sheet detachment from surfaces of TCPS grafted with PIPAAm is slow, occurring gradually from the sheet periphery toward the interior. Thus, significant time at reduced temperature is required to recover an intact cell sheet completely. Rapid detachment of cultured cell sheets is a very important recovery method that permits facile manipulation of the sheet and prevents functional damage. The rate limiting step to cell sheet recovery is the hydration of the underlying PIPAAm grafted surface. Previously, we have reported that the use of porous membranes grafted with PIPAAm as culture substrates facilitates the rapid cell sheet detachment.[7]

Also we have fabricated porous membranes co-grafted with PEO chains and PIPAAm to achieve much more rapid cell sheet detachment than porous membrane grafted with only PIPAAm. It allowed rapid access and diffusion of water molecules from the beneath as well as peripheral to cultured cell sheets, facilitating rapid hydration of grafted PIPAAm chains and cell sheet detachment.[8]

To accelerate the hydration of the hydrophobized PIPAAm segments interacting to the cell sheet, grafting of a highly hygroscopic polymer under PIPAAm layer, which is able to provide water molecules to help to hydrate of PIPAAm layer is optimal. In the present study, the PHEMA was grafted firstly onto TCPS dishes and then PIPAAm was grafted onto PHEMA-TCPS dish surfaces to achieve much more rapid cell sheets detachment than TCPS dishes grafted with PIPAAm only.

2. Materials and Methods

2.1 Materials

N-Isopropylacrylamide (IPAAm) was kindly provided by Kohjin (Tokyo, Japan) and used after recrystallization from *n*-hexane. 2-Hydroxyethyl methacrylate (HEMA) was purchased from Aldrich (Milwaukee, WI). Tissue culture grade polystyrene dishes (TCPS, Falcon 3001) were purchased from Becton Dickinson Labware (Oxnard, CA, USA). Trypsin-EDTA solution, streptomycin, Dulbecco's modified Eagle's medium (DMEM) and penicillin were bought from Gibco BRL (Grand Island, NY, USA).

2.2 Preparation of PIPAAm-PHEMA-TCPS Dish

PIPAAm-PHEMA-TCPS dishes used for single cells and cell sheet recovery were prepared as follows: HEMA monomer dissolved in 2-propanol to be 20~60 wt% concentrations. This monomer solution (30 μL) was spread uniformly onto TCPS dishes and then electron beam irradiated using an Area Beam Electron Processing System (Curetron EBC-200-AA2, Nissin-High Voltage Co. Ltd., Kyoto, Japan) at a radiation dose of 0.3 MGy (acceleration voltage of 150 kV under 1.0×10^{-4} Pa). Unreacted monomer and ungrafted polymers were washed out with distilled water. And then thermo-responsive PIPAAm was grafted onto PHEMA-grafted TCPS dishes as follows: IPAAm monomer dissolved in 2-propanol to be 60 wt% concentrations. A 60 μL of monomer solution was spread uniformly onto PHEMA-grafted TCPS dishes and then electron beam irradiated as previously mentioned. Unreacted monomer and ungrafted polymers were removed by extensive washing with cold distilled water, and the PIPAAm-PHEMA-TCPS dishes were dried *in vacuo* at room temperature.

2.3 Surface Characterization

The amounts of PHEMA and PIPAAm grafted onto TCPS dishes were determined by ATR-FTIR (JASCO Valor-III, Tokyo, Japan).[4,7,8] The control substrates, ungrafted TCPS dishes, have strong absorption band attributed to aromatic group at 1600 cm^{-1}. As PHEMA was grafted onto TCPS dishes, an ester absorption band appeared in the region of 1720 cm^{-1}. As PIPAAm was grafted onto PHEMA-TCPS dishes, an amide absorption band appeared in the region of 1650 cm^{-1}. The peak intensity ratios of $I_{1720/1600}$ and $I_{1650/1600}$ were used to determine the amount of grafted PHEMA and PIPAAm, respectively. All the sample surfaces were also analyzed using ESCA (PHI 5800 ESCA system, Physical Electronics, Chanhassen, MN, USA). Survey spectra were acquired with a take-off angle of 90° and surface elemental compositions were calculated from integrated peak areas for each element.

2.4 Contact Angle Measurements

Four samples each of ungrafted TCPS, PIPAAm-TCPS, PIPAAm-PHEMA(20 wt%)-TCPS, PIPAAm-PHEMA(40 wt%)-TCPS, and PIPAAm-PHEMA(60 wt%)-TCPS dishes were cut in size (1.5×0.8 cm) to measure water contact angles. Water contact angles were determined by sessile drop method at 20 and 37°C with a FACE contact angle meter (Image processing type CA-X, Kyowa Interface Science, Niiza, Saitama, Japan). All samples were measured five times and averaged. Contact angles at 20 and 37°C were presented as a mean value (n=5) with standard deviation.

2.5 Cell Culture

Bovine aortic endothelial cells (BAECs) were purchased from Clonetics Co. (MD, USA) and cultured on TCPS dishes in DMEM supplemented with 10% FBS, 100 units/mL of penicillin, 100 μg/mL of streptomycin at 37°C in a humidified atmosphere with 5% CO_2. BAECs were recovered from

ungrafted TCPS dishes by treatment with 0.25% trypsin-0.26 mM EDTA in PBS and subcultured on ungrafted TCPS dishes, and each modified surface. Cell morphology was monitored and photographed periodically under phase-contrast microscopy (ET300, Nikon, Tokyo, Japan).

2.6 Detachment of Single Endothelial Cells

Detachment of single endothelial cells was achieved using low temperature treatment after incubation at 37°C for 3 hr. BAECs were plated on each surface at a density of 3×10^4 cells/cm^2 and cultured for 3 hr to allow attachment and spreading on each polymer-grafted surface. For low temperature treatment, spread cells were transferred to a CO_2 incubator equipped with a cooling unit fixed at 20°C. After 5, 10, 15, 20, 30 and 45 min incubation, cell morphology was observed using a phase-contrast microscope and photographed. Both rounded and spread cells in the photographs were counted, and the percentage of rounded cells to the total cells counted were presented as a mean value and standard deviation. Ungrafted TCPS dishes were used as controls. The percent recovery of single cells from PIPAAm-PHEMA-TCPS dishes was compared to PIPAAm-TCPS dishes and ungrafted TCPS dishes.

2.7 Detachment of Confluently Cultured Endothelial Cell Sheets

For cell sheet detachment experiment, BAECs were plated onto each surface at a density of 1.3 times confluency (1.3×10^6 cells/dish) and cultured at 37°C. After 24 hr incubation, unattached cells were removed by medium exchange. Cell sheets were cultured for 8 days after reaching confluency, and each plate was transferred to the CO_2 incubator equipped with a cooling unit fixed at 20°C, and periodically taken out from incubator to acquire photographs during detachment. The photographs were scanned into a computer system for analysis. Software, NIH Image (ver. 1.61) was used to measure the area of each detached cell sheet. Areas of detached cell sheets relative to *in situ* confluently cultured cell sheet area were calculated and averaged from four photographs of each sample.

3. Results and Discussion

3.1 Surface Characterization

Elemental analyses of TCPS dishes grafted with PIPAAm, PHEMA and PIPAAm, and ungrafted TCPS dishes were carried out using ESCA, and surface composition results are summarized in Table 1. Increased atomic percent of nitrogen was observed on PIPAAm-TCPS and PIPAAm-PHEMA-TCPS dish surfaces after electron beam irradiation. Because control TCPS dish surface does not contain nitrogen atom in their chemical structures, these results directly support PIPAAm grafting onto TCPS dishes by electron beam irradiation. Also, grafting of PHEMA on TCPS dishes was confirmed by the increase in oxygen content and decrease in carbon content on PIPAAm-PHEMA-TCPS dish surfaces as a function of concentration of HEMA solution used for grafting. Amounts of PHEMA and PIPAAm grafted onto TCPS dishes determined by comparison with ATR-FTIR standard curves are presented in Table 2. The amounts of grafted PHEMA on TCPS dishes were increased with increasing concentration of HEMA monomer solutions used for grafting. All the samples had consistent surface amount of grafted PIPAAm except PIPAAm-PHEMA(60 wt%)-TCPS dish, which enabled direct comparison of detachment of cells and cell sheets from each modified surface. The amount of grafted PIPAAm on PIPAAm-PHEMA(60 wt%)-TCPS dish is higher than the other surfaces probably due to the increased penetration of IPAAm monomer

Table 1. Amounts of PHEMA and PIPAAm grafted on TCPS dishes determined by ATR-FTIR.

Substrate	PHEMA	PIPAAm
	($\mu g/cm^2$)	
Ungrafted TCPS dish	0	0
PIPAAm-TCPS dish	0	1.9±0.5
PIPAAm-PHEMA (20 wt%)-TCPS dish	28±4	1.8±0.4
PIPAAm-PHEMA (40 wt%)-TCPS dish	42±5	2.0±0.5
PIPAAm-PHEMA (60 wt%)-TCPS dish	73±8	3.1±0.5

Table 2. Atomic composition of each surface determined by ESCA.

Substrates	Atom (%)		
	C	N	O
Ungrafted TCPS dish	87.9	10.8	1.3
PIPAAm-TCPS dish	85.8	7.8	6.4
PIPAAm-PHEMA (20 wt%)-TCPS dish	72.7	16.3	6.0
PIPAAm-PHEMA (40 wt%)-TCPS dish	68.8	18.1	7.1
PIPAAm-PHEMA (60 wt%)-TCPS dish	65.0	21.6	7.4

Table 3. Water contact angle(°) of each surface measured by sessile drop method.

Substrates	20	37
Ungrafted TCPS dish	66.2±0.8	66.4±3.0
PIPAAm-TCPS dish	54.0±1.8	66.0±2.5
PIPAAm-PHEMA (20 wt%)-TCPS dish	50.0±2.2	64.0±3.5
PIPAAm-PHEMA (40 wt%)-TCPS dish	48.7±2.6	64.1±2.9
PIPAAm-PHEMA (60 wt%)-TCPS dish	47.7±3.8	60.3±3.6

into PHEMA layer and formation of interpenetrated structure.

Table 3 shows water contact angle data from each surface using the sessile drop method at 20 and 37°C. PIPAAm-grafted surfaces exhibited decreasing contact angles by lowering temperature from 37 to 20°C, while ungrafted TCPS dishes had negligible contact angle changes with changing temperature. This result indicates that PIPAAm surfaces, hydrophobic at the higher temperature, became markedly more hydrophilic in response to temperature reduction due to spontaneous hydration of surface-grafted PIPAAm.[1-5] Water contact angle on PIPAAm-PHEMA-TCPS dishes a little bit decreased than PIPAAm-TCPS dishes at both 20 and 37°C, and water contact angle slightly decreased as the PHEMA content increases.

We previously reported a quite large contact angle changes of PIPAAm-modified glass plate surfaces.[9] Large contact angle changes observed for PIPAAm-grafted surfaces were due to the existence of free mobile chain end of linear PIPAAm grafts on the surfaces. Electron beam irradiation used for PIPAAm grafted culture dish preparation caused cross-linking reaction within the grafted PIPAAm chains in part, restricting PIPAAm chain mobility. Thus, the contact angle changes observed on PIPAAm-PHEMA-TCPS dishes were at the most 15°. This difference is sufficient to enable cell detachment by low temperature treatment. Tamada and Ikada[10] reported contact angles of material surfaces strongly influenced on cell attachment behavior, and showed maximum cell attachment around 60° of contact angle. In our system, contact angle at 37°C should be suitable for cell attachment, hydration of the surface grafted PIPAAm chains might affect to detach cultured cells. There is negligible contact angle difference between PIPAAm-PHEMA(20 wt%)-TCPS and PIPAAm-PHEMA(40 wt%)-TCPS dish surfaces above and below the LCST, despite of introduction of different amount of PHEMA chains.

3.2 Cell Culture

Seeded BAECs were well attached and spread on ungrafted TCPS, PIPAAm-TCPS and PIPAAm-PHEMA-TCPS dish surfaces at 37°C. After 3 hr incubation at 37°C, almost all of seeded cells were attached and spread on those surfaces. Cell attachment and spreading on each surface was nearly identical (data not shown). PHEMA is well known as a highly hygroscopic polymer and cross-linked PHEMA hydrogel is utilized as soft contact lens.[11] Hydrophilic polymer surfaces, such as PEG-containing surfaces are received wide recognition as a biomaterial because of its unique adhesive resistant properties for biomolecules and cells.[12,13] Its lack of interaction with proteins and other biological entities makes it a promising material for use in many biomedical applications.[14-17] In this study, however, even hygroscopic PHEMA chains were grafted on TCPS dishes, cells were well attached and spread on each surface. This is probably because PIPAAm chains were existed onto the surface of PHEMA layers. Cells recognized only hydrophobic PIPAAm layer not PHEMA layer at above its LCST.

3.3 Single Cell Detachment

When the culture temperature was reduced to 20°C after almost all of seeded cells were attached and spread on those surfaces (3 hr incubation), spread cells were rounded and detached from all the surfaces containing PIPAAm layer. This is because that PIPAAm is hydrated below its LCST, producing an expanded, swollen, hydrophilic surface. This surface property change weakened cellular adhesion, resulting in spontaneous cell detachment.

Fig 1 shows the percentage of detached single cells from the surface of PIPPAm-TCPS, PIPAAm-PHEMA(20 wt%)-TCPS, PIPAAm-PHEMA(40 wt%)-TCPS and PIPAAm-PHEMA(60 wt%)-TCPS dishes as a function of incubation time at 20°C. Detached cells are not observed from ungrafted control TCPS dishes because of unchange of surface property by reducing temperature. (data not shown). Spread cells cultured on the PIPAAm-PHEMA-TCPS dish surfaces detached more rapidly than those on PIPAAm-TCPS dishes.

In case of PIPAAm-PHEMA-TCPS dishes, water molecules for hydration of PIPAAm are supplied through underneath of adherent cells as well as from the periphery of each cell, while

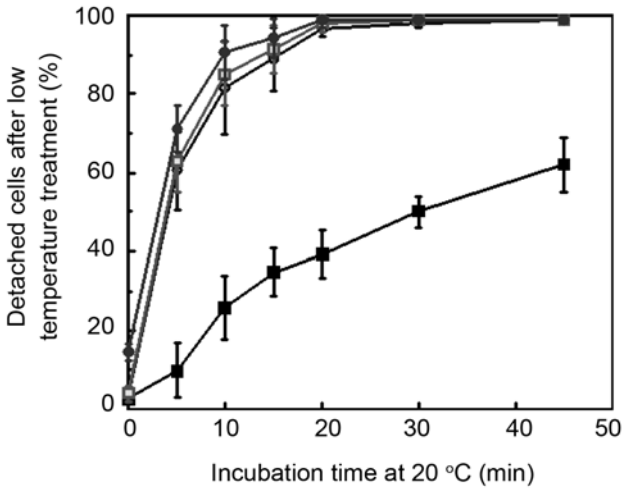


Figure 1. Percentage of detached single cells from the surfaces of PIPAAm-TCPS (■), PIPAAm-PHEMA (20 wt%)-TCPS (○), PIPAAm-PHEMA (40 wt%)-TCPS (□) and PIPAAm-PHEMA (60 wt%)-TCPS (●) dishes as a function of incubation time at 20°C.

water molecules to hydrate PIPAAm chains on PIPAAm-TCPS dishes can be penetrated the culture matrix from only the periphery of each cell to the interface between the cell and grafted PIPAAm chains.

Approximately 20 min incubation at below the LCST, nearly all of spread cells were detached from the surface of PIPAAm-PHEMA-TCPS dishes, while only 60% of cultured cells were detached from PIPAAm-TCPS dishes. PHEMA layers of highly hydrophilic and hygroscopic nature are able to trap enough water and supply water molecules to entire PIPAAm grafts. Rapid access of bulk water to PIPAAm grafts by the existence of PHEMA layers should accelerate single cells detachment.

Cultured single cells were detached more rapidly as the amounts of grafted PHEMA increases. Increasing amount of PHEMA chains of highly hydrophilic and hygroscopic nature are able to enhance water diffusion much more rapidly to entire PIPAAm grafts above the PHEMA layer, i.e.; much more rapid water supply is achieved by grafting of increasing amounts of PHEMA chains.

Detached cells adhere to and grow readily on other surfaces at 37°C. We already reported that cultured single hepatocytes were detached with pre-adsorbed fibronectin matrix by low temperature treatment from PIPAAm-TCPS surfaces as a result of physical cell contractile forces,[18] and detached cells maintain their differentiated function, i.e., albumin secretion, more than cells detached by trypsinization after re-adhesion because membrane proteins readily degraded by enzymatic digestion which resulted in extremely low function to adhere and grow on the new surfaces.[2] Recovered extracellular matrices including fibronectin with cells are considered to enhance re-adhesion of recovered cells to other surfaces.

3.4 Cell Sheet Detachment

When culture temperature is decreased below the LCST, after cells proliferate to confluency, cells readily detach from the temperature-responsive PIPAAm grafted surfaces with intact cell-cell junctions. Generally used enzymatic digestion method dissociated monolayer cell sheet to single cells, because membrane proteins and extracellular matrices are susceptible to destruct by usual enzymatic digestion. For example, even though the keratinocytes can be obtained in the membrane of sheet configuration for transplantation to burned patients in clinical application, recovery of cultured sheets are usually carried out with dispase, which induces significant destruction of cadherin and extracellular matrix proteins.[19]

Figure 2 shows changes in detached area of cell sheets on PIPAAm-TCPS, PIPAAm-PHEMA(20 wt%)-TCPS and

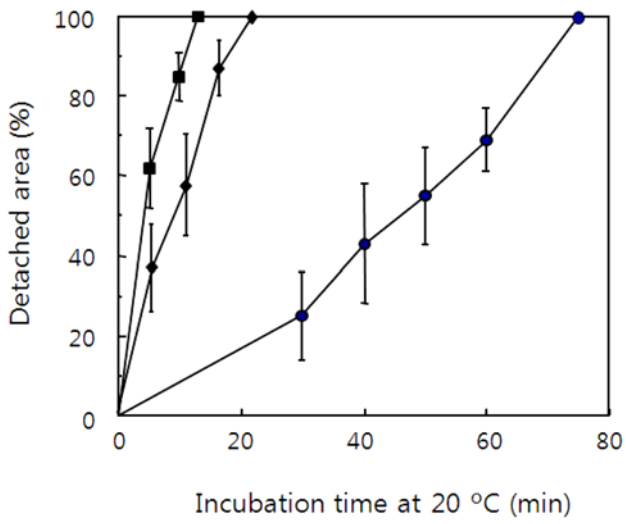
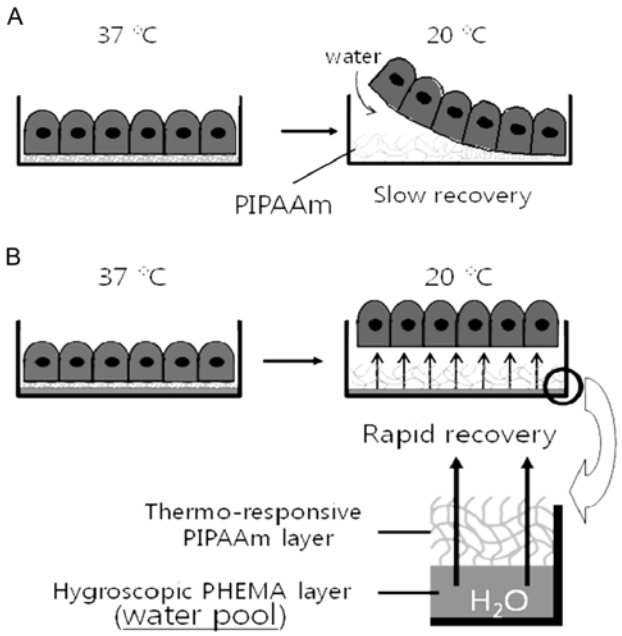


Figure 2. Detached area (%) of cell sheet from the surfaces of PIPAAm-TCPS (●), PIPAAm-PHEMA(20 wt%)-TCPS (◆) and PIPAAm-PHEMA (40 wt%)-TCPS (■) dishes as a function of incubation time at 20°C.

PIPAAm-PHEMA(40 wt%)-TCPS dishes as a function of reduced temperature treatment time. In the case of PIPAAm-PHEMA(60 wt%)-TCPS dishes, cells did not reach to confluency probably due to the relatively hydrophilic property at 37°C than other surfaces as shown in Table 3. Cell-cell junction was not enough to recover as an intact cell sheet.

Cell sheets are completely detached from PIPAAm-TCPS, PIPAAm-PHEMA(20 wt%)-TCPS and PIPAAm-PHEMA(40 wt%)-TCPS dish surfaces within 75, 22, and 13 min-incubation, respectively, with culture area of 9.8 cm^2 under quiescent culture conditions and reduced culture temperature. Considering that the amount of PIPAAm grafted on each surface is similar, and therefore, the degree of hydration force produced to detach adhered cells is also similar, the observed rapid cell sheet detachment from PIPAAm-PHEMA-TCPS dish surfaces is significant. Cell sheet are detached much more rapidly as the content of PHEMA increases.

These results support that accelerated detachment of cell sheets is due to the existence of PHEMA layer on TCPS dish surface. Cell sheets on PIPAAm-TCPS dish surfaces initially detached slowly, probably because water required to hydrate grafted PIPAAm could only penetrate from the cell sheet periphery. (Scheme 1A) Cell sheets on PIPAAm-PHEMA-TCPS dish surfaces detached more rapidly with onset of low temperature treatment, suggesting that PIPAAm-PHEMA-TCPS dish surfaces permit rapid re-hydration of PIPAAm layer by effective and simultaneous supply of essential water molecules through beneath as well as peripheral of cell sheets



Scheme 1. Illustration of cell sheet detachment by different types of water supply to (A) PIPAAm-TCPS and (B) PIPAAm-PHEMA-TCPS dish surfaces.

at 20°C. (Scheme 1B) This contrasting mechanism between each grafted surface is schematically depicted in Scheme 1.

Until now, we used the PIPAAm-grafted TCPS surfaces to detach cultured cell sheets at 20°C. Cell sheets formed on PIPAAm-grafted TCPS surfaces detach slowly and gradually, beginning from sheet edges and moving toward the cell sheet interior, presumably because water penetrates the interface between cell sheets and PIPAAm-grafted dish surfaces mainly from the periphery of cells (Scheme 1A). Time required to recover cell sheets completely from TCPS surfaces grafted with PIPAAm is therefore impractical. Hypothermic storage of cells is known to induce apoptosis,[20-22] which was mainly dependent on the storage time of cells or tissue at reduced temperature. Longer treatment time at reduced temperature to recover cell sheets might have negative effects on cell functions and viability. Therefore, rapid detachment of cultured cell sheet at reduced temperature is very important to prevent cells from functional damage and retain their viability. Chitin membranes were used to detach and transfer cell sheets to other surfaces with maintained cell polarity.[4] When the cell sheets are detached from the culture surfaces, they readily tend to contract because of strong cell-cell interaction,[23] therefore the use of chitin membranes as supporting materials enabled transferring of detached cell sheets without shrinkage. This idea also applied to achieve rapid detachment of cultured cell sheets.[24,25] While differentiated a renal epithelial cell line, Madin-Darby canine kidney (MDCK) cell sheets are detached from PIPAAm grafted TCPS dishes merely by reduced temperature after 4 week of culture, such cell sheet detachment were greatly repressed in the early stages of culture (up to three weeks) probably because the contractile force of MDCK cells is weak. Recently, we succeeded in the rapid harvest of confluent MDCK cell sheet and intact transfer to other culture dishes by utilizing hydrophilic poly(vinylidene difluoride) (PVDF) membrane as a cell sheet mover.[24] The whole MDCK cell sheet was harvested together with the PVDF membrane because the PVDF membrane stick to apical cell surfaces by physical attraction force. Below the LCST, the interaction between apical cell surfaces and the PVDF membrane should be higher than that between basal cell surfaces and hydrated PIPAAm surfaces. In the present report, however, we tried to achieve rapid cell sheet detachment under quiescent culture condition and reduced temperature without any supporting materials. If some supporting materials like a PVDF membrane are used, cell sheets might detach much more rapidly than those under quiescent culture condition from the PIPAAm-PHEMA-TCPS dish surfaces.

Previously, we have succeeded in fabricating stratified cell sheet culture by 2-dimensional cell sheet manipulation to construct tissue-like structures.[26] In natural organs, the parenchyma comprise intimately associated cell sheets. Liver comprises basically two sheets of hepatocytes and endothelial cells those are interconnected to form a continuous three-dimensional tissue. To construct liver lobule structure, basic unit of liver, hepatocytes and endothelial cells were cultured separately on PIPAAm-grafted TCPS dishes. Cultured confluent monolayer cell sheets of hepatocytes were detached by low temperature treatment and placed to a confluent endothelial cell sheet. Stratified cell sheets are highly resembled liver lobules histologically. Intact, viable cell sheet detachment by lower temperature treatment could prove useful to construct 3-dimensional tissue-like structures by fabricating sandwiches of cell sheets and associated ECM for application to tissue restoration, as a transplant material, or for construction of artificial organs. Thus, rapid detachment and recovery methods are needed to maintain cell phenotype and biological functions. TCPS dishes successively grafted with PHEMA and thermo-responsive PIPAAm are shown capable of achieving such dramatically rapid 2-dimensional cell sheet detachment.

4. Conclusions

The TCPS dishes successively grafted with PHEMA and

PIPAAm have been effectively applied to accelerate detachment of viable bovine aortic endothelial cell sheets from culture surfaces. Introduction of hydrophilic and hygroscopic PHEMA layer on TCPS culture surfaces had no adverse effect on cell attachment or proliferation. Introduction of hygroscopic PHEMA layer between TCPS dish surface and PIPAAm layer allows rapid and simultaneous supply of water molecules from beneath as well as peripheral to cultured cell sheets, facilitating rapid hydration of grafted PIPAAm molecules and cell sheet detachment. Rapid 2-dimensional intact cell sheet recovery with no damage by reducing temperature should prove interesting to fabricate 3-dimensional tissue-like structures by piling up of various types of cell sheets.

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